Nanoprecipitation in Quartz Nanopipettes and Application in the Crystallization of Inorganic Salts

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NANOPRECIPITATION IN QUARTZ NANOPIPETTES AND APPLICATION IN THE CRYSTALLIZATION OF INORGANIC SALTS

by

WARREN DACOSTA HUGH BROWN

Under the Direction of Dr. Gangli Wang.

ABSTRACT

The high surface to volume ratio which is a property of nanoscale devices means the interfacial effects from these devices on the mass transport of analyte can be significant. Quartz nanopipette effect on the mass transport behavior of inorganic monovalent salts such as potassium chloride is shown to differ from those of conical nanopore. Quartz nanopipettes demonstrate a more significant interfacial impact on the mass transport behavior of inorganic salts. This is evidenced by significant impacts on ionic transport even at high electrolyte concentration where nanopore interfacial effects do not significantly impact the ion transport. Nanopipettes have been use to precipitate salts such as lithium chloride in bulk concentrations three orders of magnitude below the saturation concentration. These novel interfacial interactions have opened new avenues for crystallization of more complex organic biomolecules using inorganic systems as model systems on which to base the approach for these more complex systems.

INDEX WORDS: Nanoprecipitation, Crystallization, Ionic Transport, Mass Transport, Nanodevices, Nanopipette, Nanopores, Current rectification, Electrochemistry
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Warren Dacosta Hugh Brown

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CRYSTALLIZATION OF INORGANIC SALTS

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Office of Graduate Studies
College of Arts and Sciences
Georgia State University
August 2012
DEDICATION

I want to dedicate this work first and foremost to my mother who made all my achievements possible. Without her there is no way I could have done any of this. Her strength and the lessons she taught me while I was growing up have been invaluable. To my brother for his calming influence. To my sister who seems to love me no matter how mean I may be to her. To my niece and nephew for calling me on Sundays to cheer me up. To my father for teaching me invaluable lessons about life. To my grandparents for whom I can find no words to describe their value in my life.
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1. INTRODUCTION

1.1 Nanoscale devices and Applications

1.1.1 Type and composition of studied nanoscale device

Nanopipettes are investigated which are composed of quartz which is a fairly well characterized material. As is typical for nanoscale devices, nanopipettes are characterized by a high surface to volume ratio. The impact of a charged surface on ionic transport becomes more significant at the nanoscale and exhibits behavior which differs significantly from that observed for larger scale devices. Larger scale apertures tend to exhibit constant resistance and a plot of the current versus potential produces a linear curve. Therefore larger scale devices exhibit behavior which is consistent with Ohm’s law. The effect of the surface charge on ