INVESTIGATION OF SURFACE-POTENTIAL CONTROLLED NUCLEATION USING AN ACOUSTIC LEVITATION APPARATUS

by

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ABSTRACT

The focus of this thesis was to investigate the effect that droplet surface potential has upon nucleation of a solute from levitated solution droplets; there being virtually no such investigations thereof in the current literature. An increase in droplet surface potential resulted in significant promotion to solute nucleation, as determined by the number of crystals observed, for sodium chloride, ammonium nitrate, α-cyano-4-hydroxycinnamic acid, and 2,4,6-trihydroxyacetophenone monohydrate solution droplets that were individually levitated using an electrodynamic levitation trap. A change in NaCl crystal habit was also observed for a population of levitated droplets once a surface charge density threshold of \( \sim 9 \times 10^{-4} \text{e} \cdot \text{nm}^{-2} \) was surpassed; but was not observed for individually levitated droplets. Since the effects of surface potential and the presence of an external electric field on solute nucleation cannot be differentiated using an electrodynamic levitation trap as a means of levitation, an acoustic levitator was designed and constructed for this purpose. For acoustically levitated NaCl solution drops, both the presence of drop surface potential (SCD_{max} \sim \pm 3.44 \times 10^{-4} \text{e} \cdot \text{nm}^{-2}) and an external electric field (E_{ext} = 6.0 \times 10^5 \text{V} \cdot \text{m}^{-1}) were each found to promote solute nucleation as determined by the increase in the number of crystals observed in each drop relative to the number of crystals observed in the absence of drop surface potential and E_{ext}. The effect of both drop surface potential and the presence of E_{ext} on the crystallization of the concomitant dimorphic m-nitrophenol and trimorphic anthranilic acid systems with respect to polymorphic fraction were investigated for acoustically levitated solution drops. For anthranilic acid trials conducted in n-butanol under conditions of negative drop surface potential, the polymorphic ratio changed from approximately 0 : 100 : 0 to 0 : 91 : 9 (form I : form II : form III). A more dramatic shift to approximately 0 : 68 : 32 was observed for the same solution drops in the presence of E_{ext}. The enhancement of form III appeared correlated to the decreases observed in drop supersaturation. No change in polymorphic ratio was observed for m-nitrophenol drops under similar conditions.
DEDICATION

To all of my family
Textbooks teach you that to lock solutions in your icebox overnight can precipitate from water, candy on a cord, words accreting meaning so that the line can end at last in the sweetest of stalactites.

Crystallization.

Christian Bök
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I would also like to express my deep gratitude and appreciation to the machine shop and electronic shop personnel without whom the levitator would only be a collection of schematics; to Dr. Ray Batchelor for several discussions concerning X-ray powder diffraction and the $\lambda/2$ effect (oh those mysterious peaks!) and to Dr. Noham Weinberg for several discussions concerning classical nucleation theory. I also gratefully appreciate Mr. Steve Bernie for taking time out of his busy schedule to kindly photograph the levitation equipment.

Finally a special thank you goes out to all of my family whose love, support, and encouragement was essential during my studies. I have no idea how I would have completed this work without their steadfast support.

Sometimes the lights are shining on me,
Other times I can barely see.
Lately it occurs to me
What a long, strange trip it’s been!

GD
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<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>$a$</td>
<td>activity</td>
</tr>
<tr>
<td>$ac$</td>
<td>alternating current</td>
</tr>
<tr>
<td>$\text{Å}$</td>
<td>Angstrom</td>
</tr>
<tr>
<td>$A_c(n)$</td>
<td>total area of embryo surface</td>
</tr>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>API</td>
<td>active pharmaceutical ingredient</td>
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<tr>
<td>$b$</td>
<td>fitting constant</td>
</tr>
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<td>$B_o$</td>
<td>Bond number</td>
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<tr>
<td>$b_{RL}$</td>
<td>mobility limit</td>
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<tr>
<td>b.p.</td>
<td>boiling point</td>
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<td>Celsius</td>
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<tr>
<td>$C$</td>
<td>concentration</td>
</tr>
<tr>
<td>$C_e$</td>
<td>solubility</td>
</tr>
<tr>
<td>$C_p$</td>
<td>specific heat capacity</td>
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<tr>
<td>$c_e$</td>
<td>operator to electric field magnitude in electrostatic free energy change expression ($\Delta G_{\text{el}}$)</td>
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<tr>
<td>CHCA</td>
<td>$\alpha$-cyano-$4$-hydroxycinnamic acid</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CNT</td>
<td>classical nucleation theory</td>
</tr>
<tr>
<td>$d$</td>
<td>diameter</td>
</tr>
<tr>
<td>$d_{hkl}$</td>
<td>spacing between Miller planes of a unit cell</td>
</tr>
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<td>D</td>
<td>Debye</td>
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</table>
$D$ diffusion coefficient

dc direct current

dB decibel

dH$_2$O distilled water

DMF dimethylformamide

DMSO dimethylsulfoxide

$E_{cr}$ critical electric field

$E_E$ Earth’s electric field

$E_{ext}$ external electric field

e charge of an electron

EDLT electrodynamic levitation trap

ERH efflorescent relative humidity

ES-AAJD electrospray assisted-aerosol jet deposition

ES-AVD electrospray assisted vapour deposition

ES-CVD electrospray assisted chemical vapour deposition

EtOH ethanol

$f$ frequency

$F_{ac}$ acoustic radiation force

fC femtoCoulomb

fps frames per second

$G$ free energy

g gram

$g$ acceleration due to gravity constant

GCR galactic cosmic rays
Hz       Hertz

i       integer number

ions_{D^+}       ions that comprise the net excess charge of a droplet

IP       induction electrode potential

IR       infrared

J       rate of nucleation

J_o       pre-exponential factor for nucleation rate

K       Kelvin

k       Boltzmann constant

kHz  kilohertz

l       length

M       molecular weight

m       mass

\dot{m}       mass flux

m       metre

MALDI       matrix assisted laser desorption ionization

MeCN       acetonitrile

mg       milligram

MHz       megahertz

MIBK       methyl isobutyl ketone

min       minute

mA       milliampere

mL       millilitre

mm       millimetre
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<thead>
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<td>millimolar</td>
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<td>mmol</td>
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<td>millipascal</td>
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<td>MS</td>
<td>mass spectrometry</td>
</tr>
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<td>megawatt</td>
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<td>milliwatt</td>
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<td>N</td>
<td>Newton</td>
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<td>N</td>
<td>Avogadro’s number</td>
</tr>
<tr>
<td>n</td>
<td>number of molecules or ion pairs</td>
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<tr>
<td>n*</td>
<td>number of molecules (or ion pairs) that constitute a critical nucleus</td>
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<tr>
<td>nC</td>
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<tr>
<td>NPLIN</td>
<td>non-photochemical laser induced nucleation</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>1-butanol</td>
</tr>
<tr>
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</tr>
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<td>picolitre</td>
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<tr>
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<td>parts per million</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
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<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>PZT</td>
<td>lead zirconate titanate</td>
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<td>radius of critical nucleus</td>
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<td>RESS</td>
<td>rapid expansion of supercritical solutions</td>
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<td>RH</td>
<td>relative humidity</td>
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<td>ROY</td>
<td>5-methyl-2-[(2-nitrophenyl)amino]3-thiophenecarbonitrile</td>
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<td>$R_w$</td>
<td>reliability factor</td>
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<td>second</td>
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<tr>
<td>$S$</td>
<td>supersaturation ratio</td>
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<td>SAM</td>
<td>self-assembled monolayer</td>
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<tr>
<td>SCD</td>
<td>surface charge density</td>
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<td>SEDSS</td>
<td>solution enhanced dispersion by supercritical solution</td>
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<td>slow expansion of supercritical solutions</td>
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<td>temperature of environment</td>
</tr>
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<td>$t$</td>
<td>time</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
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<td>-------------</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<td>THAP</td>
<td>2,4,6-trihydroxyacetophenone monohydrate</td>
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<td>$U$</td>
<td>applied potential</td>
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<td>$V$</td>
<td>volt</td>
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<tr>
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</tr>
<tr>
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<tr>
<td>$V_n$</td>
<td>volume occupied by critical nucleus</td>
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<tr>
<td>$V_o$</td>
<td>volume occupied by monomer or ion pair</td>
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<td>$V_{O-P}$</td>
<td>over-potential voltage</td>
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<td>$W$</td>
<td>work</td>
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<td>$W^*$</td>
<td>energy barrier to nucleation</td>
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<td>XRPD</td>
<td>X-ray powder diffraction</td>
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<td>$Y$</td>
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<td>one dimensional</td>
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<td>pi</td>
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<tr>
<td>$\Delta \mu$</td>
<td>supersaturation</td>
</tr>
<tr>
<td>$\Delta G_E$</td>
<td>change in electrostatic energy when $n$ ion pairs or molecules transform from solution to a cluster in the presence of an electric field</td>
</tr>
<tr>
<td>$\Delta G_f$</td>
<td>free energy of formation</td>
</tr>
<tr>
<td>$\Delta G_f^*$</td>
<td>free energy of formation of critical nucleus</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>adiabatic index</td>
</tr>
<tr>
<td>$\beta$</td>
<td>evaporation rate constant</td>
</tr>
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</table>
\( \delta \) permeability
\( \varsigma \) dynamic viscosity
\( \eta \) refractive index
\( \kappa \) wavenumber
\( \sigma \) interfacial tension
\( \sigma_{\text{eff}} \) effective interfacial tension
\( \omega \) radial frequency
\( \tau \) heat conductivity
\( \gamma_{i} \) surface energy
\( \varepsilon_{o} \) permittivity of vacuum
\( \varepsilon_{c} \) dielectric constant of solute
\( \varepsilon_{d} \) dielectric constant of solution drop
\( ^\circ \) degree
\( \rho \) density
\( \lambda \) wavelength
\( \mu \) dipole moment
\( \mu_{s} \) chemical potential of a molecule in solution
\( \mu_{c} \) chemical potential of a molecule in the bulk of a crystal phase
\( \mu_{L} \) microlitre
\( \mu_{m} \) micrometre
CHAPTER 1

INTRODUCTION

1.1 CRYSTAL ENGINEERING: NUCLEATION OF CRYSTALS FROM SOLUTION

The nucleation of crystals from solution is a phenomenon of widespread importance with many biological, environmental, atmospheric, and industrial implications. From a manufacturing point of view, crystallization is very attractive because this single process combines both purification and particle formation, whereby the desired, distinct physiochemical properties of a chemical material can be obtained by controlling crystallite preparation.\textsuperscript{1-4} For example, most pharmaceutical products contain bioactive compounds and excipients (i.e. pharmacologically inactive substances used as carriers for the active ingredients of a medication) that are crystalline, while the different crystalline forms of an active compound can vary in solubility, absorption, and stability properties.\textsuperscript{4-7} As such, control of the crystallization process is one of the most valuable techniques used in separation and purification processes employed by a variety of industries, including, not only pharmaceuticals, but agrochemicals, pigments, foods, photographic materials, and explosives.\textsuperscript{2,8-10}

Since nucleation plays such an important role in determining crystal form and size distribution, an increase of fundamental knowledge with regard to solute nucleation would be beneficial towards the achievement of control over these properties.\textsuperscript{4} However, the nature of solution crystallization is a complex operation governed by both thermodynamic and kinetic factors which can make the process highly variable and difficult to control. Similar to a chemical reaction, nucleation is an activated process with a transition state. Unlike the formation of covalent bonds and a transition state molecular complex that occurs for a chemical reaction, a cluster comprised of a few tens of molecules, held together by weak intermolecular forces and packed in some regular arrangement, is considered the transition state for nucleation.\textsuperscript{2}
From a mechanistic point of view, solute crystallization is a two-step process; a result derived from the bulk diffusion of growth units through a mass transfer boundary layer and their subsequent incorporation into a growing crystal lattice.\textsuperscript{1,2,11,12} Despite the fact that these steps are typically discussed as unconnected events, they are actually interdependent; as nucleation and growth proceed, the supersaturation of the solution (a requirement for nucleation to actually occur whereby the concentration of the solute in solution is greater than its solubility) decreases which slows down the system kinetics. As the system moves towards equilibrium, thermodynamic factors outweigh kinetic factors and crystal growth ceases.

Crystallization can be thought of as the combined result of nucleation and crystal growth, whereby the factors affecting one or both processes can be expected to have an effect on the overall crystallization behavior.\textsuperscript{13} Although both steps are governed by different mechanisms, they are both influenced by several experimental variables such as temperature, pH, ionic strength, solvent evaporation rate, solution stirring rate, the addition of nucleation agents and stabilizers and even the influence of magnetic fields.\textsuperscript{14-20} Changes in the experimental variables result in changes to the rates of nucleation and crystal growth, which influence changes in crystal habit and morphology. Experimental variables far less studied are the influence of electric fields and non-electrically neutral environments on solute nucleation and crystal growth.\textsuperscript{10,21-41}

The effect of an external electric field on nucleation kinetics and crystal growth in saturated solutions was first investigated by both Shubnikov and Kozlovskii in the early 1960s. Shubnikov studied the effect of an external electric field on the nucleation of ammonium chloride by placing drops of aqueous ammonium chloride in a static electric field and letting the solvent slowly evaporate.\textsuperscript{21} He observed that the number of NH\textsubscript{4}Cl crystals observed in a given time increased for drops in presence of the external electric field, regardless of field orientation, as compared to the NH\textsubscript{4}Cl nucleation rate for drops in the absence of an external electric field.\textsuperscript{21} In an independent series of experiments using NH\textsubscript{4}Cl, Kozlovskii observed that the increased NH\textsubscript{4}Cl nucleation rate was a function of the square of the external electric field for a given NH\textsubscript{4}Cl supersaturation.\textsuperscript{24,25} He also observed the same phenomenon for other halide salts such as NaCl, KCl, KI, NH\textsubscript{4}Br, NH\textsubscript{4}I and CdI while observing no nucleation rate increase for NiSO\textsubscript{4}, and
CuSO$_4$. In a separate set of experiments, Kozlovskii also observed that a spark discharge to a supersaturated solution of inorganic salt produces nuclei whereby the number of nuclei produced was a function of the strength and polarity of the electric field for a given supersaturation. No mention was made as to whether or not nucleation occurred primarily at the drop surface under conditions of a spark discharge.

Twenty years later, Evans grew camphor crystals from saturated CCl$_4$ solutions situated in between the parallel plates of a capacitor while under a non-uniform external electric field ($E_{\text{ext}} \sim 5 \times 10^4$ V⋅m$^{-1}$). Evans asserted that this phenomenon was the reciprocal of the thermodielectric effect postulated by Costa Ribero and could be used as a new crystal growing technique. Costa Ribeiro had previously observed that electric charges are always produced at an interface between a solid phase and other phases of matter in dielectrics and that an electric current is produced when a phase change is occurring. The production of charge was observed not only in the case of melting and solidification, but also in other changes of physical state in which one phase was a solid (i.e. sublimation) and in the precipitation of substrates from saturated solution.

More recently, Saban et al. observed that electric field strengths of greater than $10^5$ V⋅m$^{-1}$ had significant controlling effects on NaCl and bis-thiourea zinc chloride nucleation, most notably at lower supersaturation ratios, from sitting solution droplets on hydrophobic glass slides that were placed between the electrodes of a capacitor (formerly referred to as a condenser) as illustrated in Figure 1.1. The experiments were conducted in air in a sealed chamber at atmospheric pressure.
Figure 1.1  Experimental set-up for a sitting drop in an external electric field. Adapted from Saban et al.\textsuperscript{31}

Although several reports confirm that externally applied electric fields have a stimulating effect on the nucleation rate of small molecule crystals in saturated solutions,\textsuperscript{32,33} a significant decrease in the number of nuclei was observed when an external electric field was applied during the crystallization of the protein lysozyme from both hanging drops and sitting drops placed between capacitors.\textsuperscript{42,43} As such, the lysozyme crystals obtained were fewer in number, larger in size, and of better crystallographic quality as quantified by a reduction in their mosaic spread upon X-ray diffraction; a most important goal for crystallogenesis studies on biological macromolecules.\textsuperscript{42-47} Interestingly, the induction time for nucleation and subsequent crystal growth was reduced from twelve to five hours despite the reduction in the number of crystals obtained.\textsuperscript{48} In a separate study, Nanev et al. observed that nucleation occurred predominantly at the cathode, that crystals are oriented about the $c$-axis, and that nucleation was stimulated during the nucleation and crystal growth of lysozyme from sitting drops placed between a capacitor.\textsuperscript{45,49} These contrasting results (especially the effects between small molecule nucleation and protein nucleation in response to external electric fields) certainly suggest that more research is necessary to better understand the mechanism of nucleation and crystal growth in the presence of electric fields.

In a related study, Sanjoh et al. developed Si devices for protein crystal growth screening whereby nucleation and crystallization sites having different surface-potentials were made from deposited thin-film semiconductor and insulating materials fabricated on
the surface of growth cells, as illustrated in Figure 1.2. Since a protein molecule is typically charged in solution as a result of the solution’s pH, it could interact strongly with a charged surface with free energies that could be easily several kT per protein molecule. Hence an oppositely charged surface to that of the protein acted as a nucleant and induced nucleation of a protein crystal.

Figure 1.2 Illustration of crystals preferentially growing on the charged surfaces of a substrate. Adapted from Sanjoh et al.

Electric field parameters and non-electrically neutral environments also play an important role in the disciplines of biomineralization and interfacial crystal growth. It is widely recognized that electrostatic interactions at charged surfaces are significant with respect to biomineralization whereby such nucleation and crystal growth is closely linked with negatively charged macromolecules. Although cation binding, localized cation concentration enhancement, and orientation-specific stabilization of nuclei are cited as roles for negatively charged sites and surfaces, crystallization occurring at these charged sites and surfaces remains poorly understood. Relatively few studies have attempted to quantitatively assess factors such as surface charge density (SCD), ion binding, and ion distributions within the Helmholtz double layer at the interface in biomineralizing systems. Better understanding of the surface electrostatic parameters would provide insight into natural phenomena such as bone, tooth, and shell formation, as well as to assist scientists and engineers with the application of biomimetic strategies towards the development of novel materials such as advanced composites and coatings for medical, chemical, optical, and electronic applications. For example, Yamashita et al. observed that the mineral apatite (calcium phosphate) grew rapidly on the negative pole, but not on
the positive pole, of polarized ferroelectric crystals.\textsuperscript{54} The rate of apatite growth on polarized substrate was observed to be six times that of non-polarized substrate due to the strong electric field ($E_{\text{ext}} = 1.2 \times 10^5$ V m$^{-1}$) present at the solution-substrate interface.\textsuperscript{54,55} These observations are important as there is considerable interest in inducing apatite nucleation on synthetic surfaces in order to promote bone attachment to orthopaedic implants.\textsuperscript{55}

Recently it was observed by Ehre \textit{et al.} that the positive surfaces of pyroelectric LiTaO$_3$ crystals and SrTiO$_3$ thin films promoted the nucleation of ice from supercooled droplets, while the same surfaces, when negatively charged, reduced the freezing temperature.\textsuperscript{56} Interestingly, powder X-ray diffraction studies demonstrated that nucleation and crystal growth on the positively charged surface originated at the substrate-liquid interface while nucleation and crystal growth on the negatively charged surface originated at the air-liquid interface.\textsuperscript{56} Ehre \textit{et al.} speculated that, since the electron density and geometry of the lone electron pairs of oxygen are very different from those of the two hydrogen atoms, the water molecules self-assembled and interacted differently with the surfaces of opposite charge. The ability to control the freezing temperature of supercooled water using auxiliaries that promote or suppress ice nucleation is a critical factor in a variety of areas such as the cryopreservation of cell and tissues, crop freezing prevention, cloud seeding, and snow making.\textsuperscript{56} It should be noted that although the aforementioned system demonstrated the effects of an electric field on nucleation at an interface, it pertained to nucleation from a melt rather than solute nucleation from solution.

Although the theoretical basis for solute nucleation in the presence of an external electric field has been described in the context of classical nucleation theory, (refer to section 1.3.3 of this work), there are few theoretical considerations concerning the parameter of non-electrically neutral environments on solute nucleation; most of them related to processes that occur in Wilson cloud chambers.\textsuperscript{32,33,57-61} Wilson was the first person to quantitatively investigate ion-induced nucleation,\textsuperscript{62,63} which has been defined as the promotion of cluster growth around an ion which acts as a heterogeneous nucleation site for the condensation of supersaturated vapors.\textsuperscript{62-66} The strong ion-dipole interaction between ions and vapor molecules lowers the activation barrier, and hence reduces the
supersaturation necessary, for the nucleation of a droplet from the vapor phase. In the microelectronics industry, Jang et al. observed that gaseous C\textsuperscript{-} ions make possible the synthesis of diamond without the co-deposition of graphite in a chemical vapor deposition process. Jang et al. suggested that the net charge reduced the critical cluster radius required for nucleation and when the cluster was sufficiently small (< 5 nm), the interfacial tension between the cluster and the surrounding gas phase was high enough to shift the metastable phase boundary making diamond more stable than graphite. Nucleation rates of silicon particles, via the condensation of neutral silicon vapor on Si\textsuperscript{-} ions in processing plasmas, were modeled using ion-induced nucleation theory. Highly anisotropic silicon nanowires were grown on Si, SiO\textsubscript{2}, and Si\textsubscript{3}N\textsubscript{4} substrates via chemical vapor deposition processing only when charged silicon clusters were employed; only porous skeletal structures were formed when the silicon cluster were neutral. Moreover, the phenomenon of ion-induced nucleation has important implications with regards to atmospheric processes where ions provide condensation sites for sulfuric acid and water vapors to form new aerosol particles that can grow into sizes needed for cloud condensation nuclei and ice nuclei, as illustrated in Figure 1.3. This in turn affects the particle size distribution and lifetimes of clouds, and hence the radiative properties that factor into atmospheric albedo (i.e. the atmosphere’s capacity to reflect radiation) forcing climate change.
Although the physics of droplets that possess net charge is a subject that continues to receive attention, the condensed phase chemistry occurring within such droplets, as a result of the net charge localized in the diffuse layer at the droplet-air interface, is not well characterized.\textsuperscript{37,38,79,80} Mass spectrometry studies of cluster ions released from charged droplets in an electrospray have identified shifts in chemical equilibria and the formation of preferred nanocrystalline structures.\textsuperscript{81-88} Electrospray assisted chemical vapour deposition (ES-CVD) has been used to prepare isolated and non-agglomerated nanoparticles of SiO\textsubscript{2}, TiO\textsubscript{2}, and ZrO\textsubscript{2}, while thin films of hexagonal crystalline ZnS and chalcopyrite crystalline CuInS\textsubscript{2} have been grown using an electrospray assisted-aerosol jet deposition (ES-AAJD) method and an electrospray assisted vapour deposition (ES-AVD) method respectively. The SCD on precursor droplets has also affected nanoparticle size and morphology, and has enhanced crystalinity and the preferred growth orientation of thin film structures grown using the ES-CVD and ES-AAJD (and ES-AVD) method respectively.\textsuperscript{39,89-91}

There is also increasing interest in creating various solid physical forms of active pharmaceuticals with the objective of enhancing their \textit{in vivo} performance and achieving desired release characteristics which are both influenced by the morphology of the constituent particles. By electrohydrodynamic spray drying molecular solutions or
suspensions, a variety of inorganic materials, biomolecules, polymers, proteins, and drug molecules have been formulated in particulate form. A schematic of a nanoparticle electrohydrodynamic spray dryer is show in Figure 1.4.\textsuperscript{92-97} As previously introduced, condensed phase chemistry occurring in charged droplets is also of particular interest to atmospheric scientists. It was observed by Pruppacher \textit{et al.} that sulfur particles, which typically act as poor ice nuclei at -20 °C, were able to cause ice formation in supercooled droplets at -8 °C when they were electrified.\textsuperscript{72,98} This charge-enhanced contact nucleation is usually described as field-induced electrofreezing; the process by which an electric field causes the freezing of a supercooled water drop.\textsuperscript{72,99-103}

In electrodynamic levitation studies with charged droplets, the Agnes group has reported the enhancement of $\alpha$-cyano-4-hydroxycinnamic acid (CHCA) co-crystallization with one or more peptides, and the promotion of NaCl precipitation; both a function of the magnitude of the droplet’s net charge.\textsuperscript{37,38} This phenomenon of promoted solute nucleation has several implications for laboratory, industrial, and natural processes involving media with net charge ranging from fundamental aspects of soft ionization for mass spectrometry to the preparation of nanophase materials with improved properties.
Clearly, there could be a wealth of knowledge regarding the chemistry that occurs in charged droplets as a result of their violation of electroneutrality.\textsuperscript{37,38,40}

The primary focus of the research discussed in this thesis was to investigate the effect that droplet surface potential (i.e.: droplet net charge) has upon the nucleation of a solute from a non-electrically neutral droplet; there being virtually no such investigations in the current literature. The goal was to be able to determine whether or not droplet surface potential could be used as a tool to facilitate controlled nucleation of a solute with respect to the nucleation rate, the resultant crystal size and habit (or shape), and the particular crystal structure adopted by the nucleus. One could picture this goal of surface potential controlled nucleation within a droplet in a similar fashion to that of surface controlled nucleation on a fabricated surface (see Figure 1.2), as illustrated in Figure 1.5.

\textbf{Figure 1.4} Schematic of an electrohydrodynamic nanospray dryer. Adapted from Chan \textit{et al}.\textsuperscript{97}
1.2 GENERAL ASPECTS OF CHARGED DROPLETS

In light of the primary goal of this thesis, some background information with regard to charged droplets and their dynamics would be useful for the investigation of how droplet surface potential could affect solute nucleation. Although droplets that carry electrical charges occur naturally, as can be found in ocean and waterfall sprays, as well as in thunderclouds, the mechanism of droplet charging and properties of charged droplets are complex subjects. Three main types of droplet charging mechanisms are diffusion charging, induction charging, and contact charging. However, regardless of the charging mechanism, a droplet’s net charge is a result of a net excess of either positive or negative ions (or electrons) found within (or at the surface) of the droplet. Typically the ions are either found in the net neutral liquid prior to droplet formation or are gas phase ions that collide with and are absorbed by the droplet. The latter case of droplet charging is an example of the diffusion charging mechanism and is the most common way to charge aerosol particles. Corona charging, whereby an electrode is brought to high potentials such that the surrounding medium (usually air) is ionized, is a type of diffusion charging. Diffusion charging is also thought to be a mechanism by which cloud droplets can become charged. Since negative ions display a higher mobility in an electric field then do positive ions, in the absence of an electric field negative ions have a greater
diffusion coefficient than positive ions and hence negative ions will diffuse onto cloud droplets at a higher rate than positive ions. As cloud droplets grow via vapour condensation, they fall into lower regions of a cloud, leaving behind positive space charge in upper cloud region explaining the charge separation present as measured in cumulonimbus clouds.

Induction charging of a droplet occurs when the droplet forms in the presence of an electric field. This has an impact on natural phenomena as the Earth’s natural electric field (or fair-weather field; $E_E \sim 130 \text{ V m}^{-1}$) is thought to be responsible for the charging of sea water droplets and waterfall droplets. The Earth’s electric field, a result of the planet’s positively charged ionosphere coupled with the negatively charged earth surface, induces negative surface charge at the water’s surface. For the case of the waterfall, as the water falls over the edge, it breaks up into small droplets creating a mist of negatively charged droplets from the water’s surface. Charged droplet production occurring via aerodynamic jet breakup in nature is known as the Lenard effect; named after the physicist who studied the increased charge density close to waterfalls. It should be noted that pure water can be formed into charged droplets via this mechanism since water is self-ionizable. Although appearing somewhat subtle, the Lenard effect is thought to greatly influence the charging of the atmosphere from the breakup of sea water bubbles at the ocean surface into charged droplets that are carried into the atmosphere via updrafts. Induction charging is also thought to be at work in the charge separation that occurs in clouds. If the level of charge carried by a population of droplets is large within a cloud, then sparking may occur; as is apparent from atmospheric lightening. Both diffusion and induction charging of atmospheric droplets and ion-induced nucleation contribute to the global electric current as depicted in Figure 1.6.
Figure 1.6  Schematic of the global electrical circuit.

The current generators are thunderstorms, which are located predominantly over tropical land masses. The return path is the global fair-weather current flowing between the ionosphere and ground which varies between 1 and 4 pA m$^{-2}$, depending on the local atmospheric columnar resistance. It is mainly determined by the surface altitude and the galactic cosmic ray (GCR) flux. A continuous current supply is required to maintain the ionospheric potential near +250 kV since the decay time constant of the ionosphere-ground spherical capacitor is only about 2 minutes. Adapted from Kirkby.78

1.2.1 Surface Charge and the Rayleigh Limit

The surface tension of the liquid that constitutes a droplet is responsible for its shape. Although they can be easily deformed, droplets tend to be pulled into a spherical shape by the cohesive forces of the surface layer. Since the liquid molecules at the surface layer of a droplet are not completely surrounded by other neighboring molecules of liquid, they are pulled inwards creating internal pressure that forces the surface layer to adopt a minimal surface area. In opposition to the inner pressure caused by surface tension, droplet surface charge causes an outward electrostatic pressure due to the repulsive forces of ions having the same polarity. In other words, surface tension forces tend to stabilize droplet shape while the electrostatic forces tend to destabilize the droplet. The criterion of instability for a charged droplet was derived by Lord Rayleigh$^{112}$:

$$Q^2 < 64r^3 \pi \sigma \varepsilon_o$$

(1.1)
Equation 1.1 relates the stability of the droplet as a function of its interfacial tension ($\sigma$) and its radius ($r$) to the magnitude of its net elementary charge ($Q$); $\varepsilon_0$ is the permittivity of vacuum. In the laboratory, the Rayleigh instability limit can be reached by the controlled evaporation of charged droplets whereby charge is not lost during evaporation until the limit is reached. Once the Rayleigh limit is reached, the charged droplet ruptures sending out one (or more) smaller daughter droplets in the form of a jet, away from the parent droplet. This droplet fission is known as a Coulomb explosion event and was first visualized by Duft et al. using a high speed camera while observing a charged ethylene glycol droplet as is shown in Figure 1.7.

![Ethylene glycol droplet undergoing a Coulomb explosion event](image)

**Figure 1.7** Ethylene glycol droplet undergoing a Coulomb explosion event (after Duft et al.).

The maximum mobility of a charged droplet moving in an electric field is obtained in the Rayleigh limit. Assuming Stokes Law for the drag force is valid, the mobility limit ($b_{RL}$) of a charged droplet is:

$$b_{RL} = \frac{Q}{6r\pi\zeta} = \frac{4\sqrt{\varepsilon_0 r \sigma}}{3\zeta}$$  \hspace{1cm} (1.2)

where $\zeta$ is the dynamic viscosity of the medium. The charge to mass ratio ($Q/m$) is another important parameter for the acceleration and manipulation of charged droplets in electric fields. At the Rayleigh limit:

$$\left(\frac{Q}{m}\right)_{RL} = \frac{6\sqrt{\varepsilon_0 \sigma}}{\rho r^3}$$  \hspace{1cm} (1.3)

where $\rho$ is the density of the droplet.
1.2.2 Electrosprays

The amount of droplet charge produced in a droplet production process is also a function of the concentration and mobility of free ions in the parent liquid. Of the liquid volume forming the droplet, a fraction of either the total number of positive or negative ions that by chance happen to be part of the forming droplet, are responsible for the droplet’s net charge.\(^\text{114}\) The hydrodynamic processes that occur during the formation of a spray influence both the droplet size and charge distribution over the population of droplets that constitutes a spray.\(^\text{104}\)

![Figure 1.8](image)

**Figure 1.8** Schematic of Lord Kelvin’s water dropping apparatus. Opposite charges build up on the two water containers until the electric field between a spark gap between the two containers is sufficient to breakdown the air, at which point a spark jumps across the gap, discharging the droppers.
This breakup of a fluid into droplets is referred to as atomization. When atomization occurs in the presence of an anthropogenic external electric field, the charging process can be strongly enhanced and is more effective than natural charging. Lord Kelvin was able to illustrate this with his, now famous, water dropping apparatus whereby he established a large potential difference (10 – 20 kV) between two droplet collectors by cross coupling the collectors and ring electrodes for two water droppers as illustrated in Figure 1.8. This type of induction charging was responsible for the explosion of three very large oil cargo tanks that had built up space potentials of several thousands of volts during water jet washing and subsequently discharged.

There are several different ways to produce an anthropogenic spray of charged droplets, or an electrospray, for a technical application. Most atomizers use mechanical energy to break up the liquid body into droplets and are of the pressure, rotary, or twin fluid type. Application of a voltage to the spraying device results in the induction of charge to the spray.

The illustration in Figure 1.9 shows a typical electrospray set up consists of conducting capillary, where high voltage can be applied, and a grounded plate. Liquid feeds through the capillary and is atomized by the field upon exit at the capillary. Depending upon set-up geometry, liquid properties (surface tension, conductivity, etc.), feed rate, and the applied potential, different modes of electrospray are observed as shown in Figure 1.10.
Figure 1.9  Schematic of a typical electrospray set up.

Figure 1.10  Different modes of electrospray. Adapted from Jaworek et al.\textsuperscript{91}
1.2.2.1 Electrospray Deposition

Since the trajectories of charged droplets can be manipulated with electric fields, electrospray is often very useful in order to direct or deposit a liquid onto a target and hence making electrospray a candidate for many types of layer and film production. This technique is very useful as the necessary equipment is inexpensive and can be run at atmospheric conditions. Crop spraying,\textsuperscript{119} automobile painting,\textsuperscript{120,121} and ink-jet printing,\textsuperscript{105} are examples of processes that employ electrospray deposition. Numerous ceramic materials are also formed via electrospray pyrolysis deposition whereby the charged droplets are deposited onto a heated substrate kept at ground potential. Film morphologies are dependent upon such processing parameters as spray production (including droplet net charge), aerosol transport, solvent composition and evaporation, and droplet discharge on the substrate.\textsuperscript{39,122,123} Applications include the formation of electrodes and electrolytes for batteries and fuel cells,\textsuperscript{122} the formation of crystalline semiconductors for optoelectronic devices and photovoltaics,\textsuperscript{39,90} the formation of catalysts,\textsuperscript{91} and as an effective route to nanotechnologies of many types.\textsuperscript{91,124} Nanoparticle production of pharmaceutical agents is a rapidly growing area of interest that also employs electrospray deposition (see Figure 1.4). Drug particles with very specific and narrow sizes ranges, that have been shown to improve inhaling efficiency in the treatment of asthma for example, can be fashioned using electrospray deposition.\textsuperscript{125} One disadvantage of the electrospray process as it relates to pharmaceuticals is the low production rate of pharmaceutical agent, typically on order of grams per hour.

1.2.2.2 Electrospray Ionization

Electrospray ionization is a powerful tool used in mass spectrometry (MS) whereby large and complex molecules can be analysed. Such molecules are dissolved in solvent that is sprayed through a capillary electrode held at fixed potential. As the solvent of the charged droplets evaporates, the surface charge density of the droplets increases until the Rayleigh limit is reached. As the droplets destabilize, small, charged satellite droplets are emitted that relaxes the negative pressure produced by the original droplets’ surface charge. Repeated Coulomb explosions result in very small charged droplets. Once the radii of the droplets shrink below 10 nm, small ions can desorb from the droplet surface into the ambient gas, carrying with them non-ionic solvent or solute molecules.\textsuperscript{126,127} For
the detection of larger ions such as proteins, it is postulated that complete evaporation of a small droplet containing a single macromolecule occurs whereby the residual charges are transferred to the macromolecule. The molecule-ion system is then transported via forced convective gas flow and focused by electrostatic lenses within a mass spectrometer for analysis. The technique of electrospray ionization MS allows for the analysis of a large variety of analytes such as inorganic ions, ionized polymers, nucleic acids, peptides, and proteins having molecular masses that range from kilos to hundreds of mega Daltons.\textsuperscript{127}

1.3 CLASSICAL NUCLEATION THEORY

In light of the primary goal of the thesis, an understanding of classical nucleation theory (CNT) would also be useful in knowing how droplet surface potential could affect solute nucleation. The formation of a new phase from within an initial phase consisting of monomers, be it atoms, ions, or molecules, commences when a small number of monomers stick together to yield small pre-nucleus clusters or embryos. Kinetically, nucleation can be thought of as a chain reaction of monomer additions to a cluster that eventually reaches macroscopic dimensions. It should be noted that growth of an embryo into macroscopic size is not a certainty as there is an equal chance for monomers to detach from the embryo.

According to classical nucleation theory, the driving force for nucleation and growth of a crystal from solution is the supersaturation ($\Delta \mu$) which is commonly expressed as follows:\textsuperscript{128}

$$\Delta \mu = \mu_s - \mu_c = kT \ln S \quad (1.4)$$

where $\mu_s$ and $\mu_c$ are the chemical potentials of a molecule in solution and in the bulk of the crystal phase, respectively, $k$ is the Boltzmann constant, $T$ is the absolute temperature, and $S$ is the supersaturation ratio which is defined as:\textsuperscript{128}

$$S = \frac{[a_1 \cdot a_2 \cdot \ldots \cdot a_j]}{[a_{1e} \cdot a_{2e} \cdot \ldots \cdot a_{je}]} \quad (1.5)$$

where $a_1, a_2, \ldots, a_j$ and $a_{1e}, a_{2e}, \ldots, a_{je}$ are the actual and equilibrium activity values of the different ions in solution that come together to make up a crystal, respectively. For non-ionic crystals this reduces to:
\[ S = \frac{a}{a_e} = \frac{C}{C_e} \]  

(1.6)

where \( C \) and \( C_e \) are actual and equilibrium concentrations respectively (concentrations can be substituted for activities for sufficiently dilute solutions). \( C_e \) is also known as the solubility. Nucleation and growth of a crystal is only possible when the solution is supersaturated (i.e.: \( \Delta \mu > 0 \) and \( S > 1 \)). Ordinarily homogeneous nucleation of a solute is established by either changing the temperature of a system in the appropriate direction (typically a temperature decrease) or by allowing the solvent to evaporate in a slow and controlled manner; both methods result in the supersaturation of the system.

If the Gibbs-Thompson equation is applied to a cluster of radius \( r \) in a supersaturated solution, then the equilibrium condition is the following:

\[ kT \ln S = \frac{2\sigma V_o}{r} \]  

(1.7)

where \( \sigma \) is the interfacial tension between the solution and the growing cluster of molecules, and \( V_o \) is the volume occupied by molecule (or ion pair) of the cluster.

According to classical nucleation theory, once the cluster reaches a critical size, this critical nucleus can grow into a macroscopic crystal. Nucleation is considered to be the process whereby initial fragments of a new and more stable phase within a metastable phase are created whereby each fragment can grow into macroscopic units of the stable phase.

Nucleation is classified into two categories: homogeneous nucleation and heterogeneous nucleation. Homogeneous nucleation refers to the spontaneous formation of critical nuclei in an initially homogeneous phase. On the other hand, heterogeneous nucleation occurs when critical nuclei form on foreign surfaces such as on container walls and impurity particles. Secondary nucleation, a type of heterogeneous nucleation, occurs when critical nuclei form on the surface of microscopic crystals of the solute already present in the mother liquor.

It should be noted that just because a solution is supersaturated, it does not imply that crystals will grow from it. In order to create a new phase, an activation barrier must be overcome known as the free energy of formation. When the supersaturation is low, the
activation barrier may not be overcome by thermodynamic energy fluctuations. This can be expressed with a solubility diagram as shown in Figure 1.11.

![Solubility Diagram](image)

**Figure 1.11** Saturation-supersaturation diagram showing the stable, metastable, and supersaturated zones for the crystallization of a solid from solution. The solid line represents the equilibrium solubility curve and the dashed line represents the maximum supersaturation or nucleation curve. The separation of the two curves represents the metastable zone width. Adapted from Davey *et al.*

The diagram is split into three regions: an undersaturated region where neither nucleation nor crystal growth is possible; a metastable region where nucleation cannot occur spontaneously but crystallites can grow; and a supersaturated region where stable nuclei can form and grow.

### 1.3.1 Homogeneous Nucleation

The formation of a new phase (a nucleus) in a homogeneous phase results in an initial increase in free energy due to the formation of an interface between phases which in turn makes this process thermodynamically unfavorable. However, due to thermodynamic fluctuations in the system, such as micro concentration, temperature, and interfacial tension fluctuations within the bulk solution for example, the nucleus can keep growing. Once it has reached a certain size, the decrease in volume free energy is larger than the
increase in free energy from surface creation and the net free energy change is negative which results in the spontaneous growth of the nucleus into a crystal. This is expressed in the free energy diagram for a spherical nucleus as shown in Figure 1.12.

![Free Energy Diagram for Spherical Nuclei](image)

**Figure 1.12** Schematic diagram of the free energy changes associated with the formation of spherical nuclei with different radii \((r)\) in solution. The overall free energy \((\Delta G)\) depends on a volume contribution \((\Delta G_V)\) that favours nuclei formation and a surface contribution \((\Delta G_S = 4\pi r^2 \sigma)\) that opposes nuclei formation. There is a free energy maximum \((\Delta G_f^*)\) and critical radius \((r^*)\) associated with nuclei formation. Adapted from McClements.  

The change in free energy as the nucleus forms \((\Delta G_f)\) with respect to nucleus radius is represented by the following equation:  

\[
\Delta G_f = 4r^2 \pi \sigma - \frac{4}{3} r^3 \Delta G_V
\]  

(1.8)

where \(\Delta G_V\) is the change in free energy due to phase formation per unit volume; the first term of equation 1.8 is the free energy change due to surface formation. With respect to monomer number \((n)\) that comprises the nucleus, the change in free energy as the nucleus forms can be written as:  

\[
\Delta G_f = -n \Delta \mu + \sigma A_V(n) = -nkT \ln S + \left(36\pi V_n^2\right)^{\frac{1}{3}} n^{\frac{2}{3}} \sigma
\]  

(1.9)
where \( A_c(n) \) is the total area of the cluster surface. As an embryo of new phase grows, the net free energy increases until it reaches a maximum and then it steadily decreases. The nucleus for which \( \Delta G_f \) is a maximum is the critical nucleus and has a radius \( (r^*) \) equal to:

\[
r^* = \frac{2\sigma}{\Delta G_V}
\]

(1.10)

The critical nucleus for which \( \Delta G_f \) is a maximum contains a critical number of monomers \( (n^*) \), either individual molecules or ion pairs for a salt, equal to:\(^{128}\)

\[
n^* = \frac{32\pi V_o^2 \sigma^3}{3k^2T^2(\ln S)^3}
\]

(1.11)

Substitution of \( r^* \) into equation 1.16 gives the free energy of formation for a critical nucleus (or the energy barrier to nucleation) \( (\Delta G_f^*) \):\(^{128,130}\)

\[
\Delta G_f^* = \frac{16\pi \sigma^3}{3(\Delta G_V)^2} = \frac{16\pi \sigma^3 V_o^2}{3k^2T^2(\ln S)^2}
\]

(1.12)

The third term of equation 1.12 is obtained by rearranging equation 1.10 and substituting for \( \Delta G_V \). Equation 1.12 indicates that the \( r^* \) and \( \Delta G_f^* \) will decrease with an increase in supersaturation ratio. The critical nucleus size is very small, typically in the 1-100 nm range.\(^{131}\)

Since the probability of finding a fluctuation in energy \( (W) \) is given by the Boltzmann factor, the equilibrium concentration of critical nuclei in solution is:\(^{128}\)

\[
C^* = C_o \exp\left(\frac{-\Delta G_f^*}{kT}\right)
\]

(1.13)

where \( C_o \) is the concentration of nucleation sites in the system and, to a good approximation, is equal to \( 1/V_o \) since every molecule in solution can provide a nucleation site.

The steady-state rate of nucleation \( J \) is equal to the number of nuclei formed per unit time per unit volume and can be expressed as:\(^{128}\)

\[
J = J_o \exp\left(\frac{-\Delta G_f^*}{kT}\right) = J_o \exp\left(\frac{-16\pi \sigma^3 V_o^2}{3kT(\ln S)^2}\right)
\]

(1.14)
where $J_o$ is a pre-exponential kinetic factor that is related to the rate of attachment of molecules to the critical nucleus and is dependent upon molecular mobility. It can be approximated as:

$$J_o = \left( \frac{kT}{V_o \sigma} \right)^{\frac{1}{2}} DC_o S \ln S$$

where $D$ is the monomer diffusion coefficient. Since the supersaturation ratio enters the exponential term in equation 1.15, the rate of nucleation is sensitive to the degree of supersaturation.

### 1.3.2 Heterogeneous Nucleation

Heterogeneous nucleation takes place in solutions containing impurity molecules, foreign microparticles, or on substrates that act as active sites for nucleation. It is approximated by considering a spherical cap with a contact or wetting angle ($\theta$). The wetting angle is dependent upon the balance of surface tension forces at the edge of the droplet as shown in Figure 1.13. This takes into account not only solution/cluster interfacial energies, but substrate/cluster and substrate/solution interfacial energies, where the substrate is comprised of foreign microparticles or even microparticles of the solute that have already crystallized.

**Figure 1.13** Illustration of cross section of a cap-shaped solution drop on a substrate showing the wetting angle ($\theta$) and the interfacial energies between solution and substrate ($\gamma_{ss}$), cluster and substrate ($\gamma_{cs}$), and solution and cluster ($\gamma$). Adapted from Kashchiev.
When the interfacial tension between the cluster and substrate is small, $\theta$ is small. Complete wetting occurs when $\theta = 0$. The interfacial tension term ($\sigma$) found in the equations in section 1.3.1 is replaced by an effective interfacial tension term ($\sigma_{\text{eff}}$) which is equal to:

$$\sigma_{\text{eff}} = \psi(\theta)^{-\frac{1}{3}} \sigma; \quad \psi(\theta) = \frac{(2 \cos \theta)(1 - \cos \theta)^2}{4} \quad (1.16)$$

The implication of using $\sigma_{\text{eff}}$ in lieu of $\sigma$ is that the energy barrier to nucleation decreases as $\theta$ decreases. Heterogeneous nucleation usually takes place on small particles where the nucleating phase adsorbs on a foreign particle to form the nucleus. If the small particle happens to be a crystal of the solute, this is known as secondary heterogeneous nucleation.

### 1.3.3 Nucleation in the Presence of External Electric Field

As previously discussed in section 1.3.1, the formation of a new crystalline phase in a homogeneous solution occurs as a result of thermodynamic fluctuations that take place in the system. As the surface of a new phase is created, the free energy of the system is increased. The growing cluster or embryo is not considered to be a nucleus until it has reached a critical size. It is the degree of supersaturation as expressed by the supersaturation ratio ($S$) that generally controls the size of the critical nucleus in terms of its radius ($r^*$) and number of molecules ($n^*$), the free energy formation for the nucleus ($\Delta G_f^*$), and the concentration of critical nuclei present. Typically to establish nucleation, either the temperature of the system is changed or the solvent of the system is allowed to slowly evaporate in order to manipulate $S$.

The theoretical examination of homogeneous nucleation from solution in the presence of a uniform external electric field in terms of the classical equations used for nucleation has been determined by Kashchiev. Under conditions of constant pressure, temperature, and supersaturation ratio, the free energy required to form an embryo in the presence of an electric field can be written as:

$$\Delta G_f = -nkT \ln S + \left(36\pi V_n^2 \right)^{\frac{1}{3}} n^{2/3} \sigma + \Delta G_E \quad (1.17)$$
where $\Delta G_E$ is the change in electrostatic energy when $n$ molecules transform from the initial phase to the new phase.

The electric field inside a spherical body, such as a droplet, in a uniform external field is represented by Figure 1.14. It should be noted that the effects of elongation in the direction of the external electric field ($E_{ext}$) have been ignored and that the electric field inside the droplet ($E_d$) is uniform everywhere and points in the direction of the unperturbed field.

![Figure 1.14](image)

**Figure 1.14** Droplet in an externally applied uniform field. Adapted from Kashchiev.\textsuperscript{133}

Assuming $n$-independence of the cluster’s dielectric constant ($\varepsilon_c$), the change in electrostatic energy of the system due to cluster formation as a function of $n$ monomers is given by:\textsuperscript{133}

$$
\Delta G_E = \left[\frac{3\varepsilon_d \varepsilon_c (\varepsilon_d - \varepsilon_c)}{2(\varepsilon_c + 2\varepsilon_d)}\right] E_{ext}^2 V_n
$$

(1.18)

where $\varepsilon_d$ is the dielectric constant of the droplet, $\varepsilon_c$ is the dielectric constant of the cluster, and $V_n$ is the volume of the cluster.

The free energy change for the formation of a nucleus within an external electric field can then be written as:

$$
\Delta G_f = -n(kT \ln S + c_e E_{ext}^2) + \left(36\pi V_n \varepsilon_c^2 \right)^{1/3} n^{2/3} \sigma
$$

(1.19)

where $c_e$ is defined as:
Since \( c_e E_{ext}^2 \) is additive to the supersaturation, the formation of a spherical nucleus in a uniform \( E_{ext} \) occurs at an effective supersaturation (i.e.: \( \Delta \mu + c_e E_{ext}^2 \)) that is a function of the field strength. Depending on the sign of \( c_e \) (i.e.: the difference of \( c_e - \epsilon_d \)), the effective supersaturation can be less than the actual supersaturation when \( c_e < 0 \) (i.e.: when \( \epsilon_c < \epsilon_d \)) or greater than the actual supersaturation when \( c_e > 0 \) (i.e.: when \( \epsilon_c > \epsilon_d \)). It should be noted that for \( \epsilon_c = \epsilon_d \), \( E_{ext} \) should have no effect on the thermodynamics of nucleation. This relationship between external field strength and sign of \( c_e \) is general and applies to any type of homogeneous nucleation. Both \( \epsilon_c \) and \( \epsilon_d \) can be determined by using the Maxwell relation \( \epsilon = \eta^2 \) where \( \eta \) is the index of refraction of the solute or solution at a particular wavelength.

In a similar fashion to equations 1.10, 1.11 and 1.12, the critical nucleus size (number of monomers and radius) and its critical free energy of formation are determined for homogeneous nucleation in the presence of \( E \):

\[
r^* = \frac{2V_o \sigma}{kT \ln S + c_e E_{ext}^2} \quad (1.21)
\]

\[
n^* = \frac{32\pi V_o^2 \sigma^3}{3(kT \ln S + c_e E_{ext}^2)^3} \quad (1.22)
\]

\[
\Delta G_f^* = \frac{16\pi \sigma^3 V_o^2}{3(kT \ln S + c_e E_{ext}^2)^2} \quad (1.23)
\]

When \( c_e > 0 \), homogeneous nucleation is stimulated by \( E_{ext} \) since \( n^* \) (and hence \( r^* \)) and \( \Delta G_f^* \) are smaller than at \( E_{ext} = 0 \), provided that the supersaturation does not change (Figure 1.15). The steady-state rate of homogeneous nucleation \( J \) in the presence of \( E_{ext} \) can be expressed as:

\[
J = J_o \exp \left( \frac{-\Delta G_f^*}{kT} \right) = J_o \exp \left( \frac{-16\pi \sigma^3 V_o^2}{3kT(kT \ln S + c_e E_{ext}^2)^2} \right) \quad (1.24)
\]

and shows that for a given supersaturation, \( J \) is a strong function of \( E_{ext} \) when the contribution to the supersaturation is significant.
Figure 1.15  Theoretical effect of external electric field ($E_{\text{ext}}$) on nucleation rate ($J$). 

a) When the dielectric constant of the solute ($\varepsilon_c$) is less than that of the solution drop ($\varepsilon_d$), $E_{\text{ext}}$ supresses solute nucleation.  
b) When $\varepsilon_c$ is greater than $\varepsilon_d$, $E_{\text{ext}}$ enhances solute nucleation.  $E_{\text{cr}}$ is the critical $E_{\text{ext}}$ necessary for either suppression or enhancement. Adapted from Kashchiev.58

The effect on $J$, be it an increase (when $c_c > 0$) or a decrease (when $c_c < 0$) is more pronounced at lower supersaturations (Figure 1.16).133

Figure 1.16  Dependence of the nucleation rate on the strength of the external electric field ($E_{\text{ext}}$) in the case where $\varepsilon_c > \varepsilon_d$ for homogeneous nucleation of a condensed phase at $T$=293 K and supersaturation ratios ($S$) equal to 1.06 and 1.07, respectively. Adapted from Kashchiev.133
In several instances found in the literature, it has been stated that an electric field stimulates nucleation under the condition of $c_c < 0$ (i.e.: when $c_c < c_d$) contrary to the theory presented above.$^{31-33,57,58,134-139}$ This is likely due to corrections made to Kashchiev’s original theoretical work that were not implemented by the cited authors; whereby a factor of three that was absent, and more importantly, inaccurate limits of integration, are both corrected for.$^{133}$

It should be noted that $E_{ext}$ may also have a direct influence the monomer diffusion coefficient ($D$) and even change the mechanism of monomer attachment, hence affecting $J_o$ and the nucleation kinetics. Experimental model systems that use charged colloidal suspensions to study crystal nucleation and structural phase transitions have recently shown that external electric fields (both ac and dc) affect the transportation of charged particles towards the electrodes by electric-field induced fluid flow allowing for the production of large and highly ordered 2-D colloidal crystals at relatively low nucleation rates.$^{140}$

1.3.4 Shortcomings of Classical Nucleation Theory

Researchers have widely applied classical nucleation theory to solution crystallization due to its simplicity. However, differences (of several orders of magnitude at times) between theoretical predictions and experimental results suggest that nucleation of solids from solution does not proceed via the classical pathway.$^{4,141,142}$ A few key shortcomings have been cited as reasons for the inadequacies of classic nucleation theory (CNT).

According to CNT, pre-nucleation clusters are modeled as spherical droplets whereby their density is independent of their size and is the same as the macroscopic density of the bulk condensed phase.$^4$ For crystallization from solution, this assumption implies that the clusters show an internal order of their molecular units; hence the molecular arrangement in a crystal’s critical cluster is identical to that in a large crystal. This assumption does not hold true for many proteins, such as for lysozyme, where critical clusters consisting of 1–10 molecules were observed and thus could not have the structure of a tetragonal lysozyme crystal.$^{142,143}$ The theoretical studies of Oxtoby with regards to first-order phase transitions have also shown that the properties of a critical nucleus can be significantly different from the new phase in composition and structure.$^{144}$
Another CNT limitation is that cluster size is the only criterion for whether concentration fluctuations become nuclei; hence CNT is considered one dimensional.\textsuperscript{145,146} CNT fails to recognize randomly oriented clusters that do not correspond to the solid crystals and organized clusters.\textsuperscript{142} At least two order parameters (density and periodic structure) are necessary to distinguish between a new and an old phase with regards to the nucleation of crystalline solids from solution. However, the CNT description allows only for a single parameter difference between a new and old phase; that of local density.\textsuperscript{142,143}

CNT also assumes that cluster growth takes place on one monomer unit at a time; collisions between existing clusters are not accounted for. Clusters are assumed to be at rest and do not undergo translational, rotational, or vibrational motion.\textsuperscript{4} However, molecular dynamic simulation experiments suggest that cluster-cluster interactions have a significant influence on nucleation.\textsuperscript{4,147} Furthermore, it has been observed using Raman and FTIR spectroscopy that many crystals have growth units other than monomers in solution and that these pre-assembled growth units can aggregate to form pre-nucleation clusters.\textsuperscript{148-150} In some cases, solvent-solute interactions affect what type of molecular growth unit(s) form and numerous research groups have exploited this phenomenon in order to control the polymorphic outcome of crystallization.\textsuperscript{4,9,142,151-153}

Other important shortcomings of CNT include the assumptions that there is no size dependence of the interfacial tension between the old and new phases, that interfacial tension is temperature independent, and the nucleation rate is time-independent (and hence is examinable using steady-state kinetics).\textsuperscript{4}

For the past several decades, scientists have worked to develop and improve nucleation models that are more accurate. Increasing evidence from both molecular simulations and experimental studies suggest that nucleation of solutes from solution is a two-step process: a dense liquid droplet is first formed which is metastable with respect to the crystalline state, followed by ordering within the dense droplet resulting in a 3-D lattice structure.\textsuperscript{4,142} Although initially proposed for the crystallization of proteins, this alternative nucleation model is also applicable to small organic molecules suggesting that this mechanism may underlie most crystallization processes from solutions.\textsuperscript{4} The rate-determining step is thought to be the organizational step within the dense droplet into the
lattice structure. This is consistent with the observation that nucleation from solution takes a longer time for large and complex molecules as it would be more difficult for such molecules to arrange themselves in their precise lattice structures due to their high degree of conformational flexibility.\textsuperscript{4}

1.4 SINGLE DROPLET EVAPORATION

As mentioned in section 1.3, homogeneous nucleation of a solute can be established by allowing the solvent to evaporate in a slow and controlled manner. As such, a discussion of the understanding of single droplet evaporation would be beneficial in light of the goal for this thesis.

The classic method that describes single droplet evaporation of a pure solvent involves the mathematical solution to the conservation equations for a motionless droplet in an infinite stagnant medium, whereby empirical correction factors are employed to account for natural or forced convection around the droplet. The evaporation of a single droplet depends upon the following parameters: droplet radius ($r$) and temperature ($T_d$), the environment temperature ($T_\infty$) that the droplet was introduced to, and the vapour mass fraction at the surface ($Y_{vap}$) of the droplet material.\textsuperscript{104} When the droplet temperature and ambient temperature are initially the same (i.e.: $T_d = T_\infty$) and the vapour pressure for the given droplet temperature corresponds to the vapour mass fraction of the environment ($Y_{vap,\infty}$), neither evaporation of the droplet, nor condensation of the surrounding vapour on the droplet will occur. When $Y_{vap,\infty}$ is increased to supersaturation (i.e.: $Y_{vap,\infty} > Y_{vap}$), condensation of vapour on the droplet will occur. Droplet evaporation will occur when $Y_{vap} > Y_{vap,\infty}$ whereby a mass flux ($\dot{m}_{vap}$) of droplet material leaves the droplet.\textsuperscript{104} The inner energy of the droplet is responsible for the latent heat of vaporization. The resultant heat flux ($\dot{q}_{liq}$) inside the droplet towards its surface causes a heat flux in the surroundings ($\dot{q}_{vap}$) to the droplet surface. This results in a temperature gradient within the droplet and a decrease of $T_d$ as shown in Figure 1.17a.\textsuperscript{104}

When the environment temperature is greater than $T_d$, the droplet begins to heat up (i.e.: $\dot{q}_{liq}$ is directed from the surface to the droplet interior) and $\dot{m}_{vap}$ increases as shown in Figure 1.17b. The increase in $\dot{m}_{vap}$ results in a $\dot{q}_{vap}$ towards the droplet that is responsible for the latent heat of vaporization for the evaporating solvent.
Simultaneously, the outward flow of the vapour in the boundary layer reduces $\dot{q}_{vap}$ to the droplet surface which in turn keeps the droplet at a temperature slightly below $T_\infty$.\(^\text{104}\)

![Diagram](image)

**Figure 1.17** Representation of the mass flux of vapour ($\dot{m}_{vap}$) and the heat fluxes in the gas phase ($\dot{q}_{vap}$) and in the liquid phase ($\dot{q}_{liq}$) of an evaporating droplet; in both diagrams the mass fraction of vapour ($Y_{vap}$) and the radial distribution of the temperature ($T$) are shown:

a) the profile is achieved, if the ambient and initial droplet temperature is equal;  
b) the profile is achieved, if the temperature of the ambience is much higher than the initial droplet temperature. Adapted from Frohn *et al.*\(^\text{104}\)

At this stage all the heat at the droplet surface is utilized for the latent heat of vaporization. A droplet evaporating at this temperature will have a linear decrease of its squared diameter ($d^2$) or radius with time:
\[
\frac{dd^2}{dt} = -\beta_d \quad \frac{dr^2}{dt} = -\beta_r 
\] (1.25)

This relationship is referred to as the \( d^2 \)-Law and is a simple model to describe evaporation of a droplet where \( \beta_d \) (or \( \beta_r \)) is the evaporation coefficient dependent on fluid properties and ambient conditions. The model is essentially a gas phase model for it does not consider \( \dot{m}_{\text{liq}} \) (liquid phase mass transport) or \( \dot{q}_{\text{liq}} \) and its major assumptions are listed in Table 1.1 as discussed by Law.\(^{154}\)

**Table 1.1** The major assumptions built into the \( d^2 \)-Law theory according to Law.\(^{154}\)

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical symmetry</td>
<td>Forced and natural convection are neglected thus reducing the analysis to one dimension</td>
</tr>
<tr>
<td>No spray effects</td>
<td>One isolated immersed droplet in an infinite environment</td>
</tr>
<tr>
<td>Diffusion is rate controlling</td>
<td></td>
</tr>
<tr>
<td>Isobaric process</td>
<td></td>
</tr>
<tr>
<td>Constant gas phase properties</td>
<td>Specific heats and thermal conductivities are constant during evaporation process</td>
</tr>
<tr>
<td>Single solvent species</td>
<td>Not necessary to analyse liquid phase mass transport</td>
</tr>
<tr>
<td>Gas-phase quasi-steadiness</td>
<td>Due to significant differences between gas and liquid densities, liquid is much slower to react to concentration and temperature changes than gas</td>
</tr>
<tr>
<td>Saturation vapour phase at droplet surface</td>
<td>Phase changes between liquid and gas are much faster than gas phase transport and hence gas phase at surface is at equilibrium at its saturation pressure</td>
</tr>
<tr>
<td>No radiation effects</td>
<td>No radiative heat transfer</td>
</tr>
<tr>
<td>No Dufour effects</td>
<td>No heat flow due to concentration gradients</td>
</tr>
<tr>
<td>No Soret effects</td>
<td>No mass flow due to temperature gradients</td>
</tr>
</tbody>
</table>

The basic equations of conservation for mass, species, and energy are as follows:\(^{104,154}\)

**Conservation of Mass** \[
\frac{d}{dr}\left(r^2 \rho_{\text{gas}} v_r \right) = 0 
\] (1.26)

**Conservation of Species** \[
\frac{d}{dr}\left[r^2 \left( \rho_{\text{gas}} v_r Y_i - \rho_{\text{gas}} D_{AB} \frac{dY_i}{dr} \right) \right] = 0 
\] (1.27)
Conservation of Energy \[ \frac{d}{dr} \left[ r^2 \left( \rho_{\text{gas}} v_r C_p (T - T_{\infty}) - \tau_{\text{gas}} \frac{dT}{dr} \right) \right] = 0 \] (1.28)

where \( r \) represents the radial coordinate, \( \rho_{\text{gas}} \) the density of the gas and \( v_r \) the radial velocity of the gas surrounding the droplet, \( C_p \) is the specific heat of the gas, \( \tau_{\text{gas}} \) is the heat conductivity of the ambient medium, \( D_{AB} \) represents the binary diffusion coefficient of the liquid with respect to the gas phase species. The analysis considers only two species, liquid and ambient gas. By definition \( \sum Y_i = 1 \) and only one conservation equation must be solved.\(^{154}\)

Upon integration of equation 1.27 and setting boundary conditions for \( r, T, \) and \( Y_i \), the following solution is obtained:\(^{104}\)

\[ \dot{m}_{\text{vap}} = 4 \pi \rho_{\text{gas,at}} D_{AB} r \ln \left[ 1 - \frac{Y_{\text{vap},s} - Y_{\text{vap},\infty}}{1 - Y_{\text{vap}}} \right] \] (1.29)

where \( \rho_{\text{gas,at}} \) is the density at the droplet surface temperature. From the conservation of mass for a liquid droplet:

\[ \dot{m}_{\text{liq}} = 4 \pi r^2 \rho_{\text{liq}} \frac{dr}{dt} = -\dot{m}_{\text{vap}} \] (1.30)

where \( \rho_{\text{liq}} \) is the density of the droplet. Substitution of equation 1.29 into equation 1.30, results in the equation describing the droplet radius as a function of time:

\[ r^2 = r_o^2 - \beta, t \] (1.31)

where \( r_o \) is the initial droplet radius. The evaporation coefficient \( \beta_r \) can be calculated:

\[ \beta_r = \frac{2 \rho_{\text{gas}} D_{AB}}{\rho_{\text{liq}}} \ln \left[ 1 - \frac{Y_{\text{vap},s} - Y_{\text{vap},\infty}}{1 - Y_{\text{vap}}} \right] \] (1.32)

When the vapour concentration at the droplet surface is very low, either due to low liquid volatility or low temperatures, the evaporation process can be described as diffusion-controlled evaporation. In this instance, it is assumed that both the droplet temperature and ambient have approximately the same value and that these temperatures are low in comparison with the droplet’s boiling point (\( T_b \)).\(^{104}\) Under these conditions, evaporation is dominated by diffusion processes in the vapour phase such that \( v_r = 0 \). The \( d^2 \)-Law is then derived by integrating equation 1.27 and substituting partial pressures for vapour concentrations (assuming ideal gas behaviour):\(^{104}\)
\[ r^2 = r_0^2 - \frac{2M_{liq}D_{AB}}{R \rho_{liq}} \left( \frac{p_s}{T_s} - \frac{p_\infty}{T_\infty} \right) * t \]  

(1.33)

where \( M_{liq} \) is the molar mass of the liquid, \( p_s \) and \( p_\infty \) are the partial pressures of the vapour at the droplet surface and in the ambient, and \( R \) is the universal gas constant. The evaporation coefficient is then given by:

\[ \beta_s = \frac{2M_{liq}D_{AB}}{R \rho_{liq}} \left( \frac{p_s}{T_s} - \frac{p_\infty}{T_\infty} \right) \]  

(1.34)

### 1.4.1 Evaporation of Droplets Containing Dissolved Solids

The evaporation of drops containing dissolved solids can be divided into two different stages as illustrated in Figure 1.18. The first stage involves evaporation of the solvent from the drop surface resulting in the decrease of drop radius (or diameter) over time along with an increase in solute mass fraction of the droplet. A second evaporative stage begins once enough solid has precipitated at the drop surface to cover it entirely while trapping liquid inside and is characterized by constant drop volume and solvent evaporation through the newly formed surface crust.

![Figure 1.18](image)

**Figure 1.18** The drying stages of an evaporating solution drop.
1.4.1.1 First Stage Evaporation

Although drops contain a dissolved component, the $d^2$-Law is followed during first stage evaporation. The amount of evaporated material remains constant over time as long as the drop surface remains completely wetted.\textsuperscript{155} Solvent migrates to the drop surface from the interior at a rate that maintains surface saturation. Depending on the nature of dissolved solid, capillary and diffusive forces are the two mechanisms thought to be responsible for observed drop evaporation behaviour.\textsuperscript{155} It should be noted that the solute has the effect of lowering the vapour pressure of the solvent.

As more and more solvent evaporates from the drop, the dissolved solute becomes more and more concentrated and a point is reached whereby the solvent migration rate to the drop surface becomes the limiting factor to evaporation; the surface wetness can no longer be maintained and the evaporation rate decreases.\textsuperscript{155} Since the rate of diffusion of the solute back into the drop is slower than the rate of solvent evaporation, crust formation of the solute at the drop surface can occur.\textsuperscript{156,157} The appearance of the crust is indicative of the end of the first stage of drying and may occur before uniform saturation throughout the drop is reached.\textsuperscript{158} The appearance of a crust is also indicative of a high nucleation rate as the crust is made up of numerous small solute crystals. That being said, it is possible to grow a small number of single crystals from a levitated drop; however, it requires careful control over the slow evaporation of the solvent.

1.4.1.2 Second-Stage Evaporation

At the end of first-stage evaporation, the evaporation rate changes as the entire drop surface is no longer entirely wetted. The wetted surface area steadily decreases until it is eventually dry. Crust formation of an evaporating drop containing dissolved solids is a function of temperature, humidity, and relative velocity of the ambient gas (i.e.: drying conditions), the surface tension and vapour pressure of the solvent, the solubility and surface activity of the solute, and the initial drop size.\textsuperscript{155,159} During this stage, the volume of the formed particle remains constant while evaporation of the remaining solvent inside of it takes place resulting in a decrease in mass and density of the particle. The heat required for evaporation is transferred through the solid, while solvent vapour moves through the solid and into the ambient air. The movement of solvent vapour through the solid decreases over time as the solid layer becomes more extensive. In spray drying
applications, this can make it difficult to create particles with specific moisture content. Since heat transfer into the solid exceeds the mass transfer through the solid for evaporation, the drop’s temperature increases resulting in possible internal vapour build-up and potential particle explosion.\textsuperscript{155}

The rate of evaporation in second-stage evaporation is largely controlled by two mechanisms: 1) the removal of solvent vapour from the solid surface due to the drying conditions in a similar manner to that of first-stage evaporation; 2) the movement of solvent vapour within the solid due to the physical nature of both the solvent and solute.\textsuperscript{158,160} A number of different mechanisms have been postulated for the movement of solvent vapour through a solid. Vapour diffusion theory asserts that a vapour concentration gradient is formed between the depths of the formed particle and its surface whereby diffusion of the vapour can occur. This kind of transport is common with non-porous solids.\textsuperscript{161} In porous solids, solvent vapour can move through interconnecting pores and channels via capillary action and not by diffusion. A meniscus of solvent is formed across each pore in the depths of the solid as solvent evaporates and the solid structure increases in size. The interfacial tension between the solvent and solid sets up capillary forces that drive the movement of solvent through the pores.\textsuperscript{161} Liquid assisted vapour transport asserts that the solvent in a porous solid migrates through the solid by successive evaporation and condensation between liquid bridges.\textsuperscript{158} Morphological changes of the forming solid, dependent upon the nature of the solute, can also affect the movement of solvent vapour through the solid. If a non-porous and pliable solute skin can form, then it is possible that particle inflation could occur due to the accumulation of vapour inside the formed particle. If the internal pressure is too great, then the skin could rupture releasing the solvent vapour.\textsuperscript{155} If a rigid and porous crust is formed, then the formed particle may crack or even explode in order to release built-up solvent vapour.

\subsection*{1.4.2 Particle Formation: Size and Morphology}

The size of a particle formed from a solution drop ($d_p$) depends upon the initial drop size ($d_o$) and the solute concentration ($C$) of the initial starting solution used as shown by the following equation:\textsuperscript{162}
\[ d_p = \left( \frac{C}{\rho_p} \right)^{1/3} d_o \]  

(1.35)

where \( \rho_p \) is the particle density. In a different study, an almost linear dependence was observed between final particle size and initial drop size.\textsuperscript{163} However, in their spray drying studies of aqueous lactose drops, Elversson \textit{et al.} observed that within the range of 5-20\% (w/w lactose/H\textsubscript{2}O), there was only a slight increase in particle size with increasing lactose content implying a lower density particle is obtained when utilizing starting solutions with lower lactose content.\textsuperscript{163} On the other hand, Masters \textit{et al.} demonstrated that a reduction in particle size occurs for film forming compounds at low concentrations.\textsuperscript{155} It should be noted that equation 1.35 is applicable for particles forming in the absence of an external field.

Initial drop size and solution concentration also affect particle morphology along with other factors such as the external drying conditions and the physical properties of the solute. It has been observed by Maa \textit{et al.} that at higher operational temperatures, protein particle deformations such as holes and dimples occur, while at lower temperatures spherical particles are obtained without deformation.\textsuperscript{164} If the solid forms a non-porous crust, then the drop may expand, collapse, or even rupture and disintegrate due to pressure build-up of trapped solvent vapour; all of which would affect final particle morphology.\textsuperscript{165} Figure 1.19 shows common particle shapes observed after spray drying.

Single drop experiments have been used by several groups to study particle morphology as it pertains to spray drying. The larger drops studied in single drop experiments are easy to observe, record, and control. Walton and Mumford conducted an extensive study on particle morphology from single drop experiments and compiled a morphological classification system; the morphologies they studied were categorized into three general groups: skin-forming, crystalline, and agglomerate materials.\textsuperscript{158} Their term ‘skin-forming’ is a generalization to describe a particle surface structure that is comprised of polymeric and microcrystalline material. They observed that higher ambient temperatures result in shorter droplet drying times but the resultant particles had a greater tendency to inflate and explode.\textsuperscript{158}
The more recent single drop experiments of Lin and Gentry demonstrated that small, dense, and regularly shaped particles are more readily obtained using low ambient temperatures with solutes that have a high latent heat of crystallization.\textsuperscript{166} They also observed that compounds having high initial solute concentration were favourable in forming dense particles with irregular shapes while compounds that formed elastic shell structures, such as ammonium chloride, were usually hollow.\textsuperscript{158} Table 1.2 gives an overview of the conditions conductive to forming solid or hollow particles.
Table 1.2  Conditions conducive for solid and hollow particle formation.

<table>
<thead>
<tr>
<th>Conditions for Solid Particle Formation</th>
<th>Conditions for Hollow Particle Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei free environment$^{167}$</td>
<td>High evaporation rate$^{167,168}$</td>
</tr>
<tr>
<td>High solubility solute$^{167}$</td>
<td>High temperature causing vapour formation$^{169}$</td>
</tr>
<tr>
<td>Smaller drop$^{168}$</td>
<td></td>
</tr>
<tr>
<td>Lower diffusivity in solvent$^{170}$</td>
<td></td>
</tr>
<tr>
<td>High viscosity$^{171}$</td>
<td></td>
</tr>
<tr>
<td>Low evaporation rate$^{166}$</td>
<td></td>
</tr>
<tr>
<td>Low initial drop saturation$^{166}$</td>
<td></td>
</tr>
</tbody>
</table>

1.5 GENERAL TECHNIQUES

1.5.1 Droplet Levitation

Levitation techniques, whereby an object has been suspended by physical forces against the force of gravity in a stable position without physical contact, have been used in both science and engineering laboratories for the past century in the study of several fundamental processes; the most famous being the Millikan oil droplet experiment that measured the elementary electric charge (i.e.: the charge of the electron). More recently, levitation techniques have been used to investigate various aspects of material processing. By isolating a material from everything except its radiation environment, the perturbation influence of container walls and impurities are removed, helping to ensure a better understanding of a material’s behaviour.$^{172}$ Levitation techniques have been previously used to measure thermophysical properties of materials such as surface tension, density, viscosity, and heat capacity.$^{173}$ They have also been used to study the solidification behaviour of undercooled melts and the drying processes of droplets. The drying of solution droplets is of particular interest in several industrial processes whereby powders are produced by spray drying.$^{174,175}$

In order to examine a sample without a container under Earth’s gravity, an upward force must be applied without interruption to counteract the sample’s weight.
Furthermore, lateral forces acting on the sample must also be counteracted to keep it in the field of view of diagnostic instruments. Several types of levitators have been developed including acoustic, electrodynamic, electromagnetic, and electrostatic levitators; each having unique capabilities and uses.\textsuperscript{172} Most levitators of all types are usually built in-house as required; the lone exception being an ultrasonic levitator manufactured by a company (tec5) that designs and manufactures systems for spectroscopy.\textsuperscript{176}

Upon entering the Agnes Group, I became familiar with an apparatus that was being used to generate atmospheric particles mimics via an electrodynamic levitation technique whereby charged solution or emulsion droplets were levitated. After the solvent(s) had evaporated from the droplet(s), the remaining particle(s) would remain levitated, until needed for experiment. In this thesis, both electrodynamic and acoustic levitation techniques are employed to process droplet samples.

1.6 RESEARCH OBJECTIVES

The general purpose of this work was to investigate the effect that droplet surface potential (or droplet net charge) had upon the nucleation of a solute from a levitated droplet; there being virtually no such investigations in the current literature. The goal was to be able to determine whether or not droplet surface potential could be used as a tool to facilitate controlled nucleation of a solute with respect to the nucleation rate, the resultant crystal size and habit (or shape), and the particular crystal structure adopted by the nucleus.

This work can be divided into five sections. The first part presents theoretical and background information with regard to the electrodynamic levitation of charged droplets. An electrodynamic levitation trap was then used for both single droplet and droplet population experiments with regard to the investigation of surface potential controlled nucleation. Two salt solutions and two solutions containing neutral organic molecules were considered and the influences of droplet evaporation and the magnitude and polarity of the droplet surface potential on the nucleation mechanism and rate, and particle habit were discussed. The results are compared to relevant experimental data in the literature.
The second part of this work presents theoretical and background information with regard to acoustic levitation and its benefits over using an electrodynamic levitation technique for sample processing. The design, construction, and assembly of an acoustic levitation apparatus are discussed. The in-house built apparatus is characterized and short-comings of the prototype apparatus are discussed.

The third section of this work summarizes previous experimentation in the literature with regard to the acoustic levitation of solution drops and the subsequent nucleation and crystal growth of solutes therein. Charged and neutral drops of sodium chloride solution are acoustically levitated and the influences of drop evaporation, the magnitude and polarity of the droplet surface potential and the effects of an external electric field on the nucleation mechanism and rate, and particle habit are discussed. The results are compared to the results presented in the first section and to the relevant experimental data in the literature.

The final section presents theoretical and background information with regard to the nucleation and growth of polymorphic compounds from solution. The effect of both drop surface-potential and the presence of an external electric field on the crystallization of two concomitant polymorphic systems with respect to the polymorphic fraction formed are investigated for acoustically levitated solution drops. The results are compared to relevant experimental data in the literature.
CHAPTER 2
SURFACE-POTENTIAL CONTROLLED NUCLEATION: PROMOTED NUCLEATION IN LEVITATED CHARGED DROPLETS

2.1 INTRODUCTION

Levitation techniques, whereby an object has been suspended by physical forces against the force of gravity in a stable position without physical contact, have been used in both science and engineering laboratories for the past century in the study of several fundamental processes; the most famous being the Millikan oil droplet experiment that measured the elementary electric charge (i.e.: the charge of the electron). Upon entering the Agnes Group, I became familiar with an apparatus that was being used to generate atmospheric particles mimics via an electrodynamic levitation technique whereby charged solution or emulsion droplets were levitated. After the solvent(s) had evaporated from the droplet(s), the remaining particle(s) would remain levitated, until needed for an experiment. The apparatus is called an electrodynamic levitation trap (EDLT) and is a modified version of a quadrupole ion trap.

2.1.1 Quadrupole Ion Trap

The quadrupole ion trap was first invented in 1953 by Paul and Steinwedel, and became incorporated into commercial mass spectrometers thirty years later. Ions are trapped by the quadrupole using a conjunction of constant dc electric fields and radio frequency oscillating ac fields. This 3-D trap consists of a metal ring electrode (where the oscillating ac is applied) positioned halfway between two hyperbolic metal electrodes (where dc potential can be applied but are usually kept at ground potential) that have their foci facing each other as illustrated in Figure 2.1.
Figure 2.1 Illustration of a quadrupole ion trap showing both end cap electrodes and the centre ring electrode.

Ions are trapped in the space between the three electrodes in a saddle-shaped electric field where they move about in a complex motion that generally involves the ions moving in a long and narrow trajectory followed by a short and wide trajectory. As the ions deviate linearly from the centre of the trap, a quadratic restoring force moves them back towards the centre. The ions oscillate between the two trajectories and hence the technique is time dependent and is referred to as electrodynamic.

2.1.2 Electrodynamic Levitation Theory

The motions of an ion in a quadrupole ion trap are governed by the numeric solutions to the second order differential Mathieu equation:

$$\frac{d^2 y}{dx^2} + [a - 2q\cos(2x)]y = 0$$  \hspace{1cm} (2.1)

Solutions to the Mathieu equation were originally used to mathematically describe the vibrations of a stretched skin in terms of regions of stability and instability.

The stability of an ion in a quadrupole ion trap is predicted by the solution to the Mathieu equation as expressed by two dimensionless parameters: $a$ and $q$:
where $e$ is the charge of the electron, $V_{dc}$ is the applied potential to the end cap electrodes, $m$ is the mass of an ion, $z_o$ is the halfway distance between the two end cap electrodes, $\omega$ is the radial frequency of the applied potential having an amplitude of $V_{ac}$ to the ring electrode.

Hence the ability for an ion to be trapped is dependent upon its mass to charge ratio (or charge to mass ratio), the frequency and amplitude of the potentials applied to the electrodes, and the size of the trap. In order for an ion to be trapped and levitated, its trajectory must be stable in both the axial and radial dimensions simultaneously. This can be visualized by superimposing the plots of $a_z$ vs. $q_z$ and $a_r$ vs. $q_r$ and locating the regions of overlap as shown in Figure 2.2. This particular bihyperboloidal trap configuration is well suited for mass spectrometry where it is kept at low atmospheric pressure in order to facilitate the formation of ions from neutral analyte molecules via electron bombardment inside the trap. For a conventional ring electrode radius of 1.0 cm and a separation distance between the hyperbolic cap electrodes of 1.5 cm, low molecular weight ions can be trapped using fixed radiofrequencies (i.e. MHz) having low (1-10 kV) amplitudes. By ramping the $V_{ac}$ of the ring electrode, ions with a particular mass to charge ratio are ejected from the quadrupole trap at their point of instability and directed to a conventional electron multiplier detector. However, if 1-100 $\mu$m-sized charged droplets are to be levitated in a quadrupole ion trap, then the frequency applied to the ring electrode needs be lowered to the 10-1000 Hz range since an increase of $V_{ac}$ much above 10 kV will cause electrical discharge at the ring electrode. Furthermore, the ac frequency should be adjustable during levitation (as opposed to fixed) to enable real-time compensation for the levitated droplet’s changing mass to charge ratios due to evaporation of the solvent. Hence in order to study particles such as $\mu$m-sized charged droplets, a modified quadrupole ion trap is necessary.
2.1.3 Electrodynamic Levitation Trap

The use of the electrodynamic levitation balance or trap (EDLT) to study microparticles (solid and/or liquid) at atmospheric pressure parallels the use of the quadrupole ion trap for ion spectroscopy at reduced pressures. The first EDLT configuration, derived from the Millikan apparatus from the oil-drop experiment, consisted of an ac ring electrode placed midway between two flat dc electrodes. Several different configurations of the EDLT have also been employed including the popular bihyperboloidal version, a modified version of the quadruple ion trap designed to work at atmospheric pressure, and the relatively simple and effective double-ring configuration introduced by Davis as shown in Figure 2.3.
Figure 2.3  Standard double ring configuration for the EDLT. Adapted from Davis et al.\textsuperscript{182}

Although charged particle mobility within the EDLT is governed by the solution set for the Mathieu equations, modifications to the equation are necessary to account for the aerodynamic drag force on the particles at atmospheric pressure. Usually the electrodes for an EDLT are housed within a chamber with viewing ports in order to reduce the loss of particles due to air currents. Most work involving a double-ring EDLT has been for liquid and/or solid particles ranging from 1-100 µm in diameter with approximate 1 cm spacing between the rings and using a 2 kV applied potential amplitude with a frequency in the 100 Hz range.\textsuperscript{180}

Unlike the quadrupole ion trap employed in MS whereby charged ions are produced inside the trap in situ, there several methods used to inject charged droplets into an EDLT. A dc pulse can be used to charge and eject droplets from a hypodermic syringe needle; the mass to charge ratio of the droplets trapped is controlled by adjustment of the ac potential and frequency in order to expel undesired satellite droplets.\textsuperscript{183} A vibrating orifice generator fitted with an induction election electrode has also been used to introduce charged droplets into an EDLT.\textsuperscript{184} For more precise droplet size and charge control, a droplet-on-demand dispenser can be used to dispense droplets that are inductively charged upon formation prior to trapping.\textsuperscript{185,186} The electric field between the induction electrode and the dispenser tip allows for a redistribution of ions in solution as the droplet forms resulting in a dispensed charged droplet as shown in Figure 2.4.
Figure 2.4  Illustration of negatively charged droplet formation from a droplet dispenser in the presence of a positively charged induction electrode.

After droplet injection into the EDLT, the charge droplet oscillates vertically at either the ac frequency or half of the ac frequency with an amplitude that is dependent upon the ac potential. If the ac field is too large, then the oscillating droplet will be ejected from the trap; if it is too small, then the droplet will not be initially trapped.

Similar to ions trapped in a quadrupole ion trap, a trapped droplet will continue to oscillate in the ac field of the EDLT. By adjusting the dc potential of the end-cap electrodes in order to balance the vertical forces, a minimally oscillating, stably trapped droplet can be obtained in the EDLT. As droplets in micron-size range are difficult to view with the naked eye, illumination via laser light scattering in combination with optical magnification make droplet motion easily observable for ac/dc field adjustment and droplet characterization. It should be noted that multiple droplets can be trapped and levitated in an EDLT and that the motion of one droplet is affected by the rest of the droplet population.¹⁸⁷
Several properties and characteristics of droplets can be measured in an EDLT. Elastic light scattering measurements can be used to determine a droplet size and refractive index. The droplet’s chemical characteristics can be obtained using a spectrometer to record Raman or fluorescent spectra via inelastic light scattering. Vapor pressures, evaporation coefficients, gas phase diffusion coefficients, droplet charge limits, water activities and activity coefficients of multicomponent systems can be determined from droplet evaporation measurements.\(^{186,188-194}\) Furthermore the thermodynamics of highly concentrated salt solutions and their nucleation and crystallization can be studied without interference from container walls in the EDLT.\(^{195-198}\)

In their previous electrodynamic levitation studies with charged droplets, the Agnes group reported the enhancement of \(\alpha\)-cyano-4-hydroxycinnamic acid (CHCA) co-crystallization with one or more peptides and the promotion of NaCl precipitation; both a function of the magnitude of the droplet’s net charge.\(^ {37,38,199}\) It is widely believed that indiscriminate co-crystallization of compounds having a wide range of properties within a host compound is a necessary requirement for optimal preparation of solid-samples for matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS).\(^ {200,201}\) MALDI MS is a laser-based soft ionization method whereby a sample is embedded in a chemical matrix that greatly facilitates the production of intact gas-phase ions from large, nonvolatile, and thermally labile compounds such as proteins, oligonucleotides, synthetic polymers, and high molecular weight inorganic compounds. The matrix plays a key role in this technique by absorbing the laser light energy and causing a small part of the target sample to vaporize and ionize.

This phenomenon of promoted solute nucleation was originally termed ion-induced nucleation \textit{in solution} by the Agnes group, having several implications for laboratory, industrial, and natural processes involving media with net charge ranging from fundamental aspects of soft ionization for mass spectrometry to the preparation of nanophase materials with improved properties.\(^ {40,89,202-204}\) However, since it was unclear that the excess ions in solution were responsible for the heterogeneous nucleation that was implied when using the term ion-induced nucleation, surface potential controlled nucleation was believed to be a more concise term to use so that heterogeneous
nucleation is not implied. Surface potential controlled nucleation will be the term used to describe the phenomenon of promoted solute nucleation within this thesis.

2.1.4 Research Objectives

The focus of the research discussed in this chapter was to characterize the threshold behaviour of surface potential controlled nucleation using an electrodynamic levitation trap for sample processing. The nucleation and crystal growth of sodium chloride from both single droplet and droplet population experiments was to be conducted under conditions of both high and low droplet charge and differing charge polarity to determine their effects on nucleation rate and the type of crystal habit adopted. The phenomenon of surface potential controlled nucleation will also be considered with two neutral organic compounds. A method that uses surface potential controlled nucleation to prepare ammonium nitrate particles to be subsequently used in in vitro toxicology studies of ambient particle types will also be examined.

2.2 EXPERIMENTAL SECTION

2.2.1 Chemicals

Reagent grade NaCl, methanol, and glycerol were purchased from BDH. The 20 nm FluoSpheres® used, purchased from Molecular Probes (Invitrogen Inc., Burlington, ON, Canada), are polystyrene-based spheres (density = 1.05 g mL⁻¹) that encapsulate ~180 fluorescein molecules per sphere. They are supplied as suspensions (2 % solids) in water. Ammonium nitrate (NH₄NO₃), 2,4,6-trihydroxyacetophenone monohydrate (THAP), and α-cyano-4-hydroxycinnamic acid (CHCA) were purchased from Aldrich. All aqueous solutions were prepared using distilled water (dH₂O).

2.2.2 Droplet Dispensing, Capture, and Levitation

The steps involved in the methodology used to dispense and characterize droplets that were levitated for time periods between 30 seconds to several hours, are described below.

A micropipette was used to load a 3 μL aliquot of a starting solution into the reservoir of an inkjet-style droplet dispenser (models MJ-AB-01-40 and MJ-AB-01-60, Microfab

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Technologies Inc., Plano, TX, USA). Activation of the piezoceramic crystal bonded to the outside of the dispenser’s reservoir generated an acoustic wave that caused a volume of liquid to pass through the nozzle as a jet that then separated from the nozzle and subsequently collapsed to form a monodisperse droplet. The nozzle of the dispenser was positioned over a 5.0 mm diameter hole cut into a flat copper electrode. A dc potential applied to this electrode using a high voltage power supply (Model PS350, Stanford Research Systems, Sunnyvale, CA) established an electric field between it and the nozzle, influencing ion mobility in the jet prior to its separation from the dispenser nozzle. This induced charge separation within the jet caused the resultant droplet to have a net excess charge. Variation of the dc potential applied to the induction electrode proportionally varied the magnitude of the image charge imparted onto the droplets. Each of these droplets then passed into an electrodynamic levitation trap (EDLT) where they were trapped and levitated.

2.2.3 The Employed Electrodynamic Levitation Trap Apparatus

Figure 2.5 illustrates the EDLT used in this study; a double ring, double disk trap that was built in-house and previously described.

Figure 2.5 Schematic of the electrodynamic levitation trap (EDLT) showing the important operational components. The inset is a representative image of laser light scattered by a population of droplets levitated in the EDLT.
Its two ring electrodes were constructed using single stranded 1 mm diameter copper wire that were each shaped into 2 cm diameter rings and mounted parallel to each other at a separation distance of approximately 6 mm. The pair of rings was positioned between two flat end-cap electrodes referred to as the induction and the bottom electrode. The induction electrode during droplet dispensing also served as the upper end-cap electrode for the EDLT during droplet levitation. Typically, the dc electric field between it and the lower end-cap flat electrode is used to balance the force of gravity acting on the droplets. However, the non-hyperbolic electrode shape and positioning used in the EDLT resulted in significant deviations from a quadrupole electric field, which permitted ongoing droplet levitation after the potentials of both the upper and lower end cap electrodes had both been reduced to 0 V while a sine wave was maintained on the ring electrodes. It should be noted that two EDLT’s were used in the course of this study: one for droplet population experiments, whereby a 60 Hz sine wave, 500-2700 V\textsubscript{O-P}, was applied to both rings, in phase, by use of an in-house constructed Variac-controlled voltage amplifier, and one for single droplet experiments, whereby a sine wave (50-700 Hz, ± 500-4000 V) was applied to both rings, produced using a function generator (Model TFG-4613, Topward Electric Instruments Co., Taipei Hsien, Taiwan) in series with a high voltage variable frequency amplifier (Model 609E-6, Trek Inc., Medina, NY, USA). Immediately after a droplet was trapped in single droplet experiments, the ac frequency applied to the rings was increased from ∼80 Hz to ∼700 Hz, in order to ensure the droplet was quiescent. By reducing the ac potential of the ring electrodes while both the upper and lower end cap electrodes were held at 0 V, the force of gravity exerted on the charged droplet caused it to fall out of the ac trap and to be deposited on the lower end-cap electrode, or target plate, at the end of each levitation experiment. This deposition procedure was strictly adhered to for singly levitated charged droplet experiments. The distance between the centre of the ring electrodes and the deposition plate was 15 mm. All electrode potentials were relative to ground potential (0 V). The droplets in the EDLTs were illuminated via forward scattering by a 4 mW green (λ = 543 nm) HeNe laser (Uniphase model 1676, Manteca, CA) that was defocused (laser spot size diameter ∼ 6 mm). Although laser light can affect nucleation from solution, several
control experiments were conducted, confirming that nucleation was unaffected by the HeNe laser. A photo of the EDLT is presented in Figure 2.6.

![Diagram of the EDLT](image)

**Figure 2.6** The electrodynamic levitation trap (EDLT) without front plate and viewing port attached.

### 2.2.4 Characterization of Droplet Residues

Each experiment involved flushing the internal reservoir of the droplet dispenser with clean solvent and then filling it with a 3 µL volume of a starting solution. Starting solutions were comprised of zero or one solute in a solvent mixture of water/glycerol, unless otherwise noted. Within ~ 2-5 s of the droplet dispensing event, the majority of the volatile solvent evaporated, leaving behind a droplet comprised of glycerol and the non-volatile solutes, referred to throughout this thesis as the droplet residue. In most experiments, a glass coverslip was positioned on top of the bottom electrode of the EDLT where the droplet residues were eventually deposited at the end of each levitation experiment. Droplet residues were characterized by optical microscopy (Model B5 Professional, Motic, Richmond, BC).

### 2.2.5 Measurement of Initial Droplet Volume and Net Charge

The initial volume of a droplet was determined using fluorescence emission microscopy. After loading a dispenser (40 µm diameter orifice) reservoir with an aqueous solution of FluoSpheres® (50 µL FluoSpheres® in 25 mL dH₂O), a predetermined number of droplets were dispensed consecutively onto a glass coverslip where they dried forming a small spot. Droplets were dispensed using a 10 V waveform
amplitude applied to the droplet dispenser at a frequency of 1 Hz. A series of these spots made up of differing numbers of droplets were deposited in a row on the same coverslip and the fluorescence emission of each spot was measured. A Zeiss Axioplan 2 (North York, Ont., Canada) optical microscope fitted with an excitation filter (BP-546/12) and emission filter (LP-590) was used to collect all images of fluorescence emission. For each sample spot, fluorescence emission was collected from a 1.50 mm × 2.00 mm area centered over the site of droplet deposition. The signal intensity of the fluorescence emission for each image was determined using Image J software (Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA) and integrated. The resultant calibration graph of fluorescence emission signal intensity vs. number of droplets is shown in Figure 2.7.

![Figure 2.7 Calibration of the initial volume of droplets dispensed using the fluorescence emission from FluoSpheres® against volumes delivered by a micropipette.](image)

The fluorescence emission from a known volume \( V = 0.020 \pm 0.005 \, \mu\text{L} \) of the same aqueous solution, as deposited by microliter syringe onto a glass coverslip, was then measured under identical conditions as those employed for the fluorescence emission measurement of the spots. By using the calibration graph, the initial dispensed droplet
volume was calculated to be 250 ± 90 pL (average radius = 39 ± 14 µm) for a 40 µm diameter orifice dispenser. It should be noted that different droplet dispenser waveform amplitudes result in different dispensed droplet volumes.

The induced droplet net charge was determined by dispensing individual charged water droplets directly onto a metal target plate connected to an electrometer (Model 6517a, Keithley Instruments, Cleveland, OH, USA). For these measurements of droplet net charge magnitude, the droplet dispenser, induction electrode, and metal target plate were situated inside a Faraday cage. The separation distance between the nozzle tip and the induction electrode was 1.25 ± 0.02 mm while the separation distance between the induction electrode and metal target plate was ~ 40 mm. The magnitude of the induced net charge per droplet was plotted, as a function of the dc potential applied to the induction electrode, in Figure 2.8.

![Figure 2.8](image)

**Figure 2.8** Measurement of the net excess charge on dispensed droplets as a function of induction potential.

Data points for dispensed droplets were the average of 100 droplets dispensed using the same 10 V waveform amplitude applied to the droplet dispenser at a frequency of 1 Hz. The polarity of the charge induced on the forming droplet was opposite to the polarity of
the dc potential applied to the induction electrode. The magnitude of the dc potential did not affect the volume of the droplet dispensed within experimental error as determined using fluorescence emission microscopy.

2.2.6 Droplet Evaporation Rate

The trajectories of multiple droplets simultaneously levitated in the EDLT (see Figure 2.5 inset) depended on several factors such as the geometry of the ring electrodes and the potentials applied to them, droplet mass, droplet net charge, and the number of droplets simultaneously levitated. For dispensed droplets of the same volume, an increase in droplet net charge should result in higher droplet mobility for a droplet population trapped at atmospheric pressure during levitation in an EDLT. Results from single droplet studies have demonstrated a substantial enhancement in the evaporation rate for stably oscillating single droplets over their stationary droplet counterparts.\textsuperscript{208,209} Hence, a population of charged droplets having higher droplet mobility should experience enhancement of the droplet evaporation rate relative to a population of charged droplets having lower droplet mobility. The effect of droplet mobility on the rate of solvent evaporation over the range of conditions used in this study was first determined before preliminary characterization of surface potential controlled took place.

A population of droplets was dispensed from a water/glycerol starting solution (99:1 v/v) using a 10 V waveform amplitude applied to the droplet dispenser (40 µm diameter orifice) at a frequency of 1 Hz. The dc potential applied to the induction electrode was kept constant for the dispensing of each droplet in a population that was levitated in the EDLT. The relative humidity and temperature of the chamber was monitored with a digital humidity meter (Model L914797, VWR International, Edmonton, AB, Canada). Over the course of this and all subsequent experiments, the temperature and relative humidity of the EDLT chamber had average values of (22 ± 1) °C and (33 ± 5) % respectively, unless otherwise noted. The same type of experiment was then repeated for single, quiescent levitated droplets in lieu of a droplet population.

2.2.7 Nucleation and Growth of NaCl from Single Quiescent Droplets

The Agnes Group previously presented evidence that control of the magnitude of the net charge (i.e.: number of ions\textsubscript{DNEG}) contained in a levitated droplet can be used to
promote the co-crystallization of an organic acid, \(\alpha\)-cyano-4-hydroxycinnamic acid (CHCA), with one or more peptides.\(^{38}\) To characterize the role of ions\(\text{DNEC}\) (i.e.: droplet net charge) in affecting crystal nucleation and growth, a series of experiments were performed to elucidate the effect of relevant and readily varied properties of levitated droplets. These experiments involving droplets having net charge were designed so that droplets would not undergo Coulomb explosion.

The effect of droplet net charge on NaCl nucleation was determined. For the following two trials, a calibrated 40 \(\mu\)m diameter orifice droplet dispenser with a 10 V applied waveform at 1 Hz was used to dispense droplets from a 298 mM NaCl aqueous starting solution containing 571 mM glycerol (water:glycerol = 97:3 v/v). NaCl was chosen as the nucleating solute due to the relative simplicity of the system and the certainty with which the identity of the ions\(\text{DNEC}\) that constitute the droplet net charge could be assigned; \(\text{Cl}^-\) ions\(\text{DNEC}\) or \(\text{Na}^+\) ions\(\text{DNEC}\) depending on the polarity of the induction electrode.\(^{37}\)

The first trial was comprised of several experiments, whereby single droplets were dispensed, trapped and levitated. Each droplet had a net charge of \(\sim -48\) fC (induction potential (IP) = 130 V). Within \(\sim 5\) seconds of droplet formation, the volume of each levitated droplet decreased significantly, due to the rapid evaporation of \(\text{H}_2\text{O}\), until equilibrium with the levitation chamber was reached. Since the chamber was kept at a relative humidity (% RH) of 0-5 %, the droplet residue volume shrank to \(\sim 7.5\) pL (average radius \(\sim 12.1 \mu\)m) and became saturated in NaCl (NaCl solubility in glycerol = 1.711 M).\(^{210}\) After 5 minutes of levitation, the quiescent droplet residue was deposited onto a glass coverslip and the number of individual NaCl crystals formed was counted. Previously described control experiments demonstrated that NaCl precipitation occurred in the levitated residues and not as a result of heterogeneous nucleation at the glass slide-residue interface; the growth of NaCl from a droplet directly deposited on a glass slide without surface potential showed significantly different morphology than NaCl grown from a levitated droplet.\(^{37}\) The crystals obtained had regular cubic habits and the length of a side of each crystal in the droplet residue was measured in order to ascertain the size of each salt crystal. Numerous repeat experiments were conducted.

The second trial was comprised of several experiments conducted under the same conditions as the first trial, with the exception of net droplet charge which was increased
to ∼ -110 fC (IP = 330 V). The total number of NaCl crystals was counted using optical microscopy and the side length for each crystal in the droplet residue was measured. Crystal sizes ranged from 1.0 µm$^3$ to 343 µm$^3$. Numerous repeat experiments were conducted.

### 2.2.8 Nucleation and Growth of NaCl from Droplet Populations

The effect of droplet net charge on NaCl nucleation was also investigated in populations of levitated droplets. While conducting initial NaCl nucleation trials with droplet populations, it was observed that the NaCl crystal habit changed from regular, well formed, cubic shapes to dome-shaped dendrites as the induction potential used to impart charge on the droplets was increased.$^{37}$ As crystal habit is controlled by the kinetics of the atomic growth process through which assembly occurs,$^3$ it was speculated that the change in droplet net charge (i.e.: change in external growth conditions) was responsible for the observed dendritic NaCl$\text{(s)}$. A set of experiments was then devised to better characterize this effect of surface potential controlled nucleation on the observed NaCl crystal habit.

A calibrated 40 µm diameter orifice droplet dispenser with a 30 V applied waveform at 1 Hz was used to dispense droplets (initial volume = 260 ± 10 pL); the calibration was performed by Samuel Bakhoum and the results have been previously published.$^{38}$ Four different starting solutions (1.1, 2.2, 3.3, or 4.4 mg of NaCl, 4, 8, 12, or 16 µL of glycerol, and 396, 392, 388, or 384 µL of dH$_2$O) were used to prepare droplet residue populations with similar NaCl concentrations with initial droplet residue radii of 8.9, 11.2, 12.8, or 14.1 µm, respectively. For a given induction potential, a population of ∼ 40-70 droplets was dispensed and levitated for 5 minutes in the EDLT. The subsequent population of droplet residues was deposited onto a glass slide where each residue was then examined via optical microscopy.

The effect of changing the net charge polarity of the droplets (i.e.: changing the polarity of the induction electrode) on the observed NaCl crystal habit was determined. By changing the polarity of the induction electrode, the ions$^{DNEC}$ were switched from Cl$^-$ to Na$^+$. A calibrated 40 µm diameter orifice droplet dispenser with a 30 V applied waveform at 1 Hz was used to dispense droplets (initial volume = 260 ± 10 pL).$^{38}$ By
dispensing individually charged droplets directly onto a metal target plate connected to an electrometer, the magnitude of the droplet net charge for droplets of opposite polarity was measured to be identical within experimental error. A starting solution comprised of 3.3 mg of NaCl, 12 µL of glycerol, and 388 µL of dH₂O was used to prepare initial glycerol droplet residues with radii of \( \sim 12.8 \, \mu m \). A population of droplets was dispensed and levitated for 5 minutes in the EDLT, followed by droplet residue deposition and examination by optical microscopy. The procedure was repeated for various induction potentials of both polarities.

The effect of changing the solvent composition of the droplet residues on the observed NaCl crystal habit was also determined. For these trials, a calibrated 60 µm diameter orifice droplet dispenser with a 20 V applied waveform at 1 Hz was used to dispense a population of droplets (initial volume = 400 ± 20 pL). To ensure the levitated droplet residues were of similar size (residue diameter \( \sim 28 \, \mu m \)) and NaCl concentration, the composition of the starting solution used was systematically altered. Four different starting solutions (9.2, 11.5, 12.0, or 12.5 µL of glycerol, and 390.8, 388.5, 388.0, or 387.5 µL of dH₂O), each containing 3.3 mg of NaCl, were used to dispense the initial droplets. By changing the relative humidity from 60 % to 10 % in the levitation chamber prior to the dispensing event, the percent glycerol in the droplet residues was varied from 68 % to 97 %.\(^{211}\) As previously described, a population of droplets was dispensed and levitated for 5 minutes in the EDLT, followed by droplet residue deposition and examination by optical microscopy. The procedure was repeated using each solution at various induction potentials.

### 2.2.9 Nucleation and Growth of THAP and CHCA

In their previous studies with charged levitated droplets, the Agnes group observed that \( \alpha \)-cyano-4-hydroxycinnamic acid co-crystallization with one or more peptides was enhanced relative to non-levitated droplets.\(^ {38}\) In order to better characterize this phenomenon of affected nucleation and growth, experiments using increased magnitudes of droplet net excess charge were conducted with two organic compounds that are typically used as MALDI MS matrices: 2,4,6-trihydroxyacetophenone monohydrate.
(THAP) and α-cyano-4-hydroxycinnamic acid (CHCA), which have different crystal growth kinetics.

For the following two preliminary trials, a calibrated 40 µm diameter orifice droplet dispenser with a 30 V applied waveform at 1 Hz was used to dispense droplet populations (initial volume = 260 ± 10 pL). Each trial involved the nucleation and crystal growth of either 4.4 mg THAP or 0.9 mg CHCA dissolved in a mixed solvent (mixed solvent composition: 12 µL of glycerol, 40 µL of acetone, 50 µL of 0.1 % trifluoroacetic acid (TFA) in dH_2O, 98 µL of dH_2O, and 200 µL of acetonitrile); one that is commonly employed during MALDI sample preparation.

2.2.10 Nucleation and Growth of NH_4NO_3

A calibrated 40 µm diameter orifice droplet dispenser with a 10 V applied waveform at 1 Hz was used to dispense single droplets from a 1.0 M NH_4NO_3 aqueous starting solution. Each single quiescent droplet was trapped and levitated for 5 minutes after which time the droplet residue was deposited onto a glass coverslip and examined with an optical microscope.

The effect of droplet net charge on NH_4NO_3 nucleation was also determined. A calibrated 40 µm diameter orifice droplet dispenser with a 10 V applied waveform at 1 Hz was used to dispense droplets from a 489 mM NH_4NO_3 aqueous starting solution containing 571 mM glycerol (water:glycerol = 97:3 v/v) into an EDLT chamber kept at a relative humidity (% RH) of 0-5 %. The polarity of the induction electrode was set such that the identity of the ions_{DNEC} that constitute the droplet net charge were NO_3^- ions_{DNEC}.

2.3 RESULTS AND DISCUSSION

2.3.1 Droplet Evaporation Rate

The evaporation of solvent from each droplet levitated led to its Coulomb instability resulting in the eventual loss of the droplet from the EDLT following a Coulomb explosion event. The size at which a levitated droplet first undergoes Coulomb explosion was estimated based on its physical and chemical description at the start of an experiment in concert with the Coulomb explosion relationship from equation 1.1.
It was assumed that the decrease in droplet diameter as a function of time was given by the $d^2$-Law as described in Section 1.3 and expressed in equation 2.1 in terms of droplet diameter:

$$
d^2 = d^2_0 - \beta_d \cdot t
$$

(2.1)

where $d_C$ is the calculated droplet residue diameter at the Coulomb explosion limit, $d_o$ is the initial droplet residue diameter following volatile solvent evaporation, $\beta_d$ is the evaporation rate constant, and $t$ is the time required for a droplet in a population to undergo Coulomb explosion as indicated by the loss of the droplet from the EDLT.

### 2.3.1.1 Levitated Droplet Populations

A population of water/glycerol droplets (99:1 v/v) was levitated and the times at which the droplets reached the Rayleigh limit (i.e. were lost from the EDLT) were recorded. This experiment was repeated for several droplet populations using different induction electrode potentials while keeping the population number constant. It was also observed that the radial distance from the centre point increased as the number of levitated droplets increased due to the like-charge repulsion of the droplets. It should be noted that during the course of each experiment, the amplitude of the ac field in the EDLT was periodically adjusted in order to maintain the droplet trajectories relatively constant. The results are plotted in Figure 2.9. The average evaporation rate constant increased in a linear fashion from $1.0 \pm 0.3$ µm$^2$ min$^{-1}$ for droplets having -61.1 ± 1.3 fC net charge to $1.7 \pm 0.6$ µm$^2$ min$^{-1}$ for droplets having -104.7 ± 0.5 fC net charge. An application of the t-test to the droplet population having the lowest net charge with the population having the highest net charge ($t = 4.65$; degrees of freedom = 42; $P < 0.0001$) demonstrated that these two mean values of $\beta_d$ were significantly different. Hence, the population of droplets with higher mobility (i.e.: droplets having higher net charge) evaporated more quickly than the droplet population having lower mobility (i.e.: droplets having lower net charge) as previously suggested. The calculated $\beta_d$ values of the glycerol droplet residues were significantly greater than those reported by Ray et al. for single, quiescent glycerol droplets in humid air streams ($\beta_d = 0.26, 0.31$ µm$^2$ min$^{-1}$ for % RH = 30, 40 respectively).
2.3.1.2 Levitated Single Quiescent Droplets

Using the same droplet dispenser settings as described above, a single droplet was dispensed, then trapped and levitated quiescently until its eventual loss from the EDLT following a Coulomb explosion event. The experiment was repeated many times using four different induction electrode potentials and the results plotted in Figure 2.10. The evaporation rate constant decreased from $1.4 \pm 0.2 \ \mu m^2 \ min^{-1}$ to $0.4 \pm 0.1 \ \mu m^2 \ min^{-1}$ as the droplet net charge was increased from $-61.1 \pm 1.3 \ fC$ to $-104.7 \pm 0.5 \ fC$. An application of the t-test to the data set for individual droplets having the lowest net charge with the data set for individual droplets having the highest net charge ($t = 21.83$; degrees of freedom = 37; $P < 0.0001$) demonstrated that these two mean values of $\beta_d$ were significantly different. Unexpectedly, not only were the four calculated $\beta_d$ values significantly greater than those reported by Ray et al. for single glycerol droplets, the observed decrease in $\beta_d$ for increasing droplet net charge was also in violation of the $d^2$-Law, which Ray reported as being strictly adhered to for single glycerol droplets at a given relative humidity.211

Figure 2.9 Evaporation rate constant of levitated droplet residue populations as a function of their net excess charge.
A third odd finding was that for the same droplet net charge of -61.1 ± 1.3 fC, the $\beta_d$ value for a quiescent droplet (1.4 ± 0.2 $\mu$m$^2$ min$^{-1}$) was significantly greater than the corresponding droplet population $\beta_d$ value (1.0 ± 0.3 $\mu$m$^2$ min$^{-1}$). It was likely that droplet-droplet interactions were responsible for the observed decrease in $\beta_d$ value for droplets of the same initial net charge levitated in a population vs. single levitated droplets. Previous investigations performed on simple multi-droplet configurations by Tian et al. demonstrated that the rate of droplet evaporation decreased as the number of droplets in the population increased and that the decrease in $\beta_d$ was dependent upon the inter-drop spacing.$^{214}$ It was assumed that no droplet-droplet collisions occurred since each droplet in a population had the same polarity and magnitude of net elementary charge.

The observed decrease in $\beta_d$, as shown in Figure 2.10, was not likely a direct function of the droplet’s net excess charge. In his work with the electrostatic application of pesticide sprays, Law demonstrated that the electric charge on evaporating liquid droplets neither altered $\beta_d$, nor was dissipated by evaporation for water droplets in humid air.$^{215}$ However, the presence of surface charge does alter the phase equilibrium between a
droplet and the surrounding vapor. For the above experiments, the origin of the ions that comprise the net excess charge of the droplet (ions\text{DNEC}) was either various impurities present in the solvents used, or arrived at via electrolysis.\textsuperscript{216} Modeling studies of such droplets suggest that ions\text{DNEC} are localized in a diffuse layer at the droplet-air interface where they collectively form an electric potential that diminishes to null in the centre of the droplet.\textsuperscript{80} The equilibrium solvent vapor pressure is modified by the chemical activity of the ions\text{DNEC} which lowers the solvent vapor pressure.\textsuperscript{217} This effect can lead to large super-saturations in small liquid droplets. The correction to the vapor pressure over a droplet due to the surface charge is given by:\textsuperscript{197}

\[
\ln \left( \frac{p}{p_o} \right) = -\frac{2Q^2 M (\varepsilon - 1)}{R T d^4 \pi \rho}
\]  

(2.2)

where \( p \) and \( p_o \) are the vapor pressures over the droplet in presence and absence of surface charge respectively, \( M \) is the molecular weight of the liquid, \( \varepsilon \) is the dielectric constant of the solution, \( \rho \) is the solution density, \( R \) is the gas constant, and \( T \) is the absolute temperature. For the glycerol droplet residue diameters (\( d \sim 10 \mu m \)) and charge (\( Q \sim 55-105 \text{ fC} \)) used in this study, the surface charge was predicted to have a negligible effect on the vapor pressure above the droplet.

In several experiments involving single droplets, Davis \textit{et al.} observed that evaporating single component droplets followed the \( d^2 \)-Law until the droplets fissioned at approximately 90\% of the theoretical Rayleigh limit of charge; whereby a small mass loss, along with a large charge loss, occurred during a Coulomb explosion event.\textsuperscript{112,191,218} Perhaps the observed decrease in \( \beta_d \) may have been due to the droplet residues undergoing Coulomb explosion several times prior to the parent droplet loss from the EDLT. Droplets having higher initial levels of net charge would probably be more susceptible to this possibility. If multiple Coulomb explosion events occurred, then \( t \) was overestimated resulting in artificially low \( \beta_d \) values for droplets with higher net charge; accounting for the lack of adherence to the \( d^2 \)-Law. Witnessing actual Coulomb explosions would be difficult as the droplets are very small while the path length they travel far exceeds the diameter, hence droplet monitoring using a high speed camera would be neither be practical nor perhaps possible. It should be noted that a droplet can be destabilized by an external electric field, whereby an instability is induced leading to a
discharge event.\textsuperscript{218} The critical field at which this occurs is known as the Taylor limit given by the following:

\[
E_c^2 = b \left( \frac{\sigma}{4\pi \varepsilon_o r} \right)
\]  \hspace{1cm} (2.3)

where \(b\) is a fitting constant whose accepted value is \(\sim 2.64\) for liquid droplets in air.\textsuperscript{219,220} The electric fields the glycerol droplet residues (\(r \sim 5 \, \mu m\)) encountered in the EDLT were several orders of magnitude smaller than the critical field (\(E_c \sim 1.7 \times 10^7 \, \text{V} \cdot \text{m}^{-1}\)) necessary to cause their premature fission.

On the other hand, if we were to assume that the single droplets having low net charge were not, in fact, completely quiescent due to small translations and/or rotations present that were not distinguished, and that as the droplet net charge was increased, these small translations and/or rotations of the droplets were reduced, then the discrepancies between the magnitude of the \(\beta_d\) values calculated and the apparent deviation to the \(d^2\)-Law may both be accounted for. Deviations from a quadrupole electric field in the ac trap, as introduced by the non-hyperbolic shape and positioning of the electrodes used in the EDLT, would lend credence to such an explanation. Nonetheless, more sophisticated light-scattering techniques, such as optical resonance spectroscopy, to monitor the evolving droplet diameter due to solvent evaporation would be necessary to further study the kinetics of droplet evaporation as presented here.\textsuperscript{112,191} It should be noted that solvent evaporation rates from a solution drop typically decrease in the presence of the solute.

### 2.3.2 Nucleation and Growth of NaCl from Single Quiescent Droplets

The results were plotted as histograms, as shown by the three representative data sets in Figure 2.11. Each histogram consisted only of the data collected during one particular day since day-to-day differences in both room temperature (up to 10 °C) and % RH (outside the levitation chamber where analysis of the droplet residues took place) were significant. It was possible that these factors affected the results. None the less, all three histograms demonstrated that the size distribution of NaCl crystals was affected by the net charge of the droplet.
Figure 2.11  Size distribution of NaCl crystals observed in single quiescent droplet residues having a net excess charges of -48 fC (blue colour) or -110 fC (red colour), respectively over the course of one day. (A) Data collected on May 31st, 2006; ambient % RH = 30. (B) Data collected on June 8th, 2006; ambient % RH = 36. (C) Data collected on June 19th, 2006; ambient % RH = 38. The two insets are representative images of NaCl crystals that had formed in single quiescent droplet residues having a net excess charge of -48 fC (left) or -110 fC (right), respectively.
For droplet residues with \( Q = -48 \) fC, the distribution of NaCl crystals that had side lengths < 2.5 \( \mu \)m were 6.2, 2.4, and 10.5 % respectively, while the distribution of NaCl crystals that had side lengths \( \geq 4.0 \) \( \mu \)m were 45.1, 42.9, and 36.5 % respectively. This distribution shifted toward smaller crystal size as the droplet residue net charge increased to -110 fC (side length < 2.5 \( \mu \)m : 28.2, 21.3, and 27.0 % respectively; side length \( \geq 4.0 \) \( \mu \)m : 10.9, 16.4, and 13.5 % respectively).

Examination of the data also showed that the average number of crystals per droplet was always slightly higher for droplets having higher net charge (-48 fC : average number of crystals / droplet = 14.1, 6.0, and 11.6 respectively; -110 fC : average number of crystals / droplet = 19.5, 8.7, and 13.1 respectively). This result suggested that increasing the magnitude of droplet net charge resulted in a promotion of solute nucleation for a given initial droplet residue NaCl concentration. It was likely that the increase in the number of nuclei present for droplets having higher net charge was responsible for the observed decrease in crystal size as a result of increased competition for solute molecules. The observations of promoted NaCl nucleation as affected by droplet net charge (i.e.: surface potential controlled nucleation) were similar to those of Saban et al., whereby the application of an external electric field enhanced the NaCl nucleation rate in large neutral droplets placed in a parallel plate capacitor. Saban et al. observed that the number of observed NaCl crystals increased while the average NaCl crystal size decreased above an electric field threshold of \( 2 \times 10^5 \) V\cdot m\(^{-1}\), especially for lower supersaturation values.\(^{31}\) It should be noted that the charged droplet residues experienced an external electric field (\( E_{\text{ext}} \)) in the EDLT in this study. It was speculated that the influence of the ions_{DNEC} had a greater influence on the NaCl nucleation than that of the EDLT’s \( E_{\text{ext}} \) since both trials were conducted using the same EDLT parameters with the exception of droplet net excess charge (i.e.: all droplet residues experienced the same \( E_{\text{ext}} \)). Assuming the quiescent levitated droplet residues were spherical and that the ions_{DNEC} were localized in a diffuse layer at the residue-air interface, the electric field at the residue surface due to ions_{DNEC} was estimated using Gauss’s Law:

\[
E = \frac{Q}{4\pi \varepsilon_0 r^2}
\]  

(2.4)
For droplet residues with $Q = -48 \text{ fC}$ and $-110 \text{ fC}$, $E \sim 2.5 \times 10^6 \text{ V/m}$ and $5.7 \times 10^6 \text{ V/m}$, respectively; values significantly larger than the $2 \times 10^5 \text{ V/m}$ required to influence NaCl nucleation rates as reported by Saban et al.; assuming that NaCl nucleation occurs at the residue interface. It should be noted that on account of Gauss’s Law, the electric field inside a spherical droplet due to surface charge would be $0 \text{ V/m}$. Furthermore, for single quiescent droplets located at the centre of the EDLT, assuming the electrode geometry is ideal, the magnitude of the external electric field generated by the EDLT is also $0 \text{ V/m}$. It was assumed that each singly levitated quiescent droplet was positioned in the same location and experienced little translational motion.

However, assuming that nucleation occurred at the residue surface due to the surface $E$ imposed by the ions $\text{DNEC}$, the observation of promoted NaCl nucleation is contrary to what is predicted by thermodynamics for the effect of $E_{\text{ext}}$ on nucleation. As described in section 1.3.3, the formation of a spherical nucleus in a uniform electric field occurs at an effective supersaturation (i.e.: $\Delta \mu + c_e E_{\text{ext}}^2$) that is a function of the field strength. According to thermodynamics, the sign of $c_e$ (i.e.: the difference of $\varepsilon_c - \varepsilon_d$) and the magnitude of $E$ determines whether the electric field enhances or inhibits nucleation. For an electrolyte solution of a highly polar solvent, the dielectric constant is less than that of the pure solvent since the formation of a solvation shell around the ions prevents the bound solvent molecules from being oriented in an external field. The bound solvent molecules are unable to contribute to the effective dipole moment of the system and therefore cause a decrease of polarization and the dielectric constant. For a droplet residue that consists mostly of glycerol ($\varepsilon_d = 42.5$ at room temperature) and NaCl ($\varepsilon_c = 6.1$ at room temperature), it can be assumed that $c_e < 0$; hence the assumed surface $E_{\text{ext}}$ should inhibit NaCl nucleation at the residue surface, rather than enhancing NaCl nucleation as was observed. It should be noted that the thermodynamic arguments employed by Saban et al. in the explanation of their observations of promoted NaCl nucleation are derived using an early hypothesis proposed by Kashichiev that predicts enhancement of nucleation in the case of $\varepsilon_c < \varepsilon_d$; contrary to Kashichiev’s corrected theory presented in section 1.4.3.

Assuming that NaCl nucleation takes place at the surface of a spherical droplet residue, the degree of nucleation inhibition ($J / J^*$) can be estimated using the following equation:
$J = J^0 \exp\left(\frac{-\Delta G_E}{kT}\right)$

(2.5)

where $J^0$ is the nucleation rate in the absence of $E_{ext}$. Substituting equation 1.18 into equation 2.5, and assuming a cubic critical nucleus with a side length of 31 Å, $e_d = 42.5$, and $\epsilon_c = 6.1$ all at $T = 296$ K, and $E_{ext} = 5.7 \times 10^6$ V·m$^{-1}$ (as calculated above for droplets having high net charge), would result in a calculated decrease in the rate of NaCl nucleation by a factor of 0.95; which would be likely undetectable under the limits of the experiment. By comparison, it is calculated that a droplet net charge of 174 fC could provide a strong enough $E_{ext}$ field at the residue surface ($E_{ext} = 1.57 \times 10^7$ V·m$^{-1}$) to decrease nucleation by a factor of 0.67.

Despite the fact that $c_e < 0$ for NaCl nucleation from glycerol solution, there are a number of possibilities that may account for the observed promotion of NaCl nucleation in the droplet residues rather than the thermodynamically predicted inhibition of NaCl nucleation. For experiments conducted, it was speculated that the origins of the ions that comprise the ions$_{SDNEC}$ were derived from the NaCl electrolyte added to the starting solution. Even though the residues contained a total of $\sim 4.5 \times 10^{13}$ dissolved ions, the influence of $\sim 7 \times 10^5$ Cl$^-$ ions$_{SDNEC}$ in droplet residues having -110 fC of net charge could have influenced the nucleation process because nucleation involves only a small number of atoms (or ion pairs) and that these ions$_{SDNEC}$ could have enhanced solute ordering in the diffuse layer at the droplet-air interface. A sufficiently high supersaturation of Cl$^-$ or Na$^+$, depending on droplet net charge polarity, in such a layer could create a driving force for nucleation and growth of NaCl$_{(s)}$. However, it is difficult to believe that such a slight difference in $S$ between droplets having -110 fC and -48 fC could result in the observed promotion in NaCl nucleation.

From equation 1.22, the rate of solute nucleation ($J$) is proportional to the cube of the interfacial tension term ($\sigma$) between the sub-nucleus solute cluster and the solvent, hence small changes in this parameter could lead to large changes in the nucleation rate. It was possible that $\sigma$ is affected by the droplet residue’s surface potential which led to enhanced NaCl nucleation. However no studies considering this effect, either theoretical or experimental, were found in the literature.
Another possible explanation for the observed increase in crystal number for droplet residues of higher surface potential is as follows: Since evaporation of the solvent from the droplet created a gradient in solute concentration that was at a maximum at the droplet-air interface,\textsuperscript{168,224} nucleation was likely to occur at or near the droplet residue surface. It was also possible that the critical clusters incorporated charge as the ions were mainly located in the diffuse layer at the droplet-air interface. If we were to assume that each growing crystal must be charged, and that the total amount of material crystallized was approximately constant between droplet residues having low and high net charge (as observed by an increase in crystal number with a decrease in crystal size for residues having high net charge when compared to residues of low net charge), then, due to reduced, like-charge repulsion, a system of many smaller and lesser charged crystals was probably more thermodynamically stable than a system of fewer, larger, and more highly charged crystals. In other words, the droplet residues having higher net charge reduced Ostwald ripening of the crystals rather than altering the nucleation rate (\(J\)).

In their investigations of electrodynamically levitated water droplets, Krämer \textit{et al.} found that small variations in the magnitude of droplet net charge did not affect the nucleation of ice for 60 \(\mu\)m diameter droplets carrying a maximum of \(\pm 0.37\) pC (surface charge density value = \(\pm 2.04 \times 10^{-4}\) e\(\cdot\)nm\(^{-2}\)).\textsuperscript{225} Although the surface charge density (SCD) values were comparable to the maximum used in these single quiescent droplet NaCl nucleation trials (SCD \(\sim \pm 3.73 \times 10^{-4}\) e\(\cdot\)nm\(^{-2}\)), the nucleating species (H\(_2\)O vs. NaCl) and system (nucleation from the melt vs. nucleation from solution) were different. Interestingly, the ice nucleation rates for Krämer’s electrodynamically levitated charged droplets were larger than those measured in cloud chamber studies, suggesting that the crystallization process was affected by the ions\textsubscript{DNEC}.\textsuperscript{77}

\subsection*{2.3.3 Nucleation and Growth of NaCl from Droplet Populations}

In droplet population trials, the number of NaCl crystals observed to have formed in droplet residues with \(Q = -325\) fC was typically twice that observed in the residues of droplets with \(Q = -135\) fC. The observed increase in crystal number for the population trials can be attributed, in part, to the increased rate of solvent evaporation observed for a
population of droplets having a high net charge. Increased solvent evaporation due to the increased mobility of a droplet population for droplets having a high surface potential was likely responsible for the promotion of the co-crystallization CHCA with one or more peptides observed by Agnes et al.\textsuperscript{38} It should be noted that the levitated droplets in a population were not located at the centre point of the EDLT (i.e.: $E_{ext} \neq 0$).

It was observed that NaCl crystals, as formed within charged levitated droplet populations, had either regular, cube-like habits or dome-shaped dendritic habits dependent upon the magnitude of the droplet surface potential. NaCl naturally forms as cubic crystals, but other habits can be produced such as flake salt which is obtained by careful surface evaporation of brine in flat pans open to the atmosphere. Dendritic salt is prepared by the evaporation of brine solutions that contain 5-20 ppm concentration of ferrocyanide ion which suppresses growth on the crystal faces while enhancing growth at the crystal edges and corners.\textsuperscript{226}

The observed NaCl dendrites formed were branched in one or more directions from a single point; each branch showing clear and distinguishable regions of curvature. Hence, it was speculated that the nucleation of dendrites occurred in the diffuse layer at the droplet-air interface, and that crystal growth occurred along the droplet residue surface where mass transport at the liquid-gas interface was thought to be larger than that of the bulk solution.\textsuperscript{227} Dendritic solute crystals with less defined shapes tend to form at higher supersaturations while crystals tend to be well formed at lower supersaturations in evaporating solution droplets.\textsuperscript{168} Perhaps this was a type of surface-activated NaCl nucleation (i.e.: nucleation and crystal growth that occurs at the liquid-vapor interface) that resulted in the dendrite formation. Surface-activated nucleation is thought to occur for ice formation on supercooled water droplets and for hydrates of nitric acid in concentrated nitric acid droplets,\textsuperscript{228,229,227,228,227,228,227,228,227,228,227,228,227,228,227,228} although this can neither be confirmed nor disregarded based upon the theoretical and laboratory evidence available.\textsuperscript{228-230} Recently, direct observation of surface-activated ice nucleation was observed in undercooled sucrose solution droplets; however none of the current theories for surface nucleation properly describe the phenomenon.\textsuperscript{231}

Figure 2.12 shows the percentage of dendritic NaCl as plotted as a function of the induction potential for droplet residues of a particular radius. The induction potential
value taken at the inflection point of each of the four trend lines plotted in Figure 2.12 was considered to be the necessary potential threshold required to incite a change in crystal habit for nucleating NaCl in a population of levitated droplets as processed in the EDLT. It should be noted that the raw data collected for Figure 2.12 was performed by a former undergraduate student of the Agnes Group, Mr. S.F. Bakhoum.

![Figure 2.12](image)

**Figure 2.12** Percentage of droplet residues with dendritic NaCl precipitates as a function of induction potential (IP) for droplet residues of different radii. The two insets are representative images of NaCl crystals formed from droplet residues having a surface charge density below or above $-9 \times 10^4$ $e\cdot$nm$^{-2}$ (bottom and top inset, respectively).

The observed threshold potential values were then expressed in terms of surface charge density (SCD) and were plotted as a function of the droplet residue radius (Figure 2.13). As indicated by the dotted line, dendritic NaCl crystal growth was consistently observed once the droplet residue SCD surpassed a threshold of $-9 \times 10^4$ $e\cdot$nm$^{-2}$. The theoretical SCD necessary for Coulomb explosion to occur was not reached in these droplet trials, as indicated by the solid black line in Figure 2.13. It should be noted that the presence of a growing crystal(s) in a droplet could affect the electric field within a droplet and SCD of
a droplet. This would occur if the growing crystal caused a change in the droplet’s shape away from that of being a sphere, and if the growing crystal was charged respectively.

![Graph showing surface charge density (SCD) of levitated droplet residues as a function of the droplet radius showing the threshold necessary to induce NaCl dendrite formation, as indicated by the dotted line. The SCD for the theoretical Coulomb explosion limit for these droplet residues is indicated by the solid black line.](image)

**Figure 2.13** Surface charge density (SCD) of levitated droplet residues as a function of the droplet radius showing the threshold necessary to induce NaCl dendrite formation, as indicated by the dotted line. The SCD for the theoretical Coulomb explosion limit for these droplet residues is indicated by the solid black line.

Dome-shaped dendritic NaCl growth was not observed in experiments conducted with single quiescent droplets at a relative humidity between 0-5 %. Thus, it suggested that for droplet populations levitated in the EDLT, the observed dendritic NaCl growth in droplets with high surface potential occurred due to an increase in droplet mobility which in turn resulted in greater droplet deformation and higher rates of solvent evaporation as compared with droplets with a lower surface potential; rather than the direct effect that ions_{DNEC} may have on interfacial tensions (liquid-solid, vapor-solid, and vapor-liquid) within the droplet residues that favor surface-activated nucleation.\(^{228}\) Furthermore, droplets with a higher net charge adopt different positions within the EDLT than droplets with a lower net charge which results in the higher net charged droplets experiencing a different anisotropic \(E_{ext}\) than the droplets at lower net charge which also can affect
nucleation. It should be noted that dendritic crystal growth generally occurs during faster rates of crystal growth whereby the excess energy of solidification at the solid interface is dissipated by convection (solution flow) rather than diffusion.

The percentage of dendritic NaCl was then plotted as a function of induction potential for droplet residues of different polarity as shown in Figure 2.14.

![Graph showing percentage of droplet residues with dendritic NaCl growth](image)

**Figure 2.14** Percentage of droplet residues with dendritic NaCl as a function of IP for droplet residues having different polarity of the net excess charge.

Dendritic NaCl was observed for droplet residues with Cl\(^-\) ions\(_{DNEC}\) at a lower induction potential threshold than that observed for droplet residues with Na\(^+\) ions\(_{DNEC}\). Differences in the hydration spheres of Cl\(^-\) and Na\(^+\) as well as the geometry and packing of the initial atomic cluster from which a NaCl nucleus grew could both be factors involved with regards to the observed results.\(^{232,233}\) Recent computational and experimental studies have shown that in neutral aqueous solutions, Cl\(^-\) ions are present in the air/water interface at enhanced concentrations while Na\(^+\) ions prefer the environment of the bulk liquid.\(^{234}\) Thus, it was not surprising that nucleation occurred at a lower induction potential threshold for droplet residues with Cl\(^-\) ions\(_{DNEC}\) than for droplet residues with Na\(^+\) ions\(_{DNEC}\), assuming that nucleation occurred at the droplet-air interface. It should be
noted that the raw data collected for Figure 2.14 was performed by a former undergraduate student of the Agnes Group, Mr. S.F. Bakhoum.

The percentage of dendritic NaCl was then plotted as a function of induction potential for droplet residues of different glycerol composition as shown in Figure 2.15.

![Figure 2.15](image)

Figure 2.15 Percentage occurrence of dendritic NaCl crystals as a function of the induction potential for droplet residue populations having different water/glycerol solvent composition.

The results were similar to those presented in Figure 2.12. Dendritic NaCl growth was consistently observed in droplet residues of various compositions once the induction potential reached a certain threshold. However in this case, the threshold was dependent on residue composition. It should be noted that the raw data collected for Figure 2.15 was performed by a former undergrad student of the Agnes Group, Mr. S.F. Bakhoum.

There was a linear increase in the SCD required for dendritic NaCl growth over the range of viscosity sampled as shown Figure 2.16. These results suggest that surface ion_{DNEC} mobility was a factor in promoting NaCl dendrite formation.
2.3.4 Nucleation and Growth of THAP and CHCA

In the first trial set that consisted of experiments with droplet populations containing THAP, the crystals formed consisted of randomly stacked rods for droplets with $Q = -135$ fC, as shown in Figure 2.17 A(i). For droplets with $Q = -325$ fC, dendritic THAP crystals were observed in the population, as indicated by the dark branching points in Figure 2.17 A(ii). Clear and distinguishable regions of crystal curvature were also observed, suggesting that nucleation occurred in the diffuse layer at the droplet-air interface, as indicated by the white arrow (inset, Figure 2.17 A(ii)). As previously observed for NaCl solutions, dendritic growth occurred only under conditions of high droplet surface potential. Thus, it was speculated that by varying the droplet net excess charge, dendritic THAP growth can be induced in each droplet of a levitated droplet population; a result of surface potential controlled nucleation.

In the second trial set, droplet surface potential affected the number and size of the observed CHCA precipitates as shown in Figure 2.17 B. By increasing $Q$ from -135 fC
to -325 fC, a significant increase to the number of CHCA precipitates with $d < 1 \mu m$ from none to an average of 48.0 per droplet respectively was observed.

Figure 2.17 Representative images of crystals of (A (i, ii)) 2,4,6-trihydroxyacetophenone monohydrate (THAP) formed in levitated droplet residues having net excess charge of (i) -135 and (ii) -325 fC. (B) Size and number of CHCA precipitates formed in levitated droplet residues as a function of droplet net charge, precipitate diameter were > 3.5 µm (red colour), 1.0 - 3.5 µm (blue colour), and < 1.0 µm (cream colour).
Over the same increase in net charge, the relative abundance of CHCA precipitates with \( d > 3.5 \mu m \) almost tripled from an average of 2.7 per droplet to an average of 7.8 per droplet, while the relative abundance of CHCA precipitates with \( 1.0 \mu m < d < 3.5 \mu m \) increased \( \sim 15 \) times from an average of 1.2 per droplet to an average of 17.0 per droplet. It should be noted that the raw data collected for Figure 2.17 was performed by a former undergraduate student of the Agnes Group, Mr. S.F. Bakhoum.

The observed increase in CHCA nucleation in droplet populations with high net charge was likely to have occurred due to the increased rate of solvent evaporation observed for droplet populations having a high net charge. The ability to influence CHCA precipitate size and number by tuning droplet SCD should be beneficial for MALDI sample preparation. Since the MALDI technique is primarily based on laser desorption of solid analyte-matrix deposits (analyte : matrix = 1 : 10000), it suffers from the disadvantage of low reproducibility between laser shots. Decreasing crystal size increases crystal homogeneity and improves spot-to-spot reproducibility, minimizing the need to search for regions that yield maximal analyte signal-to-noise in the sample. Furthermore, the formation of smaller crystals has the advantage of smaller sample size and improved mass accuracy and resolution. In a previous study, Agnes et al. observed that peptide ion signal-to-noise ratios obtained by MALDI-MS from sample spots created from droplets that had high net excess charge were consistently greater than those detected from sample spots created from droplets that had low net excess charge.38

2.3.5 Nucleation and Growth of Ammonium Nitrate

A common constituent of atmospheric aerosols is ammonium nitrate, the vast majority of which is of anthropogenic origin.235,236 Ammonium nitrate forms in the atmosphere when the hydroxyl radical oxidizes NO\(_x\) to nitric acid, which in the presence of ammonia, is neutralized to ammonium nitrate. In the absence of sunlight, nitric acid can be produced by the heterogeneous conversion of N\(_2\)O\(_5\), formed from the reaction of NO\(_2\) with NO\(_3\), by hydrated aerosols.236 Unlike most of the other hygroscopic salts that are found in the atmosphere, ammonium nitrate, not only has a large efflorescence barrier, but has a relatively high vapor pressure.237-239 As such, ammonium nitrate has been the subject of a few rather limited studies of its hydration and dehydration properties. To this
end, the use of surface potential controlled nucleation to form ammonium nitrate particles from levitated droplets is demonstrated.

Spherical polycrystalline NH$_4$NO$_3$ particles were successfully formed at both low (-48 fC) and high (-110 fC) droplet surface potential when the relative humidity of the EDLT chamber was kept below 31%. For RH $\geq$ 31, only small droplets were observed, even when the levitation time was increased to 24 hours under conditions of both low and high droplet surface potential.

The efflorescence relative humidity (ERH) of 31% observed for NH$_4$NO$_3$ was similar to that reported by Chan et al. and within the range reported by Tang (ERH ~ 30 %, and 25-32 % respectively). In contrast, several groups have observed anhydrous NH$_4$NO$_3$ particles in a liquid-like state that fail to effloresce. Based upon their experiments with NH$_4$NO$_3$ particles that were repeatedly cycled through deliquescence and efflorescence, Lighthouse et al. confirmed the existence of anhydrous liquid NH$_4$NO$_3$ and suggested that the wide range of efflorescent points previously reported was due to heterogeneous nucleation on unknown impurities present in all the water sources used, regardless of treatment. Although these studies employed the use of an electrodynamic balance, the magnitude(s) of droplet surface potential(s) used, along with surface potential polarity, were not reported. Perhaps surface potential controlled nucleation should be a factor to be considered with respect to the observed discrepancies in the NH$_4$NO$_3$ efflorescence point since a highly supersaturated liquid-like state of NH$_4$NO$_3$ was reported to be very sensitive to foreign nuclei; readily and reproducibly crystallizing in the more stable anhydrous state in their presence.

Two trials were conducted whereby single quiescent droplets containing NH$_4$NO$_3$ were dispensed, trapped and levitated. Each droplet had either $Q = -48$ fC (IP = 130 V) or $Q = -110$ fC (IP = 330 V). After 5 minutes of levitation, the droplet residue was deposited onto a glass coverslip and the number of individual NH$_4$NO$_3$ crystals formed was counted. The crystals observed in the droplet residues had regular bladed habits and the area of each was measured in order to ascertain its size.

The results were plotted as histograms, as shown by the three representative examples in Figure 2.18.
Figure 2.18  Size distribution of NH$_4$NO$_3$ crystals observed in single quiescent levitated droplet residues having a net excess charge of -48 fC (blue colour) or -110 fC (red colour), respectively over the course of one day. (A) Data collected on July 31st, 2006; ambient % RH = 33. (B) Data collected on August 1st, 2006; ambient % RH = 38. (C) Data collected on August 2nd, 2006; ambient % RH = 43. The two insets are representative images of NH$_4$NO$_3$ crystals formed from single quiescent droplets having a net excess charge of -48 fC (left) or -110 fC (right), respectively.
Each histogram consisted only of the data collected during one particular day since day-to-day differences in both room temperature and % RH (outside the levitation chamber where analysis of the droplet residues took place) were significant. Similar to the results obtained for single NaCl solution droplets, the histograms demonstrated that the size distribution and number of NH$_4$NO$_3$ crystals were affected by the droplet surface potential. For droplet residues with $Q = -48$ fC, the distribution of NH$_4$NO$_3$ crystals that had areas $\leq 50 \, \mu m^2$ were 11.1, 14.3, and 20.4 % respectively, while the distribution of NH$_4$NO$_3$ crystals that had areas $> 125 \, \mu m^2$ were 31.8, 31.2, and 32.3 % respectively. The distribution shifted toward smaller crystal size when droplet $Q = -110$ fC (area $\leq 50 \, \mu m^2$: 40.8, 48.5, and 42.5 % respectively; side length $> 125 \, \mu m^2$: 8.8, 6.4, and 11.3 % respectively). Examination of the data also showed that the average number of crystals per droplet almost doubled for droplets having higher net charge (-48 fC: number of crystals / droplet = 9.0, 10.2 and 8.5 respectively; -110 fC: number of crystals / droplet = 17.9, 17.0, and 14.5 respectively). This result suggested that increasing the magnitude of droplet net charge resulted in the promotion of solute nucleation for a given initial droplet residue NH$_4$NO$_3$ concentration. It was likely that the increase in the number of crystals present for droplets having higher net charge was responsible for the observed decrease in crystal size as a result of increased competition for solute molecules.

NH$_4$NO$_3$ particle formation was also investigated for droplet populations under conditions of high relative humidity (i.e.: % RH > 35). A calibrated 60 $\mu m$ orifice droplet dispenser with a 50 V applied waveform at 120 Hz was used to dispense droplet populations from a 1 M aqueous solution of NH$_4$NO$_3$. The relative humidity of the EDLT chamber was $\sim 45 \%$. In order to generate NH$_4$NO$_3$ particles at this relative humidity, the temperature of the levitation chamber was elevated to 50 °C, at which point differences in the levitation time necessary for particle formation, dependent on the magnitude of the levitated droplet’s net charge, were observed. The results are plotted in Figure 2.19. An increase in IP from 150 V to 500 V resulted in decreasing the induction time for NH$_4$NO$_3$ particle formation from 30 minutes to 20 minutes. It should be noted that the NH$_4$NO$_3$ particles were formed using a different EDLT from the one used in the previous trial sets; droplet surface charge had not been calibrated as a function of induction potential for this EDLT. The NH$_4$NO$_3$ particles formed were to be utilized to
perform dose-response trials to measure pro-inflammatory mediator differentiated expression by A549 cells; the \( \text{NH}_4\text{NO}_3 \) particles used as a proxy for an ambient tropospheric particle type.\(^{243}\) It should be noted that charged droplets that were dispensed onto a sheet of parafilm and placed in an incubator (\( \%\text{RH} = 45; \ T = 50 \ ^\circ\text{C} \)) showed no precipitation of \( \text{NH}_4\text{NO}_3 \) after 24 hours.

![Figure 2.19](image)

**Figure 2.19** Percentage of droplet residues in which \( \text{NH}_4\text{NO}_3 \) was observed for initial droplets having different surface potential (IP = 150 V (blue bars); IP = 500 V (red bars)) as a function of time. The inset is a representative image of an ammonium nitrate particle formed in the EDLT.

### 2.4 CONCLUSIONS

The nucleation and growth of sodium chloride in both single quiescent charged droplets and charged droplet populations that were levitated in an electrodynamic levitation trap were investigated. In both cases, the magnitude of a droplet’s net excess charge influenced NaCl nucleation and growth, albeit in different capacities as a result of surface potential controlled nucleation. For single quiescent levitated droplets, an increase in drop surface potential resulted in a significant promotion of NaCl nucleation, as determined by the number of crystals observed. For levitated droplet populations, a change in NaCl crystal habit from regular cubic shapes to dome-shaped dendrites was
observed once a surface charge density threshold of $\sim 9 \times 10^{-4} \text{e-nm}^{-2}$ was surpassed. Although promotion of NaCl nucleation was also observed for droplet population experiments, this was attributed to the increased rate of solvent evaporation observed for levitated droplet populations with high surface potential as result of their increased mobility in the EDLT. Promotion of solute nucleation was also observed for charged solution droplets of 2,4,6-trihydroxyacetophenone monohydrate and $\alpha$-cyano-4-hydroxycinnamic acid. These results are of direct relevance to processes that occur in both soft ionization techniques for mass spectrometry and to a variety of industrial processes. To this end, the use of surface-potential controlled nucleation to form ammonium nitrate particles from levitated droplets to be used in \textit{in vitro} toxicology studies of ambient particle types was demonstrated.
CHAPTER 3
ACOUSTIC LEVITATION:
BUILDING AN APPARATUS TO INVESTIGATE SURFACE-POTENTIAL CONTROLLED NUCLEATION

3.1 INTRODUCTION

Although electrodynamic levitation traps are able to levitate small particles (liquid and/or solid), there is an upper mass limit of only approximately $10^{-6}$ g that can be levitated. This limitation is determined by the breakdown voltage of the gas found in the EDLT chamber and is also dependent upon the charge-to-mass ratio.$^{179}$ If the breakdown voltage is reached, then charge loss is rapid and the particle is lost from the trap. In order to levitate larger particles such as millimeter-sized drops, a more robust levitation technique is required. Since part of this study was to be able to determine whether or not droplet surface potential could be used as a tool to facilitate controlled nucleation of a solute with respect to particular crystal structure adopted by a nucleus, it was thought necessary to augment the levitable sample size during experiments such that enough crystalline material would remain after solvent evaporation in order to conduct powder X-ray diffraction analysis on the generated solute particles. By comparing the powder X-ray diffraction patterns of the samples to generated powder diffraction patterns from crystal structure data, the crystal structure (or morphology) of the samples can be determined. The minimum sample size for powder X-ray diffraction requires tenths of a gram; four orders of magnitude greater than what is obtainable from an EDLT droplet. The technique of powder X-ray diffraction analysis will be further described in section 5.1.2.

A brief summary of the different techniques for droplet levitation are found in Table 3.1.
Table 3.1 Comparison of various levitation methods. Adapted from Welter.\textsuperscript{244}

<table>
<thead>
<tr>
<th>Levitation Method</th>
<th>Levitation Force</th>
<th>Levitable Volumes</th>
<th>Gradients in Levitating Force</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical</td>
<td>Radiation force from intense light source (laser)</td>
<td>&lt; 50 nL</td>
<td>Yes; vertical and horizontal</td>
<td>Non-absorbing samples</td>
</tr>
<tr>
<td>Aerodynamic</td>
<td>Aerodynamic force from a vertical gas stream</td>
<td>~ 1-175 µL</td>
<td>Depending on device design</td>
<td>Many sample types with the exception of liquids with low viscosity and/or surface tension</td>
</tr>
<tr>
<td>Electrostatic</td>
<td>Electrostatic fields</td>
<td>~ 1-175 µL</td>
<td>No</td>
<td>Ability to carry electrostatic charge</td>
</tr>
<tr>
<td>Electrodynaminc</td>
<td>Electrodynamic fields</td>
<td>&lt; 50 nL</td>
<td>Yes, vertical and horizontal</td>
<td>Ability to carry electrostatic charge</td>
</tr>
<tr>
<td>Diamagnetic</td>
<td>Very strong magnetic fields</td>
<td>up to 1800 mL</td>
<td>No</td>
<td>Diamagnetic</td>
</tr>
<tr>
<td>Acoustic</td>
<td>Acoustic radiation pressure from ultrasonic source</td>
<td>~ 1-175 µL</td>
<td>Yes; vertical and horizontal</td>
<td>Many sample types with the exception of liquids with low viscosity and/or surface tension</td>
</tr>
</tbody>
</table>
Ideally, there are five important requirements for the use of a levitation technique: 1) stable sample positioning with easy adjustment and measuring (i.e.: a vertical and horizontal gradient of the levitating force is desirable); 2) easy access to the sample; 3) no special sample properties required; 4) a wide range of levitable sample volumes; 5) low cost for supply and operation. As can be seen from Table 3.1, acoustic levitation has considerable advantage over other techniques; not only can drops that range in diameter from 40 – 4000 µm be levitated, but specific physical properties of the sample are not required; all solid and liquid samples are acoustically levitable whereby the maximum allowable diameter of the sample is about half the wavelength of the standing acoustic wave at atmospheric pressure. These advantages make acoustic levitation the preferred levitation technique for studying the effect of droplet surface potential on solute nucleation for not only can larger droplets be levitated, but charged droplets can be levitated acoustically without an external electric field. Although the magnitude of the external electric field within an EDLT having ideal electrode geometry at the null point is 0 V m\(^{-2}\), the actual field strength was likely non-zero due to the field effects of the wire that extended from each ring electrode and connected them electrically (see Figure 2.3); this may have had an effect on nucleation of the solutes. Unlike the experiments conducted in chapter 2 using the EDLT, the effect of droplet surface potential can be differentiated from the effect of an external electric field with regard to solute nucleation using the technique of acoustic levitation.

Acoustic or ultrasonic levitation draws its origins from the Kundt’s tube experiment in acoustics. Kundt’s tube is an acoustical apparatus that was used to measure the speed of sound in a gas (or a solid rod) and is useful in demonstrating acoustical forces and standing waves, as illustrated in Figure 3.1.
A small amount of fine powder is placed inside the transparent tube and a single frequency sound source is placed at one end of the tube. The other tube end is blocked by a movable piston that is used to adjust its length until the tube is at resonance; observable as a large increase in sound intensity. At resonance, the tube length is some multiple of the sound wavelength and the sound waves inside the tube are standing waves.

At resonance, the relationship between the sound frequency \( f \), wavelength \( \lambda \), and length of the tube \( l \) is as follows:

\[
f = \frac{v_{\text{air}}}{\lambda} = \frac{i v_{\text{air}}}{2l} \quad i = 1, 2, 3 \ldots
\]

where \( v_{\text{air}} \) is the speed of sound in the ambient medium (for air, \( v_{\text{air}} \approx 343 \text{ ms}^{-1} \)) and \( i \) is an integer. The powder in the tube collects in the nodal regions along the tube where the amplitude of the air vibrations is equal to zero. The distance between powder piles is equal to the half wavelength of the sound.

Acoustic levitation uses intense sound waves travelling through a fluid, usually air, to balance the force of gravity in order to suspend an object in a medium. In a similar manner as Knudt’s tube, a system of standing sound waves is created by using an oscillating surface as a sound transmitter (i.e.: an acoustic driver) with reflector, separated by an integral number of half wavelengths and oriented parallel to the pull of gravity. Figure 3.2 shows a schematic the standing wave produced by an acoustic levitator.
The net force on an object subjected to an acoustic field of this type is due to the nonlinear relationship between the instantaneous pressure and velocity of the medium. The interferences between gas compressions and rarefactions give rise to portions of the standing wave that have a constant downwards pressure and other portions that have a constant upwards pressure, with little pressure found at the nodal points; thus making them the ideal positions in space to levitate an object. A first description of acoustic levitation was published by King in 1934. It was not until the late 1970’s that both the American and European space agencies further developed the idea into a reliable tool for containerless processing under microgravity conditions in order to conduct various scientific experiments in the fields of physics and chemistry. These ideas were also applied to terrestrial levitation under regular gravity conditions. Table 3.2 presents a sample of published applications for acoustic levitation except those that involve droplet drying and particle formation which will be discussed in section 4.1.
Table 3.2 Experimental research in material science, physics and biotechnology using an acoustic levitation system.

<table>
<thead>
<tr>
<th>Author</th>
<th>Ref.</th>
<th>Research Area</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trinh</td>
<td>250</td>
<td>Density measurements</td>
<td>Use of two different techniques based on the static equilibrium position of levitated samples and on the dynamic interaction of a levitated sample and the acoustic field</td>
</tr>
<tr>
<td>Frost</td>
<td>251</td>
<td>Surface tension of monolayer</td>
<td>Formation of liquid-condensed phase of 1-octadecanol thin films on the surface of levitated drops</td>
</tr>
<tr>
<td>Tuckermann</td>
<td>252</td>
<td>Surface tension of monolayers</td>
<td>Investigation of the liquid-condensed state of surfactant monolayers of levitated droplets using an infra-red camera</td>
</tr>
<tr>
<td>Welter, Eberhardt, Rohling</td>
<td>244,253,254</td>
<td>Micro and trace analysis</td>
<td>Levitation of analytes contained in liquid droplets in combination with absorption and fluorescence measurements for acid-base titrations with absorption and fluorescence indicator.</td>
</tr>
<tr>
<td>Oshaka</td>
<td>255</td>
<td>Viscosity measurements of liquids</td>
<td>Levitation of liquid samples and elongation of drops by rotating them beyond the point of bifurcation. After the drop was allowed to restore by surface tension driven relaxation, the viscosity was determined via a relaxation model.</td>
</tr>
<tr>
<td>Oshaka</td>
<td>256</td>
<td>Thermal diffusion coefficients</td>
<td>Levitation of laser-heated droplet with subsequent natural cooling by heat loss from the surface. Infra-red camera measurement of the cooling rate in combination with a radial heat conduction model enables the calculation of the diffusion coefficient.</td>
</tr>
<tr>
<td>Kavouras</td>
<td>257</td>
<td>Gas-solid reactions</td>
<td>Levitation of single Ca(OH)(_2)(_4)(_3) particles and exposition to a defined gas atmosphere. Measurement of the particle weight change to calculate SO(_2)(_3) and HCl(_4)(_3) absorption.</td>
</tr>
<tr>
<td>Weis</td>
<td>258</td>
<td>Enzyme kinetics</td>
<td>Measurement of the rate of product formation of alkaline phosphatase-catalyzed hydrolysis of 4-methylum-belliferone phosphate in super-cooled levitated droplets</td>
</tr>
</tbody>
</table>

### 3.1.1 Acoustic Levitation Theory

In order to obtain expressions for the effect of an acoustic field on a spherical object placed within it, it is necessary to solve the 3-D wave equation for the superposition of the incident planar standing wave and the divergent scattered wave (due to the presence
of the object) with application of the boundary conditions at the surface of the sphere. Expressions of this type have been derived by a number of authors. The acoustic radiation force \( F_{ac} \) on a rigid sphere placed in such a standing wave is determined by integrating the time-averaged pressure deviation due to the acoustic field over the entire surface of the sphere. A very useful approximate analytical expression for \( F_{ac} \) has been derived by King and can be approximated by:

\[
F_{ac} = \frac{p_1^2 \pi}{\rho_{gas} v_{air}^2} \kappa \cdot r^3 \frac{\rho + 2/3 (\rho - \rho_{gas})}{2\rho + \rho_{gas}} \sin(2\kappa z) \tag{3.2}
\]

where \( p_1 \) is the pressure amplitude, \( \kappa \) is the wavenumber of the sound wave and is equal to \( 2\pi / \lambda \), \( \rho_{gas} \) is the density of the ambient medium, \( \rho \) is the density of the object to be levitated, and \( z \) is the distance between the nodal plane of the standing wave and the centre of the object. It should be noted that equation 3.2 is valid under conditions where the object’s radius is significantly less than the acoustic wavelength. Assuming that \( \rho \gg \rho_{gas} \), one obtains:

\[
F_{ac} = \frac{5p_1^2 \pi}{6\rho_{gas} v_{air}^3} \kappa \cdot r^3 \sin(2\kappa z) \tag{3.3}
\]

This result has been verified and found to be consistent with the expression for \( F_{ac} \) as derived using the exact expression for the wave equation and appropriate boundary conditions. As shown by equation 3.3, \( F_{ac} \) varies as the cube of the object’s radius and since mass also varies as the cube of the radius for a homogeneous sphere, spheres having the same density will be accelerated equally under the influence of the acoustic field.

Under microgravity conditions, the only force acting on the suspended object is that of the acoustic field. Stable positions for object suspension exist for \( z = \pi / 2\kappa, 3\pi / 2\kappa, 5\pi / 2\kappa, \ldots \) which correspond to the pressure nodes (or velocity antinodes) of the standing wave. However under terrestrial conditions, the object’s mass is compensated for at a position slightly below the pressure nodes. Since \( F_{ac} \) must be equal to or greater than the force of gravity for levitation to occur:

\[
\frac{5p_1^2 \pi}{6\rho_{gas} v_{air}^3} \kappa \cdot r^3 \sin(2\kappa z) \geq \frac{4}{3} \pi r^3 g \rho \tag{3.4}
\]
where \( g \) is the acceleration constant due to gravity (\( g = 9.81 \text{ m/s}^2 \)). An interesting point stemming from equation 3.3 is that \( F_{ac} \) varies sinusoidally with \( z \) and hence there are two points within each wavelength for which the \( F_{ac} \) is maximized, corresponding to \( \kappa z = \pi/4, \) and \( 3\pi/4. \)

However, only the upper equilibrium position is stable. Small perturbations in position around the upper equilibrium position result in restorative acoustic forces on an object, moving it back to its original position; but for perturbations about the lower equilibrium position, acoustic forces are non-restorative causing the object to be lost from the acoustic field. This stable upper equilibrium position at a region of minimum potential energy exists at a distance of approximately \( \frac{1}{8} \lambda \) below a node; heavier objects sit lower, while lighter objects will sit nearer to the node.

Figure 3.3 illustrates the vertical stability of a spherical object in an acoustic field in the presence of gravity.

![Figure 3.3](image.png)

**Figure 3.3** Vertical stability of a spherical object in an acoustic field in the presence of gravity. Equilibrium is achieved when the acoustic force on the sphere just cancels the gravitational force the object experiences. In the stable region (i.e.: the upper equilibrium location), the gradient of the acoustic force field is such that a sphere perturbed from the upper equilibrium location experiences a restoring force back to the same location. The reverse is true for a sphere located in the lower equilibrium position.
In order to avoid overlapping successive nodes, the object to be levitated should have a diameter equal to or less than $\frac{1}{2} \lambda$. Objects having $d \geq \frac{2}{3} \lambda$ cannot be levitated, while objects with $d = \frac{1}{3} \lambda$ have the optimal diameter for which minimal acoustic power is required for levitation.\textsuperscript{246} Table 3.3 shows the upper limits of the levitation range for water drops in air at several different frequencies and the minimum sound pressure levels required for levitation.

**Table 3.3** Upper limits of the levitation range for water droplets in air at different frequencies. Adapted from Lierke.\textsuperscript{246}

<table>
<thead>
<tr>
<th>$f$ (kHz)</th>
<th>$\lambda$ (mm)</th>
<th>$d_{\text{max}}$ (mm)</th>
<th>$SPL_{\text{min}}$ (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>17.2</td>
<td>6.4</td>
<td>161</td>
</tr>
<tr>
<td>40</td>
<td>8.6</td>
<td>3.2</td>
<td>159</td>
</tr>
<tr>
<td>60</td>
<td>5.7</td>
<td>2.1</td>
<td>156</td>
</tr>
<tr>
<td>80</td>
<td>4.3</td>
<td>1.6</td>
<td>155</td>
</tr>
<tr>
<td>100</td>
<td>3.4</td>
<td>1.3</td>
<td>154</td>
</tr>
</tbody>
</table>

Due to the relationship between sound frequency and wavelength, as described in equation 3.1, when the sound frequency is decreased for a constant speed of sound, the sound wavelength increases; hence the maximum levitable size also increases. Sound pressure level ($SPL$) is a logarithmic measure of the sound pressure as it relates to a reference sound pressure level (or noise):

$$SPL = 20 \log \frac{p}{p_{\text{ref}}}; \quad p_{\text{ref}} = 2.0 \times 10^{-5} \text{ Nm}^{-2}$$ \hfill (3.5)

Since a standing wave is not a perfect plane wave and shows slight divergence, a symmetrical radial force exists that serves to centre the suspended object along the levitator axis. The radial force is approximately an order of magnitude less than the axial levitation force.\textsuperscript{246} Thus, if deformable objects are levitated, such as liquids, solutions or suspensions, then the resultant object is deformed into an oblate ellipsoid as is shown in the micrographs in Figure 3.4.
The degree of deformation is proportional to the $SPL$. With increasing $SPL$ above the $SPL_{\text{min}}$ for levitation, a drop will have continuous growth of its horizontal diameter. At very high values of acoustic force, drop deformation becomes increasingly severe, whereby the drop is flattened, until the surface tension forces that hold the drop together are overcome and the droplet shatters as demonstrated in micrographs of Figure 3.5\textsuperscript{246,247}

Several authors over the past thirty years have predicted the shapes of drops levitated in an acoustic field with increasing consistency between theoretical and experimental data.\textsuperscript{246,263-266} Yarin \textit{et al.} successfully used a boundary element method to predict both

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**Figure 3.4** Two micrographs of the same levitated water drop using: a) minimal amount of acoustic force; b) acoustic force above the minimum threshold.

**Figure 3.5** Shattering of a water drop due to increasing acoustic force that exceeds its surface tension.
drop shape and centre position relative to the pressure node with good accuracy when compared with experimental results, establishing a practical link between SPL and drop shape in a levitator.\textsuperscript{259}

In Lierke’s résumé of terrestrial levitation for liquid drops and solid particles in single axis acoustic standing wave levitators, a normalized theoretical description of drop displacement and deformation was presented. Lierke determined that liquids with a Bond number ($B_o$) greater than 1.4 cannot be stably levitated.\textsuperscript{246} A liquid’s $B_o$ relates its surface tension, density, and size in the context of gravity and the surrounding fluid:

$$B_o = \frac{gr^2\rho}{\sigma}$$

An overview of different liquids and their maximum levitable drop diameter under terrestrial conditions is presented in Table 3.4. The values correspond to the critical Bond number ($B_o = 1.4$) and are irrespective of the acoustic frequency employed. It should be noted that for solution drops, both the drop surface tension and density would be altered due to the presence of the solute; for an acoustically levitating, evaporating solution drop, both these parameters would be changing until a particle was formed.

\textbf{Table 3.4} Overview of different liquids and their maximum diameter with $B_o = 1.4$ for acoustic levitation. Adapted from Lierke.\textsuperscript{246}

<table>
<thead>
<tr>
<th>Liquid</th>
<th>$\sigma$ (dynes cm(^{-1}))</th>
<th>$\rho$ (g cm(^{-3}))</th>
<th>$d_{\text{max}}$ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>22.3</td>
<td>0.7894</td>
<td>4.16</td>
</tr>
<tr>
<td>Acetone</td>
<td>23.3</td>
<td>0.7910</td>
<td>4.24</td>
</tr>
<tr>
<td>Benzene</td>
<td>28.9</td>
<td>0.8790</td>
<td>4.48</td>
</tr>
<tr>
<td>Glycerine</td>
<td>65.7</td>
<td>1.2610</td>
<td>5.64</td>
</tr>
<tr>
<td>Mercury</td>
<td>465</td>
<td>13.546</td>
<td>4.58</td>
</tr>
<tr>
<td>Carbon Tetrachloride</td>
<td>26.8</td>
<td>1.5940</td>
<td>3.20</td>
</tr>
<tr>
<td>Water</td>
<td>72.8</td>
<td>0.9982</td>
<td>6.67</td>
</tr>
</tbody>
</table>
3.1.2 Research Objectives

This chapter is focused on the building and characterization of an acoustic levitation apparatus whose purpose is to process drop samples for the investigation of surface potential upon solute nucleation and crystal growth.

3.2 ACOUSTIC LEVITATOR HARDWARE: IN-HOUSE DESIGN AND CONSTRUCTION

The purpose of the acoustic levitator is to create and maintain a stable acoustic standing wave that is sufficiently strong to levitate liquid droplets up to a few millimetres in diameter. Droplets of this size are referred to as drops. Such an acoustic field can be generated by an acoustic driver and a reflector which define its boundary. The acoustic driver generates periodic compression waves in the ambient medium by means of a mechanical displacement achieved by excitation via an electrical signal that varies sinusoidally. Upon reaching the reflector, the compression waves are scattered back towards the acoustic driver and a standing wave is created when the separation distance between transducer and reflector is equal to an integral number of half wavelengths of the compression waves. Since the scattered waves arrive at the driver in phase, the acoustic field is maintained. Thus, equilibrium between the energy supplied by the acoustic driver and the energy dissipated by the ambient medium occurs. The acoustic field in this case consists of alternating nodal and antinodal planes that are parallel to the surface of the transducer. By placing the acoustic driver and reflector several wavelengths apart, a number of nodal planes are created; each of which are suitable for the levitation of drops.

As observed in Table 3.2, the choice of operating frequency of the transducer directly affects the levitable object diameter. Although a lower frequency results in a larger levitable object size, it also results in larger physical dimensions necessary for the levitator due to an increase in wavelength. Furthermore, it would be prudent to restrict the frequency to be outside the range of human hearing (20 Hz – 20 kHz) for comfort and safety concerns. Hence, it was determined to keep the operational frequency in the ultrasonic region above 23 kHz.

The acoustic compression wave is supplied by an acoustic driver that resonates at a frequency determined by its physical dimensions. The acoustic driver consists of three
elements: 1) an actuator that converts an electrical signal into mechanical displacement; 2) a resonator that amplifies the mechanical displacement; 3) a transmitter that converts energy stored in the resonator into a directed acoustic compression wave. A schematic of the acoustic driver is presented in Figure 3.6.

![Figure 3.6](image)

**Figure 3.6** Definitions of the acoustic driver elements including the functional and the corresponding pictorial description. The driver converts a high voltage sinusoidal signal into an acoustic compression wave having the same frequency as the excitation signal.

It is quite common to consider both the actuator and resonator as a single unit called a transducer. The acoustic driver presented in this chapter is based upon previous designs by Trinh and Lupi.²⁴⁷,²⁴⁹

### 3.2.1 Ultrasonic Transducer

The most common transducer geometry is the Langevin, or sandwich type as shown in Figure 3.7. This style of transducer was chosen for its ease of manufacture and assembly.²⁴⁹,²⁶⁷,²⁶⁸ The transducer consists of a pair of piezoelectric ceramics crystals (the actuator) that are compressed between two metal sections of the resonator and held together by a central steel bolt. This type of transducer is a $\frac{1}{2} \lambda$ resonator that is restricted to the extensional mode of vibration.
Figure 3.7  Schematic of a Langevin transducer.

The piezoelectric ceramics crystals produce a displacement that is directly proportional to the instantaneous voltage placed across them (i.e.: they convert electrical energy into mechanical energy). This energy conversion is dependent upon the composition, the alignment of crystals within the ceramic, and the piezoelectric ceramic dimensions. The most common type of piezoelectric ceramics used in the manufacture of ultrasonic transducers is composed of lead zirconate titanate (PZT). Stock item PZT piezoelectric ceramic rings (Piezo Kinetics Inc., Bellefonte, PA) having an outer diameter of 3.81 cm (1.5 inches), inner diameter of 1.59 cm (0.625 inches), and thickness of 0.51 cm (0.2 inches) were used in the assembly of the transducer. The resonant frequency(ies) of the PZT rings determine the operating frequency of the transducer for standing wave generation.

The resonator section of the transducer is comprised of two metal cylindrical blocks whose dimensions are determined by the desired frequency of the acoustic driver and is dependent the longitudinal speed of sound in the metal and the diameter of the
piezoceramic rings. The length of the metal cylinders \((l_m)\) is determined using the following equation:

\[
l_m = \frac{v_m}{4f} - l_p
\]

where \(v_m\) is the speed of sound in the metal, and \(l_p\) is the thickness of the PZT ring.\(^{267}\)

Equation 3.6 ensures that the metal cylinders are \(\frac{1}{4}\) \(\lambda\) of the desired frequency while including the thickness of the PZT rings in the \(\frac{1}{4}\) \(\lambda\) distance.

When a sinusoidal potential is placed across the PZT rings, they oscillate with a piston-like motion and compression (or rarefaction) waves emanate along the cylindrical resonator blocks. When the acoustic wave reaches the metal/ambient air interface having travelled \(\frac{1}{4}\) \(\lambda\), the majority of it is reflected back towards the origin having undergone a 180° phase shift.\(^{267}\) The amount of acoustic wave reflected versus transmitted into the surrounding medium is related to the differences in the density and speed of sound between the resonator material and the ambient medium:

\[
\frac{P_t}{P_r} = \frac{2\rho_a v_{air}}{\rho_m v_m + \rho_a v_{air}}
\]

where \(P_t\) and \(P_r\) is the acoustic power transmitted and reflected respectively; \(\rho_m\) and \(\rho_a\) is the density of the metal resonator section and of air respectively; and \(c_m\) and \(c_a\) is the speed of sound in the metal resonator section and in air respectively. For an aluminium resonator section in air, \(P_t/P_r = 6.5 \times 10^{-5}\) and most of the acoustic is reflected \((c_m = 2700 \text{ m s}^{-1}, \rho_m = 5060 \text{ kg m}^{-3}; c_a = 343 \text{ m s}^{-1}, \rho_a = 1.29 \text{ kg m}^{-3})\). When the acoustic wave returns to the origin, it has travelled \(\frac{1}{2}\) \(\lambda\) and constructively interferes with the acoustic wave emitted in the same direction having the same phase and frequency; thus having larger amplitude. Operation of the transducer at a frequency outside of the resonance frequency results in destructive interference of the acoustics waves along the transducer and no emission of ultrasound.

It should be noted that the resonator sections of the transducer need not be of the same metal. It is common to make the rear resonator section and central bolt of the same material. This is done to avoid strains due to differential thermal expansion that would occur if the transducer is driven hard enough to generate appreciable heat.\(^{268}\) Since the central bolt needs to be fashioned from steel to provide high tensile strength necessary for
the pre-compression of the transducer, stainless steel \((c_m = 5790 \text{ m s}^{-1})\) was used for the rear resonator section. Aluminium 6061 \((c_m = 5060 \text{ m s}^{-1})\) was used for the front resonator section.

### 3.2.2 Ultrasonic Horn

In order to focus and amplify the ultrasound emanating from the transducer, an additional resonator section is attached to the transducer; referred to as an ultrasonic horn. The horn is typically fabricated from the same material as the front resonator section of the transducer. The most simple and effective horn reduces the diameter from that of the transducer in a stepped manner using two \(\frac{1}{4} \lambda\) sections such that the amplitude of the standing wave is maximized at the end of the horn. In order to provide good acoustic coupling, the first \(\frac{1}{4} \lambda\) section of the horn should have the same diameter as the transducer. A decrease in diameter prior to this would result in energy losses and unwanted internal stresses.\(^{267}\) The horn step should be located at the end of first \(\frac{1}{4} \lambda\) section of the horn. Equation 3.8 describes the amplification for a stepped horn:

\[
\frac{v_1}{v_2} = \left(\frac{d_2}{d_1}\right)^2
\]

(3.8)

where \(v_1, d_1,\) and \(v_2, d_2\) are the displacement amplitudes and diameters for the transmitting and opposite end of the horn, respectively. As can been seen from equation 3.8, the greater the difference in diameters of the horn ends results in a greater amplification as shown by the increase in the displacement amplitude ratio. Hence, the stepped diameter should be as small as possible without compromising its mechanical strength.\(^{267}\) The horn is easily attached to the transducer via the central bolt. The diameter ratio across the horn was 40 mm : 10 mm for the fabricated horn resulting in a displacement amplification of 16.

Conical and exponential tapers for horns are also commonly employed but result in a lesser displacement amplitude at the transmission end. Although the stepped horn is capable of providing greater displacement amplification, its sharp discontinuity results in a slight dissipation of mechanical energy into heat; a phenomenon not as pronounced for the other two tapers. This loss in efficiency can be compensated by supplying more
electrical power to the transducer. Figure 3.8 illustrates the three most popular horn types.

![Common resonator horn geometries.](image)

**Figure 3.8** Common resonator horn geometries.

### 3.2.3 Radiating Plate

In order to better couple the ultrasound emanating from the ultrasonic horn to the surrounding ambient air, a circular radiating plate was attached to the end of the stepped horn. The plate acts as the transmitter for the acoustic driver and is comprised of the same material as that of the resonator in order to achieve good coupling to the horn. In order to maintain the acoustic driver frequency and minimize energy losses, the radiating plate is placed at the end of the horn such that its top surface at the ambient interface is included in the $\frac{1}{4} \lambda$ of the stepped horn. It was held in place to the horn by a small section of threaded stainless steel rod.

For good transmission efficiency, the diameter of the plate should be approximately half the resonator section diameter of the transducer.\(^{267}\) A plate diameter similar to the
resonator section diameter results in a defocusing of the ultrasound whereby the radial acoustic force is significantly reduced resulting in the loss of lateral stability of the levitated object. The radiating plate should neither be so thick that it nullifies the amplification provided by the horn, nor be so thin that it experiences stress fracturing while operating.

It should be noted that the radiating plate does not exhibit piston-like behaviour, but exhibits flexural-type behaviour. As such, the plate vibrates with an axisymmetric (0,2) Bessel-shaped mode. Within the nodal circle, approximately equal to half the radius of the plate, the pressure waves produced are sufficiently planar so that a standing wave acoustic field can be created. Furthermore, this mode shape is responsible for the secondary effects in the acoustic field responsible for the radial component of the acoustic force which drives levitating objects towards the axis of symmetry.

### 3.2.4 Assembly of the Acoustic Driver

In order for the Langevin transducer to function properly, the piezoelectric ceramics crystals must be aligned with their poles in opposite directions to ensure the correct phasing necessary for maximum ultrasound production. The sinusoidal potential was applied to a thin stainless steel electrode that separated the two ceramic crystals and served as the nodal plate for the assembly. As a result, both PZT rings expanded with a positive applied voltage and contracted with a negative applied voltage. Both metal sections of the resonator acted as the ground for the acoustic driver; electrical continuity was established via the central steel bolt. The bolt was shielded from the nodal plate and the PZT rings by placing a polyethylene sheath around the centre of the bolt (see Figure 3.7).

By fabricating the nodal plate with a larger diameter than the rest of the transducer, it served as the place of contact whereby the entire acoustic driver was supported to the levitation chamber and it served as the positive terminal for the transducer. Four polyethylene posts were used as supports, as well as providing electrical insulation between the nodal plate and the ground, for the acoustic driver. Support of the acoustic driver at the nodal plate minimized the transmission of unwanted noise and vibration to
the support posts; an important detail as large amounts of energy can be lost from acoustic wave focus and transmission through its incorrect support.\textsuperscript{247}

If the acoustic driver was to vibrate as a whole, then it was important to have good mechanical coupling between all of the various sections of the acoustic driver. Although adhesives or non-oxidizable metal film inserts can be used to fill up crevices and correct any lack of parallelism, the best method was to assemble the pieces dry and without inserts.\textsuperscript{268} Hence all mating surfaces needed to be machined flat and perpendicular to the driver axis as well as aligning the PZT rings parallel. In order to aid in the assembly of the acoustic driver, the nodal plate was slightly grooved on both sides so that the PZT rings would be aligned along the central axis and would not move laterally during transducer assembly. The resonator sections were purposely fabricated with a slightly larger diameter than the PZT rings so that the two resonator sections were slightly grooved to accommodate the PZT rings and prevent them from moving laterally during transducer assembly. Before transducer assembly, all components were cleaned with isopropyl alcohol. The nodal plate, metals sections of the transducer, central steel bolt, ultrasonic horn, radiating plate, and polyethylene support posts were fabricated by the Simon Fraser University Faculty of Science machine shop.

A bias pressure, typically in the range of 7000 – 15000 psi, was applied to the piezoelectric ceramics crystals, via torqueing of the central bolt, to achieve good acoustic coupling to the metal sections and to prevent material fatigue.\textsuperscript{267} The frequency of the emitted ultrasound is directly affected by the amount of bias pressure applied to the actuator; an increase in bias pressure results in an increase in the resonance frequency.\textsuperscript{269,270} It was important upon pre-compression of the PZT rings, that the nodal plate was shorted to ground in order to dissipate the static charge created in the PZT rings due to their pre-compression. For the same reason, the nodal plate needed to be shorted to ground upon disassembly of the transducer. It should be noted that the amount of torque applied via the central bolt was never measured but the number of turns applied to the bolt after it was finger tight (approximately one and a quarter rotations) was kept constant. Over torquing resulted in the breakage of the PZT rings. The total length of the assembled acoustic driver was 137.4 mm.
3.2.5 Reflector

In order to establish a standing wave, a reflector was needed; aligned along the acoustic driver’s axis facing the radial plate. The effectiveness of the reflector is determined by both its position relative to the radiating plate and its geometry. Employment of a flat reflector results in a standing wave whereby objects of an appropriate size can be levitated slightly below the nodal points as discussed in section 3.1.1. The position of the reflector must be such that the distance between the radiating plate and reflector is equal to an integer number of half wavelengths of the ultrasound emitted by the acoustic driver for stable levitation to occur. This can be summarized by the following equation:

\[ H = -\frac{nv_{air}}{2f} \quad n = 2,3,4,5\ldots \]  

(3.9)

where \( H \) is the distance between the surface of the radiating plate and the reflector surface.\(^{271}\) Thus, by changing the value of \( H \) for the standing wave, a different number of nodal points can be created (i.e. acoustic levitation cavities).

Deviations greater than \( \pm 20 \mu m \) in \( H \) can result in an object’s positional instability (both laterally and vertically) or simply that levitation cannot occur. In equation 3.9, \( v_{air} \) is the only variable that is subject to changes in temperature, pressure, and relative humidity; hence the reflector required the ability to be vertically adjusted as needed to ensure stable levitation. Ideally the reflector should be smooth and rigid to best reflect approaching acoustic waves.

Although a flat shape reflector is the most simple reflector shape to machine that can be used to create the necessary standing wave acoustic field for levitation, other reflector geometries have proven more effective.\(^{247,249,267,271-275}\) It was determined theoretically that a spherically concave reflector with a radius of curvature of twice the wavelength of the emitted ultrasound in air was the most effective reflector shape for maintaining levitation and keeping lateral stability of a drop; as depicted in Figure 3.9.\(^{271}\)
Figure 3.9 Sketch of the semi-confocal set-up of the ultrasonic levitator with a radius of curvature of twice the transducer-reflector distance.

It was thought that such a reflector focuses more ultrasound along the levitation axis, without destroying the planar nature of the field, in a manner analogous to mode structure and optical cavity design in laser fabrication with the use of different parameters.\textsuperscript{267,276} In order to minimize energy loss to the environment, the diameter of the reflector should be approximately the same diameter as the transducer and not the radiating plate. It should be noted that using a curved reflector results in a more complex nodal pattern within the acoustic field which has slight deviations to the integer values of $n$.\textsuperscript{267,271} The reflector was fabricated from aluminium by Hansen Industries, Vancouver, BC. Its surface exposed to the acoustic field was polished. The reflector was centred along the axis of levitation and was attached to a piston that was manually adjustable along the levitation axis by means of a micrometre screw. A polyethylene spacer threaded on both sides separated the reflector and piston, ensuring electrical isolation of the reflector.

### 3.3 ELECTRONICS

In order to drive the transducer, a function generator, an amplifier, and an impedance matching transformer were needed. A sine wave output from a digital function generator (Waveteck 20 MHz Synthesized Arbitrary Function Generator Model 95, San Francisco,
CA, USA) was used to create a piston-like motion from the PZT rings of the transducer. The amplifier (Krohn-Hite Model 7500, Avon, MA, USA) and impedance matching transformer (Krohn-Hite Model MT-56R, Avon, MA, USA) were used to provide enough electrical power for acoustic levitation.

An ultra-miniature condenser microphone (Nexxttech Model 3303013, Barrie, ON, Canada) was used to determine the resonance frequency of the acoustic driver and to initially monitor the acoustic driver output via the amplitude of the microphone signal. The microphone was positioned close to the radiating plate and its signal was observed using an oscilloscope (Tektronix Model 485, Beaverton, OR, USA). By manually scanning the ultrasound frequency between 20-50 kHz and observing the amplitude of the microphone signal, it was determined that the acoustic driver had a sharp resonance at 45.64 kHz. It was observed that the amplitude of the microphone signal was greatly diminished when the impedance matching transformer was employed during resonant acoustic field formation; hence the impedance matching transformer was not used. Since the resonance frequency varied slightly as a function of temperature, it was necessary to tune the transducer to resonance prior to levitation by adjusting the frequency of the input signal until the microphone signal reached a maximum.\textsuperscript{258}

By applying a potential to the electrically isolated reflector, an electric field was generated between the reflector and the grounded radiating plate of the acoustic driver. A high voltage power amplifier (Trek Model 20/20C, Medina, NY, USA) was used to apply potential to the reflector. It should be noted that arcing between the reflector electrode and a levitating drop was observed for electrode potentials equal or greater than 18 kV. Experiments involving the application of an external electric field were limited to an electrode applied voltage of 16 kV in order to ensure that arcing did not occur.

3.4 LEVITATION CHAMBER

In order to control the surroundings of the resonant acoustic field with respect to temperature and humidity, and to serve as a support for the acoustic driver and reflector hardware, a box-shaped chamber was designed to enclose the resonant field. The chamber \((w \times h \times d : 17.0 \text{ cm} \times 14.5 \text{ cm} \times 18.5 \text{ cm})\) was assembled from four aluminium plates (top, bottom, and two opposing sides) and two Plexiglas plates (remaining two
opposite sides) that allowed optical access for the photographic equipment used to monitor the location and size of the levitated object. The chamber was mounted onto four stainless steel posts. The levitation hardware was introduced through portals in the top and bottom walls of the chamber. The reflector assembly was mounted to the bottom plate of the chamber while the acoustic driver was mounted, via the four support posts attached to the nodal plate, to the top wall of the chamber. This particular orientation was chosen to facilitate sample recovery from the chamber.

Temperature control of the levitation chamber was achieved using a peltier cooler (TC-NTC-1 Direct-Air Assembly, SuperCool, Stromstad, Sweden) and controller (PR-59 peltier control module, SuperCool, Stromstad, Sweden) that was bolted onto the outside of one of the aluminium side walls. Desired temperatures were adjusted and maintained between 15 °C and 40 °C to within approximately ± 0.5 °C. An additional four portals were placed on the opposing aluminium wall to accommodate a feed-through port for the cable connected to the microphone, a digital temperature/humidity meter (Model L914797, VWR International, Edmonton, AB, Canada), a gas inlet, and a separate apparatus for droplet injection described elsewhere (section 3.5). A low flow of extra dry compressed air was used to manually adjust the humidity of the chamber. The chamber walls were insulated with 1.25 cm thick polyethylene foam to minimize heat transfer to and from the chamber.

3.5 DROP INJECTION METHOD

Although liquid samples were originally introduced to the resonant acoustic field using a hand-held microliter syringe, this method lacked consistency and reproducibility as approximately seventy per cent of drops introduced in this manner failed to be levitated for more than a few seconds. Instead, a more reliable drop injection method was adopted. A 250 µL syringe with PTFE Luer Lock termination fitted with PEEK tubing, dual RN coupler, and a 6 cm Kel-F Hub needle (Hamilton Company, Reno, NV, USA) was mounted to a syringe pump (Harvard Apparatus, Model 22, South Natick, MA, USA) that dispensed liquid samples. The coupler with needle was mounted to a piston that was positioned using an XYZ stage that could manually slide along an optics rail (ThorLabs, Newton, NJ, USA).
A liquid sample was deployed once the needle tip was positioned slightly above a pressure node. Detachment of the drop from the needle tip was facilitated by slightly increasing acoustic field by adjusting the output power of the amplifier. The increase of acoustic force overcame the surface tension between the needle and drop, pulling the droplet into the pressure node. After drop detachment, the levitating drop underwent oscillations that were damped by adjusting either the reflector height via the micrometre and/or the ultrasonic power level. Upon successful drop deployment, the stage was pulled away along the rail and the injection portal to the chamber was closed.

In order to obtain charged drops for levitation, a potential was applied to the needle during drop deployment using a high voltage power supply (Stanford Research Systems Model PS350, Sunnyvale, CA, USA). The drop net charge is related to the potential applied to the needle \(U\) and the initial drop diameter \(d_0\):

\[
Q = 2\pi\epsilon_0 d_0 U
\]

(3.9)

It should be noted that solid samples were introduced to the pressure nodes manually using a pair of tweezers. This technique was employed to levitate small pieces of Styrofoam and polystyrene beads that had been used in the initial steps of commissioning the apparatus for determining the pressure node positions (described in section 3.7).

### 3.6 DROP MONITORING AND DATA COLLECTION

A high speed CMOS camera (Prosilica C640C IEEE 1394 C-mount camera, Burnaby, BC, Canada) coupled with a 1.25 × imaging lens system (TechSpec MMS OBJ-9/R-3, Edmund Optics, Barrington, NJ, USA) was used to view the levitation cavity during experiments in real time. Levitated drops were illuminated using a fibre optic light unit (Jenalux 20, SFBN Mechanik GmbH, Bucharest, Germany) that was oriented parallel to at an angle of 0° relative to the camera lens. The two arms of the light unit were pointed towards (but not directly at) the levitated drop and onto the Plexiglas wall that had been fitted with a white glossy panel of construction paper on the outside of the chamber; hence the drop was illuminated with scattered light. Illumination intensity was adjusted such that the outline of the levitated object was not obscured. A levitated drop was displayed in real time by streaming images taken by the camera (shutter rate: 35 frames per second) via LabView (National Instruments, Austin, TX, USA). A LabView 8.0
program written in-house and named *DropMon* displayed collected, and saved data generated from images taken by the camera. The program was also used to control the high voltage supply when applying potential to the reflector.

While the *DropMon* program was running, two real time images of a levitated drop were displayed: a false colour image and a binary image. The program used the false colour image to create the binary image of a levitated droplet. Using the binary image data, the horizontal and vertical diameter and 2-D area of a levitated drop were measured in real-time. The system was calibrated against standardized polypropylene spheres \((d = 1.85 \pm 0.05 \text{ mm})\). Data collection (start/stop, collection rate, location and name and location of saved file) was controlled through the operator interface and consisted of drop diameter (horizontal and vertical), droplet 2-D area, and chamber temperature. The real-time false colour image generated by the program was also used to assist with fine tuning of the standing wave during drop injection into the pressure node of acoustic field by the operator. A screen shot of the *DropMon* program user interface is shown in Figure 3.10.

![Figure 3.10](image)

**Figure 3.10** User interface screen of the *DropMon* LabView program.
A separate LabView 8.0 program named *MakinMovies* (also written in-house) was used to collect levitated drop images when required for descriptive purposes.

### 3.7 APPARATUS VERIFICATION

#### 3.7.1 Drop Levitation and Data Collection

Figure 3.11 is a cartoon of the proposed design for the acoustic levitation apparatus showing the necessary hardware (transducer, reflector, chamber, drop injector) and electronics (function generator, amplifier, microphone, humidity/temperature probe, CMOS camera, and computer) as discussed in the previous sections 3.2-3.6. Figures 3.12-3.14 are photographs depicting the built and functioning acoustic levitation apparatus based upon the design presented in Figure 3.12.

**Figure 3.11** Representation of the acoustic apparatus for particle levitation.
Figure 3.12 Photograph of the acoustic levitation apparatus including drop injection hardware.

Figure 3.13 Inside the acoustic levitation chamber.
The cost of the levitator hardware (i.e. transducer, reflector, and chamber) was inexpensive (~ $3000 CDN); however the peripheral equipment necessary for operation, monitoring, and data collection (i.e., function generator, signal amplifier, two high voltage power supplies, computer, LabView software, camera and lens, Peltier cooler, syringe pump, and translation stage) cost significantly more (~ $17500 CDN).

As described in section 3.2.5, the reflector curvature, along with the modal pattern generated by the flexural plate, focused the acoustic field so that horizontal stability was achieved whereby samples were directed towards the levitator axis. The optimal separation distance between the radiating plate and the reflector ($H$) was found to be $(25 \pm 1)$ mm; as measured from the plate to the centre of the curved reflector. Although five serviceable pressure nodes were observed at this $H$ value, only the inner three were found suitable for stable levitation of liquid samples.
Figure 3.15  Micrographs of the drop launching sequence. After a needle was positioned above a levitation cavity, the syringe pump was activated until a drop formed on the needle tip. The acoustic force was slowly increased in order to pull the drop into the levitation cavity, breaking the surface tension between the needle tip and drop.
The centre pressure node was found to provide the most stable levitation and was used for all subsequent experiments. The pressure nodes were approximately \((4.0 \pm 0.5)\) mm apart.

The micrographs in Figure 3.15 depict the manual drop injection method as described in section 3.5. Upon viewing the micrographs (beginning from top left and ending at bottom right) a drop that formed on the syringe needle was pulled into the centre pressure node of the acoustic field by increasing the SPL of the field until the surface tension of the drop-needle interface was overcome. The drop then translationally oscillated for a short time \((\approx 1-8\, \text{s})\) until the horizontal restoring forces of the acoustic field dampened its motion. The most notable effect of the acoustic field on a levitated drop was to deform it into an oblate spheroid (see Figure 3.4). The extent of deformation was dependent upon the size and surface tension of the drop, and the strength of the acoustic field. Small shape distortion of the drop was not expected to affect levitation performance. Interestingly, such shape distortions of an acoustically levitated drop corresponded, to a first order approximation, the distortions of a free falling rain drop.\(^{247,278}\)

Once the levitated drop stopped oscillating following its injection into the acoustic field, data collection was manually initiated via the \textit{DropMon} program (as described in section 3.6) and was automatically terminated upon stopping the control program. The data was subsequently plotted.

It should be noted that precision of sample measurements was dependent upon the reproduction scale, the resolution of the CCD-chip, the intensity of the back illumination and the system calibration, as well as the dynamics of the levitated drop. Care was taken to ensure that the levitation system was precisely adjusted in order to minimize out-of-focus pictures that can result from translational drop oscillations due to acoustic standing wave drift. Interior drop oscillations also occurred that resulted in a continuous change in drop aspect ratio. This resulted in a larger standard deviation for drop diameter measurements than was found when levitating solid samples. It was also observed that measured drop diameters decreased with an increase in illumination intensity upon reaching an intensity threshold.
3.7.2 Drop Evaporation

Evaporating one component solvent drops levitated by the acoustic levitator were expected to follow the $d^2$-Law as mentioned in section 1.4. There were a number of ways to plot data obtained from drop evaporation experiments as shown in Figure 3.16 for an evaporating water drop. The plot of squared diameter with time yielded an approximate straight line.

Figure 3.16 Different diagrams of dH$_2$O drop size as a function of evaporation time. The drop was levitated at a temperature of 23.0 °C and relative humidity of 3.0%. The initial drop size was 1.15 µL.

Upon differentiating equation 1.31 (which has diameter substituted for radius), the change in drop diameter with time is:

$$\frac{dd}{dt} = -\left(\frac{\beta_d}{2d}\right)$$  \hspace{1cm} (3.10)

For a constant $\beta_d$ the change of diameter per unit time is dependent only upon the momentary diameter ($d$). If the $d^2$-Law is sufficient in describing the evaporative phenomena, then the influence of initial droplet size can be accounted for by dividing
equation 1.31 by the initial diameter \((d_0)\). Hence, the evaporation process can be described by:

\[
\frac{d^2}{d_o^2} = 1 - \beta_d \left( \frac{t}{d_o^2} \right)
\]  

(3.11)

The evaporation rate constant \((\beta_d)\) is the negative slope for a plot of \(d^2/d_o^2\) vs. \(t/d_o^2\) and is dependent upon the thermodynamic properties of the drop, ambient temperature, and relative humidity for water and/or hygroscopic drops.

Figure 3.17 shows the evaporation rate plot for a dH\(_2\)O drop. Since acoustically levitated drops were oblate ellipsoids, direct determination of drop diameter was not possible.

![Figure 3.17](image)

**Figure 3.17** Evaporation rate plot for a dH\(_2\)O drop at \(T = 23\) °C and \(\%RH = 0\). The diameter of the drop was calculated from a volume equivalent sphere. The linear fit indicated for the data series shows adherence to the \(d^2\)-Law whereby the slope is equal to the evaporation rate coefficient \((\beta_d)\).

The drop diameter was calculated from the drop volume of an equivalent sphere:

\[
V_{\text{drop}} = \frac{4}{3} \pi r_{\text{hor}}^2 r_{\text{vert}} \pi = \frac{1}{6} d_{\text{hor}}^2 d_{\text{vert}} \pi
\]  

(3.12)
Although the slope of the \( d^2 \) line in Figure 3.17 had an overall linear profile, there was scatter present in the horizontal and vertical drop diameter data collected; likely the result of internal oscillations and/or translational oscillations of the drop during levitation. As shown in Figure 3.17, \( \beta_d \) was calculated as \( 1.74 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1} \) and was comparable to the values \( (\beta_d = (1.75-1.83) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}) \) calculated by Tuckermann et al. for dH\(_2\)O drops acoustically levitated (resonant frequency: \( f = 58 \text{ kHz} \)) at \( T = 21 \text{ °C} \) and %RH = 5.0.\(^{279}\) In order to extend the levitating water drop’s lifetime from that shown in Figure 3.17 (approximately 10 minutes), the humidity of the chamber would simply need to be increased.

It should be noted that evaporation of multicomponent drops results in deviations from the \( d^2 \)-Law as evidenced by the non-linear slope derived from a \( d^2/d_o^2 \) vs. \( t/d_o^2 \) plot; several investigators have reported this effect in their studies of binary component drop evaporation.\(^{154,175,280}\)

### 3.7.3 Phase Change Phenomena

In order to demonstrate the capability of the acoustic levitation apparatus to support an object under changing conditions, a phase change experiment was conducted and repeated several times. A solution drop containing anthranilic acid was injected into the acoustic field and the solvent was left to evaporate at a constant chamber temperature (\( T = 24 \text{ °C} \)). Over the course of the experiment, the excitation frequency and reflector position were adjusted to ensure stable levitation of the drop. The CMOS camera captured images during the course of the experiment at a rate of 35 fps. Images of the evaporating drop are shown in Figure 3.18. Once a critical supersaturation is reached, nucleation and subsequent crystal growth were observed to occur quickly. In less than a second, the formally transparent drop became opaque as it appeared that the entire surface of the drop was covered with a crust of solid anthranilic acid. The anthranilic acid particle retained a spherical shape and remained levitated.
Figure 3.18  Particle formation of an anthranilic acid solution drop with 84.3 mg mL⁻¹ solid content at 23 °C and %RH = 0. The initial drop volume was approximately 0.9 μL.
3.8 APPARATUS SHORT-COMINGS: CONTROL ISSUES

Although the levitation apparatus constructed herein was an effective tool for conducting experiments to probe the effect of drop surface potential on solute nucleation, a number of apparatus inconveniences were observed after its extensive use. The issues were noticed during times of large temperature changes (± 10 °C) from room temperature (23 °C).

3.8.1 Manual Acoustic Field Control

It was important that the excitation voltage delivered to the transducer closely matched the frequency of the transducer resonance. A difference of 5 Hz (or approximately 0.010 % of the operational frequency) resulted in a significant decrease in power delivered to the transducer and hence a significantly weaker acoustic field was generated. Several effects caused the resonant frequency to shift making it necessary to manually adjust the resonant frequency and/or the reflector distance during levitation. Drift in the resonant frequency was primarily due to thermal effects. Changes in temperature of both the actuator and transmission elements of the transducer due to ambient temperature fluctuations and/or dissipation of mechanical energy into heat altered the speed of sound through elements of the transducer. This in turn shifted the resonance of the elements such that the excitation frequency needed to be slightly adjusted.

During the summer months, it was common for room temperature to increase to 35 °C on warm days. Although the acoustic field was contained in the temperature controlled levitation chamber, the transducer actuator was found outside of the chamber. The transducer resonant frequency was significantly altered by this ~12 °C temperature change and resulted in a critically weak acoustic field strength whereby drops (d ~ 1.0 mm) could not be levitated without excitation voltage adjustment. Active control of the excitation frequency based upon a feedback loop would have been more consistent and efficient than monitoring each individual experiment and manually adjusting the excitation voltage frequency when deemed necessary; as was performed throughout this thesis. A room with better temperature control and air conditioning would have provided an improvement to the degree of levitation stability.
3.8.2 Manual Reflector Control

It was also important that the separation distance \( H \) between the transducer and reflector be controllable in order to construct the acoustic standing wave necessary for levitation. From equation 3.9, \( H \) is dependent upon both the frequency \( f \) of sound emitted by the transducer and the speed of sound in air \( c_a \) within the acoustic region which is also temperature dependent:

\[
c_a = \left( \frac{\alpha_a RT}{M_a} \right)^{1/2}
\]

(3.10)

where \( \alpha_a \) is the adiabatic index for air \( (\alpha_a = 1.402) \)\(^{281} \) and \( M_a \) is the molar mass for air.

Thermal gradients in the acoustic region and shifting operational frequency of the transducer required adjustments to the reflector position. Although the temperature of the chamber was controlled to ± 0.5 °C, manual adjustment of the reflector position made certain experiments impractical, such as inducing solute nucleation in a drop by means of slowly lowering chamber (and hence drop) temperature. Thus the experiments conducted with the acoustic levitator in this thesis were constrained to those involving solvent evaporation to induce solute nucleation. For similar reasons as mentioned above, active control of the reflector position based upon a feedback loop would have been a more consistent and efficient control method rather than monitoring each experiment and manually performing adjustments to the reflector position when deemed necessary. Active reflector position control would allow for practical investigation of temperature induced nucleation.

3.8.3 Chamber Temperature and Humidity Control

Although both the levitation chamber temperature and humidity were controlled, the degree of control over both control variables was limited. The levitation chamber set-point temperature was controlled via a temperature probe and feedback loop to the peltier cooler. The range of chamber temperatures achievable was between 15 °C and 40 °C; a set-point temperature was chosen for the levitation chamber and the peltier cooler achieved and roughly maintained it to approximately ± 0.5 °C. The peltier cooler’s software would need to be integrated into a LabView interface or even the LabView drop monitoring program in order to realize automatic temperature ramping that could be used
for temperature induced nucleation experiments. Furthermore, a more robust manner of chamber heating/cooling would have enlarged the range of solute/solvent systems and experimental conditions that could have been examined with the acoustic levitation apparatus.

Chamber humidity was also controlled manually. If the chamber humidity needed to be reduced from the ambient, then dry air from a gas cylinder was introduced to the chamber. If the chamber humidity needed to be increased from the ambient, a small Petri dish of distilled water was introduced inside the chamber. At best, this allowed for crude humidity control. An automatic feedback control of the chamber humidity would have provided greater experimental range and control during nucleation experiments.

### 3.9 CONCLUSIONS

An acoustic levitation device whose purpose was to process drop samples for the investigation of drop surface potential upon solute nucleation and crystal growth was designed and built and whose capabilities were demonstrated. Liquid samples as large as 2 mm in diameter were levitated. Levitated drops of this size were significantly distorted into oblate spheroids due to the effect of acoustic forces. The maximum levitable drop size was dependent on the resonant frequency of the transducer \( f = 45.64 \text{ kHz} \); in order to levitate larger drops, a longer acoustic wavelength with a corresponding lower resonant frequency would have to be employed. The acoustic levitation apparatus constructed for this work was designed to operate above the range of human hearing for operator safety and comfort. The ability to study nucleation and crystal growth was demonstrated using the acoustic levitation apparatus whereby stability of the levitated particle after solvent evaporation was maintained.

Although the constructed acoustic levitation apparatus was an effective tool for manually conducting experiments to probe the effect of drop surface potential on solute nucleation, the addition of feedback systems, to actively control the excitation resonance frequency and the separation distance of between the reflector and flexural plate of the transducer in order to compensate for thermal drifting, would significantly improve levitation stability by removing manual adjustments from the operator’s domain. In combination with a more robust and active temperature/humidity control system, the
acoustic levitation apparatus would be then amenable to additional experimentation such as temperature induced nucleation studies.
CHAPTER 4
SURFACE-POTENTIAL CONTROLLED NUCLEATION: PROMOTION OF NUCLEATION IN LEVITATED DROPLETS USING AN ACOUSTIC LEVITATION APPARATUS

4.1 INTRODUCTION

As detailed in chapter 2, the magnitude of an electrodynamically levitated droplet’s net excess charge (ion$_{DNEC}$) influenced NaCl nucleation and growth as a result of surface potential controlled nucleation. An increase in ion$_{DNEC}$ resulted in a significant promotion of NaCl nucleation, as determined by the number of crystals observed in single quiescent droplet experiments. In order to obtain larger sample sizes deemed necessary for morphological studies, the technique of acoustic levitation has been adopted in lieu of electrodynamic levitation as detailed in chapter 3. Unlike the experiments conducted using the EDLT, the effect of droplet surface potential can be differentiated from the effect of an external electric field with regard to solute nucleation using the technique of acoustic levitation. Drops (charged or uncharged) can be levitated acoustically without the use of an external electric field. However, an external electric field can be applied between the flexural plate and reflector of the acoustic levitator in order to probe its effect on solute nucleation. As mentioned in section 1.4.3, an electric field can be a factor that affects nucleation. Since an electric field is a carrier of energy, it can modify the free energy of nucleation ($\Delta G_f^*$). If the original phase and newly forming phase have different dielectric constant values ($\varepsilon$), then an electric field will influence the organization of charges, including charge migration and electric dipole reorientation (i.e.: the electric displacement), for each phase in a different manner. This potential modification of free energy therefore affects the conditions under which the phases are stable. It should be noted that the use of an external electric field during nucleation and crystal growth may also result in changes to the kinetics of nucleation.
since nucleation has a dependence on molecular transport. Hence the presence of an electric field may also result in changes to crystal habit and morphology. The experiments described in this chapter have served to examine both the effect of surface-potential controlled nucleation for acoustically levitated drops as it relates to the observations made using electrodynamically levitated droplets in chapter 2 and the effect of an external electric field on nucleation for acoustically levitated drops.

**4.1.1 Drop Interactions with the Acoustic Field**

As discussed in section 2.3, droplets experience rapid, periodic translational movement while being levitated in the EDLT due to the influence of the EDLT’s dynamic electric field. Even during levitation of single quiescent droplets where translational movement appeared to be minimized, it is likely that a levitated droplet experienced rapid rotation in the dynamic electric field due to imperfections in the ring electrode configuration. The influence of the dynamic electric field affected droplet evaporation kinetics and hence effected solute nucleation.

A drop suspended in an acoustic field is influenced by the standing acoustic wave in a number of different ways. As mentioned in section 3.1.1, a levitated drop can be deformed into an oblate spheroid due to a difference in strength between axial and radial acoustic forces. Other influences include: 1) acoustic streaming around the levitated drop; 2) heating of the acoustic cavity; 3) the change of SPL during drop evaporation; all of which affect drop evaporation kinetics.

**4.1.1.1 Acoustic Streaming**

According to Yarin, acoustic steaming includes both inner and outer streaming as shown in Figure 4.1. At the surface of an acoustically levitated drop, a boundary layer is formed due to inner acoustic streaming which results in acoustic convection. Yarin showed that the acoustic boundary layer is approximately an order of magnitude smaller than the diffusion boundary layer for a levitated drop. The convection acts similar to an external air flow around the drop and increases the drop evaporation rate. In turn, this induces internal circulation within the drop.
Figure 4.1 Acoustic streaming field near a levitated droplet with the system of outer toroidal vortices. The inner acoustic streaming is positioned directly at the acoustic boundary layer, whereas the outer acoustic streaming (outer toroidal vortices) are emerging in space of the levitator. Adapted from Yarin et al.\textsuperscript{282}

In contrast to increased mass transfer and evaporation rate as a result of inner acoustic streaming, a decrease in mass transfer and evaporation rate occurs as a result of outer acoustic streaming. Outer acoustic streaming forms a series of toroidal vortices that accumulate solvent vapour from the evaporating levitated drop that, if considered a closed system, mass transfer from the vortices to the ambient is then repressed.\textsuperscript{282} The reduction in evaporation rate due to outer acoustic streaming can be eliminated by directing an external airstream towards the levitated drop such that the airstream does not have enough velocity to reach the drop but is sufficient enough to blow out the vortices.\textsuperscript{282}

4.1.1.2 Heating of the Acoustic Cavity

Due to the oscillations of the transducer, as mentioned in section 3.8.3, the temperature in the acoustic field increases which results in an increase to the evaporation rate. The temperature increase was measured to be approximately 2 °C at a chamber set-point temperature of 24 °C. This factor is important to consider for experiments conducted at temperatures significantly below room temperature without the use of an
external convective airstream. For experiments conducted at elevated temperature, this thermal effect can be neglected.

4.1.1.3 Change in Sound Pressure Level During Drop Evaporation

As a levitated drop evaporates, the ratio of its horizontal radius to vertical radius changes (recall that the levitated drop is an oblate spheroid) due to the internal increase in pressure; a result of the general shrinking of the drop. The decrease in drop volume affects the acoustic standing wave since the standing wave during a levitation event is partially composed of a scattered wave component due to the levitating drop. As the drop decreases in size, the wave reflections at the drop surface change resulting in less scattering of the standing wave and hence an increase in SPL. Yarin developed a method to calculate the effective SPL from a drop volume in combination with its aspect ratio. Tuckermann et al. found that acoustic streaming was scaled by the intensity of the ultrasonic field and found that the evaporation rate linearly increased with SPL.

4.1.2 Evaporation of Acoustically Levitated Drops

As mentioned in section 3.1, the technique of acoustic levitation has been used in the study of drop evaporation which is important with respect to this study of surface potential controlled nucleation in levitated drops. Amongst the first published studies were those of Burdukov et al. who studied the effect of an acoustic field on the evaporation and mass transfer of naphthalene spheres. They calculated the mass transfer rate at the surface of a small naphthalene sphere positioned in a standing wave. Seaver et al. described the evaporation of volatile non-ideal liquid mixtures, the condensation of water vapour onto evaporating drops of 1-butanol, and the remote thermometry of water drops using an acoustic levitator in a free-jet wind tunnel. Drops having a diameter range between 150-3000 µm were levitated in laminar air streams whereby evaporation measurements of water drops agreed closely to calculated predictions together with a correlation to take the effects of mass transfer by forced convection into account. Gopinath et al. examined convective heat transfer from a sphere due to acoustic streaming of an ultrasonic levitator using large Reynolds numbers; the results obtained were important for thermal analysis of containerless processing in space. The Reynolds number is a dimensional number used in fluid mechanics which
is the ratio of inertial forces to viscous forces and it can be used to characterize different flow regimes. A large Reynolds number is indicative of turbulent flow where inertial forces are dominant resulting in flow instabilities such as eddies and vortices, whereas a low Reynolds number is indicative of laminar flow where viscous flows are dominant resulting in smooth and constant fluid motion. Tian et al. studied the various phenomena associated with drop arrays and the influence of an acoustic field on the evaporation of single droplets.\textsuperscript{214} They observed that the perturbation of the acoustic field to the droplet evaporation process is negligible. In a similar study, Yarin et al. demonstrated the influence of acoustic streaming on Sherwood number (a dimensionless number that represents the ratio between convective and diffusive mass transport) and mass transfer, as well as an enrichment of gas at the outer boundary of the acoustic boundary layer by liquid vapour; in disagreement with Tian’s conclusions.\textsuperscript{282} The opposite effects of increased mass transfer due to acoustic streaming and decreased evaporation rate due to the enrichment of liquid vapour around the drop surface compensate each other and hence may have led to Tian’s statement regarding the evaporation process equal to that of undisturbed droplet.\textsuperscript{159} Kastner et al. have also investigated the influence of the acoustic field on evaporating drops. Using an acoustic tube levitator, single drop drying of solvents and suspensions under various drying conditions were investigated for which the experimental data supported a multistage mechanism with regards to the evaporation process of both binary mixtures of solvent drops and suspension drops.\textsuperscript{159} Yarin et al. also studied pure and binary mixtures of solvents, and suspension drops while investigating the effect of drop surface oscillations on evaporation rates.\textsuperscript{174} Oscillations of the drop surface resulted in an increased evaporation rate in all cases. Tuckermann et al. investigated the evaporation rates of alkanes and alkanols while monitoring drop surface temperature using an acoustic levitator along with an IR-thermography system.\textsuperscript{279} During drop evaporation, Tuckermann et al. observed that water from a humid environment (\%RH = 5 – 80) was condensed on the drop surface and in the case of n-pentane, the condensed water froze as a result of the evaporative cooling.\textsuperscript{279}
4.1.3 Nucleation and Crystal Growth in Acoustically Levitated Drops

The technique of acoustic levitation has been previously employed in the study of nucleation and crystal growth. The previous studies conducted that examined crystallization can be separated into two groups: crystallization from the melt and crystallization from solution. Amongst the first studies published were those of Whymark who examined containerless melting and solidification of aluminium, glass, plastics, and benzophenone in an acoustic levitator. Whymark’s acoustic levitator was adapted to be used for position control and sample processing during orbital space experiments. Trinh et al. used an acoustic levitator to undercool and solidify indium, gallium, and o-terphenyl drops. Due to a lack of container contamination, a large supercooling (> 35 °C) was observed for Trinh’s et al. levitated drops which indicated a high sample purity; they also observed the onset of o-terphenyl nucleation at the drop surface. Biswas also studied o-terphenyl nucleation from acoustically levitated drops and monitored the process using inelastic Raman scattering to assess the kinetics of the phase transformation; o-terphenyl growth rates as a function of undercooling were inferred from a changing peak intensity of a Raman feature. Ohsaka et al. subjected succinonitrile drops to thermal cycling in order to observe drop melting and solidification in an acoustic levitator. Succinonitrile crystallization was observed to occur along the drop surface; as speculated by the temperature gradient that develops in the drop. Furthermore, the solidified drop surface was observed to be dendritic. Bauerecker et al. used an acoustic levitator as a cold gas trap to form ice particles and snowflakes from ice aerosols whereby the process was to be used for the atmospheric physics study of precipitation formation and transport phenomena of water that included radiation. Jacob et al. designed and constructed an acoustic levitator for the freezing of aqueous drops to simulate and investigate cloud processes such as the uptake of gaseous H₂O₂ by a water drop and the subsequent loss of dissolved H₂O₂ upon drop freezing. Etter et al. studied the freezing of single sulphuric acid drops suspended by an acoustic levitator in the presence of particulate contamination. Depending upon sulphuric acid concentration and the type of impurity, the polluted drops froze between -11 °C and -35 °C whereas homogeneous acid drops froze 40 degrees below the acid-ice solution thermodynamic equilibrium curve. Lü et al. observed that water drop supercooling and ice nucleation
was influenced by the SPL of an acoustic levitator. The enlargement of surface area for a drop under higher SPL was found to significantly decrease the supercooling of a water drop and that the occurrence of ice nucleation is mainly confined to the drop surface.\(^{293}\)

Surprisingly, there are only a few studies in the literature with regards to acoustic levitation and the nucleation of inorganic solutes such as NaCl. Lü et al. examined levitated supercooled aqueous NaCl and KCl solution drops. They demonstrated that drop supercooling showed a linear rise with increasing salt concentration due to dissolved Na\(^+\) and K\(^+\) ions affecting the orientation of water molecules extending into the bulk structure. They also observed that for identical concentrations, NaCl showed a larger supercooling than that of KCl.\(^{294}\) Leiterer et al. followed the evaporative-induced crystallization of NaCl by energy dispersive X-ray diffraction from acoustically levitated solution drops placed within a synchrotron beam.\(^{295}\) Wolf et al. conducted the first \textit{in situ} X-ray study of contact-free homogeneous precipitation of CaCO\(_3\) in acoustically levitated drops. They observed that CaCO\(_3\) formed via an amorphous liquid-like state that served as a template for the crystallization of calcite.\(^{296}\) Saha et al. have investigated the evaporation-induced crystallization of cerium nitrate from acoustically levitated aqueous drops. Porous nanoceria precipitates were formed at low temperatures, following cerium nitrate crystallization, when the drop was heated with a radiative CO\(_2\) laser having an output power of less than 2.5 MW m\(^{-2}\).\(^{297}\) Tuckermann et al. investigated the evaporation-induced crystallization of both (NH\(_4\))\(_2\)SO\(_4\) and Na\(_2\)SO\(_4\) from acoustically levitated aqueous solution drops. The SO\(_4^{2-}\) Raman band of (NH\(_4\))\(_2\)SO\(_4\) was observed to red shift from 982 cm\(^{-1}\) to 976 cm\(^{-1}\) \textit{in situ} during the molecular transformation between solution drop and the crystalline state and that (NH\(_4\))\(_2\)SO\(_4\) was found to simultaneously exist in the aqueous and crystalline state as evidenced by the appearance both aforementioned SO\(_4^{2-}\) Raman bands. More interestingly, an acoustically levitated solution drop of Na\(_2\)SO\(_4\) was observed to pass through an intermediate state of Na\(_2\)SO\(_4\)\(\cdot10\) H\(_2\)O before reaching anhydrous crystalline Na\(_2\)SO\(_4\); in contrast to the case for electrodynamically levitated droplets where Na\(_2\)SO\(_4\)\(\cdot10\) H\(_2\)O was never observed.\(^{298}\) There are also several studies in the literature with regards to acoustic levitation and the nucleation of organic solutes as will be described in section 5.1.
4.1.4 Research Objectives

The focus of this chapter was to characterize the threshold behaviour of surface potential controlled nucleation using an acoustic levitation apparatus for sample processing. The main goals of these investigations were two-fold: first, to compare and contrast the surface potential effects on nucleation as presented in chapter 2 using acoustic levitation with the same solution compositions as conducted using the EDLT; second, to differentiate between surface potential effects and external electric field effects with regard to nucleation of a solute from a solution drop (i.e. conduct independent experiments with levitated NaCl solution drops having surface potential and with levitated NaCl solution drops under the influence of an external electric field).

4.2 EXPERIMENTAL SECTION

4.2.1 Chemicals and Starting Solutions

Reagent grade NaCl and glycerol were purchased from BDH. All aqueous solutions were prepared using distilled water (dH$_2$O). Starting solutions were comprised of NaCl in a solvent mixture of water/glycerol, unless otherwise noted. For the following trials, a 565 mM NaCl aqueous starting solution containing 548 mM glycerol was used. It should be noted that the NaCl concentration used was approximately double to that of the analogous starting solution mentioned in section 2.2.7 as it reduced the time required for each trial by approximately 8 minutes. Despite the increase in [NaCl], the initial solution remained undersaturated.

4.2.2 Acoustic Levitation Apparatus Parameters

The acoustic levitation apparatus was operated under the parameters as described in chapter 3 and summarized in Table 4.1. Prior to each experiment, the levitation chamber temperature was conditioned to be at room temperature (23 °C) and a relative humidity of 0 %. This initial relative humidity was chosen for all experiments because it was a simple and reliable humidity setting to control. Dry air flow to the chamber was terminated prior to drop injection so that the external gas flow would not affect solvent evaporation rates. During the course of a single experiment, the final humidity reached in the chamber, due to the evaporation of a solution drop, was never greater than 7 %.
Table 4.1  Operating specifications of the acoustic levitator.

<table>
<thead>
<tr>
<th>Operating Specifications</th>
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</thead>
<tbody>
<tr>
<td>Resonance Frequency ( (f) )</td>
</tr>
<tr>
<td>Reflector Separation Distance from Transmission Plate ( (H) )</td>
</tr>
<tr>
<td>Wavelength of Standing Acoustic Wave ( (\lambda) )</td>
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<tr>
<td>Chamber Temperature ( (T) )</td>
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<tr>
<td>Initial Chamber Relative Humidity ( (% \text{ RH}) )</td>
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<tr>
<td>Maximum Electrode Voltage</td>
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<tr>
<td>Applied Voltage to Syringe Needle</td>
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4.2.3 Drop Injection and Levitation

The steps involved in the methodology used to inject and levitate individual drops were described in section 3.5. Prior to conducting experiments, the syringe, tubing and needle were emptied of solvent, followed by a priming (i.e.: flushing the syringe, tubing, and needle three times) and loading with starting solution. Before each single drop experiment, the needle tip was cleaned with solvent and five drops of solution were pumped out of the syringe outside the chamber prior to drop injection. Following drop injection, the needle tip was wrapped with parafilm to prevent solvent evaporation from the needle tip. These two procedures were followed to minimize potential crystal seeding from the needle tip between experiments. At the end of each day, any remaining solution left in the syringe, tubing, and needle was discarded. The syringe, tubing and needle were then rinsed three times with fresh solvent (for the experiments reported in chapter 4, the solvent is dH\(_2\)O) and were left filled with solvent until the next experiments were conducted.

Once an injected drop was stably levitated, data collection was manually initiated (and terminated). Adjustments of reflector position and/or the SPL were made by the operator when deemed necessary to maintain stable levitation during the course of an experiment. The excitation voltage frequency was tuned before and between experiments (not during the course of an experiment) because the transducer required a continuous excitation voltage for acoustic levitation to occur; while the function generator’s voltage signal was discontinuous when its frequency was altered, resulting in a momentary loss of the acoustic field and levitable ability. After 450 seconds of levitation, the drop residue was
deposited onto a glass coverslip and the number of individual NaCl crystals formed was then counted. The crystals formed had regular cubic habits. Numerous repeat experiments (~ 90-100 trials each for the four experimental conditions: absence of both drop charge and $E_{ext}$; absence of drop charge and presence of $E_{ext}$; absence of $E_{ext}$ and presence of positive or negative drop charge) were conducted. Since it was determined in section 2.3.2 that the increase in the number of nuclei present for droplets having higher net charge was likely responsible for the observed decrease in crystal size as a result of increased competition for solute molecules, the sizes of the NaCl crystals obtained in the following trials were not quantitatively ascertained.

### 4.2.4 Characterization of Drop Residues

The deposited drop residues were characterized by optical microscopy (Model B5 Professional, Motic, Richmond, BC).

### 4.3 RESULTS AND DISCUSSION

#### 4.3.1 Drop Evaporation

Evolving drop diameter was monitored during the course of each trial and the evaporation rate constant ($\beta_d$) was calculated using equation 3.11 and the method of least squares; an example is presented in Figure 4.2. Drop diameters were calculated from the drop volume of equivalent spheres (see equation 3.12). When the levitator was properly tuned, the $d^2$-Law was adhered to for the duration of the experiment as evidenced by the value of the coefficient of determination ($R^2$), despite the fact that the drops were composed of both water and glycerol and contained NaCl; as shown by the red data series in Figure 4.2 ($\beta_d = 1.596 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}; R^2 = 0.997$). However, if during an experiment the resonance frequency of the levitator drifted beyond a certain degree (due to reasons previously described in section 3.8), then the levitated drop became destabilized resulting in the necessity for manual adjustments to the $SPL$ and/or the reflector distance to maintain drop levitation.
Figure 4.2  Reduction of drop diameter due to solvent evaporation during acoustic levitation of an aqueous drop ([NaCl] = 565 mM; [glycerol] = 548 mM); two individual trials are shown. The diameter of each drop was calculated from a volume equivalent sphere. The linear fit indicated for the red data series showed adherence to the $d^2$-Law whereby the slope was equal to the evaporation rate coefficient ($\beta_d$) while the blue data series showed deviations to the $d^2$-Law as evidenced by $R^2 = 0.955$. When drop destabilization was not minimal and occurred several times during a trial, strict adherence of the $d^2$-Law was compromised as evidenced by the poor calculated $R^2$ value as shown by the blue data series in Figure 4.2 ($\beta_d = 1.862 \times 10^{-3} \text{ mm}^2 \text{s}^{-1}$; $R^2 = 0.955$). Prior to data analysis, all trials having an $R^2$ value less than 0.990 were excluded from the data to safely eliminate those trials compromised by drop destabilization. It should be noted that for all trials conducted in this chapter, the SPL was kept slightly above the minimum necessary for levitation in order to minimize acoustic streaming which increases the evaporation rate. For acoustically levitated, net neutral drops in the absence of an external electric field, 33.2 % of trials had an $R^2 < 0.990$ and were filtered from the data. For acoustically levitated, net neutral drops in the presence of an external electric field, 58.0 % of trials had an $R^2 < 0.990$ and were filtered from the data. For
acoustically levitated, drops having net charge in the absence of an external electric field ($E_{ext}$) 32.3 % and 45.1 % of trials had an $R^2 < 0.990$ and were filtered from the data for net positive and net negative drop charge, respectively. Overall, the calculated percentages of filtered data trials indicated that active control of both the resonance frequency and the reflector distance via feedback loop in real time would offer increased drop levitation control (i.e.: a reduction in drop destabilization) which would likely improve data quality and decrease the number of necessary trials required for a data set. The surprisingly high percentage of drop destabilization observed in external electric field trials was likely due in part to prolate drop deformation as imposed by the $E_{ext}$; a phenomenon first described by Taylor and dependent upon the ratios of drop resistivity, permittivity, and viscosity to those of the medium, the drop diameter, and the interfacial tension.$^{299}$ While residing in a standing wave levitation node, a drop is deformed into an oblate spheroid partly to ensure that the drop does not overlap with anti-nodal regions (i.e.: non-levitable zones) above and below it. Hence, prolate deformation causes drop destabilization dependent on the magnitude of $E_{ext}$ and the drop’s physical properties, including its diameter. It was predicted that this type of drop destabilization would be greater at the onset of a trial when the drop diameter was at a maximum. It was unclear as to why acoustically levitated drops having a net negative charge tended to experience more frequent drop destabilization than drops having net positive charge or net neutral drops in both the presence and absence of $E_{ext}$.

The evaporation rate constant average and standard deviation for all trials having an $R^2 > 0.989$ showed a Gaussian distribution and was calculated to be $(1.64 \pm 0.07) \times 10^{-3}$ mm$^2$ s$^{-1}$. This value was slightly less than that found for pure water (see Figure 3.17; $\beta_d = 1.743 \times 10^{-3}$ mm$^2$ s$^{-1}$) as expected for a drop that is 4 % (v/v) glycerol; a low volatility liquid.

4.3.2 Nucleation and Growth of NaCl in Acoustically Levitated Drops

During the course of experimentation, it was determined that there were two distinct types of error that had a significant effect upon the evaporation rate of acoustically levitated drops. Since prior to conducting each trial the levitation chamber was manually purged with dry air until RH % = 0, it was possible that the dry air valve was left open
during a trial due to human error resulting in a significant increase to the evaporation rate; thus increasing the nucleation rate of NaCl. Evaporation rate data for this type of manual error is shown in Figure 4.3.

The red data series was collected with the chamber drying gas valve closed during the trial and showed good adherence to the $d^2$-Law ($R^2 = 0.997$) with a $\beta_d = 1.596 \times 10^{-3}$ mm$^2$ s$^{-1}$; only a single NaCl crystal was observed at the end of the trial. The blue data series in Figure 4.3 was collected with the chamber drying gas valve left open during the trial and also showed good adherence to the $d^2$-Law ($R^2 = 0.995$). However the linear regression data showed a significant increase in evaporation rate constant ($\beta_d = 1.853 \times 10^{-3}$ mm$^2$ s$^{-1}$) and a large number of NaCl crystals (> 200) observed at the end of the trial. Since the SPL was under manual control, drop levitation may have differed significantly from trial
to trial which also would have affected the evaporation rate. Drop evaporation constants should be very similar for every trial so that meaningful results can be extracted from the data collected in order to determine the effects of $E_{\text{ext}}$ and drop net charge on the nucleation rate of NaCl. Thus it was deemed necessary to further filter the data by excluding trials whose $\beta_d$ values were less than or greater than one standard deviation from the $\beta_d$ average value. In other words, only the data from trials having a $\beta_d$ value between $(1.570 - 1.710) \times 10^{-3}$ mm$^2$ s$^{-1}$ were included in the study.

The distribution of the number of NaCl crystals found in an acoustically levitated drop was plotted as a histogram as shown in Figure 4.4.

![Figure 4.4](image)

**Figure 4.4** Distribution of NaCl crystals formed in an acoustically levitated drop normalized by the total number of trials.

The pale red series was filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$. The red series was filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 - 1.710) \times 10^{-3}$ mm$^2$ s$^{-1}$. [NaCl] = 585 mM; initial drop diameter range: 0.97 – 1.00 mm.

Two data series are shown: the pale red series is comprised of data that had been filtered using evaporation rate constants whose linear fits had $R^2 > 0.989$, while the red
series is comprised of the same data as the pale red series but filtered using evaporation rate constants whose linear fits had $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 - 1.710) \times 10^{-3}$ mm$^2$ s$^{-1}$. Each series was normalized with respect to the total number of trials and the data sets are shown as a per cent occurrence of the number of NaCl crystals formed per drop. For acoustically levitated drops having no net charge in the absence of an external electric field, the distribution of NaCl crystals between the two data series was very similar, showing little significant difference between each method of data processing; save the number of trials (pale red data series: 185 trials; red data series: 140 trials). Both series showed that no crystals formed in approximately 40% of the trials, one to ten crystals formed in 37% of the trials, and eleven to twenty crystals formed in 12% of the trials. Only 10% of the trials conducted showed more than twenty formed NaCl crystals in a drop. The crystals observed had regular cubic habits as shown in the micrograph of Figure 4.5.

![Micrograph of an acoustically levitated NaCl solution drop after deposition onto a glass slide showing NaCl crystals of regular cubic habits.](image)

**Figure 4.5** Micrograph of an acoustically levitated NaCl solution drop after deposition onto a glass slide showing NaCl crystals of regular cubic habits.

### 4.3.2.1 Presence of an External Electric Field

The distribution of the number of NaCl crystals found in acoustically levitated drops while in the presence an external electric field ($E_{ext} \sim 6.0 \times 10^5$ V m$^{-1}$) was plotted as a histogram as shown in Figure 4.6. Two data series are shown: the pale blue series is comprised of data that has been filtered using evaporation rate constants whose linear fits had $R^2 > 0.989$ (# of trials: 70), while the blue series is comprised of the same data as the
pale blue series but filtered using evaporation rate constants whose linear fits had $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 \times 10^{-3})$ mm$^2$ s$^{-1}$ (# of trials: 41). Each series was normalized with respect to the total number of trials and the two data series are shown as a per cent occurrence of the number of NaCl crystals formed per drop. Unlike the pair of data series presented in the previous section, filtering the data for acoustically levitated drops in the presence of an electric field with respect to evaporation rate constant showed significant differences in the distribution of the number of NaCl crystals found in a levitated drop; most notably for trials where one to ten crystals formed (a $\sim$ 10 % increase from 33 % to 43 %), where eleven to twenty crystals formed (a $\sim$ 5 % decrease from 10 % to 5 %), and where greater than sixty crystals formed (a $\sim$ 7 % decrease from 27 % to 20 %).

Figure 4.6  Distribution of NaCl crystals formed in an acoustically levitated drop, in the presence of $E_{ext}$, and normalized by the total number of trials. The pale blue series was filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$. The blue series was filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 \times 10^{-3})$ mm$^2$ s$^{-1}$. $[\text{NaCl}] = 585$ mM; initial drop diameter range: 0.97 – 1.00 mm.
Figure 4.7 compares the distribution of the number of NaCl crystals formed in a drop between acoustically levitated drops in the absence (red series) and presence (blue series) of an external electric field using data that was filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 - 1.710) \times 10^{-3}$ mm² s⁻¹. The sets were normalized with respect to the total number of trials and the data sets are shown as a per cent occurrence of the number of NaCl crystals formed per drop.

The general trend observed in Figure 4.7 is that nucleation of NaCl in acoustically levitated drops was enhanced in the presence of an external electric field, as evidenced by the significant upwards shift in the distribution of trials where crystals formed. In the absence of $E_{ext}$, crystals were observed in 60 % of trials while in the presence of $E_{ext}$, crystals were observed in 83 % of trials. The distribution shift was significantly noted for
instances where one to ten crystals formed (a ~ 7% increase from 37% to 44%), where thirty to forty crystals formed (a ~ 5% increase from 2% to 7%), and where greater than sixty crystals formed (a ~ 17% increase from 3% to 20%) in a drop. It should be noted that the observed crystal size was much smaller for drops containing a significantly greater number of crystals formed than for drops containing relatively few crystals; as observed for similar experiments conducted using the EDLT for levitation in section 2.3.

As mentioned in chapter 1, Kozlovskii observed that an electric field promoted NaCl nucleation.24,25 The observation of promoted NaCl nucleation in acoustically levitated drops as affected by an external electric field were similar to that of Saban et al., whereby the application of an external electric field enhanced the NaCl nucleation rate in large neutral droplets placed in a parallel plate capacitor. Saban et al. also found that the number of observed NaCl crystals increased while the average NaCl crystal size decreased above an electric field threshold of $2 \times 10^5$ V m$^{-1}$;31 a threshold value less than what was used in this work ($E_{\text{ext}} \sim 6.0 \times 10^5$ V m$^{-1}$). Kozlovskii also observed promotion of nucleation when a spark discharge to a supersaturated solution of inorganic salt occurred.24,25 It should be noted that although no visible arcing between the charged reflector and drop was observed for $E_{\text{ext}} \sim 6.0 \times 10^5$ V m$^{-1}$, it was possible that a nonvisible discharge to the drop may have occurred resulting in the promotion of NaCl nucleation.

Recently, Alexander et al. demonstrated the spatial and temporal control of KCl nucleation by non-photochemical laser-induced nucleation of aqueous solutions supersaturated in KCl upon reaching a laser power threshold of 6.4 MW cm$^{-2}$.300 This threshold peak power density corresponded to a peak electric field of $E = 6.9 \times 10^5$ V m$^{-1}$. The group postulates that the peak electric field of the light modified the free energy of the subcritical clusters of KCl via electronic polarization which enhanced KCl nucleation and growth.300,301 The technique has been used to control the special and temporal nucleation of KCl in agrose gels.301 In their work with glycine crystallization, Aber et al. also claim that charged solute clusters that have not yet become fully crystalline in a supersaturated solution can be aligned by an electric field, causing an organization of the clusters and moving them along the path to crystallization.10
As mentioned in section 2.3.2, the observed promotion of NaCl nucleation is contrary to what is predicted by thermodynamics for this solute/solvent system. According to thermodynamics, the sign of $c_e$ (i.e., the difference of $\varepsilon_c - \varepsilon_d$) and the magnitude of $E_{\text{ext}}$ determines whether the electric field enhances or inhibits nucleation. For a drop that consists mostly of water ($\varepsilon_d = 80.4$ at room temperature) and NaCl ($\varepsilon_c = 6.1$ at room temperature), it can be assumed that $c_e < 0$; hence the external electric field should hinder NaCl nucleation rather than enhancing NaCl nucleation. As mentioned in section 2.3.2, the degree of nucleation inhibition ($J / J^*$) is estimated by substituting equation 1.18 into equation 2.5, and assuming a cubic critical nucleus with a side length of 31 Å, $\varepsilon_d = 80.4$, and $\varepsilon_c = 6.1$ all at $T = 296$ K, and $E = 6.0 \times 10^5$ V·m$^{-1}$. The resultant calculated decrease in the rate of NaCl nucleation is by a factor of 0.99; an undetectable decrease under the limits of the experiment. It should be noted that for the works of Alexander et al. involving KCl nucleation, the group considers $c_e > 0$ and hence $E_{\text{ext}}$ to enhance nucleation theoretically. Dielectric constant values are wavelength dependent and can be related to the index of refraction ($\eta$):

$$\varepsilon = \frac{\eta^2}{\delta}$$

(4.1)

where $\delta$ is the permeability of the material. Alexander et al. measured the indices of refraction for water and KCl at $\lambda = 1064$ nm (the wavelength of the laser used) and calculated the dielectric constants for water ($\varepsilon_d$) and KCl ($\varepsilon_c$) to be 1.7535 and 2.1897 respectively.

Despite the fact that $c_e < 0$ for NaCl nucleation from solution, the external electric field applied may have changed the process kinetics; hence accounting for the observed promotion of NaCl nucleation in the acoustically levitated drops. Bulk diffusion through the mass transfer boundary layer (i.e.: the diffusion step) and incorporation of growth units into a crystal lattice (i.e.: the integration step) constitute the two main processes involved in crystallization; each governed by different mechanisms and influenced by external conditions. Thus $E_{\text{ext}}$ may have a direct influence the monomer diffusion coefficient ($D$) and even change the mechanism of monomer attachment, hence affecting $J_o$ and the nucleation kinetics. In his work involving boric acid crystallization, Sahin, observed that at room temperature, the rate of surface integration significantly increased
along with the overall growth rate of boric acid from solution in a fluidized bed crystallizer, in the presence of an external electric field ($E_{\text{ext}} \sim 5.0 \, \text{V m}^{-1}$) that was several orders of magnitude less than was used in this work.\textsuperscript{36} In a theoretical study, Kadota \textit{et al.} have investigated the ionic motions at the solid-liquid interface of supersaturated NaCl solutions by molecular dynamic simulations to better understand crystallization processes. They found that the diffusion coefficients of both Na\textsuperscript{+} and Cl\textsuperscript{-} ions in solution rapidly increase after a threshold of $E_{\text{ext}} \sim 2.20 \times 10^8 \, \text{V m}^{-1}$ has been reached.\textsuperscript{303,304} Prior to reaching the $E_{\text{ext}}$ threshold, the Cl\textsuperscript{-} ion diffusion coefficient is greater than that of Na\textsuperscript{+} ion for a given $E_{\text{ext}}$; however, after the threshold is reached, the Na\textsuperscript{+} ion diffusion coefficient becomes greater than that of the Cl\textsuperscript{-} ion. They postulate that the external electric field disturbs the hydration of the Na\textsuperscript{+} ion near the solid-liquid interface; hence the activation energy required for the dehydration of Na\textsuperscript{+} ions is reduced allowing them to be more quickly incorporated to a growing NaCl surface, promoting nucleation and crystal growth.\textsuperscript{303,304} It should be noted that the $E_{\text{ext}}$ used in their molecular dynamic simulations was approximately two orders of magnitude greater than that used in this work.

### 4.3.2.2 Drops Having Net Charge

The distribution of the number of NaCl crystals found in acoustically levitated drops having net charge is plotted as a histogram as shown in Figure 4.8. Four data series are shown: the pale orange and pale green series are comprised of data that has been filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$ (# of trials: pale orange 52; pale green 53), while the orange and green series are comprised of the same data as the pale orange and pale green series but having been filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$ and whose $\beta_d$ values range from $(1.570 - 1.710) \times 10^{-3} \, \text{mm}^2 \text{s}^{-1}$ (# of trials: orange 42; green 45). Each data series was normalized with respect to the total number of trials and the data sets are shown as a per cent occurrence of the number of NaCl crystals formed per drop. The average drop charge ($Q_{\text{Avg}}$) for the orange and green data sets is $(43.2 \pm 0.5) \, \text{pC}$ and $(-43.3 \pm 0.4) \, \text{pC}$ respectively as calculated using equation 3.9. For the trials with drops having $Q_{\text{Avg}} = 43.2$
pC, the distribution of NaCl crystals between the two orange data series was very similar showing little significant difference between each method of data processing.

**Figure 4.8** Distribution of NaCl crystals formed in an acoustically levitated drops having positive net charge (pale orange and orange data series) or net negative net charge (pale green and green data series).

Data was normalized by the total number of trials. The pale orange and pale green series were filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$. The orange and green data series were filtered using evaporation rate constants whose linear fits have $R^2 > 0.989$ and whose $\beta_d$ values range from $(1.570 - 1.710) \times 10^{-3}$ mm$^2$ s$^{-1}$. [NaCl] = 585 mM; initial drop diameter range: 0.97 – 1.00 mm.

For the trials with drops having $Q_{Avg} = -43.3$ pC, the distribution of NaCl crystals between the two green data series was also similar with the exception for trials where greater than sixty crystals formed whereby an approximate 7% decrease from 9% to 2% was observed. Figure 4.9 compares the distribution of the number of NaCl crystals formed in a drop between acoustically levitated drops having no net charge (red series) and those having a positive or negative net charge (orange and green series, respectively) in the absence of an external electric field. Each data set was comprised of data filtered...
using evaporation rate constants whose linear fits had $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 - 1.710) \times 10^{-3}$ mm$^2$ s$^{-1}$.

Figure 4.9  Distribution of NaCl crystals formed in acoustically levitated drops with no net charge (red series, 140 trials), positive net charge (orange series, 42 trials), and negative net charge (green series, 45 trials).

The data was normalized by the total number of trials and was filtered using evaporation rate constants whose linear fits had $R^2 > 0.989$ and whose $\beta_d$ values ranged from $(1.570 - 1.710) \times 10^{-3}$ mm$^2$ s$^{-1}$. [NaCl] = 585 mM; initial drop diameter range: 0.97 – 1.00 mm.

The data sets were normalized with respect to the total number of trials and are shown as a per cent occurrence of the number of NaCl crystals formed per drop. The general trend observed in Figure 4.9 is that nucleation of NaCl in acoustically levitated drops is enhanced for drops having either a net positive charge or net negative charge as evidenced by the significant upwards shift in the distribution of trials where crystals formed in both cases. For drops having no net charge, crystals were observed in 60 % of trials while for drops having net positive and net negative charge, crystals were observed in 90 % and 84 % of trials, respectively. The distribution shift was most noted for instances where one to ten crystals formed (a ~ 8 % and 16 % increase for net positive
and net negative charge, respectively) where thirty to forty crystals formed (a ~ 10 % and 9 % increase for net positive and net negative charge respectively), and where greater than sixty crystals formed for drops having a net positive charge (a ~ 16 % increase from 3 % to 19 %). It should be noted that the observed crystal size was much smaller for drops containing a significantly greater number of crystals formed than drops containing relatively few crystals; as observed for similar experiments conducted using the EDLT for levitation in section 2.3.

These general trends were quite similar to those presented in the previous section for drop trials in the presence of an external electric field, whereby a significant upwards shift in the distribution of trials where NaCl crystals formed was also observed. If one assumed that the ions $DNEC$ that compromise the drop net charge were localized in a diffuse layer at the drop-air interface, then the electric field at the residue surface due to $DNEC$ can be estimated according to Gauss’s Law using the drop radius as calculated from an equivalent sphere. Since the average drop charge was calculated at approximately ± 43.2 pC, the electric field at the drop-air interface ranged between ± 1.6 $\times$ 10$^6$ V·m$^{-1}$ at the beginning of the trial ($r_d$ ~ 0.5 mm) to ± 2.7 $\times$ 10$^7$ V·m$^{-1}$ by the end of the trial ($r_d$ ~ 0.25 mm). Assuming that NaCl nucleation occurred at the drop-air interface, both of these $E_{ext}$ values are larger than the 6.0 $\times$ 10$^5$ V·m$^{-1}$ external electric field employed in the previous section whereby NaCl nucleation was observed to be enhanced. It should be noted that if NaCl nucleation is thought to occur at the drop-air interface as a result of the electric field produced by the $DNEC$, then similar discussions apply with respect to the thermodynamic and kinetics arguments as discussed previously in section 2.3.2 for electrodynamically levitated droplets and in section 4.3.2.1 for acoustically levitated drops. It is unclear as to why drops having a negative charge show no increase in the number of trials where greater than sixty crystals formed per drop while drops having a net positive charge show a dramatic increase. Perhaps the reason for the large increase in nucleation sites where more than 60 crystals are observed can be linked to the molecular dynamic simulations of Kadota et al. who found the Na$^+$ ion diffusion coefficient to be greater than that of the Cl$^-$ ion and that the activation energy required for the dehydration of Na$^+$ ions was reduced allowing Na$^+$ ions to be more quickly incorporated to a growing NaCl surface, promoting nucleation and crystal growth in the
Interestingly in the field of colloid particle self-assembly, a few instances were found in the literature whereby the surface charge polarity of a drop affected the end result of a nucleation experiment. In their work with mixed colloids, Sen et al. found that the buckling-driven sphere to deformed-doughnut like morphological transformation of mixed colloidal suspension droplets during evaporation induced self-assembly can be arrested by tuning of the droplet surface charge from a net negative droplet charge to net positive droplet charge; in spite of the observations being contrary to the existing hypothesis of buckling for colloidal suspension droplets that contain only one type of particle. Lee et al. have also used droplet surface potential to direct the particle morphology of colloids from porous particle to hollow particle when the net droplet charge goes from negative to positive respectively, as the surface charge influenced the distribution of nearby ions in the polar medium.

As mentioned in section 2.3.2, Krämer et al., found that small variations in the magnitude of droplet net charge did not affect the nucleation of ice for 60 µm diameter droplets carrying a maximum of ± 0.37 pC (surface charge density value = ± 2.04 × 10^{-4} e·nm^{-2}) for droplets levitated in an electrodynamic balance. Although the surface charge density (SCD) values were comparable to the maximum used in these NaCl nucleation trials of acoustically levitated drops (SCD_{max} ± 3.44 × 10^{-4} e·nm^{-2}), the nucleating species (H₂O vs. NaCl) and system (nucleation from the melt vs. nucleation from solution) was different. Since the trials of Krämer et al. involved supercooling liquid H₂O to induce ice nucleation, their SCD values were considered as constant under the assumption that their droplet radius does not significantly change during the course of an experiment. Since the trials conducted for this chapter involved evaporating solvent to induce NaCl nucleation, the SCD values increased during the course of the experiment.

Assuming that NaCl nucleation takes place at the drop-air interface, it was possible that the ions_{SDNEC} increase the supersaturation (S) of the system. Since the nucleation rate is very sensitive to S (refer to equations 1.14 and 1.24), a small increase to the value of S would result in promotion of NaCl nucleation. The nucleation rate is also sensitive to the interfacial tension term (σ) whereby a decrease in σ value would result in the promotion of NaCl nucleation. It is unknown how ions_{SDNEC} would affect the interfacial tension between the solution and the subcritical nuclei.
4.3.3 Acoustic vs. Electrodynamic Levitation

Unlike experiments conducted with the EDLT, use of the acoustic levitator allowed for differentiation between the experimental conditions of levitated drops carrying (or not carrying) drop surface charge and the presence (or absence) an external electric field. The experiments conducted using the EDLT in section 2.3.2 showed promotion of NaCl nucleation when droplet surface potential increased by a factor of ~2.3 (SCD range: \(-1.63 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\) to \(-3.73 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\)) while in the presence of a dynamic electric field. The experiments conducted using the acoustic levitator showed promotion of NaCl nucleation for drops having a SCD\(_{\text{max}}\) value of \(\pm 3.44 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\) as compared to drops without surface potential in the absence of an electric field. Although the SCD\(_{\text{max}}\) value employed in acoustically levitated drop trials \(\pm 3.44 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\) was comparable to those employed in electrodynamically levitated droplet trials of single droplets carrying a high net charge (i.e.: for \(Q = -110 \text{ fC}\); droplet SCD = \(-3.73 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\)), it should be noted that the acoustically levitated drops had diameters that were approximately three orders of magnitude greater than those levitated in the EDLT and hence carried a significantly greater number of ions\(_{\text{DNEC}}\).

The percent distribution of NaCl crystals per drop for the experiments conducted using the EDLT appeared very similar to the percent distributions presented in the previous section, whereby the distribution shifted towards a greater number of crystals observed per drop upon elevating droplet SCD from \(-1.63 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\) to \(-3.73 \times 10^{-4} \text{e}\cdot\text{nm}^{-2}\) (see Figure 4.10). However, the range for the number of crystals observed in a levitated droplet was significantly less when the EDLT was used in lieu of the acoustic levitator (total number of crystals observed per drop: between 1 and 29; between 0 and 200 for experiments conducted in the EDLT and acoustic levitator respectively). This reduction in range was likely due to the decreased size of the droplets levitated in the EDLT; the amount of total solute available to form individual crystals was significantly less in the smaller initial droplets. It was also interesting to note that when using the EDLT, crystals formed in every levitated droplet while crystals failed to form in 10% and 15% of drops having positive and negative surface potential respectively for trials conducted using the acoustic levitator.
Figure 4.10 Distribution of NaCl crystals formed in electrodynamically levitated droplets having low surface potential (red series) and high surface potential (blue series). The data was normalized by the total number of trials.

Experiments conducted using the acoustic levitator also showed promotion of NaCl nucleation in net neutral drops in the presence of an external electric field \( E_{\text{ext}} = 6.0 \times 10^5 \text{ V} \cdot \text{m}^{-1} \) when compared to net neutral drops in the absence of \( E_{\text{ext}} \); a nucleation parameter that could not be individually probed for experiments conducted using the EDLT. Thus, it was likely that promotion of NaCl nucleation in samples processed by the EDLT likely occurred as a result of both effects. A few NaCl nucleation trials were conducted whereby drops carrying surface charge were acoustically levitated in the presence of an external electric field. This line of investigation was short lived due to drop stabilization issues. As a drop carrying surface charge (SCD\(_{\text{max}}\) of \( \sim 3.44 \times 10^{-4} \text{ } e \cdot \text{nm}^2 \)) evaporated in the presence of an external electric field (\( E_{\text{ext}} = 6.0 \times 10^5 \text{ V} \cdot \text{m}^{-1} \)), the force exerted on charged drop by \( E_{\text{ext}} \) eventually extracted the drop from the acoustic levitation cavity. In order to achieve stable drop levitation under the conditions of both drop surface potential and \( E_{\text{ext}} \) for an evaporating drop, the magnitude of \( E_{\text{ext}} \) would need
to be decreased relative to the rate of solvent removal. Hence, this line of investigation was not pursued since $E_{\text{ext}}$ could not be held at a constant value. Proper investigation of charged drops in the presence of a decreasing $E_{\text{ext}}$ would require a feedback loop between drop position and $E_{\text{ext}}$ to remove it from the manual adjustment domain. It is as yet unknown whether or not the presence of drop surface potential in the presence $E_{\text{ext}}$ would result in more extensive promotion of NaCl nucleation than the each of the individual NaCl nucleation promotions observed.

4.4 CONCLUSIONS

The nucleation and growth of sodium chloride in acoustically levitated drops has been investigated. Acoustic levitation afforded the differentiation between the effects of drop surface potential and the presence of an external electric field on the nucleation of sodium chloride from levitated drops; an advantage over processing the similar solution drops with the EDLT. It was observed that the presence of drop surface potential (SCD$_{\text{max}}$ ~ ± 3.44 × 10$^{-4}$ e·nm$^{-2}$) and the presence of an external electric field ($E_{\text{ext}} = 6.0 \times 10^5$ V·m$^{-1}$) each individually resulted in a significant promotion of sodium chloride nucleation from acoustically levitated drops as determined by the increase in the number of crystals observed in each drop relative to the number of crystals observed in the absence of drop surface potential and the presence of $E_{\text{ext}}$. Promotion of NaCl nucleation occurred in the presence of $E_{\text{ext}}$ despite the fact that theoretical thermodynamics predicts its inhibition since the NaCl dielectric constant value of NaCl is significantly less than the dielectric constant value of the drop solution. The presence of $E_{\text{ext}}$ was thought to have increased the monomer diffusion coefficient hence affecting nucleation kinetics; thereby enhancing of NaCl nucleation. The crystal habits of sodium chloride grown from acoustically levitated drops were regular with cubic shapes for the trials conducted in the levitator including those conducted with a drop a SCD$_{\text{max}}$ of ~ ± 3.44 × 10$^{-4}$ e·nm$^{-2}$. This was in contrast to the dome-shaped sodium chloride crystals grown upon reaching a SCD threshold of ~ 9 × 10$^{-4}$ e·nm$^{-2}$ when using the EDLT for the sample processing of droplet populations.
CHAPTER 5
THE USE OF SURFACE-POTENTIAL
CONTROLLED NUCLEATION TO AFFECT
ON POLYMORPHOLOGY

5.1 INTRODUCTION

As mentioned in both chapters 3 and 4, the technique of acoustic levitation has been previously employed in the study of particle formation by several research groups. The majority of studies conducted with regard to acoustic levitation and nucleation of solutes stems mainly from the pharmaceutical industry. Recently, the pharmaceutical industry has taken advantage of acoustic levitation processing of solution drops to shorten times for the development of new pharmaceuticals that are processed via spray drying, be it the active compounds themselves or more complex formulations containing both active and excipient compounds. Spray drying involves the spraying of a liquid feed formulation into air whereby the droplets formed via atomization form solid particles that are collected as a dry powder. \(^{155,165}\) Spray drying is also employed extensively by food manufacturing, paints and pigments, and ceramic materials industries. Applications of the process include production of different compounds such as inorganic salts, foodstuffs, enzymes and pharmaceuticals. The process is very flexible and allows good control over various powders properties such as a well-defined particle size, particle morphology and residual solvent content.

Sloth \textit{et al.} used an acoustic levitator to dry single aqueous solution drops of maltodextrin and trehalose. They were able to successfully use the drying kinetics for their single solution drops to model the spray drying process for the production of pharmaceutical preparations containing maltodextrin or trehalose excipients.\(^{307}\) Schiffter \textit{et al.} subsequently studied the drying kinetics and particle formation of aqueous manitol, and trehalose drops that were acoustically levitated whereby a strong similarity was observed between the morphology of the levitated particles to those that were spray dried.\(^{308}\) Leiterer \textit{et al.} examined the nucleation and crystallization of ascorbic acid,
acetylsalicylic acid, and colloidal gold aqueous drops by both small and wide angle X-ray scattering measurements during acoustic levitation which allowed access to novel insights into aggregation and crystallization phenomena.\textsuperscript{309} Benmore \textit{et al.} demonstrated that amorphous forms of various organic compounds having different glass forming abilities that have enhanced solubility can be produced from acoustically levitated solution drops.\textsuperscript{310} In their studies with acoustically levitated pharmaceuticals, Weber \textit{et al.} even observed that in several cases, solute crystallization from a levitated solution drop did not occur following the loss of solvent. The remaining drop of viscous liquid could be transformed into a glass via drop cooling as was the case for probucol, a cholesterol-reducing drug, dissolved in acetone.\textsuperscript{311}

In their work that couples surface-enhanced Raman scattering detection with acoustically levitated drops, Santesson \textit{et al.} observed the formation of two crystal modifications for both the pharmaceutical precursor benzamide and the anti-inflammatory drug indothemacin.\textsuperscript{312} This appears to be the first reported instance in the literature where the unusual conditions of acoustic levitation (i.e.: no container; ultrasonic field) resulted in the formation of crystal forms not produced during conventional crystallization. Leiterer \textit{et al.} used time-resolved synchrotron small- and wide-angle X-ray scattering measurements to follow the crystallization process of acetylsalicylic acid in situ by continuously increasing the concentration of the samples via solvent evaporation.\textsuperscript{309} Although acetylsalicylic acid is known to have two polymorphs, only the common form I was observed during repeated evaporative crystallization experiments. Following these experiments, Leiterer \textit{et al.} used the same technique to study in situ the crystallization of caffeine from acoustically levitated aqueous drops where they were surprised to observe that only the metastable $\alpha$-polymorph formed in contrast to the mixture of $\alpha$- and $\beta$-polymorphs that form from evaporating sessile drops placed on solid surfaces; akin to the ring deposits formed by coffee drops on a table top.\textsuperscript{313} Recently, acquisition of simultaneous X-ray diffraction and Raman data acquisition within a twenty second time period enabled Kilmakow \textit{et al.} to investigate polymorphic phase transitions during crystallization via solvent evaporation of acoustically levitated drops in situ. They observed that the crystallization process of the active pharmaceutical ingredient (API)
nifedipine exhibited a strong solvent dependence; transitioning between two intermediate phases (β and glassy) prior to the α-polymorph transformation.\textsuperscript{153}

Acoustic levitation has also been used to study nucleation and crystallization of biomolecules. Chung \textit{et al.} developed a hybrid acoustic levitator to grow protein crystals in a containerless environment whereby both lysozyme and thaumatin crystals were grown under controlled crystallization conditions that mimic aspects of growth in a low-gravity environment.\textsuperscript{314} Santesson \textit{et al.} developed a screening method based on acoustically levitated drops for the study of precipitation of proteins for crystallisation purposes. They demonstrated that an acoustically levitated drop is easily varied with regards to protein, additives, and crystallization agent concentrations allowing for a wide concentration range to be screened using a single protein containing levitated drop.\textsuperscript{315}

Electric fields are known to promote nucleation and affect polymorph formation.\textsuperscript{10,316,317} As demonstrated in chapter 4, for a 1.0 mm diameter acoustically levitated drop, a $\text{SCD}_{\text{max}}$ value of $\pm 3.44 \times 10^{-4}$ e·nm\textsuperscript{-2} corresponds to an initial electric field at the drop-air interface of $\pm 1.6 \times 10^6$ V·m\textsuperscript{-1}. As this field is similar in magnitude as is that required ($6.0 \times 10^5$ V·m\textsuperscript{-1}) for affecting polymorph formation, drop surface potential would also appear to be a likely candidate to influence the polymorph fractional outcome of evaporation-driven crystallization in an acoustically levitated drop.\textsuperscript{10,207}

\section*{5.1.1 The Phenomenon of Polymorphism}

When forming crystals some molecules are able to adopt more than one crystal structure; that is they exhibit polymorphism, as illustrated in Figure 5.1. When preparing materials via crystallization, it is important to be able to control this phenomenon because although identical in chemical composition, polymorphs have unique mechanical, thermal, and physical properties including solubility and bioavailability, dissolution rate, melting point, colour, filterability, density, and flow behaviour.\textsuperscript{318} Hence it comes as no surprise that the production of specific and well defined polymorphs is crucial in chemical manufacture.\textsuperscript{2} A hundred years ago, this was important in the development of azo pigments and copper phthalocyanide for the dye industry.\textsuperscript{319}
Currently, the polymorphism of drugs has been the subject of great interest in the pharmaceutical industry since the variation in solubility between different polymorphic pharmaceuticals affects drug efficacy, bioavailability, and safety.\textsuperscript{318,320-324} Although differences in bioavailability of polymorphs of the same compound are usually related to differences in solubility or dissolution kinetics, in some cases one polymorph may have a pronounced beneficial therapeutic effect over the other polymorph(s) despite having very similar solubilities as was found for glycine in the treatment of genetically cataleptic rats; both $\alpha$- and $\gamma$-glycine are quite soluble, however, $\gamma$-glycine showed significantly better therapeutic effects than that of $\alpha$-glycine.\textsuperscript{325}

The famous American chemical analyst Walter C. McCrone commented in 1965 that virtually all compounds are polymorphic; the number of polymorphs of a material is in direct proportion to the time and money spent looking for them.\textsuperscript{326} Since then, development of solid state chemistry has exemplified this statement as is shown by some common examples in Table 5.1.
Table 5.1  Common examples of polymorphic chemical compounds.  Adapted from Davey et al.²

<table>
<thead>
<tr>
<th>Chemical Compound</th>
<th># of Polymorphs</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylsalicylic acid</td>
<td>2</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>3</td>
<td>Pigment</td>
</tr>
<tr>
<td>Ammonium nitrate</td>
<td>5</td>
<td>Fertilizer</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>2</td>
<td>Food additive (sweetener)</td>
</tr>
<tr>
<td>Titanium trichloride</td>
<td>4</td>
<td>Catalyst</td>
</tr>
<tr>
<td>Lead azide</td>
<td>2</td>
<td>Explosive</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>4</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Indigo</td>
<td>2</td>
<td>Dye</td>
</tr>
</tbody>
</table>

Due to the demand for high yields and high production rates, many chemical processes are conducted far from equilibrium which increases the possibility of forming polymorphs. However, these structures are not the most stable and can undergo transformations to more stable phases. It should be noted that only one polymorph is thermodynamically stable at a specific pressure and temperature; hence the observation of other forms implies them as kinetically favored.³²⁷ Under such non-equilibrium conditions as supersaturation, kinetically favored polymorphs crystallize more rapidly than the thermodynamically favored polymorph. The initial polymorphs formed during crystallization are referred to as metastable forms, as originally described by Ostwald’s Rule of Stages.²³²⁸ This rule, first stated by Wilhelm Ostwald in 1897 to account for numerous experiments whereby an unstable solid was first formed from solution or melt, later to be replaced by a more stable form, postulates that in such a process, the system progresses from the supersaturated state to equilibrium in stages, where each stage represents the least amount of change in free energy of the system. Thus in a polymorphic system, metastable polymorphs form before crystals of the lowest energy form.² It should also be noted that Ostwald’s Rule of Stages are not always strictly followed.³²⁹

Given that only one polymorph corresponds to the thermodynamic minimum, both at one temperature and one specific pressure, strategies for controlling polymorphism must rely on the careful manipulation of nucleation and crystal growth kinetics.³¹⁹ In general, fast crystallization processes, that have timescales of seconds to hours, have a greater
tendency to form metastable polymorphs than slow processes having time scales of days to months.\textsuperscript{318} The traditional methods of polymorphic control are summarized in Figure 5.2 along with their time scales and propensities to forming either stable or metastable polymorphs.\textsuperscript{150,318}

Although the conditions mentioned above are widely used to control particular polymorph formation, control is not always easy to achieve or maintain. Thus, there have been several recent advances in the methods used for polymorph control. Crystal seeding is the common practice whereby small crystallites of a compound are added to a solution or melt to induce crystallization; the typically slow, rate-limiting nucleation step can thus be accelerated.

![Figure 5.2](image-url)  
**Figure 5.2** Crystallization experiments showing the timescales that can be employed to favour stable or metastable polymorphs. Adapted from Llinàs \textit{et al.}\textsuperscript{318}

For a system of polymorphs, the intentional seeding of one particular form is often the most successful manner of producing it rather than the other form(s); assuming seed crystals of the wanted form are available.\textsuperscript{329} Tamura \textit{et al.} have induced both the growth of the $\gamma$-form of a racemic sulphate and the enrichment of enantiomers from racemic mixtures by way of seeding.\textsuperscript{330,331}
Another successful control strategy involves the addition of a molecule that inhibits the formation of a particular polymorph, leaving the other polymorph(s) free to form, by interfering with either its nucleation or growth.\textsuperscript{318,332} Such an additive is chosen via its ability to mimic specific motifs of the desired polymorph while disrupting growth of the other polymorph(s); such as inhibiting the fastest growing crystal faces of the unwanted polymorph. Molecular modelling and Monte Carlo crystal growth simulations have been useful in determining the appropriate choice of additive. By modelling the interactions of L-glutamic acid, Davey \textit{et al.} successfully inhibited the stable $\beta$-form in favour of the metastable $\alpha$-form with both glutaric acid and 2-methylglutaric acid additives.\textsuperscript{333}

An additive compound can also be added to a polymorphic system in order to directly enhance the growth of a specific form; this control strategy is known as templating. Mitchell \textit{et al.} used pimelic acid as a substrate for the selective heterogeneous nucleation and subsequent growth of the metastable YN-form of 5-methyl-2-[(2-nitrophenyl)amino]3-thiophenecarbonitrile (better known as ROY; an API precursor) due to good epitaxial matching of the (101) pimelic acid crystal face to the YN-form.\textsuperscript{334} Self-assembled monolayers (SAMs) and polymers have also been used as templates for polymorph selection through heterogeneous nucleation. Lang \textit{et al.} demonstrated that the metastable orthorhombic form of acetaminophen could be grown from aqueous solutions on several different polymer substrates.\textsuperscript{335}

The use of supercritical fluids to assist in polymorphism control has shown considerable promise. Both rapid (or slow) expansion of supercritical solutions (RESS and SESS respectively) and solution enhanced dispersion by supercritical solution (SEDSS) have successfully been shown to precisely control crystallization of a desired polymorph along with precise control of crystal habit and size.\textsuperscript{318} By controlling temperature, pressure, flow rate, and solvent used, different polymorphs can be formed isolated such as four forms of sulfathiazole from methanol using SEDSS and three forms of tolbutamide using RESS.\textsuperscript{336,337}

Differing types of solution confinement have also been successfully used to control polymorph formation.\textsuperscript{318} By confining a small amount (1-50 $\mu$L) of solution in a capillary tube, less stable polymorphs are able to grow without competition from more stable forms since the small solution volume is rarely able to accommodate more than a
single nucleation site. Seven polymorphs of ROY have been isolated using this technique. Nanoporous polymers and glass matrices have also been used to confine solution in order to direct polymorph formation. Ha et al. could selectively grow the anthranilic acid metastable form II polymorph within nanoporous glass beads having a 7.5 nm pore size. Upon changing the pore size to 55 nm, only form III could be obtained. It is thought that the critical nucleus size for both forms I and III are too large to form in the smaller pore size glass beads.

The influence of ultrasound on polymorphism has been recently investigated. Ultrasound irradiation was found to generate the more stable polymorph form(s) along with smaller, more uniform crystal size and less crystal agglomeration. The mechanisms for these effects are not well understood.

Infrared radiation can also affect polymorph formation as mentioned in section 2.2.3. The technique of non-photochemical laser induced nucleation (NPLIN) was introduced by Garetz et al. who observed that a short (9 ns), intense laser pulse induced nucleation in supersaturated urea solutions; lowering the induction time by a factor of $10^{13}$. Interestingly, when NPLIN was applied to supersaturated glycine solutions, it was observed that linear and circular polarizations induce the nucleation of the $\gamma$-form and $\alpha$-form of glycine respectively within a particular region of supersaturation. The group postulates that the peak electric field of the light modifies the free energy of the subcritical clusters of the solute causing an organization of the clusters through the electric field induced alignment of solute molecules within the cluster.

Assuming that nucleation occurs at the drop-air interface for a levitated drop, the electric field due to the surface potential of the charged levitated drops employed in chapter 4 is $\sim 2 \times 10^6 \text{ V}\cdot\text{m}^{-1}$, a value greater than $6 \times 10^5 \text{ V}\cdot\text{m}^{-1}$ used by Aber et al. to induce nucleation of the $\gamma$-form of glycine from supersaturated glycine solutions in a test tubes. Aber et al. postulate the same mechanism for nucleation induction and control as that of NPLIN. Since it was demonstrated in both chapters 2 and 4 that the surface potential of a solution drop can significantly promote solute nucleation, perhaps drop surface potential could also influence the polymorphic outcome of evaporation-driven crystallization in an acoustically levitated drop and hence could be adopted as a technique for polymorph control.
5.1.1.1 Thermodynamics

If one considers a dimorphic system comprised of polymorph I and II, the more stable polymorph at specific temperature $T$ has the lowest free energy $G$:

$$G_I < G_{II}$$  \hspace{1cm} (5.1)

In this case, polymorph II is more stable. This implies that the chemical potential ($\mu$) of polymorph II is also less than that of polymorph I. At equilibrium, the chemical potential of the solid is equal to that of the species in solution:

$$\mu_c = \mu_s = \mu^o + RT \ln a_s$$  \hspace{1cm} (5.2)

where $\mu_c$ and $\mu_s$ are the chemical potentials of a species in the solid crystal phase and in solution respectively, $\mu^o$ is the standard chemical potential, and $a_s$ is the activity a species in solution. Then for the case of the dimorphic system:

$$\mu^o + RT \ln a_{II} < \mu^o + RT \ln a_{II}$$  \hspace{1cm} (5.3)

and hence:

$$a_{II} < a_{II} = C_{II} < C_{II}$$  \hspace{1cm} (5.4)

where $C_c$ is the molar concentration of a species in solution in equilibrium with the solid phase (i.e.: the solubility). Equation 5.4 demonstrates that the most stable polymorph always has the lowest solubility. Typically the most stable polymorph also has the highest density; this is known as the density rule.

In general, two possible situations are encountered in a dimorphic system as illustrated in Figure 5.3. If the solubility curves do not cross in solution, then the system is considered to be monotropic; the most stable polymorph in Figure 5.3a is polymorph II. If the solubility curves of the two polymorphs cross each other below the melting points of each polymorph as shown in Figure 5.3b, then the system is considered enantiotropic; the point of crossing is referred to as the transition temperature ($T_{tr}$). Below $T_{tr}$, polymorph II is less soluble and hence more stable; while above $T_{tr}$, polymorph I is less soluble and hence more stable. It should be noted that the boiling point of the solvent limits the temperature range of the solubility curves in solution.
5.1.1.2 Kinetics

A traditional energy-reaction coordinate diagram is useful when examining the kinetic factors of a polymorphic system. If one considers the same dimorphic system from the previous section (i.e.: $G_H < G_I$), then a representation of a reaction coordinate for the crystallization of the dimorphic system can be drawn showing the activation barriers for the formation of each polymorph as illustrated in Figure 5.4. The initial system is comprised of supersaturated solute in a solvent (free energy = $G_0$) that transforms into either polymorph I or polymorph II via a transition state and an activation free energy, which are involved in the relative rates of formation for each polymorph. However, unlike a chemical reaction, the transition state for crystallization does not involve a simple bi- or trimolecular process where a covalent bond is formed. Rather, crystallization involves a collection of self-assembled molecules into a specific packing arrangement that belongs to a new separate phase. In his kinetic theory of nucleation from homogeneous solutions, Volmer recognized that the magnitudes of the activation barriers are dependent upon the size (or surface-to-volume ratio of the new phase) of the supramolecular assembly (or crystal nucleus). As discussed in section 1.31, the critical nucleus size ($n^*$ or $r^*$) is dependent upon the degree of supersaturation; the higher the degree supersaturation, the smaller is the critical size of the nucleus for crystallization.

**Figure 5.3** Solubility curves in a) monotropic and b) enantiotropic polymorphic systems. Adapted from Davey et al.²
Although the supersaturation of polymorph II \((G^o - G_{II})\) is greater than polymorph I \((G^o - G_I)\) in Figure 5.4, if the critical size of polymorph I is less than that of polymorph II for a given solution composition, then the activation barrier for polymorph I is lower than that of polymorph II and kinetics will favour the initial crystallization of polymorph I.\(^9,320\) However, the metastable polymorph I will ultimately transform into polymorph II as previously mentioned. The probability of forming a particular polymorph depends on both its free energy of formation and the rate of some kinetic process related to its molecular aggregation into a crystal. According to equation 1.14, the classical nucleation rate \((J)\) is a function of the supersaturation ratio \((S)\), the interfacial energy between the solution and the newly forming solid phase \((\sigma)\), the system temperature \((T)\), and both the solubility \((C_e)\) and the diffusivity \((D)\) which appear in the pre-exponential factor \((J_o)\) in equation 1.15. The trade-off between thermodynamics and kinetics can make predicting the outcome of a crystallization event difficult even in a simple monotropic system as depicted in Figure 5.5. For any solution compositions and temperatures that correspond to the region between the two solubility curves of polymorph I and II in Figure 5.5, only
polymorph II can crystallize. However, if an isothermal crystallization is undertaken from point 1 to point 2, as indicated in Figure 5.5, the outcome of crystallization is not clear. In this case, the solution is supersaturated with respect to both polymorphs whereby polymorph I is the kinetically favoured product and polymorph II is the thermodynamically favoured polymorph.9,320

![Figure 5.5](image)

**Figure 5.5** Schematic solubility diagram for a dimorphic system (polymorphs I and II) showing a hypothetical crystallization pathway at constant temperature.

At point 2 there are a couple of crystallization outcome possibilities: 1) polymorph II is present in solution and appears simultaneously with polymorph I (or by heterogeneous nucleation on polymorph I) and grows at the expense of polymorph I that eventually disappears (it should be noted that the presence of solvent surrounding the system often promotes this type of phase transformation); 2) only crystals of metastable polymorph I are present.322 Both of the aforementioned scenarios would be in agreement with Ostwald’s rule of stages. It should be noted that the kinetics of transformation from polymorph I to II is limited by the kinetics of dissolution for polymorph I or the kinetics of growth for polymorph II.322
5.1.2 Concomitant Polymorphs: Controlling the Form Obtained

It is remarkable that a collection upwards of $10^{20}$ molecules randomly distributed in solution can coalesce to form regular solids with a limited number of well-defined structures as is the case with polymorphs. The collection of experimental conditions, (such as solvent(s) used, temperature, and rate of evaporation for example) under which a substance crystallizes, is referred to as the occurrence domain and its contents are usually never completely known. For a polymorphic system, the contents of the occurrence domain are not necessarily unique. This results in regions of domain overlap, whereby multiple polymorphs can crystallize under identical conditions. The determination of unique domain regions within a polymorphic system allows for control over which particular polymorph is formed. If a particular crystallizing procedure yields more than one form under identical conditions, then there is an overlap in occurrence domains; the polymorphs obtained are termed concomitant. Concomitant polymorphism implies the simultaneous nucleation and growth of multiple forms; it should be not be confused with solvent mediated transformation of one form to another as mentioned in the previous section. Furthermore, concomitant polymorphism defies Ostwald’s rule of stages since both the stable phase and metastable phase(s) nucleate and grow simultaneously. When the nucleation and growth rates of the stable and metastable phases are very similar in value, the probability of appearance for each phase is nearly identical; hence concomitant polymorphism will occur.

The experimental conditions relative to both thermodynamic conditions and kinetic processes give rise to the situations in which polymorphs concomitantly crystallize. In a strict thermodynamic sense, polymorphs can only coexist in solution at the thermodynamic transition temperature ($T_{tr}$; where the $G$ curves cross) in an enantiotropic system. However, since such a crystallization would necessarily have to occur at a specific $T_{tr}$, the probability of such a precise crystallization occurring would be low and hence the kinetics of the process likely plays a role in this type of crystallization. Davey et al. theoretically demonstrated that even in a monotropic dimorphic system, concomitant polymorph formation is possible; dependent on both the nucleation rate and supersaturation of each polymorph. A concomitant system that remains in contact via the solution will inevitably change towards the most stable form as mentioned previously.
(i.e.: solvent mediated transformation); although this could take a long period of time. Furthermore, a solid state transformation is also possible. Since concomitant polymorphs are close to being energetically equivalent structures, fine tuning of the crystallization conditions can be used to control the form obtained. The most common conditions varied are temperature, concentration, choice of solvent, and the rate of solvent evaporation; of which numerous examples can be found in the literature.

Since drop surface potential has been shown to affect nucleation rates, perhaps drop surface potential could also influence the polymorphic outcome of evaporation-driven crystallization and hence could be adopted as a technique for polymorph control. A concomitant polymorphic system seems well suited for such an investigation.

5.1.3 Choice of Concomitant Polymorphic System for Investigation

The literature values concerning the prevalence of polymorphs for small organic molecules (i.e.: molecular weights less than 600 g mol⁻¹) is approximately 51%. Upon examination of the Agnes lab chemical inventory, two simple organic compounds known to form concomitant polymorphs were located and were found to be suitable candidates for drop surface potential experiments.

5.1.3.1 m-nitrophenol

The dimorphism of m-nitrophenol was first observed by Groth at the turn of the century whereby he described two distinct melting points for the compound (93 °C and 96 °C); however, he stated that this was simply an acceptable melting point range and concluded the compound showed no polymorphism. He also observed that the compound crystallized as centrosymmetric monoclinic prismatic crystals.

Several decades later, the crystal structure of m-nitrophenol was determined by Pandaere et al. to belong to the common centrosymmetric monoclinic P2₁/n space group. However, Shigorin et al. observed that the compound exhibited non-linear optical properties having a strong second harmonic one hundred times more efficient than quartz; a physical property that requires crystals to belong to a non-centrosymmetric space group. Upon re-examination of their batch of crystals, Pandaere et al. discovered that approximately 20% of the crystals belonged to the non-centrosymmetric
orthorhombic space group $P2_12_12_1$ which accounted for the observed second harmonic effect.$^{356,357}$

The $m$-nitrophenol system is enantiomorphic. At room temperature, orthorhombic crystals are stable; upon heating they undergo a phase transition to the monoclinic form at 356 K, having a transition enthalpy of 200 J mol$^{-1}$, which is irreversible upon cooling.$^{357}$ $M$-nitrophenol melts at 370 K with a melting enthalpy two orders of magnitude greater than that of the transition enthalpy ($\sim 20$ kJ mol$^{-1}$). The metastable monoclinic phase can also be grown from the melt and forms a very long lasting phase below the transition temperature due to the slow kinetics of the phase transformation.$^{358}$ The phase situation of $m$-nitrophenol is presented in Figure 5.6.

**Figure 5.6** Phase situation for $m$-nitrophenol. Adapted from Wójcik.$^{358}$

Both crystalline phases of $m$-nitrophenol show similar molecular arrangements as shown in Figure 5.7. In both polymorphs, 1-D molecular ribbons of coplanar $m$-nitrophenol molecules are formed via hydrogen bonding between the nitro group acceptor in one molecule and the hydroxyl donor of another. However, the 1-D molecular ribbons are arranged differently in each polymorph: either centrosymmetrically for the monoclinic phase (Figure 5.7a), whereby the adjacent molecular ribbons are aligned in an antiparallel fashion, or non-centrosymmetrically for the orthorhombic phase (Figure 5.7b), whereby the adjacent molecular ribbons are aligned in a parallel manner.$^{357,359}$ Weak hydrogen bonds ($O-H-O > 3.4$ Å) and weak interactions of both the C-H···O and C-H···π type between the 1-D molecular ribbons are responsible for the 3-D herringbone-type structures formed in both polymorphs.

Due to the polarity of $m$-nitrophenol molecule ($\mu = 5.81$ D), it is thought that both the ions $\text{DNEC}^+$ and an external electric field could affect $m$-nitrophenol crystallization. It is
hypothesized that the interaction of ions\textsubscript{DNEC} with the molecular dipoles of \textit{m}-nitrophenol could cause an organization of the subcritical nucleation clusters, such as the aforementioned 1-D polar molecular chains, in a different manner than that which occurs in a neutral \textit{m}-nitrophenol solution drop; the result being manifested in shift of polymorphic ratio between acoustically levitated drops with and without surface charge.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure57}
\caption{Adjacent 1-D molecular ribbons of \textit{m}-nitrophenol for a) the monoclinic phase and b) the orthorhombic phase. Colour scheme: O red; N pale blue; C grey; H white. Hydrogen bonds are denoted by the purple dotted lines.}
\end{figure}

In a similar manner, it is hypothesized that an external electric field could also cause organizational changes of the 1-D polar molecular \textit{m}-nitrophenol chains. This modification of the free energy of the subcritical clusters of \textit{m}-nitrophenol via electronic polarization could enhance the nucleation and growth of one polymorph form over the other.
*M*-nitrophenol is used mainly to make dyes, paint coloring, rubber chemicals, fungicides, and pharmaceuticals such as the anti-inflammatory drug mesalazine (5-aminosalicylic acid).\textsuperscript{360,361}

### 5.1.3.2 Anthranilic Acid

The enantiotropic trimorphic system of anthranilic acid (or \(o\)-aminobenzoic acid) has been widely studied and crystallizes in either form I (orthorhombic space group \(P2_1cn\)), form II (orthorhombic space group \(Pbca\)), or form III (monoclinic space group \(P2_1/c\)).\textsuperscript{329}

Upon heating forms I and II in air, a phase transformation occurs at \(T_r = 90.2 \, ^\circ\text{C}\) to form III; the transformation of form II to III occurs substantially slower than the transformation of I to III. The heating of form III in air showed no phase transformation before its melting point.\textsuperscript{362} From their antisolvent (water / ethanol) crystallization studies of anthranilic acid at room temperature, Jiang \textit{et al.} proposed a solubility diagram for anthranilic acid as depicted in Figure 5.8.\textsuperscript{345,363}

![Figure 5.8 Proposed solubility diagram for the three phases of anthranilic acid. Adapted from Jiang \textit{et al.}\textsuperscript{363}](image)

Form I was found to be enantiotropically related to forms II and III while forms II and III are monotropically related. At room temperature, form I was found to be the stable form in solution and that concomitantly crystallized form II could rapidly transform to it
while in aqueous solution. Pure metastable form II is usually formed from rapid crystallization from supersaturated solutions that are subsequently filtered and dried, while pure form III can be obtained either from supersaturated solutions that are kept above 53 °C for greater than 800 minutes, or via sublimation. Interestingly, all three polymorphs have a melting point range between 147-148 °C.

The unit cell of the non-centrosymmetric form I consists of two independent molecules, one neutral and one zwitterionic, while both centrosymmetric forms II and III are comprised only of neutral molecules. As a result, form I shows significantly different molecular packing than both forms II and III due in large part to the different hydrogen bonding networks formed between anthranilic acid molecules. The crystal structures of all three polymorphs show dimerization of anthranilic acid molecules as depicted in Figure 5.9.

![Figure 5.9](image)

**Figure 5.9** Hydrogen-bonded molecular dimers from form I, form II, and form III of anthranilic acid. Note the dimer of form I consists of a zwitterion and a neutral molecule associated in an anti-configuration while the dimers of both forms II and III are associated in a syn-configuration. Colour scheme: O red; N pale blue; C grey; H white. Hydrogen bonds are denoted by the purple dotted lines.

The molecular dimers of both forms II and III are associated in a syn-configuration and stack together in layers to form 3-D herringbone structures; albeit in slightly different ways. Each dimer of form II hydrogen bonds to four other individual dimers via four separate contacts (N-H···O = 3.121 Å) to generate a 2-D dimeric sheet as shown in
Figure 5.10. Short contacts of the C-H⋯π type cause the 2-D sheets to stack into the 3-D dimeric herringbone structure.

Figure 5.10 2-D dimeric sheet packing diagram for form II of anthranilic acid whereby every anthranilic dimer is hydrogen bonded to four different anthranilic dimers. Colour scheme: O red; N pale blue; C grey; H white. Hydrogen bonds are denoted by the purple dotted lines.

On the other hand, each dimer of form III is bonded to two other individual dimers via four weak hydrogen bonds (N-H⋯OH = 3.385 Å) to form a 1-D chain of dimers. 2-D sheets of form III are formed via weak C-H⋯O interactions (3.422 Å) between the 1-D dimeric chains as illustrated in Figure 5.11. Short contacts of the C-H⋯π type cause the 2-D sheets of form III to stack into a 3-D dimeric herringbone structure in a similar manner to that of form II. Although form III has fewer hydrogen bond interactions overall when compared with form II, it has a more efficient packing density (ρ_{III} = 1390 kg m^{-3}) than form II (ρ_{II} = 1372 kg m^{-3}) at room temperature. 366,367
Anthranilic acid dimers weakly hydrogen bond to form 1-D dimeric chains that are in turn assembled into a 2-D sheet via weak C-H···O interactions. Colour scheme: O red; N pale blue; C grey; H white. Hydrogen bonds are denoted by the purple dotted lines while C-H···O interactions are denoted by the green dotted lines.

Unlike forms II and III, form I of anthranilic acid has a more complex 2-D hydrogen bonding network. The anti-configured dimers of form I assemble into a 2-D dimeric sheet via four hydrogen bonds: the zwitterion of a dimer forms two hydrogen bonds to two individual zwitterions from different dimers (N⁺-H···O⁻ = 2.758 Å); the neutral molecule of a dimer also forms two hydrogen bonds to two individual neutral molecules of different dimers (N-H···O = 2.894 Å) as illustrated in Figure 5.12. The 2-D dimeric sheets assemble into a 3-D network via nitrogen donor/acceptor hydrogen bonds between a zwitterion and neutral molecule from different dimers (N-H···N = 2.894 Å) which as a packing density ($\rho_I = 1409$ kg m$^{-3}$) greater than both forms II and III.$^{368}$
It is thought that both the ion$_{\text{DNEC}}$ and an external electric field could affect anthranilic acid crystallization. It is hypothesized that the interaction of ion$_{\text{DNEC}}$ with the molecular dipoles of anthranilic acid could cause an organization of the subcritical nucleation clusters, such as the aforementioned molecular dimers or 2-D hydrogen bonded network, in a different manner than that which occurs in an electrically neutral anthranilic acid solution drop; the result being manifested in shift of polymorphic ratio between acoustically levitated drops with and without surface charge. In a similar manner, it is hypothesized that an external electric field could also cause organizational changes in the formation of the 2-D anthranilic acid sheets that would favor the formation of form I over forms II and III being that form I crystallizes in a polar space group. This modification of the free energy of the subcritical clusters of anthranilic acid via electronic polarization could enhance the nucleation and growth of form I over the both forms II and III.
Anthranilic acid is used as an intermediate for production of dyes, pigments, and saccharin, while its esters are used in preparing perfumes (imitation jasmine and orange fragrance) and several pharmaceuticals.\textsuperscript{369,370} Anthranilic acid is also an intermediary in the human body’s synthesis of tryptophan.\textsuperscript{371}

5.1.4 X-ray Powder Diffraction: Using Powder Patterns to Determine Polymorphic Form

In most cases, X-ray crystallographic methods can be definitive in the identification and characterization of polymorphs. A distinction is usually made between single crystal and powder methods in the study of polymorphic systems. The former have been used to determine the detailed molecular structure (i.e.: bond lengths, bond angles, intermolecular interactions etc...) of each individual form of a polymorphic system, while the later have been typically used to identify and qualify the polymorphic form(s) present in a sample.\textsuperscript{329}

A crystal is comprised of a regular arrangement of small imaginary boxes referred to as the unit cell which contain atoms in a specific spatial arrangement; unit cells are packed together in three-dimensional space to form the crystal.\textsuperscript{2} An X-ray diffraction experiment examines the contents of the unit cell. The unit cell is a parallelepiped whose side lengths (a, b, c) and angles (α, β, γ) are known, as shown in Figure 5.13, and it can be divided up into Miller planes (or hkl-planes) that are located at a fixed distance (d\textsubscript{hkl}) from the unit cell origin. In an X-ray diffraction experiment, a crystal is irradiated with an X-ray beam of known wavelength (λ) and the constituent atoms of the crystal cause the beam to diffract. The diffraction pattern is recorded as a function of the angle (2θ) between the transmitted and diffracted beam. The location of reflections is governed by Bragg’s Law (equation 5.5) and the unit cell is determined based upon the diffraction angles:

\[
n \cdot \lambda = 2 \cdot d_{hkl} \cdot \sin(\theta_{hkl})
\]  

(5.5)

The values of d\textsubscript{hkl} reflect the dimensions of the unit cell while the intensities while the diffraction intensities are dependent upon the contents of the unit cell (i.e.: the specific atoms) and their arrangement.\textsuperscript{329}
Figure 5.13  Unit cell definition using a parallelepiped having side lengths of $a$, $b$, and $c$ and angles between the sides of $\alpha$, $\beta$, and $\gamma$.

A successful single crystal X-ray diffraction experiment determines the atomic positions of all the atoms in the unit cell except for hydrogen atoms; the location of each reflection is dependent upon the four angles that determine the Miller plane, the detector, and the incident X-ray beam. Furthermore, the sample should consist of a single crystal and not a polycrystalline sample.

X-ray powder diffraction (XRPD) is usually the most definitive method for the identification of polymorphs and distinguishing between them.\(^{329}\) In an XRPD experiment, a polycrystalline sample can be employed, but it is at the expense of a loss in atomic connectivity information. Since a powder sample is polycrystalline, all orientations of a crystal are present simultaneously leaving only the Bragg angle constant. Fortunately, this information loss affects only the possible structural information available for a compound whose crystal structure is unknown. A XRPD experiment provides a simple analysis for determining the composition of a polymorphic system whose polymorphic structures are known. A powder diffractogram is essentially a unique fingerprint for crystalline matter and is routinely used to monitor phase purity of a polymorphic sample as each form has a unique powder pattern as shown for the
monoclinic phase of \( m \)-nitrophenol in Figure 5.14. The diffractogram of a polymorphic sample is comprised of the weighted combination of each individual polymorph’s diffractogram. Furthermore, an X-ray powder diffractogram can be used to identify new polymorphs whose structures are unknown.

In order conduct qualitative and quantitative XRPD, reference diffraction patterns are required of the each form in the polymorphic system. Fortunately, powder diffraction patterns can be calculated from single crystal data using public domain software. For the quantitative analysis of polymorphic phases, the Rietveld method is employed whereby the entire diffraction pattern for each solid phase is used for comparison; standard diffraction patterns are refined against the experimental powder pattern of the sample to obtain the relative amounts of the polymorphs.  

![Figure 5.14 X-ray powder diffractogram of the monoclinic phase of \( m \)-nitrophenol.](image)

### 5.1.5 Research Objectives

The focus of this chapter was to determine the effect of surface-potential controlled nucleation during the crystallization of a polymorphic system while using an acoustic levitation apparatus for sample processing. This initial investigation examined the
concomitant polymorphic systems of dimorphic $m$-nitrophenol and trimorphic anthranilic acid. Sample phase composition was determined using XRPD and subsequent Rietveld analysis. Independent experiments for levitated drops under the influence of an external electric field were also conducted.

5.2 EXPERIMENTAL SECTION

5.2.1 Chemicals and Starting Solutions

Reagent grade ethanol (EtOH), 1-butanol ($n$-BuOH), 1-pentanol ($n$-PeOH), acetone, acetonitrile (MeCN), methyl isobutyl ketone (MIBK), glacial acetic acid (AcOH), dimethylsulfoxide (DMSO), and dimethylformamide (DMF), were purchased from BDH. All aqueous solutions were prepared using distilled water (dH$_2$O).

$m$-nitrophenol was purchased from Sigma-Aldrich. Approximately 15.0 g of $m$-nitrophenol was dissolved in a minimum amount of hot acetone and filtered. The filtrate was left to slowly cool and recrystallize. The crystals were collected via vacuum filtration, rinsed with a small portion of cold acetone, and left to dry. Starting solutions were made using the 13.9 g of yellow crystals that were recovered.

Anthranilic acid was purchased from Sigma-Aldrich. 15.0 g of anthranilic acid was dissolved in a minimum of hot anhydrous ethanol (EtOH) and filtered. After the filtrate had cooled to room temperature, the solvent was left to slowly evaporate. Recrystallized anthranilic acid was collected via vacuum filtration, rinsed with a small portion of cold EtOH, and left to dry. Starting solutions were made using the 10.9 g of pale coloured crystals that were recovered.

Starting solutions of both $m$-nitrophenol and anthranilic acid were prepared by the following procedure unless otherwise noted. A 20 mL vial was rinsed three times with a small portion of the solvent to be used in the starting solution. A measured mass of solute was added to the vial along with a measured volume of solvent at room temperature. The vials were placed in an ultrasonic bath (Branson Model 1510 MT) for 30 minutes at room temperature in order to hasten the dissolution of the solute. Descriptions of the starting solutions are listed in Table 5.2. It should be noted that the concentration of each solution is undersaturated in solute so that crystallization was not likely to occur during solution storage and drop injection.
Table 5.2 Composition of \( m \)-nitrophenol and anthranilic acid starting solutions.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>Dielectric Constant (( \varepsilon ))</th>
<th>Vapour Pressure (mmHg @ 20°C)</th>
<th>Concentration (mg mL(^{-1})) ( m )-nitrophenol</th>
<th>Concentration (mg mL(^{-1})) anthranilic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(_2)O</td>
<td>100.0</td>
<td>78.5</td>
<td>233.7</td>
<td>10.4</td>
<td>4.8</td>
</tr>
<tr>
<td>EtOH</td>
<td>78.4</td>
<td>24.6</td>
<td>44.6</td>
<td>112.7</td>
<td>59.0</td>
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<tr>
<td>MeCN</td>
<td>82.0</td>
<td>37.5</td>
<td>72.8</td>
<td>112.7</td>
<td>33.9</td>
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<tr>
<td>DMSO</td>
<td>189.0</td>
<td>47.0</td>
<td>0.417</td>
<td>112.7</td>
<td>100.0</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>118.0</td>
<td>17.8</td>
<td>4.2</td>
<td>112.6</td>
<td>95.8</td>
</tr>
<tr>
<td>n-PeOH</td>
<td>137-139</td>
<td>13.9</td>
<td>1.6</td>
<td>112.6</td>
<td>84.3</td>
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<td>DMF</td>
<td>152-154</td>
<td>36.7</td>
<td>2.7</td>
<td>112.7</td>
<td>96.3</td>
</tr>
<tr>
<td>AcOH</td>
<td>118-119</td>
<td>6.2</td>
<td>9.7</td>
<td>112.6</td>
<td>---</td>
</tr>
<tr>
<td>MIBK</td>
<td>117-118</td>
<td>13.1</td>
<td>15.7</td>
<td>113.0</td>
<td>---</td>
</tr>
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</table>

5.2.2 Acoustic Levitation Apparatus Parameters

The acoustic levitation apparatus is operated under the parameters as described in chapter 3 and summarized in Table 5.3. Prior to each experiment, the levitation chamber temperature is conditioned to be at room temperature (23 °C) and a relative humidity of 0 %. This initial relative humidity is chosen for all experiments because it is a simple and reliable humidity setting to control. Dry air flow to the chamber is terminated prior to drop injection so that the external gas flow would not affect solvent evaporation rates. During the course of a single experiment, the final humidity reached from an evaporating solution drop was never greater than 15 %.

For each solvent employed, a set of four trials, consisting of levitated drops under differing conditions, were conducted: 1) \( E_{ext} = 0 \) V m\(^{-1}\), \( Q = 0 \) C; 2) \( E_{ext} = 0 \) V m\(^{-1}\), \( Q = +ve \) C; 3) \( E_{ext} = 0 \) V m\(^{-1}\), \( Q = -ve \) C; 4) \( E_{ext} = 6.0 \times 10^5 \) V m\(^{-1}\), \( Q = 0 \) C. Each trial was repeated approximately ten times. A control trial consisting of a non-levitated solution drop placed on a glass slide inside the levitation chamber that was kept at the same temperature and relative humidity used during the levitated drop trials (\( T = 23 \) °C; % RH = 0) was conducted for each solvent used in order to ascertain the effect of acoustic levitation on solute crystallization.
Table 5.3 Operating specifications of the acoustic levitator.

<table>
<thead>
<tr>
<th>Operating Specifications</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resonance Frequency ((f))</td>
<td>45.64 kHz</td>
</tr>
<tr>
<td>Reflector Separation Distance from Transmission Plate ((H))</td>
<td>25 mm</td>
</tr>
<tr>
<td>Wavelength of Standing Acoustic Wave ((\lambda))</td>
<td>7.5 mm</td>
</tr>
<tr>
<td>Chamber Temperature ((T))</td>
<td>23 ºC</td>
</tr>
<tr>
<td>Initial Chamber Relative Humidity (% RH)</td>
<td>0 %</td>
</tr>
<tr>
<td>Maximum Electrode Voltage</td>
<td>16 kV</td>
</tr>
<tr>
<td>Applied Voltage to Syringe Needle</td>
<td>± 800 V</td>
</tr>
</tbody>
</table>

5.2.3 Polymorph Characterization

The deposited drop residues were characterized by optical microscopy (Model B5 Professional, Motic, Richmond, BC).

X-ray powder diffractograms were collected on a Rigaku RAXIS rapid curved area plate detector with a graphite monochromated Cu-Kα radiation source operated at 1.9 kW (46 kV, 42 mA) using a 0.5 µm collimator at room temperature. Ten minute scans were taken with a goniometer spinning speed of 5 ° s\(^{-1}\), while the goniometer was held at \(\omega = 90 \, ^\circ\) and \(\chi = 0 \, ^\circ\). Samples were adhered to a thin glass fibre with a small amount of vacuum grease. Samples were not grind up using a mortar and pestle as sample grinding is known to affect the polymorphic fraction.\(^{365}\) Peak positions were located in WINPLOTR.\(^{372}\) Reference powder diffractograms of each polymorph for both \(m\)-nitrophenol and anthranilic acid were generated from single crystal data taken from the Cambridge Structural Database using the program MERCURY.\(^{373}\) Quantitative analysis of sample phase composition was conducted using the Rietveld method with the Material Analysis Using Diffraction (MAUD) program.\(^{374}\)

5.3 RESULTS AND DISCUSSION

5.3.1 Nucleation and Growth of \(m\)-Nitrophenol in Acoustically Levitated Droplets

5.3.1.1 Solvent Systems

After a conducting a few initial crystallization trials, a number of \(m\)-nitrophenol / solvent systems were deemed impractical to investigate. Due to its low solubility in H\(_2\)O,
the amount of \( m \)-nitrophenol crystallized from a single large (\( d_i = 2.0 \) mm) aqueous drop was insufficient to generate a strong enough XRPD signal in order to create a diffractogram. Hence, the \( m \)-nitrophenol / \( \text{H}_2\text{O} \) system was not investigated with regards to the potential surface-charge effect on the polymorphism of \( m \)-nitrophenol.

Although the solubility of \( m \)-nitrophenol is significantly greater in both EtOH and MeCN relative to \( \text{H}_2\text{O} \), the onset of crystallization following drop injection occurred very quickly (~ 10 s) at room temperature due to the low boiling point of each solvent (b.p.: 78.4 °C, 82.0 °C for EtOH and MeCN respectively). This induction time was found to be too short in duration to prevent crystallization from occurring on the needle tip during drop injection at room temperature for both EtOH and MeCN. Hence, low boiling point solvents (b.p. < 100 °C) were deemed impractical for use in the experiments conducted during these investigations.

At the other end of the scale, \( m \)-nitrophenol / DMSO solution drops had a very long induction time (> 1 day). Due to the induction time being quite long in duration, uncorrected instrumental drift caused drop instability, frequently resulting in drop ejection from the acoustic levitation cavity prior to \( m \)-nitrophenol crystallization. Hence, high boiling point solvents (b.p. > 160 °C) were also deemed impractical to solvate \( m \)-nitrophenol during these investigations. Solvents whose boiling points were in the range 100 - 160 °C were found to be good candidates for \( m \)-nitrophenol crystallization from levitated drop trials since the induction times were neither too short, which result in solute crystallization prior to levitation, nor too long, which result in levitation stability issues, in duration.

Three different types of solvents were to be employed in this study: non-polar, polar protic, and polar aprotic. The interactions (i.e.: hydrogen bonding, Van der Waals forces) between solvent and solute molecules are different for each solvent type used and hence the molecular packing of the solute molecules could possibly be affected. This in turn could influence the polymorphic distribution upon crystallization of \( m \)-nitrophenol. Being a polar molecule, \( m \)-nitrophenol is not appreciably soluble in medium boiling (i.e.: b.p.: 100 - 160 °C), non-polar solvents. Although hot benzene has been used to grow crystals of \( m \)-nitrophenol, it was deemed impractical to use since it has a low boiling point (b.p.: 80 °C) and is a known human carcinogen.\textsuperscript{358} Attempts at using toluene (b.p.:
111 °C) in lieu of benzene as a suitable non-polar solvent proved unsuccessful due to its poor solvation of $m$-nitrophenol. MIBK was chosen as a suitable aprotic polar solvent while $n$-BuOH, $n$-PeOH, and AcOH were chosen as suitable protic polar solvents for $m$-nitrophenol (refer to Table 5.2). The solvents were found in the Agnes lab chemical inventory.

5.3.1.2 Drop Evaporation and Particle Formation

Unlike the one stage evaporation observed for NaCl solution drop trials conducted in chapter 4, a normalized plot of the square of the drop diameter vs. time for $m$-nitrophenol containing drops showed two-stage evaporation for all four solvents employed. An example of this two stage evaporation is shown in Figure 5.15 for $m$-nitrophenol dissolved in AcOH. The first evaporation stage showed an approximately constant rate of evaporation until $t/d_o^2 \approx 148$ s mm$^{-2}$ whereby the surface of the drop was observed to rapidly solidify, in a similar fashion as depicted for anthranilic acid in Figure 3.18. The solidification of the drop signified the beginning of stage two evaporation, as indicated by the dotted line in Figure 5.15. After a short period where the $m$-nitrophenol particle slightly increased its diameter (between $t/d_o^2 = 148 – 165$ s mm$^{-2}$, the diameter of the particle formed remained essentially constant. This small increase in particle diameter could be the result of particle expansion due to heat released from the crystallization of solute.
Figure 5.15  Reduction of drop diameter due to solvent evaporation during acoustic levitation of an AcOH drop containing 112.6 g mL$^{-1}$ of $m$-nitrophenol showing two-stage evaporation. The chamber was kept at room temperature and RH% = 0.

The particle was levitated for approximately an additional twenty minutes to ensure that solvent that may have been trapped inside had an opportunity to evaporate. Slight oscillations and/or rotations of the particle were observed during stage two evaporation, as evidenced by the increase in particle diameter variation (i.e.: a broader $d^2$ line observed in stage two when compared to the $d^2$ line in stage one) seen in Figure 5.15.

An example of how the evaporation rate constant ($\beta_d$) was calculated for each trial is also shown in Figure 5.15. $\beta_d$ is equal to slope of the $d^2/d_o^2$ vs. $t/d_o^2$ plot during stage one evaporation as discussed in section 3.7.2. The first evaporation stage showed a constant rate of evaporation until approximately $t/d_o^2 = 110$ s mm$^{-2}$, at which time the slope of the plot began to significantly change from linearity; hence, only the upper portion of the $d^2$ line was used for the $\beta_d$ calculation ($\beta_d = 5.56 \times 10^{-3}$ mm$^2$ s$^{-1}$) as indicated by the solid dark blue line in Figure 5.15.
Since the $d^2$-Law has been derived for spherically shaped, pure solvent drops (refer to Table 1.1 for $d^2$-Law assumptions), it is not surprising that deviations from linearity of the $d^2$ line with time was observed during stage one evaporation; the drop being an oblate spheroid and composed of a solution. The effect of solute concentration variation on 1) the mass fraction of solvent vapour at the drop surface ($Y_{vap,s}$; refer to equation 1.32); 2) the solution density ($\rho_{liq}$; refer to equation 1.32) are known to change over time and hence change the slope of the $d^2$ line with time; most significantly at the late stages of drop evaporation.\(^{375}\) This type of non-linearity in the $d^2$ line was not observed with experiments conducted in chapter 4 since those trials were very short in duration. It should be noted that the once a shell of precipitated solute has formed at the drop surface, the $d^2$-Law is no longer valid. During their studies involving the behaviour of drops of a commercial agricultural spray, Taflin et al. observed a non-linear dependence of $d^2$ with time for an aqueous solution of hexazinone in dry nitrogen.\(^{191}\) Eslamian et al. also report observing a variable $\beta_d$ for suspended aqueous solution drops of NaCl, MgSO$_4$, and zirconium hydroxychloride at both atmospheric and reduced pressures.\(^{375}\)

Acoustic streaming around a drop is also known to change the slope of the $d^2$ line with time since it can provide a convective mechanism much stronger than natural convection alters convection and hence affects the mass transfer of solvent from the drop. Both Yarin et al. and Brenn have correlated experimental $d^2(t)$ data of evaporating drops in acoustic fields by a function reminiscent of the $d^2$-Law and found that even acoustically driven evaporation dependencies of pure solvent drops can show slight non-linearity in the $d^2$ line.\(^{174,175,282,376,377}\) They also found that multicomponent liquid-liquid mixtures and solutions show significant deviations of the $d^2$ line from linearity.\(^{174,376}\)

The plot in Figure 5.16 is an example of the $d^2$ curves for each solvent employed whereby non-linearity of the $d^2$ line was observed in all cases. The dotted vertical lines indicate the transition to stage two evaporation for each solvent. It should be noted that oscillatory movement of the $d^2$ line during stage two evaporation for $m$-nitrophenol dissolved in $n$-BuOH was observed in this trial. The same type of observation was made on occasion for each solvent used and can be accounted for in the following manner.
Figure 5.16  Solution drop evaporation rate data for samples containing 112.6 g mL$^{-1}$ \(m\)-nitrophenol. The dotted lines indicate the transition from stage one evaporation (left hand side) to stage two evaporation (right hand side). Deviations from the $d^2$-Law are noticeable near the end of first-stage drying for each solvent used. The chamber was kept at room temperature and RH\% = 0.

The particle that forms at stage two evaporation can have a significant difference in its diameter along the \(x\)- and \(y\)-dimensions due to how the particle specifically formed. The diameter value used in the evaporation rate plot is calculated using a calculated drop volume (see equation 3.12) which assumes that the diameters along both the \(x\)- and \(y\)-dimensions are equivalent (i.e.: \(d_{\text{hor}}\)). If a particle were to have differing horizontal diameters, then a periodic rotation around the \(z\)-axis in the levitation cavity would manifest in the data as an oscillating particle diameter. With a second drop data collection camera set up at right angles to the first, both horizontal diameter values of a levitated drop can be collected and this systematic error can be corrected for.

A summary of the calculated drop evaporation rates for each set of trials using each solvent is presented in Table 5.4. \(Q\) values and \(\beta_d\) values listed in Table 5.4 are the average values of at least five individual experiments and their standard deviations.
Table 5.4  *M*-nitrophenol solution drop evaporation rates ($\beta_d$) for each set of trials.  $Q$ and $\beta_d$ values reported are the average values of several repeat experiments.  The initial drop concentration was ~ 112.6 mg mL$^{-1}$ for each solvent used.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$E_{\text{ext}}$ (V m$^{-1}$)</th>
<th>$Q$ (pC)</th>
<th>$\beta_d \times 10^3$ (mm$^2$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIBK</td>
<td>0</td>
<td>0</td>
<td>6.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>81 ± 3</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-76.0 ± 1.2</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>AcOH</td>
<td>0</td>
<td>0</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>77 ± 5</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-81 ± 3</td>
<td>5.50 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>$n$-BuOH</td>
<td>0</td>
<td>0</td>
<td>2.25 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>83 ± 3</td>
<td>2.43 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-81 ± 3</td>
<td>2.33 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>2.49 ± 0.10</td>
</tr>
<tr>
<td>$n$-PeOH</td>
<td>0</td>
<td>0</td>
<td>1.12 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>80 ± 2</td>
<td>1.18 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-79.8 ± 1.5</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>1.32 ± 0.05</td>
</tr>
</tbody>
</table>

Three of the four solvents used (MIBK, AcOH and $n$-BuOH) had very similar boiling points (b.p.: ~ 117 °C), but their $\beta_d$ values are significantly different; indicative of their differing vapour pressures at $T = 23$ °C (refer to Table 5.2).  The evaporation rates of levitated *m*-nitrophenol solution drops followed the same order (from highest to lowest value) as solvent vapour pressures: MIBK > AcOH > $n$-BuOH > $n$-PeOH.

In general there was little difference in the average $\beta_d$ values found for each set of trials that used a particular solvent.  Although it does appear that for trials conducted in the presence of an external electric field, $\beta_d$ was found to increase slightly relative to trials in the absence of $E_{\text{ext}}$ for each solvent, the standard deviation of $\beta_d$ for each set prevents this observation from being definitive; notwithstanding the $\beta_d$ values found for $n$-PeOH.  However as discussed in section 4.3.1, $E_{\text{ext}}$ affects the levitated drop shape by making it more prolate which alters the acoustic streaming that a levitated drop experiences relative to a drop levitated in the absence of $E_{\text{ext}}$, which in turn affects the $\beta_d$ value.  As noted in section 4.3.1, an increase in drop destabilization (i.e.: internal oscillations and translational oscillations), due to the presence of $E_{\text{ext}}$, may also result in
an increase in \( \beta_d \) value. It should be noted that a trend was observed for standard deviation values of \( \beta_d \): as \( \beta_d \) value increased, its associated standard deviation value also increased. The cause of this trend is likely due to the significant difference in the number of data points used to create the linear regression plots between rapidly evaporating and more slowly evaporating solvents (~ 190, 340, 840, and 1700 data points for MIBK, AcOH, \( n \)-BuOH, and \( n \)-PeOH respectively). The \( \beta_d \) values observed for \( n \)-BuOH and \( n \)-PeOH solution drops were comparable to \( \beta_d \) values observed by Tuckermann et al. for pure \( n \)-BuOH and \( n \)-PeOH drops that evaporated at \( T = 21 \, ^\circ \text{C} \) and \( \%\text{RH} = 38 \) (\( \beta_d \times 10^{-3} \) (mm\(^2\) s\(^{-1}\)) = 2.23, 1.27 for \( n \)-BuOH and \( n \)-PeOH respectively).\(^{279}\)

Since the amount of charge a drop obtained is dependent upon its diameter at the point which the drop breaks the surface tension from the needle tip (see equation 3.9), a variation in \( Q \) values occurred between each trial due to the variation in initial drop diameter upon drop injection.

The \( m \)-nitrophenol particles formed in all trials appeared to be of the same type: solid spheroid, as depicted in Figure 5.17 whereby the canary yellow coloured \( m \)-nitrophenol particle was formed from an acoustically levitated \( n \)-BuOH solution drop. The dense particle was an agglomeration of numerous \( m \)-nitrophenol crystals having a small block type habit as shown in Figure 5.18.

**Figure 5.17** Solid spheroid \( m \)-nitrophenol particle as formed from an acoustically levitated \( n \)-BuOH solution drop.
Figure 5.18  Crystal blocks that comprise an $m$-nitrophenol particle formed via during acoustic levitation of a solution drop. a) Several small crystals broken off a particle; b) A enlarged crystal from a).

5.3.1.3 X-ray Powder Diffraction Analysis

The X-ray powder diffractograms generated by MERCURY using single crystal data from the Cambridge Structural Database for the two $m$-nitrophenol polymorphs (monoclinic, $P2_1/n$ and orthorhombic, $P2_12_12_1$) are shown in Figures 5.19 and 5.20 respectively. The X-ray diffractograms of the two polymorphs are similar with respect to peak positions (but not peak intensities) along the $2\theta$ axis as would be expected for polymorphs of such similar structure. However, there are a number of easily identifiable unique peaks for each form, as labelled with asterisks in Figures 5.19 and 5.20, that were useful to determine the presence of each polymorph at a quick glance in the sample diffractograms. It should be noted that polymorphic ratio were determined by MAUD using the entire diffractogram.
Figure 5.19  X-ray powder diffractogram of monoclinic (\(P2_1/n\)) \(m\)-nitrophenol as generated from single crystal data obtained from the Cambridge Structural Database. Identifying peaks (\(2\theta = 15.1^\circ, 20.5^\circ, 25.3^\circ\)) are labelled with an asterisk.

Figure 5.20  X-ray powder diffractogram of orthorhombic (\(P2_12_12_1\)) \(m\)-nitrophenol as generated from single crystal data obtained from the Cambridge Structural Database. Identifying peaks (\(2\theta = 13.5^\circ, 15.5^\circ, 23.5^\circ, 27.9^\circ\)) are labelled with an asterisk.
Sample XRPD data was first taken of the bottle contents and of the recrystallized $m$-nitrophenol used in the subsequent acoustically levitated drop experiments as shown in Figure 5.21.

Figure 5.21  X-ray powder diffractograms of purchased (light blue solid line) and recrystallized (dark blue solid line) $m$-nitrophenol superimposed on the generated diffractogram of the monoclinic form (red dotted line). The numbered arrows indicate five sample diffraction peaks, appearing in both samples that do not correspond to peaks of the monoclinic form.

At first glance, the sample X-ray powder diffractograms show the presence of the monoclinic form as the major component that crystallized in both the purchased $m$-nitrophenol and the recrystallized product; as visually indicated by how close the sample diffractograms (solid light and dark blue lines in Figure 5.21) match the generated diffractogram (red dotted line in Figure 5.21). Interestingly, both sample diffractograms show five additional peaks, as indicated by the arrows in Figure 5.21 ($2\theta = 7.5^\circ, 8.8^\circ, 9.6^\circ, 10.2^\circ, 13.5^\circ$), that do not appear in the generated monoclinic diffractogram. In fact, all $m$-nitrophenol sample diffractograms obtained show the same five peaks with the same relative intensity. The first four additional peaks ($2\theta = 7.5^\circ, 8.8^\circ, 9.6^\circ, 10.2^\circ$) appear in a $2\theta$ region that corresponds to systematic absences (i.e.: no signal intensity) for
both polymorphs while the fifth additional peak (2θ = 13.5°) corresponds to an identifiable peak for the orthorhombic phase; however, no other identifiable peaks associated with the orthorhombic phase (2θ = 15.4°, 19.2°, 23.5°) are present.

The Rietveld analysis using MAUD determined the composition of both samples to be 100.0 ± 1.0 % monoclinic form despite the presence of the 2θ = 13.5° peak associated with the orthorhombic phase. This result is not surprising since Rietveld analysis models the entire sample diffractogram against the two generated diffractograms of m-nitrophenol and no other orthorhombic phase peaks were detected. There are two possible explanations for the observed additional five diffractogram peaks: 1) the presence of a new phase or crystalline impurity; 2) the λ/2–effect. It is possible that either a new phase or a crystalline impurity has been detected by XRPD as unidentified peaks would be present in the sample diffractograms. However, the presence of only five unaccounted for peaks appearing at low 2θ angles seems suspect as the diffractogram of both a new phase or crystalline impurity should be comprised of more than five peaks that are spread out over the 2θ range of 5.0° to 50.0°. The λ/2–effect can occur when large strongly diffracting samples are used for analysis. With graphite monochromatization, as was used by the Rigaku diffractometer, a small quantity of λ/2-radiation (approximately 0.1-0.3 % of the total) is included with the Kα radiation being sought. When the Bragg equation is considered (equation 5.5), a strongly diffracting set of planes (2h, 2k, 2l for example) in the crystal can then give a reflection in the position where the first order h,k,l is expected; such reflections with odd indices frequently correspond to systematic absences. Since the monoclinic form shows strong peaks at 2θ = 15.1°, 19.3°, 20.5°, 27.1°, a strong λ/2–effect would show peaks at 2θ = 7.5°, 9.7°, 10.3°, 13.6°, which correspond with four of the observed additional peaks (2θ = 7.5°, 9.6°, 10.2°, 13.5) in both samples. Hence, the λ/2–effect appears to be reason behind the additional peaks observed. The cause for the small observed peak at 2θ = 8.7° remains unclear. It should be noted that although the system is concomitant, no significant amount of orthorhombic phase was detected from both the stock bottle and recrystallized sample.

Since both generated XRPD diffractograms of m-nitrophenol show no peaks between 2θ = (5-11)°, Rietveld analyses were conducted on sample diffractograms between 2θ =
(11-50)° so as not to include four of the five peaks associated with the aforementioned \( \lambda/2 \)-effect in order to obtain more accurate polymorphic ratios. Figure 5.22 shows the graphical result from a Rietveld analysis using MAUD of an \( m \)-nitrophenol sample as formed from a levitating solution drop.

![Figure 5.22](image)

Figure 5.22  Typical Rietveld analysis graphical result for a \( m \)-nitrophenol sample. The blue trace shows the experimental XRPD data. The black trace is the fitted data from the analysis. The two rows below the diffractograms indicate the positions expected for the diffraction peaks of each form. The bottom trace shows the root mean square deviation resulting from the refinement of the combination of the full patterns for the two forms against the measured pattern.

The upper blue trace shows the experimental XRPD diffractogram. The upper black trace is the fitted data from the Rietveld analysis that models the experimental data. The two rows below the diffractograms indicate the positions expected for the diffraction peaks of each form. The bottom trace shows the root mean square deviation resulting from the refinement of the combination of the full patterns for the two forms against the measured pattern. For all sample data sets, the reliability factor, \( R_w \), which is a measure of the agreement between the crystallographic model and the XRPD data, was found to
be between \( R_w (%) = 1.40 – 8.20 \). These \( R_w \) values demonstrate excellent agreement between the model and experimental data sets.

A summary of the \( m \)-nitrophenol polymorph ratios, as calculated using MAUD, for each set of trials using each solvent set is presented in Table 5.5.

**Table 5.5** \( M \)-nitrophenol polymorphic ratios. \( \text{SCD}_{\text{max}} \) and polymorphic ratio values reported are the average values of several repeat experiments. The initial drop concentration was \( \sim 112.6 \text{ mg mL}^{-1} \) for each solvent used.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( E_{\text{ext}} ) (V m(^{-1}))</th>
<th>( \text{SCD}_{\text{max}} \times 10^4 ) (e nm(^2))</th>
<th>( C_f \times 10^2 ) (mg mL(^{-1}))</th>
<th>Monoclinic Form (%)</th>
<th>Orthorhombic Form (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIBK</td>
<td>0</td>
<td>0</td>
<td>6.6 ± 0.7</td>
<td>100.0 ± 1.1</td>
<td>0.06 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.62 ± 0.12</td>
<td>6.8 ± 0.3</td>
<td>99.9 ± 1.0</td>
<td>0.09 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-1.61 ± 0.06</td>
<td>6.2 ± 0.4</td>
<td>100.0 ± 1.0</td>
<td>0.08 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>6.0 \times 10^5</td>
<td>0</td>
<td>6.3 ± 0.7</td>
<td>100.0 ± 1.0</td>
<td>0.03 ± 0.09</td>
</tr>
<tr>
<td>AcOH</td>
<td>0</td>
<td>0</td>
<td>7.2 ± 1.1</td>
<td>100.0 ± 1.3</td>
<td>0.04 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.72 ± 0.16</td>
<td>7.0 ± 1.0</td>
<td>100.0 ± 1.1</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-1.71 ± 0.08</td>
<td>7.5 ± 0.6</td>
<td>100.0 ± 1.1</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>6.0 \times 10^5</td>
<td>0</td>
<td>6.9 ± 0.6</td>
<td>99.9 ± 1.1</td>
<td>0.36 ± 0.18</td>
</tr>
<tr>
<td>( n )-BuOH</td>
<td>0</td>
<td>0</td>
<td>8.5 ± 0.5</td>
<td>99.9 ± 1.0</td>
<td>0.08 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.82 ± 0.08</td>
<td>8.5 ± 1.2</td>
<td>99.9 ± 1.0</td>
<td>0.15 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-1.79 ± 0.09</td>
<td>7.9 ± 0.6</td>
<td>99.9 ± 1.0</td>
<td>0.16 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>6.0 \times 10^5</td>
<td>0</td>
<td>8.5 ± 0.5</td>
<td>99.9 ± 1.0</td>
<td>0.15 ± 0.16</td>
</tr>
<tr>
<td>( n )-PeOH</td>
<td>0</td>
<td>0</td>
<td>8.9 ± 0.4</td>
<td>100.0 ± 0.9</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.91 ± 0.14</td>
<td>8.6 ± 0.8</td>
<td>99.6 ± 1.0</td>
<td>0.59 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-1.89 ± 0.10</td>
<td>8.5 ± 0.6</td>
<td>99.6 ± 0.9</td>
<td>0.45 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>6.0 \times 10^5</td>
<td>0</td>
<td>7.8 ± 0.5</td>
<td>100.0 ± 0.9</td>
<td>0.06 ± 0.07</td>
</tr>
</tbody>
</table>

Both the maximum surface charge density (\( \text{SCD}_{\text{max}} \)) values and polymorphic ratios listed in Table 5.5 are the average values of at least five individual experiments and their associated standard deviations. The \( \text{SCD}_{\text{max}} \) values were calculated using the drop diameter value that corresponded to the end of stage one evaporation. The \( \text{SCD}_{\text{max}} \) values for the charged drop trials were observed to follow the opposite trend as that of the evaporation rate constant values; they increased in value as the corresponding evaporation rate decreased for each solvent used (i.e.: MIBK < AcOH < \( n \)-BuOH < \( n \)-PeOH). Since the amount of charge acquired by the drops upon their placement in the levitation cavity was very similar for each solvent used, the increasing values of \( \text{SCD}_{\text{max}} \) was indicative of the decreasing drop diameter measured just prior to rapid \( m \)-nitrophenol
crystallization for the different solvents used (i.e.: \textit{n}-PeOH solution drops were smaller at onset of crystallization than their MIBK solution drop counterparts). This is likely related to the difference in solubilities of \textit{m}-nitrophenol in each individual solvent, for which no values in the literature could be identified with the solvents employed in this work.

The final solute concentration ($C_f$) in each drop was also calculated and listed in Table 5.5. In general, as the solvent evaporation rate decreased, the $C_f$ value increased. However, this increase $C_f$ value must be examined only in relation to the \textit{m}-nitrophenol solubility ($C_e$) in the solvent used (i.e.: the drop supersaturation) in order to make any meaningful comparisons between differing trial sets, since it is the supersaturation ($S$) and not the $C_f$ value that exerts an effect on nucleation and can affect the particular polymorph ratio obtained. This is somewhat of a moot point for \textit{m}-nitrophenol trials as no change in polymorph ratio was observed.

The polymorphic ratios for \textit{m}-nitrophenol of each trial set, as expressed by percentage in Table 5.5, using all four solvents employed showed that the monoclinic form was overwhelmingly preferred over the orthorhombic form under acoustic levitation conditions both with and without drop surface charge and with and without the presence of an external electric field. Interestingly, for a dimorphic polymorphic system that is claimed to be concomitant, only a single polymorph was appreciably detected in the four solute-solution systems employed. The only possible exception would be the trials conducted in \textit{n}-PeOH, whereby the orthorhombic form increased slightly from (0.01 ± 0.03) % to ~ (0.50 ± 0.10) % for both samples having surface charge. However, even though Rietveld analysis of XRPD data uses the entire spectrum of diffraction peaks to determine the polymorphic ratio and is generally considered the most accurate quantitative XRPD method, it is usually only found to be accurate to within ± 1 %; making the aforementioned result inconclusive.\textsuperscript{329} An orthorhombic phase increase of approximately 0.5 % would have to be verified using a different and more sensitive technique such as measuring each sample’s single harmonic generation whereby the intensity of the signal generated would be proportional to the amount of non-centrosymmetric orthorhombic phase present. The monoclinic phase, which belongs to a centrosymmetric space group, would show not any single harmonic generation.
Initially, it was hypothesized that the presence of drop surface charge or an external electric field could affect the polymorphic ratio by aligning polar 1-D molecular chains of $m$-nitrophenol in a different manner than in the absence of either ions $DNEC$ or $E_{ext}$. Although the proposed alignment could be taking place in both cases, the effect had no bearing on the polymorphic ratio for the $m$-nitrophenol-solvent systems examined of acoustically levitated drops using an evaporative crystallization technique. It should be noted that individual drops of each solute-solvent system placed on glass cover slips inside the levitation chamber and left to crystallize also showed only the monoclinic phase present using Rietveld analysis of XRPD data. Since solute crystallization took place in terms of minutes for all the solvents employed, it was likely that kinetic factors remained dominant over any drop surface charge or $E_{ext}$ effect; hence resulting in the metastable monoclinic phase being preferred over the thermodynamically stable orthorhombic phase. The vast number of crystals that constitute the $m$-nitrophenol particles formed (see Figure 5.18) supported this notion. In order to move away from a kinetic regime, a higher boiling point solvent such as DMSO in order to lower the drop evaporation rate would be necessary. A DMSO solution drop would have been investigated if not for the levitation stability issues experienced as mentioned in section 5.3.1.1. Conducting the crystallization trials at lower temperatures would be another way of moving away from the kinetic regime.

It is also possible that the amount of charge allocated to drops during the trials was below the necessary threshold required to affect polymorph nucleation; hence no change in the polymorphic ratio was detected in drops having surface charge. An increase in NaCl nucleation rate was observed for droplets levitated in the EDLT when the $SCD_{max}$ values increased from $\pm 1.68 \times 10^{-4} \text{ e nm}^{-2}$ to $\pm 3.73 \times 10^{-4} \text{ e nm}^{-2}$ and for drops levitated in the acoustic levitator that had a $SCD_{max}$ value of $\pm 3.44 \times 10^{-4} \text{ e nm}^{-2}$. The $SCD_{max}$ values reached in the acoustically levitated $m$-nitrophenol drops ranged only from $\sim \pm 1.61 \times 10^{-4} \text{ e nm}^{-2}$ (MIBK solution drops) to $\sim \pm 1.90 \times 10^{-4} \text{ e nm}^{-2}$ (n- PeOH solution drops). As the surface tension for the organic solvents used were approximately three times less than that of water ($0.0240$, $0.0288$, $0.0242$, and $0.0257 \text{ N m}^{-1}$ for MIBK, AcOH, $n$-BuOH, and $n$-PeOH respectively; $0.0720 \text{ N m}^{-1}$ for H$_2$O) the $SCD_{max}$ values had an upper limit of $\sim \pm 2.4 \times 10^{-4} \text{ e nm}^{-2}$ prior to Coulomb explosion since the amount of
charge a drop can carry before Coulomb explosion occurs is related not only to its radius, but also to its surface tension (see equation 1.1). If the results from the surface charge trials using $n$-PeOH are to be believed, then perhaps a $SCD_{\text{max}} \sim 1.90 \times 10^{-4} \, e \, \text{nm}^{-2}$ is at the cusp of such a surface charge threshold required to observe a change in polymorph distribution. It is certainly possible that surface charge could affect the nucleation rate of one polymorph differently than the other resulting in a change of polymorph distribution. However, the increase in NaCl nucleation rates observed in sections 2.3.2 and 4.3.2 when SCD values of $\pm 3.44 \times 10^{-4} \, e \, \text{nm}^{-2}$ are reached do not necessarily imply that a change in polymorphic distribution should occur when a different solute-solvent system, such as neutral molecular $m$-nitrophenol in an organic solvent, is examined.

As mentioned in section 2.3.2, the observed promotion of NaCl nucleation is contrary to what is predicted by thermodynamics for this solute/solvent system. According to thermodynamics, the sign of $c_\epsilon$ (i.e.: the difference of $\varepsilon_c - \varepsilon_d$) and the magnitude of $E$ determines whether the electric field enhances or inhibits nucleation. For a drop that consists mostly of water ($\varepsilon_d = 80.4$ at room temperature) and NaCl ($\varepsilon_c = 6.1$ at room temperature), it can be assumed that $c_\epsilon < 0$; hence the external electric field should hinder NaCl nucleation rather than enhancing NaCl nucleation.

It should be noted that an attempt was made to compare induction times between the $m$-nitrophenol data sets in order to assess the $m$-nitrophenol nucleation rate(s) in a particular solvent. However, as the number of individual repeat trials were low (between five and ten) and the initial drop size significantly varied between trials affecting the induction time, no accurate means of nucleation rate comparison between differing trials could be ascertained. Furthermore, when crystallization did occur, the subsequent crystal growth was so rapid that in a few seconds only a spheroidal particle of $m$-nitrophenol was obtained consisting of a large number of crystallites that were deemed impractical to count; unlike the NaCl crystals counted in sections 2.3.2 and 4.3.2.
5.3.2 Nucleation and Growth of Anthranilic Acid in Acoustically Levitated Droplets

5.3.2.1 Solvent Systems

H₂O, MeCN, EtOH, and DMSO were not used as solvents for anthranilic acid trials for the same reasons as previously discussed in section 5.3.1.1 with regards to the m-nitrophenol trials.¹⁸⁵

Anthranilic acid is a polar molecule (µ (Debye) = 1.17) that is not appreciably soluble in medium boiling (i.e.: b.p.: 100 - 160 °C), non-polar solvents. Although hot benzene has been used to grow crystals of anthranilic acid, it was deemed impractical to use since it has a low boiling point (b.p.: 80 °C) and is a known human carcinogen.³⁵⁸ Attempts at using toluene (b.p.: 111 °C) in lieu of benzene as a suitable non-polar solvent proved unsuccessful due to its poor solvation of anthranilic acid. n-BuOH and n-PeOH were chosen as suitable protic polar medium boiling point solvents for anthranilic acid. Since MIBK had a high evaporation rate as observed in the m-nitrophenol trials, DMF was chosen as the aprotic polar solvent in the hopes that its higher boiling point (b.p.: ~ 153 °C) would not bias the system towards metastable polymorphs. After using AcOH as a solvent for m-nitrophenol, it was observed that the evaporation of a single small AcOH drop (d ~ 2.0 mm) in the levitation chamber was enough to contaminate the lab air with pungent AcOH vapour. Hence, it was not used as a solvent for anthranilic acid since a fume hood was not available for the levitation apparatus to be place in during conduction of the sample trials. The solvents used were found in the Agnes lab chemical inventory.

5.3.2.2 Drop Evaporation and Particle Formation

A normalized plot of the square of the drop diameter vs. time for anthranilic acid containing drops showed two-stage evaporation for all three solvents employed as expected. The plot in Figure 5.23 is an example of the d² curves for each solvent employed whereby non-linearity of the d² line was observed for n-BuOH and DMF solvents as expected for non-spherical solution drops. Interestingly, for all n-PeOH solution drop trials, only small deviations in linearity were observed in the d² curves; unlike the results observed with m-nitrophenol. As drop shape and diameter are comparable in both anthranilic acid and m-nitrophenol trials, this difference in d² curve shape is likely due to the differences in the solute-solvent interactions (i.e.: anthranilic
acid–n-PeOH vs. m-nitrophenol–n-PeOH). The sharp deviations from linearity in the upper regions of the $d^2$ curves during stage one evaporation observed for the DMF and n-PeOH trials (for example, at $d^2/d_o^2 \sim 0.6$) were a direct result of manual adjustments to the levitation cavity during drop levitation. Trials conducted in DMF showed a large deviation from linearity in their $d^2$ curves resulting in a significantly increased duration for stage one evaporation, as $\beta_d$ was reduced by at least half of its value, as shown in Figure 5.23 after $t/d_o^2 \sim 380$ s mm$^{-2}$. Observations of the levitated drop after $t/d_o^2 \sim 380$ s mm$^{-2}$ showed that no visible crust had formed on the surface of the drop until $t/d_o^2 \sim 860$ s mm$^{-2}$.

![Figure 5.23](image)

**Figure 5.23** Solution drop evaporation rate data for samples containing anthranilic acid concentrations of 95.8, 96.3, and 84.3 mg mL$^{-1}$ for n-BuOH, n-PeOH, and DMF respectively. The dotted lines indicate the transition from stage one evaporation (left hand side) to stage two evaporation (right hand side). Deviations from the $d^2$-Law are noticeable near the end of first-stage drying for n-BuOH and DMF. The chamber was kept at room temperature and RH% = 0.

As mentioned in section 5.3.1.2, deviations in the $d^2$-Law are to be expected for levitated solution drops; however the magnitude of change in $\beta_d$ for anthranilic acid-DMF.
solution drops was unexpected. Since the initial size and shape of all solutions drops were similar, as was the acoustic power used for drop levitation, the acoustic streaming of all solution drops was also thought to be similar and hence unlikely to be the cause for such a dramatic down shift in $\beta_d$ value for DMF solution drops. Increased solute-solvent interactions for anthranilic acid in DMF, relative to anthranilic acid- $n$-BuOH (or $n$-PeOH) were thought to be responsible for this observation.

A summary of the calculated drop evaporation rates for each set of trials using each solvent is presented in Table 5.6. $Q$ values and $\beta_d$ values listed in Table 5.6 are the average values of at least twelve individual experiments and their standard deviations.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$E_{\text{ext}}$ (V m$^{-1}$)</th>
<th>$Q$ (pC)</th>
<th>$\beta_d \times 10^{-3}$ (mm$^2$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$-BuOH</td>
<td>0</td>
<td>0</td>
<td>2.66 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>83 ± 2</td>
<td>2.62 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-83 ± 3</td>
<td>2.69 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>2.82 ± 0.10</td>
</tr>
<tr>
<td>$n$-PeOH</td>
<td>0</td>
<td>0</td>
<td>1.35 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>78 ± 4</td>
<td>1.32 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-80.4 ± 1.5</td>
<td>1.30 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>1.46 ± 0.11</td>
</tr>
<tr>
<td>DMF</td>
<td>0</td>
<td>0</td>
<td>1.58 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>78 ± 3</td>
<td>1.60 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-75 ± 5</td>
<td>1.57 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>0</td>
<td>1.59 ± 0.15</td>
</tr>
</tbody>
</table>

The evaporation rates of levitated anthranilic acid solution drops followed the same order (from highest to lowest value) as solvent vapour pressures (refer to Table 5.2): $n$-BuOH $>$ DMF $>$ $n$-PeOH. In general there was little difference in the average $\beta_d$ values found for each set of trials that used a particular solvent. Although it does appear that for trials conducted in the presence of an external electric field, $\beta_d$ was found to increase slightly relative to trials in the absence of $E_{\text{ext}}$ for both $n$-BuOH and $n$-PeOH. However as discussed in section 4.3.1, $E_{\text{ext}}$ affects the levitated drop shape by making it more prolate.
which alters the acoustic streaming that a levitated drop experiences relative to a drop levitated in the absence of $E_{ext}$, which in turn affects the $\beta_d$ value. As noted in section 4.3.1, an increase in drop destabilization (i.e.: internal oscillations and translational oscillations), due to the presence of $E_{ext}$, may also result in an increase in $\beta_d$ value. The presence of $E_{ext}$ had no effect on the $\beta_d$ value for trials conducted in DMF. The $\beta_d$ values for $n$-BuOH and $n$-PeOH anthranilic acid solutions were observed to be slightly higher than the $\beta_d$ values found for the corresponding $m$-nitrophenol solutions (see Table 5.4). Since $m$-nitrophenol is more polar than anthranilic acid, it was likely that the increase in $\beta_d$ values observed for the anthranilic acid $n$-BuOH and $n$-PeOH solutions was a result of the diminished strength in solute-solvent interactions.

Since the amount of charge a drop obtained is dependent upon its diameter at the point which the drop breaks the surface tension from the needle tip (see equation 3.9), a variation in $Q$ values occurred between each trial due to the variation in initial drop diameter upon drop injection.

Each anthranilic solution drop was observed to rapidly solidify into a particle after reaching stage two evaporation. The diameter of the newly formed anthranilic acid particles from each solvent was observed to slightly increase over a short time period before becoming constant; as was observed for the newly formed $m$-nitrophenol particles as described in section 5.3.1.2. This small increase in particle diameter could be the result of particle expansion due to heat released from the crystallization of solute. Anthranilic acid particles were levitated for approximately an additional twenty minutes to ensure that solvent which may have been trapped inside the particle had an opportunity to evaporate. Slight oscillations and/or rotations of the particle were observed during stage two evaporation, as evidenced by the increase in particle diameter variation (i.e.: a broader $d^2$ line observed in stage two when compared to the $d^2$ line in stage one) seen in Figure 5.23. During stage two evaporation, periodic oscillations were occasionally observed for trials in all three solvents, as shown in Figure 5.23 for the DMF $d^2$ curve. A likely explanation for this observation was presented in section 5.3.2.1.

The anthranilic acid particles formed in all trials appeared to be of the same type: solid spheroid, as depicted in Figure 5.24. The pale coloured anthranilic acid particle in Figure 5.24 was formed from an acoustically levitated $n$-BuOH solution drop and was cleaved
by scalpel in order to determine that it was, in fact, solid. The dense particle was an agglomeration of numerous anthranilic acid crystals having a fine needle/platelet type habit as shown in Figure 5.25. The majority of the crystal needle/platelets constituting the particle appeared to be aligned lengthwise in a radial manner.

Figure 5.24 Solid spheroid anthranilic acid particle as formed from an acoustically levitated n-BuOH solution drop. The particle was cleaved in order to determine that it was solid.

Figure 5.25 Crystal needle/platelets that comprise an anthranilic acid particle formed via during acoustic levitation of a solution drop.
5.3.2.3 X-ray Powder Diffraction Analysis

The X-ray powder diffractograms generated by MERCURY using single crystal data from the Cambridge Structural Database for the three anthranilic acid polymorphs (Form I, orthorhombic, $P_{2_1}cn$; Form II, orthorhombic, $Pbca$; Form III, monoclinic, $P2_1/c$) are shown in Figures 5.26, 5.27, and 5.28 respectively.

![X-ray powder diffractogram of Form I anthranilic acid](image)

**Figure 5.26** X-ray powder diffractogram of Form I anthranilic acid as generated from single crystal data obtained from the Cambridge Structural Database. Identifying peaks ($2\theta = 10.9^\circ, 18.7^\circ, 24.3^\circ, 30.5^\circ$) are labelled with an asterisk.
Figure 5.27  X-ray powder diffractogram of Form II anthranilic acid as generated from single crystal data obtained from the Cambridge Structural Database. Identifying peaks ($2\theta = 11.1^\circ, 15.3^\circ, 26.7^\circ$) are labelled with an asterisk.

Figure 5.28  X-ray powder diffractogram of Form III anthranilic acid as generated from single crystal data obtained from the Cambridge Structural Database. Identifying peaks ($2\theta = 11.5^\circ, 14.7^\circ, 28.1^\circ$) are labelled with an asterisk.
There are a number of easily identifiable unique peaks for each form, as labelled with asterisks in Figures 5.26-5.28 that were useful to determine the presence of each polymorph at a quick glance in the sample diffractograms. It should be noted that polymorphic ratios were determined by MAUD using the entire diffractogram. Sample XRPD data was first taken of the bottle contents and of the recrystallized anthranilic acid used in the subsequent acoustically levitated drop experiments as shown in Figure 5.29. At first glance, the sample X-ray powder diffractograms show the presence of the form I as the major component that crystallized in both the purchased anthranilic acid and the recrystallized product from EtOH; as visually indicated by how close the sample diffractograms match the generated diffractogram (i.e.: solid light and dark blue lines are superimposable on the red dotted line in Figure 5.29).

![Figure 5.29](image)

**Figure 5.29** X-ray powder diffractograms of purchased (light blue solid line) and recrystallized (dark blue solid line) anthranilic acid superimposed on the generated diffractogram of the monoclinic form (red dotted line). The numbered arrows indicate five sample diffraction peaks, appearing in both samples that do not correspond to peaks of the monoclinic form.

As observed with the stock bottle and recrystallized XRPD samples of \( m \)-nitrophenol, both anthranilic acid sample diffractograms show five additional peaks, as indicated by
the arrows in Figure 5.29 ($2\theta = 6.9^\circ$, $8.1^\circ$, $9.3^\circ$, $12.1^\circ$, $15.2^\circ$), that do not appear in the generated form I diffractogram. In fact, all anthranilic acid sample diffractograms obtained show the same five peaks having the same relative intensity. The first three additional peaks ($2\theta = 6.9^\circ$, $8.1^\circ$, $9.3^\circ$) appear in a $2\theta$ region that corresponds to systematic absences (i.e.: no signal intensity) for all three polymorphs while the fifth additional peak ($2\theta = 15.2^\circ$) corresponds to an identifiable peak of form II; however, no other identifiable peaks associated with this phase ($2\theta = 11.1^\circ$ and $26.7^\circ$) are present.

The Rietveld analysis using MAUD determined the composition of both samples to be $100.0 \pm 1.0\%$ form I despite the presence of the $2\theta = 15.2^\circ$ peak associated with form II. The fourth peak ($2\theta = 12.1^\circ$) does not correspond to any peak found in any of the three polymorphs. As previously discussed in section 5.3.1.3, the additionally observed five diffractogram peaks are present due to the $\lambda/2$–effect. Since form I shows strong peaks at $2\theta = 13.8^\circ$, $16.4^\circ$, $18.6^\circ$, $24.3^\circ$, and $30.5^\circ$, a strong $\lambda/2$–effect would show peaks at $6.9^\circ$, $8.1^\circ$, $9.3^\circ$, $12.1^\circ$, $15.2^\circ$ which correspond with the five observed additional peaks in both samples of Figure 5.29. It should be noted that although the trimorphic system is concomitant, no significant amount of form II or form III was detected from both the stock bottle and recrystallized sample. This is not unusual as form I is the thermodynamically stable polymorph at room temperature as mentioned in section 5.1.3.2. Concomitantly crystallized form II and III can easily undergo a phase change while in solution at room temperature to form I when the solution temperature drops below $50^\circ$C (see Figure 5.8). While all three polymorphs may have concomitantly formed during the recrystallization of anthranilic acid from hot EtOH (b.p.: $78^\circ$C), any form II or III present would have likely been converted to form I as evidenced by the Rietveld analysis of the XRPD data. It is also likely that the purchased source material was crystallized from a saturated solution below $50^\circ$C since only form I was observed. It should be noted that for all anthranilic acid sample data sets, Rietveld analysis using MAUD resulted in $R_w(\%) = 1.40 – 8.20$. These $R_w$ values demonstrate excellent agreement between the model and experimental data sets.

A summary of the anthranilic acid polymorph ratios, as calculated using MAUD, for each set of trials using each solvent set is presented in Table 5.7. SCD$_{max}$ values, $C_f$ values, and polymorphic ratio values listed in Table 5.7 are the average values of at least
ten individual experiments and their associated standard deviations. The $SCD_{max}$ and $C_f$ values were calculated using the drop diameter value that corresponded to the end of stage one evaporation. The $SCD_{max}$ values for the $n$-BuOH and $n$-PeOH solution drop trials were essentially the same while they were slightly greater for DMF solution drop trials. As the average $Q$ values for trials conducted in the three different solvents were similar (see Table 5.6), the slight observed increase in $SCD_{max}$ value for DMF solution drops was indicative of a smaller drop diameter measured just prior to anthranilic acid crystallization. This is likely related to the difference in solubilities of $m$-nitrophenol in each individual solvent, for which no values in the literature could be identified for DMF, $n$-BuOH, and $n$-PeOH. The final solute concentration ($C_f$) in each drop was also calculated and listed in Table 5.7.

**Table 5.7** Anthranilic acid polymorphic ratios. $SCD_{max}$, $C_f$, and polymorphic ratio values reported are the average values of several repeat experiments. The initial drop concentrations were 95.8, 84.3, and 96.3 mg mL$^{-1}$ for $n$-BuOH, DMF, and $n$-PeOH respectively.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$E_{ext}$ (V m$^{-1}$)</th>
<th>$SCD_{max} \times 10^{-4}$ (e nm$^2$)</th>
<th>$C_f \times 10^2$ (mg mL$^{-1}$)</th>
<th>Form I (%)</th>
<th>Form II (%)</th>
<th>Form III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-BuOH</td>
<td>0</td>
<td>0</td>
<td>7.8 ± 0.8</td>
<td>99.94 ± 0.11</td>
<td>0.06 ± 0.11</td>
<td>0.006 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.2</td>
<td>7.3 ± 1.3</td>
<td>0.04 ± 0.09</td>
<td>99.7 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.7 ± 0.2</td>
<td>6.7 ± 0.9</td>
<td>0.04 ± 0.09</td>
<td>91 ± 9</td>
<td>9 ± 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^3$</td>
<td>6.0 ± 1.0</td>
<td>0.03 ± 0.09</td>
<td>68 ± 11</td>
<td>32 ± 11</td>
<td></td>
</tr>
<tr>
<td>DMF</td>
<td>0</td>
<td>0</td>
<td>7.8 ± 0.5</td>
<td>99.7 ± 0.4</td>
<td>0.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.99 ± 0.17</td>
<td>7.6 ± 0.7</td>
<td>0.14 ± 0.15</td>
<td>99.86 ± 0.15</td>
<td>0.010 ± 0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2.03 ± 0.15</td>
<td>7.4 ± 0.9</td>
<td>0.12 ± 0.18</td>
<td>99.88 ± 0.18</td>
<td>0.010 ± 0.012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^5$</td>
<td>7.7 ± 0.3</td>
<td>0.07 ± 0.09</td>
<td>99.91 ± 0.10</td>
<td>0.05 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>n-POH</td>
<td>0</td>
<td>0</td>
<td>5.0 ± 0.9</td>
<td>98 ± 4</td>
<td>2 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.4</td>
<td>5.5 ± 1.0</td>
<td>0.05 ± 0.07</td>
<td>97 ± 4</td>
<td>3 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.7 ± 0.3</td>
<td>5.4 ± 1.0</td>
<td>0.11 ± 0.08</td>
<td>99.0 ± 0.7</td>
<td>1.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0 × 10$^3$</td>
<td>5.0 ± 0.6</td>
<td>0.01 ± 0.02</td>
<td>98 ± 4</td>
<td>2 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

For the DMF trials, the $C_f$ values were similar for each data set whereby $C_f \sim 760$ mg mL$^{-1}$. For the $n$-PeOH trials, the $C_f$ values were also similar for each data set whereby $C_f \sim 520$ mg mL$^{-1}$. The lower $C_f$ values found for $n$-PeOH data sets relative to the DMF data sets are likely a result of lower solubility of anthranilic acid in $n$-PeOH than in DMF. Interestingly, the $C_f$ values observed for $n$-BuOH trials show differences between their
trial sets unlike the trial sets conducted in both DMF and n-PeOH, and all of the m-nitrophenol trial sets. It was observed that the $C_f$ value decreased slightly when $n$-BuOH solution drops carried negative surface charge or when placed in an $E_{ext}$. It should be noted that $C_f$ value must be examined only in relation to the anthranilic acid solubility ($C_e$) in the solvent used (i.e.: the drop supersaturation) in order to make any meaningful comparisons between differing trial sets, since it is the supersaturation ($S$) and not the $C_f$ that exerts an effect on nucleation and can affect the particular polymorph ratio obtained.

The polymorphic ratios of anthranilic acid for the DMF trial sets, as expressed by percentage in Table 5.7, showed that form II was the only polymorph obtained to any appreciable degree under acoustic levitation conditions both with and without drop surface charge and with and without the presence of an external electric field. This result was not unusual as pure, metastable form II is typically formed by rapid crystallization from supersaturated solutions that are subsequently filtered and dried.\textsuperscript{363} Ojala \textit{et al.} successfully crystallized form II with a polymorphic fraction of 1 using H$_2$O, EtOH, AcOH, MeCN, and nitrobenzene as solvents under conditions of rapid evaporation or rapid cooling of anthranilic acid solutions.\textsuperscript{364} The result also correlates well with what was observed during solution drop evaporation and subsequent particle formation; a very large number of small needle/platelet crystals were formed rapidly, indicative of a kinetically favoured, metastable polymorph (i.e.: form II). Jiang \textit{et al.} observed that needle type crystals of form II anthranilic acid were obtained as the sole product from H$_2$O/EtOH solutions when $S > 2.3$.\textsuperscript{345} Since the advent of stage two evaporation was always readily apparent and was followed by a horizontal $d^2$ curve in all trials (the periodic oscillations in diameter notwithstanding), it was likely that any solvent remaining at the transition between the two evaporative stages, evaporated rapidly. Hence, phase transition from form II to form I was arrested leaving form II as the sole polymorph detected by XRPD. Interestingly, form II was also obtained with a polymorphic ratio of 100% from a non-levitated DMF solution drop placed on glass slide and left to evaporate inside the levitation chamber; unlike the stationary anthranilic acid solution drops of $n$-BuOH whereby only form I was obtained.

Contrary to the hypothesis presented section 5.1.3.2, neither surface charge nor external electric field was observed to affect the anthranilic acid polymorphic ratio for
acoustically levitated DMF drops. It was originally thought that both parameters could potentially shift the polymorphic ratio towards form I. If one were to assume that half of all the form I anthranilic acid molecules dissolved in DMF are zwitterions, than one would expect that a layer of ions_{DNEC} at the drop surface would affect the alignment of the zwitterions in solution; perhaps acting as a heterogeneous nucleation site in the formation of form I. A solution whereby half of the anthranilic acid molecules are zwitterions would also be affected by an $E_{\text{ext}}$, albeit in a different manner than ions_{DNEC}, as the polarity of the field would dictate the direction of zwitterion alignment and assist the formation of form I crystals which are inherently polar (i.e.: $P_{2_1}cn$ belongs to the polar sub set of space groups). This $E_{\text{ext}}$-induced orientation of highly polar zwitterions into large pre-existing solute clusters is attributed by Aber et al. to the selective formation of $\gamma$-glycine under conditions of a strong $E_{\text{ext}}$ (aqueous glycine solutions exposed to $E_{\text{ext}} = 6.0 \times 10^5$ V m$^{-1}$ for 10 minutes). It should be noted that all three polymorphs of glycine consist exclusively of zwitterions in the solid state unlike form I anthranilic acid which is comprised of 50 % zwitterions and 50 % neutral molecules. Perhaps the alignment of zwitterions by either ions_{DNEC} or $E_{\text{ext}}$ expedited the formation of syn-dimers (see Figure 5.9) with the remaining half of the neutral anthranilic acid molecules, in lieu of forming zwitterion/neutral molecule anti-dimers necessary for the formation of the form I polymorph; hence assisting the formation of the form II polymorph.

It was also possible that the fractional speciation of anthranilic acid molecules (zwitterions : neutral molecules) changed upon dissolution in DMF from the original 50 : 50 present in the solid; invalidating the previous assumption. In their investigations on the equilibrium of anthranilic acid in aqueous solutions and in two-phase (aromatic solvent / H$_2$O) systems, Zapala et al. claimed that the neutral form of anthranilic acid was predominant in organic solvents (even in methanol) over the zwitterionic form. As determined using spectrophotometric techniques, they detected no anthranilic zwitterions in any of the organic aromatic solvents they examined; only the neutral molecule was found present. If the speciation of anthranilic acid in DMF followed the observations of Zapala et al., then the form II polymorph would have a greater probability of forming vs. the form I polymorph. The observation that form II was also exclusively obtained from slow evaporation of a non-levitated DMF solution drop placed on a glass slide in
the levitation chamber would lend credence to a lack of zwitterion species present in DMF, as the form I polymorph can usually be crystallized via slow evaporation from a variety of polar solvents.\textsuperscript{364} However, it should be noted that the speciation in solution does not necessarily preclude which polymorph will predominantly crystallize as observed by Towler \textit{et al.} in their investigations of supersaturated solutions of both anthranilic acid and indomethacin.\textsuperscript{380} Regardless of the explanation, for acoustically levitated anthranilic-DMF solution drops, kinetic factors remained dominant over any surface charge or $E_{\text{ext}}$ effect resulting in the metastable form II phase being preferred over the thermodynamically stable form I phase at room temperature.

The polymorphic ratios of anthranilic acid for the $n$-PeOH trial sets, as expressed by percentage in Table 5.7, showed that form II was the dominant polymorph obtained under acoustic levitation conditions both with and without drop surface charge and with and without the presence of an external electric field. This result was not unusual as pure, metastable form II is typically formed by rapid crystallization from supersaturated solutions that are subsequently filtered and dried; similar to what was observed for levitating $n$-PeOH solution drops.\textsuperscript{365} What was unusual about the findings was the small amount of form III polymorph detected in each of the trial sets. Until recently, the form III polymorph of anthranilic acid was attainable only via sublimation or crystallization from the melt whereby form III was always found concomitant with form II; seeding a supersaturated anthranilic acid solution with form III had never resulted in its growth from solution.\textsuperscript{339,364,381} Recently, pure form III was obtained via neat grinding, grinding in the presence of a few drops of chloroform, transformation from both forms I and II in hot air ($T = 90 \, ^\circ \text{C}$), and transformation from both forms I and II in H$_2$O/EtOH solutions of varying compositions when $T > 50 \, ^\circ \text{C}$.\textsuperscript{345,362,363,365} The form III observed in this work marks the first time it has been grown from solution when $T < 50 \, ^\circ \text{C}$; albeit in very small amounts. It should be noted that form II was exclusively formed from a non-levitated $n$-PeOH solution drop placed on glass slide and left to evaporate inside the levitation chamber.

Even more remarkable were the results obtained from the trial sets conducted in $n$-BuOH as summarized in Table 5.7. As with the trials conducted in DMF, form II was the only polymorph obtained to any appreciable degree under acoustic levitation conditions
without any drop surface charge and with drops carrying positive surface charge. However, for trial sets with drops that carried a negative surface charge (SCD_{max} \sim -1.7 \times 10^{-4} e \text{ nm}^{-2}), and for trial sets conducted in an external electric field (E_{ext} = 6.0 \times 10^5 \text{ V m}^{-1}), significant amounts of form III were observed (9 \pm 8 \%, 32 \pm 11 \% for drops carrying negative surface charge and drops in the presence of E_{ext} respectively). It appeared that for this particular solute-solvent system that both negative drop surface potential and E_{ext} have influenced the polymorph fractional ratio from being comprised exclusively of form II.

Although form III is not expected to crystallize at T < 50 ^{\circ}C, it is important to consider that Jiang et al. used a mixed H_{2}O/EtOH solvent system which significantly differed than what was used in this work. According to Zapala et al., the dominant anthranilic acid species present in H_{2}O and aqueous solutions was the zwitterion at the isoelectric point. Only the neutral species was ever observed in aromatic organic solvents of differing polarity. Assuming the speciation of anthranilic acid in DMF, n-PeOH, and n-BuOH was similar to that found in the aromatic organic solvents employed by Zapala et al., the formation of form I would be likely be arrested since the zwitterions were simply not present in any appreciable amount; necessary for the formation of form I. This would effectively reduce the trimorphic anthranilic acid system to a dimorphic system consisting of forms II and III. It should be noted that although form II anthranilic acid was obtained from both the DMF and n-PeOH drops that were placed on glass slides inside the chamber, form I was exclusively formed from n-BuOH solution drops under the same conditions. This was found to be unusual and is as yet unexplained. Water absorption by n-BuOH solution drops from the ambient could have provided the necessary conditions for zwitterion formation necessary for form I crystallization; however, as the \% RH in the chamber was kept below 5 \%, this was thought to be unlikely.

As mentioned in section 5.1.3.2, forms II and III have very similar packing structures and are monotropically related (see Figure 5.8). Form III is considered thermodynamically to be the most stable form since it has a higher packing density than form II (\rho = 1390 \text{ kg m}^{-3}, 1372 \text{ kg m}^{-3} for forms III and II respectively). However, in their investigations of anthranilic acid using terahertz spectroscopy and solid-state
density functional theory simulations, Delany et al. have determined that form I has the
lowest relative energy followed by form II and then form III. After correcting for
London dispersion forces, they found the difference in energy between forms II and III
was only ~0.36 kJ mol\(^{-1}\); significantly less than the difference in energy between forms I
and II (~3.00 kJ mol\(^{-1}\)) and ambient room temperature energy (~2.45 kJ mol\(^{-1}\)). Such
a small energy difference (and structural packing difference) between forms II and III
would make a dimorphic system of forms II and III quite sensitive to changes in
crystallization conditions. It is also known that slight changes in the crystallization
conditions is known to affect the form taken by anthranilic acid; hence, a change in
crystallization conditions is known to affect the form taken by anthranilic acid; hence, a change in
polymorphic ratio as observed for \(n\)-BuOH solution drops under conditions of negative
surface potential and external electric field was observed.

It is possible that differences in solution supersaturation (\(S\)) could account for the
increase in polymorphic ratio of form III observed for \(n\)-BuOH drops under conditions of
\(E_{ext}\) and, to a lesser extent, negative surface potential. The value of \(S\) is known to affect
the solid phase fraction of a polymorphic system whereby metastable forms become
dominant at high values of supersaturation. For example, Aber et al. observed that in
changing the value of \(S\) from 1.90 to 1.85, the percentages of \(\alpha : \gamma : (\alpha + \gamma)\) of glycine
forms that spontaneously crystallized changed from 13 : 60 : 27 to 86 : 14 : 0. Although
the solubilities of anthranilic acid were not measured in the solvents used, there
was a difference in \(S\) values at the time of crystallization from \(n\)-BuOH drops in the trial
sets. As shown in Table 5.7, the \(C_f\) values significantly decreased for \(n\)-BuOH solution
drops from ~780 mg mL\(^{-1}\) for acoustically levitated neutral drops to ~670 mg mL\(^{-1}\) for
acoustically levitated drops having negative surface potential, and to ~600 mg mL\(^{-1}\) for
acoustically levitated drops in the presence of \(E_{ext}\). If we assume the solubility of
anthranilic acid at room temperature is ~120 mg mL\(^{-1}\) in \(n\)-BuOH, as based on the
extrapolation of data collected by Lazzell et al., then by using equation 1.6, the \(S\) value
decreases from 6.5 to 5.6 for drops with negative ions\(_{DNEC}\) and to 5.0 for drops in the
presence of \(E_{ext}\). The change in \(S\) value for the two trial sets (-0.9 and -1.5 for for
drops with negative ions\(_{DNEC}\) and for drops in the presence of \(E_{ext}\)), when compared with
the \(S\) value for neutral drops in the absence of \(E_{ext}\) were much greater than that observed
by Aber et al. necessary for the enhancement of \(\alpha\)-glycine (\(\Delta S = -0.05\)). Thus, a decrease
in the supersaturation value is a tenable explanation for the observed form III enhancement in levitated \( n \)-BuOH drops under conditions of negative surface potential and the presence of \( E_{\text{ext}} \). This suggests that the rate of form III nucleation increased relative to that of form II under the conditions of negative drop surface potential and the presence of \( E_{\text{ext}} \) since crystallization occurred at lower supersaturation values.

The observation that only negative ions\( \text{DNEC} \) affected the polymorphic ratio of anthranilic acid in \( n \)-BuOH drops was perhaps suggestive of a type of directed selectivity towards form III. Carter \textit{et al.} demonstrated that the polymorphic ratio of anthranilic acid could be affected by altering the composition of the self-assembled film monolayers on the substrate on which anthranilic acid crystals were grown via sublimation.\(^{381}\) They found that when the substrate surface consisted of acceptor atoms for hydrogen bonds such as hydroxyl-groups from trimethoxysilane, competitive binding of the substrate acceptors with \( \text{NH}_2 \) donor groups of anthranilic acid in the early stage of nucleation would interfere with the intermolecular N-H\( \cdots \text{O} \) hydrogen bonding in the nuclei of form II, suppressing its formation. Nucleation of form III was enhanced by the hydrogen bonding of the substrate to free \( \text{NH}_2 \) donor groups; lowering the surface energy needed for its nucleation.\(^{381}\) Perhaps the negative ions\( \text{DNEC} \) directed selectivity towards the form III polymorph in a similar fashion to the monolayers of Carter \textit{et al.}; this would also explain why the positive ions\( \text{DNEC} \) did not show enhancement of form III since they would be less likely to hydrogen bond with free \( \text{NH}_2 \) donor groups of anthranilic acid. This possible directed selectivity by negative ions\( \text{DNEC} \) at the drop surface was also reminiscent of the selective crystallization of calcium carbonate polymorphs using steric acid monolayers at the solution air-liquid interface as discussed by Rapaport \textit{et al.}\(^{384}\)

Polymorph selectivity as caused by the presence of \( E_{\text{ext}} \) is typically a result of polar molecules being induced by \( E_{\text{ext}} \) to orient in large pre-existing solute clusters; helping them organize into a crystalline structure.\(^{10}\) The neutral anthranilic acid molecule does possess a weak dipole (\( \mu = 1.17 \text{ D} \)), as does \( n \)-BuOH (\( \mu = 1.68 \text{ D} \)), and hence both solute and solvent would be oriented by a strong \( E_{\text{ext}} \).\(^{385}\) Perhaps this orientation of anthranilic acid and \( n \)-BuOH molecules by \( E_{\text{ext}} \) affects the hydrogen bonding networks formed of anthranilic acid; resulting either enhanced formation of form III over form II (or suppressed formation of form II over form III).
It is unclear as to why the polymorphic ratio results observed for trial sets conducted in \( n\)-PeOH were not similar to those conducted in \( n\)-BuOH. The \( SCD_{\text{max}} \) values for both positively and negatively charged \( n\)-BuOH and \( n\)-PeOH drops were practically the same. Both solvents are \( n\)-aliphatic alcohols with similar dipole moments (\( \mu = 1.70 \) D, \( 1.68 \) D for \( n\)-PeOH and \( n\)-BuOH respectively) that differ in chain length by a single methylene group; hence it was originally thought that their interactions (i.e.: hydrogen bonding and London dispersion forces) with the solute would be similar. However, this proved not to be the case. Most investigators agree that soluble molecular species organize into a supramolecular assembly (i.e.: a crystal) dictated through nonbonding interactions mediated by the solvent.\(^{148}\) As varying nonbonding interactions mediated by the solvent play a significant role in stabilizing the structure adopted, the soluble molecular species (supramolecular synthons such as \( \text{anti-} \) and \( \text{syn-} \) dimers) formed under specific conditions dictate their assembly and hence the crystal packing. Symmetry is also an important structural director in many systems.\(^{148}\) Perhaps the increase chain length affected the solute-solvent hydrogen bonding networks formed in anthranilic acid-\( n\)-PeOH to such a degree that they became the dominating factor over both negative drop surface potential and the presence \( E_{\text{ext}} \) with respect to the polymorphic fraction observed; hence each trial set conducted in \( n\)-PeOH showed a similar result whereby form III was present at \( \sim 2\% \).

Another difference observed between the two solvent systems was that the \( C_f \) values for trial sets conducted in \( n\)-PeOH were quite all similar (\( C_f \sim 520 \) mg mL\(^{-1}\)), unlike those observed for \( n\)-BuOH which decreased when the drops carried negative surface potential and when the drop were in the presence of \( E_{\text{ext}} \). If we assume the solubility of anthranilic acid at room temperature is \( \sim 120 \) mg mL\(^{-1}\), then the range of \( S \) values would be between 4.1 - 4.5 and \( \Delta S_{\text{max}} = 0.4; \) a value significantly less than the \( \Delta S \) in values observed in the two \( n\)-BuOH trial sets whereby form III was enhanced. It should be noted that drop supersaturation was not a controlled variable in the conducted experiments. However, the results appear to suggest that potential effects on polymorphic ratio by drop surface charge and of \( E_{\text{ext}} \) are very dependent on drop supersaturation as theory suggests and that further experimentation employing more varied \( S \) values is warranted.

It should be noted that sometimes the appearance of a more stable polymorph can change the environment in which the compound is found resulting in a displacement of
the metastable form. Unintentional crystal seeding with a very stable form is often cited as an explanation for a disappearing polymorph even though such seeds have never been actually observed. This disappearing polymorph phenomenon does occur and has been well documented in some cases.\textsuperscript{386,387} On the other hand, unintentional seeding can occur even when small amounts of the unwanted polymorph are present as contaminants; as the following example illustrates. A critical nucleus can be comprised of a few tens to a few millions of molecules. If a particle size of $10^{-6}$ g for the compound is assumed (which is close to the limit of its visible detection), then approximately $10^{10}$ nuclei could potentially be made for a compound having a molecular weight of 100 g mol\(^{-1}\) (assuming the critical nucleus is comprised of a $10^6$ molecules). In other words, once a polymorph is formed, the presence of such seed crystals in the local environment is often unavoidable.\textsuperscript{329}

Furthermore, small particles of foreign material can also act as nucleation agents in the promotion of nucleation, albeit heterogeneously, and thus can play a significant role in polymorph formation in the crystallization process. Hence control over crystallization is rarely a sure-fire procedure, especially when one is attempting to produce a particular polymorph; it still can have the nature of an art.\textsuperscript{329} This being stated, a sceptic could argue that contamination of the system by a form III seed crystal caused the appearance of form III in the \textit{n}-PeOH drop trials (all four conditions) and in the \textit{n}-BuOH trials (conditions of negative surface potential and the presence of $E_{ext}$). Such a seed crystal could have formed in the solution vial(s), in the syringe assembly, on the needle tip at the moment of drop injection, or even have formed on the drop surface by an undetected electrical discharge from the reflector at high potential. If a seed crystal causing heterogeneous nucleation can be so small as to be virtually undetectable, then this claim is difficult to refute. However, since the individual experiments were conducted in a random order (i.e. trial 1: $E_{ext} = 0$ V m\(^{-1}\), $Q = 0$ C; trial 2: $E_{ext} = 0$ V m\(^{-1}\), $Q = +ve$ C; trial 3: $E_{ext} = 0$ V m\(^{-1}\), $Q = -ve$ C; trial 4: $E_{ext} = 6.0 \times 10^5$ V m\(^{-1}\), $Q = 0$ C etc.) such seeding does appear unlikely.

5.4 CONCLUSIONS

The effect of both drop surface-potential and the presence of an external electric field on the crystallization of the concomitant dimorphic \textit{m}-nitrophenol and the trimorphic
anthranilic acid systems with respect to polymorphic fraction were investigated for acoustically levitated solution drops.

For solution drops containing $m$-nitrophenol, no change to the polymorphic ratio was observed under both conditions of drop surface potential ($SCD_{\text{max}} = (1.91 \pm 0.14) \times 10^{-4} \text{ e nm}^2$) and the presence of $E_{\text{ext}}$ when MIBK, AcOH, $n$-BuOH, and $n$-PeOH were used as solvents. In all trials, only the monoclinic polymorph was formed; as determined using Rietveld analysis on XRPD data. Since crystallization of the solute took place in terms of minutes for all the solvents employed, it was likely that kinetic factors remained dominant over any drop surface charge or $E_{\text{ext}}$ effect resulting in the metastable monoclinic phase being preferred over the thermodynamically stable orthorhombic phase. The particles formed from the acoustically levitated $m$-nitrophenol drops under the conditions examined were of the solid spheroid type; comprised of numerous small, yellow crystals with a block-type habit. The vast number of crystals formed supported the notion that $m$-nitrophenol crystallization took place under a kinetic regime.

The particles formed from the acoustically levitated anthranilic acid drops under the conditions examined were of the solid spheroid type; comprised of numerous small, pale-coloured crystals having a platelet habit. The vast number of crystals formed was indicative that anthranilic acid crystallization took place under a kinetic regime.

Originally, it was speculated that crystallization of form I would be enhanced in the presence of $E_{\text{ext}}$ since such a field would align anthranilic acid zwitterions promoting the nucleation of this polar polymorph. However, form I was never observed in the trial sets; likely due to the unfavourable conditions of anthranilic acid zwitterion formation in the solvents employed. For solution drops containing anthranilic acid in DMF, no change to the polymorphic ratio was observed in the presence of either drop surface potential or $E_{\text{ext}}$. The metastable form II was the sole polymorph crystallized in each trial set conducted in DMF; as determined using Rietveld analysis on XRPD data. For each trial set conducted in $n$-PeOH, the polymorphic ratio of forms I : II : III was observed to be approximately 0 : 98 : 2 whereby no change to the polymorphic ratio was observed in the presence of either drop surface potential or $E_{\text{ext}}$. However, when trials were conducted in $n$-BuOH under conditions of negative drop surface potential, the polymorphic ratio changed from approximately 0 : 100 : 0 to 0 : 91 : 9 (form I : form II : form III). Only
form II was detected from $n$-BuOH solution drops carrying positive drop surface charge. A more dramatic shift in anthranilic acid polymorphic ratio to approximately $0 : 68 : 32$ (form I : form II : form III) was observed for $n$-BuOH solution drops in the presence of $E_{ext}$. It appeared as though the observed enhancement of form III was correlated to the decreases observed in drop supersaturation; however further experimentation employing more varied $S$ values would be necessary for conformation. With regards to the literature, this was the first incidence that form III had been formed from solution at room temperature.
CHAPTER 6
CONCLUSIONS AND FUTURE DIRECTIONS

Control over the crystallization process allows manufacturers to obtain products with desired and reproducible properties; most importantly size, purity, morphology, and crystal structure.\textsuperscript{4,324,327,329,388} For example, the pharmaceutical industry requires production of a particular crystal form (polymorph) to ensure its necessary bioavailability.\textsuperscript{4,324,327,329,388} In solution crystallization, nucleation plays an important role in determining crystal structure and size distribution; hence understanding nucleation is vital to achieve control over these properties. This thesis is concerned with the investigation of droplet surface potential (i.e.: droplet net charge) on the nucleation of a solute from a levitated non-electrically neutral droplet; there being virtually no such investigations in the current literature. As techniques such as electrospray deposition are becoming more popular for both micro and nano particle production and thin film production, such investigations are warranted. Based upon initial evidence, it is proposed that droplet surface potential could be used as a tool to facilitate controlled nucleation of a solute with respect to the nucleation rate, the resultant crystal size and habit (or shape), and the particular crystal structure adopted by the nucleus.

6.1 CONCLUSIONS

Initial investigations involved the electrodynamic droplet levitation of individual quiescent droplets. For solution droplets carrying a high surface potential (SCD $\sim \pm 3.73 \times 10^{-4} \, e \cdot \text{nm}^2$), a significant promotion in solute nucleation, as determined by the number of crystals observed per droplet, for sodium chloride and ammonium nitrate solution droplets, was observed relative to solution droplets of the same size and composition but carrying a lower surface potential (SCD $\sim \pm 1.68 \times 10^{-4} \, e \cdot \text{nm}^2$). As the number of crystals per droplet increased for droplets carrying a high surface potential, the size of the crystals observed were significantly smaller than those observed in droplets carrying a lower surface potential.
A change in NaCl crystal habit from regular cube-like to dome-shaped and dendritic was observed for a population of levitated solution droplets once a SCD threshold of ~ 9 \times 10^{-4} \text{e} \cdot \text{nm}^{-2} was surpassed. This SCD threshold value was independent of droplet size (d (\mu m): 10 – 16) but was slightly dependent on droplet viscosity whereby decreased droplet viscosity resulted in a slight decrease to the SCD threshold value. Changing the SCD polarity also resulted in a slight decrease to the threshold value necessary to form dendritic NaCl. It appeared that NaCl nucleation above the SCD threshold took place at the droplet-air interface since the dendritic crystal growth follows the contours of the droplet. Elucidation of where solute nucleation occurs (either at the droplet surface or within its bulk) for droplet populations below the SCD threshold and for single droplet experiments has not been determined. The change in crystal habit was not observed for single droplet experiments; however the SCD values employed for these experiments never surpassed the SCD threshold observed in the droplet population experiments.

Dendritic crystal growth from the droplet surface was also observed for 2,4,6-trihydroxyacetophenone monohydrate (THAP) droplet populations upon reaching a SCD threshold but was unobserved for levitated charged droplets below it. Since the THAP crystals formed in the trials were numerous and needle shaped, a comparison of nucleation rate as determined via counting the number of crystals formed in each droplet was untenable. Although dendritic crystal growth was not observed in levitated populations of \(\alpha\)-cyano-4-hydroxycinnamic acid solution droplets, an increased nucleation rate was rate observed as indicated by the increased number of crystals and their accompanying smaller sizes. These results are of direct relevance to processes that occur in soft ionization techniques for mass spectrometry as both THAP and CHCA are MALDI-MS matrix compounds. Decreasing crystal size increases crystal homogeneity and improves the mass accuracy, resolution, and reproducibility, as well as decreasing the amount of analyte required, for an analysis by MALDI-MS.\(^{38,205}\)

Above a SCD threshold, ammonium nitrate effloresced from droplet populations kept at high humidity values while droplet populations having low SCD values showed no efflorescence. The ammonium nitrate particles formed were to be used in in vitro toxicology studies of ambient particle types.
It is important to note that an increase in nucleation rate due to an increase to the magnitude of the surface potential was also observed with levitated droplet populations. Although promotion of nucleation was observed in these experiments, the increased rate of solvent evaporation observed for droplets with high surface potential due to their increased mobility in the EDLT made differentiation between the effects of increased evaporation rate and increased droplet surface potential on solute nucleation impossible. Although both effects are thought to have contributed to the nucleation enhancement of solute from the electrodynamically levitated droplet populations, it thought that the evaporative effects dominate over surface potential effects in these experiments.

Despite the evidence that high values of droplet surface potential (∼ 0.36 of the critical $Q$ that result in Coulomb explosion) promotes solute nucleation, the possibility remains that the external electric field ($E_{\text{ext}}$) generated by the EDLT was responsible for the aforementioned observations, as $E_{\text{ext}}$ is known to affect solute nucleation.\textsuperscript{28,29} In order to differentiate between droplet surface potential effects and those of an $E_{\text{ext}}$ on solute nucleation, a different levitation technique is necessary. Acoustic levitation is ideally suited for this as charged droplets can be levitated acoustically without the need of an external electric field. An additional benefit of acoustic levitation is that larger drops ($d = 40 – 4000 \ \mu m$) are levitable; solid samples formed from such drops are large enough to be analysed via X-ray powder diffraction and the effects of drop surface potential on the adopted crystal structure (i.e.: morphology) can easily be investigated. To this end, an acoustic levitation apparatus was designed and constructed in-house, based upon previous designs found in the literature.\textsuperscript{247,249,389} The capabilities of the assembled manual acoustic levitator were then characterized. The resonance frequency of the levitator was observed to be 45.64 kHz; above the range of human hearing for operator safety and comfort. Liquid samples as large as 2 mm in diameter were levitated; however, levitated drops of this size were distorted into oblate spheroids due to the effect of acoustic forces. Solution drops were introduced into the acoustic levitation cavity via syringe controlled by a syringe pump. A drop formed on the syringe needle placed above the levitation cavity was pulled into it by increasing the sound pressure level of the acoustic field until the surface tension of the drop-needle interface was overcome. Drops were charged by placing a potential on the needle during drop injection into the levitation cavity. Data
was successfully collected of the evolving drop diameter during levitation in order to
determine drop evaporation rates. Once a critical supersaturation was reached, nucleation
and subsequent crystal growth of a solution drop were observed to occur rapidly;
levitation stability was maintained during the phase change. Additional experiments
investigating the effects of an $E_{\text{ext}}$ on solute nucleation could now also be conducted
using the acoustic levitator since a potential could be placed on the levitator’s reflector.
Although the acoustic levitation apparatus constructed herein was an effective tool for
conducting experiments to probe the effect of drop surface potential (and $E_{\text{ext}}$) on solute
nucleation, a number of apparatus short-comings were observed after its extensive use.
They will be addressed in section 6.2.1.

A sodium chloride solution similar to the one used in the droplet experiments
conducted in the EDLT, was used for the initial investigations of drop surface potential
on nucleation conducted in the acoustic levitator. For the experiments conducted, the
evaporation of acoustically levitated drops followed the $d^2$-Law quite closely when drop
levitation was stable. The degree of deviation of the observed evaporation rate curves
was related to the degree of drop instability in the levitator; the greater the drop
instability, the greater the deviation from the $d^2$-Law. Trials showing a coefficient of
determination ($R^2$) < 0.990 in the calculated evaporation rate constant ($\beta_d$) (an indication
of significant drop destabilization) were excluded from data analysis as significant spatial
oscillations of the drop occur during destabilization that affect drop evaporation and
hence solute nucleation. Trials conducted in the presence of $E_{\text{ext}}$ showed a greater
propensity towards drop destabilization likely due to drop prolation by $E_{\text{ext}}$ in the acoustic
cavity. The experiment duration was not long enough to observe the onset of second
stage evaporation from the $d^2$ data plots. Drop surface charge and the presence of $E_{\text{ext}}$
had no significant effect on the evaporation rate constant; $\beta_d = (1.64 \pm 0.07) \times 10^{-3}$ mm$^2$ s$^{-1}$

It was observed that the presence of drop surface potential ($SCD_{\text{max}} \sim \pm 3.44 \times 10^{-4}$
e$\cdot$nm$^{-2}$) resulted in a significant promotion of sodium chloride nucleation from
acoustically levitated drops as determined by the increase in the number of crystals
observed in each drop relative to the number of crystals observed in the absence of drop
surface potential. The SCD values used were comparable to those of the experiments
conducted in the EDLT. According to the literature, drop surface potential has never been investigated as a parameter in a nucleation experiment from an acoustically levitated drop. Two candidates for the mechanism by which the excess ions that constitute drop surface potential enhance nucleation were proposed. The net excess ions located near the drop surface could have acted as heterogeneous nucleation sites for NaCl; surface enhanced nucleation is known to have a lower activation energy than nucleation occurring in the bulk. The second mechanism assumes that NaCl nucleation occurs at the drop surface and hence the increased electric field due to the net excess ions at the surface ($E \sim 2 \times 10^7$ V·m$^{-1}$) functioned as a solute nucleation promoter.

Promotion of NaCl nucleation also occurred in acoustically levitated drops in the presence of an external electric field ($E_{\text{ext}} = 6.0 \times 10^5$ V·m$^{-1}$), determined as previously described the drop surface potential experiments. Enhancement of NaCl nucleation in the presence of $E_{\text{ext}}$ occurred despite the fact that classical nucleation thermodynamics predicts its inhibition since the dielectric constant value of NaCl ($\varepsilon_r = 6.1$) is less than the dielectric constant value of the drop ($\varepsilon_d \sim 45$). Hence, it was likely that the presence of $E_{\text{ext}}$ affected the nucleation kinetics thereby enhancing of NaCl nucleation, as $E_{\text{ext}}$ is known to increase ion diffusion coefficients in solution which can be the rate limiting step regarding solute nucleation. Based upon these observations, it is possible that the enhanced NaCl nucleation observed in single droplets levitated in the EDLT occurred via a combination of both droplet surface potential and $E_{\text{ext}}$ effects.

The crystal habits of sodium chloride grown from acoustically levitated drops were regular with cubic shapes for all trials; a change in NaCl crystal habit was not observed for the SCD values or the electric field values employed. It could not be ascertained in these experiments whether or not nucleation took place at the air-liquid interface or within the bulk of the drop since the optics were unable to resolve the small crystal size.

As both drop surface potential and the presence of an $E_{\text{ext}}$ promoted NaCl nucleation in acoustically levitated solution drops, perhaps both nucleation parameters could be utilized to influence (or alter) the polymorphic ratio of a concomitant polymorphic system (i.e.: a compound that can nucleate and crystallize in more than one possible structure simultaneously under the same conditions). Regulating polymorphism is essential to technological sectors such as food, pharmaceuticals, explosives, and dyes, for
which regulatory compliance and functional performance depend on polymorph identity.\textsuperscript{4,318,324} As electrospray is becoming a more common technique to generate particles from solution, especially in the pharmaceutical industry whereby approximately 40 \% of active ingredients express polymorphism, an investigation to determine whether or not drop surface potential and/or $E_{\text{ext}}$ could be adopted as a technique for polymorph control was warranted.\textsuperscript{91,94} The effect of both drop surface-potential and the presence of an external electric field on the crystallization of the concomitant dimorphic $m$-nitrophenol and the trimorphic anthranilic acid systems, with respect to polymorphic fraction, were investigated for acoustically levitated solution drops. Both organic compounds were selected, not only because they are concomitant, but because they are both easily obtainable pharmaceutical precursors that exhibit one type polymorphism (packing) with polymorphs having similar energies of stability, and that both show molecular synthons that should be affected by both net excess ions and $E_{\text{ext}}$.

For solution drops containing $m$-nitrophenol, no change to the polymorphic ratio was observed under both conditions of drop surface potential ($\text{SCD}_{\text{max}} \sim \pm (1.6 - 1.9) \times 10^{-4}$ e nm\(^{-2}\)) and the presence of an external electric field ($E_{\text{ext}} = 6.0 \times 10^{5}$ V\cdot m\(^{-1}\)) when MIBK, AcOH, $n$-BuOH, and $n$-PeOH were used as solvents. In all trials (including control trials whereby solution drops were not levitated), only the monoclinic polymorph was formed; as determined from the Rietveld analysis of XRPD data. As the two structures only differ in the packing of the same 1-D molecular polar chains, it was thought that either (or both) the net excess ions or $E_{\text{ext}}$ would have had a stronger influence with respect to the crystallization of the orthorhombic structure whose 1-D chains are aligned parallel in the same direction. However, since crystallization of the solute was rapid in every solvent employed, it was likely that kinetic factors were dominant over any drop surface charge or $E_{\text{ext}}$ alignment effect which resulted in the metastable monoclinic phase being preferred over the thermodynamically stable orthorhombic phase. The particles formed from the acoustically levitated $m$-nitrophenol drops under the conditions examined were of the solid spheroid type; comprised of numerous small, yellow crystals with a block-type habit. The vast number of crystals formed supported the notion that $m$-nitrophenol crystallization took place under a kinetic regime.
The particles formed from the acoustically levitated anthranilic acid drops under the conditions examined were of the solid spheroid type and were comprised of numerous small, pale-coloured crystals having a needle/platelet habit. The vast number of crystals formed was indicative that anthranilic acid crystallization also took place under a kinetic regime. Original speculation assumed that crystallization of form I would be enhanced in the presence of $E_{\text{ext}}$ since such a field would align the dipolar anthranilic acid zwitterions promoting the nucleation of this polar polymorph. However, form I was never observed in the trial sets, likely due to the unfavourable conditions of anthranilic acid zwitterion formation in the three solvents employed. This effectively reduced the trimorphic anthranilic acid system to a dimorphic system consisting of forms II and III.

No change to the anthranilic acid polymorphic ratio was observed using DMF solution drops either for drops having surface potential or for drops in the presence of $E_{\text{ext}}$ as compared to acoustically levitated drops in the absence of drop surface potential and $E_{\text{ext}}$. The sole anthranilic acid polymorph that crystallized from DMF in each trial set was the metastable form II, as determined from the Rietveld analyses of XRPD data. For each trial set conducted in $n$-PeOH, the polymorphic ratio of forms I : II : III was observed to be approximately 0 : 98 : 2; no change to the polymorphic ratio was observed in the presence of either drop surface potential or $E_{\text{ext}}$. The form III observed marks the first time it has been grown from solution when $T < 50 \, ^\circ\text{C}$; albeit in very small amounts. It is generally believed that soluble molecular species organize into a supramolecular assembly dictated through nonbonding interactions mediated by the solvent. As both forms II and III have very similar stability energies, it is not surprising that a change in solvent could change how the supramolecular assembly of the anthranilic acid synthons was directed resulting in the appearance of form II since both the solvent’s polarity and hydrogen bonding capacities are different. It would appear that DMF is an exceptional form II director while $n$-PeOH is slightly less so.

Interestingly, for trials conducted in $n$-BuOH under conditions of negative drop surface potential, the anthranilic acid polymorphic ratio changed from approximately 0 : 100 : 0 to 0 : 91 : 9 (form I : form II : form III). For $n$-BuOH solution drops carrying positive drop surface charge, the polymorphic ratio remained unchanged (0 : 100 : 0) and only form II was observed. It was thought that nucleation of form III was enhanced by
hydrogen bonding of the negative ions $\text{DNEC}^{-}$ to free $\text{NH}_2$ donor groups; lowering the surface energy needed for its nucleation. Trials with positive ions $\text{DNEC}^{+}$ did not show enhancement of form III since they would be less likely to hydrogen bond with free $\text{NH}_2$ donor groups of anthranilic acid.

A more dramatic shift in anthranilic acid polymorphic ratio to approximately 0 : 68 : 32 was observed for $n$-BuOH solution drops in the presence of $E_{\text{ext}}$. It was proposed that the presence of $E_{\text{ext}}$ altered the arrangement of polar anthranilic acid synthons via nonbonding interactions so that nucleation of form III was enhanced significantly. It also appeared as though the observed enhancement of form III was correlated to the decreases observed in drop supersaturation ratio ($S$); however further experimentation employing more varied $S$ values would be necessary for conformation. With regards to the literature, this was the first incidence of form III being formed from solution at room temperature.

The initial investigations conducted in this thesis demonstrate that both droplet surface potential and the presence of $E_{\text{ext}}$ has potential to be used as a tool to facilitate controlled nucleation of a solute with respect to the nucleation rate, the resultant crystal size and habit (or shape), and the particular crystal structure adopted by the nucleus. Clearly, there is a wealth of knowledge regarding the chemistry that occurs in charged droplets as a result of their violation of electroneutrality. The effects of drop surface potential and $E_{\text{ext}}$ on solute nucleation certainly have several implications for laboratory, industrial, and natural processes involving media with net charge ranging from fundamental aspects of soft ionization for mass spectrometry to the preparation of nanophase materials with improved properties.

### 6.2 FUTURE DIRECTIONS

#### 6.2.1 Recommendations For Future Development Of Apparatus

Although the constructed acoustic levitation apparatus was an effective tool for manually conducting experiments to probe the effect of drop surface potential on solute nucleation, a number of apparatus inconveniences were observed after its extensive use as detailed in section 3.8. The addition of feedback systems to actively control the excitation resonance frequency and the separation distance between the reflector and
flexural plate of the transducer to compensate for thermal drifting would significantly improve levitation stability by removing manual adjustments from the operator’s domain and allow for experiments at different temperatures from the ambient (i.e.: temperature induced nucleation experiments).

Active control of the excitation resonance frequency could be achieved by monitoring the relative phase shift of the current signal that travels through the driver. At resonance, the relative phase shift is zero and becomes non-zero when the excitation frequency does not match the resonance frequency of the driver. Furthermore, the direction of the phase shift indicates whether the excitation frequency is too high or too low. A phased lock loop circuit can use the phase difference between the voltage applied to the driver and the current running through it to automatically adjust the frequency of the excitation signal to maintain resonance.

Active control of the separation distance could be achieved by using the electrical signal from the microphone placed inside the acoustic field. Separation distance between reflector and flexural plate of the transducer could be adjusted via a small electric motor that controls the reflector position. A potentiometer coupled to the motor would serve to monitor the reflector position. As the relative phase of the microphone signal shifts considerably in relation to the relative phase of the driver excitation signal when the flexural plate-reflector separation passes through a resonance spacing, the phase difference could be employed as a control signal for the electric motor. The polarity of the phase difference would determine whether or not the separation distance needs to be increased or decreased. This active control method using relative phase difference is advantageous over employing the magnitude of the microphone signal as the motor control signal because it avoids potential saturation of the microphone signal by the SPL.

In order for the acoustic levitation apparatus to be more amenable to additional experimentation such as temperature induced nucleation studies, a more robust and active temperature/humidity control system would be beneficial. A controlled evaporation mixer (CEM) seems ideally suited for this task. It is a liquid delivery system that can be employed in both atmospheric and vacuum processes and consists of a liquid flow controller, a mass flow controller for the carrier gas, and a temperature controlled mixing
and evaporation device. With such a commercially available CEM, the chamber temperature could be controlled from room temperature up to 80 °C with a % RH from a few ppm up 100 % depending upon the air flow rate.\textsuperscript{308,390} Alternatively, the entire levitation assembly could be placed in an environmental chamber capable of adjusting and maintaining a specific temperature and humidity. Commercial chambers allow for a greater temperature range ($T$ (°C) = -50 – 200) than a CEM.

A second camera with a far greater frame capture rate would be useful to collect additional drop diameter data in order to obtain more precise drop size evolution and to correctly determine whether solute nucleation specifically occurs in the bulk drop or at the drop surface with less (or without) speculation.

Several remote and non-invasive detection techniques are compatible with acoustic levitation and would be beneficial to study nucleation and crystallization from levitated solution drops. Right-angle light-scattering detection was applied by Santesson \textit{et al}. to the study of precipitation of proteins for crystallisation purposes.\textsuperscript{391} Biswas \textit{et al}. and described solidification of acoustically levitated crystals using Raman spectroscopy.\textsuperscript{392} Santesson \textit{et al}. have also used Raman spectroscopy for studies of crystallization processes in levitated drops. Their crystallization studies on the model compounds benzamide and indomethacin resulted in the detection of two new crystal modifications for each compound, suggesting that this methodology is useful for the investigation of polymorphs.\textsuperscript{312} Acoustically levitated drops of liquid and solid (powder) samples have also been kept in an X-ray beam for sufficient time to allow collection of the X-ray powder diffraction pattern.\textsuperscript{309,310,393} This would not only cut down the time required for XRPD data collection but would allow for in situ monitoring of crystal formation.\textsuperscript{391}

Equipment improvements notwithstanding, the acoustic levitator is a promising tool to investigate the evaporation behaviour for solvents and solutions, and solute particle formation from charged (or uncharged) drops. These results and the possibility to set almost every possible condition within a given temperature range make the levitator an interesting tool to perform preliminary tests for electrospray deposition research, especially if there is only little amount of substance available.
6.2.2 Potential Future Experiments

The number of possible solute-solvent systems that could be investigated with regards to nucleation and crystallization of acoustically levitated drops under conditions of drop surface charge and/or external electric field is virtually limitless. Hence, the following sections describe general avenues of investigation rather than individual experiments.

6.2.2.1 Temperature-Induced Crystallization Studies

Once a more robust means of apparatus temperature control has been established, solution supersaturation would also be achievable via reducing the drop temperature as opposed to evaporating the solvent. This would potentially allow for better control over the superstation ratio, assuming the solubility of the compound is known for a given final temperature. Loss of solvent due to evaporation could be nullified by the controlled addition of solvent to the levitated drop via a droplet dispenser as required; as determined by the monitored drop diameter values. Such a temperature-induced crystallization study would be useful to determine the effect of drop surface charge and $E_{ext}$ on the polymorphic fraction of anthranilic acid crystallized from $n$-BuOH under different regimes of supersaturation for example.

The supercooling and nucleation of water have aroused much research interest due to their importance in atmosphere science, biology, and environmental science.\textsuperscript{293} The nucleation of ice inside the water drops in a cloud relates closely to the radiative properties of ice particles and affects global climate. Although many theoretical and experimental studies on the nucleation of ice in supercooled water have been performed, the physical process of ice nucleation is still not well understood.\textsuperscript{293} Very few ice crystallization experiments have been performed under conditions of an external electric field and/or drop surface potential.\textsuperscript{225,293,394-396} Using an upgraded acoustic levitation apparatus, this line of investigation could be explored.

6.2.2.2 Conformational Polymorphism

The two polymorphic systems studied in chapter 5 were examples of packing polymorphism whereby the polymorphism existed as a result of differences in the crystal packing of the individual molecules. Polymorphism can also result from the existence of different conformers of the same molecule. In conformational polymorphism, flexible
molecules are able to fold into different shapes that can then pack into different 3-D structures. For example, at least four different crystalline forms of 1,3-bis(m-nitrophenyl) urea (MNPU), a classic molecular crystal system, are known to crystallize from solution in various concomitant combinations. Hiremath et al. demonstrated that three of the MNPU phases α, β, and γ can each be selectively nucleated and reproducibly grown on appropriately functionalized mercaptobiphenyl self-assembled monolayers (SAM) templates whereby control over nucleation is rationalized on the basis of favorable intermolecular and geometric interactions at the SAM/crystal interface. Perhaps the presence of drop surface charge or the presence of $E_{\text{ext}}$ would also affect the conformation adopted by MNPU molecules in a particular solvent and hence affect the form taken by the nucleus offering a degree of control over the phase obtained.

6.2.2.3 Protein Crystallization Studies

Proteins are intimately involved in dictating cellular function. While proteomics and bioinformatics can identify proteins differentially expressed in different cellular states, knowledge of a protein’s structure and function remains a fundamental necessity in the development of therapeutic compounds to inhibit the activity of a targeted protein. Deduction of a protein’s crystal structure can lead to detailed understanding of that protein’s function. For proteins whose structures remains unknown, growing single crystals is a great challenge as they are typically difficult to crystallize. As the presence of $E_{\text{ext}}$ has been shown to induce nucleation in some proteins such as lysozyme and thaumatin, perhaps protein crystal nucleation and growth can be promoted in drops through controlled variation of the drop SCD. The protein lysozyme would serve as an excellent starting point for this type of investigation as it is easily crystallized; it also happens to be polymorphic.

6.2.2.4 General Studies

The acoustic levitator could also be used to perform a number of different investigations involving drop surface charge (and or the presence of $E_{\text{ext}}$). For example, one could study how drop SCD affects: 1) the dissolution of gases into a liquid matrix; 2) the reaction kinetics of liquid-liquid and liquid-gas systems; 3) the heat and mass transfer between drops and their environment; 4) the combustion of single fuel drops.
The conduction of experiments to probe the nature of ions_{DNEC} and to control what species they consist of would greatly assist the design of controlled nucleation experiments at the air-drop interface akin to SAM controlled nucleation on a flat substrate.
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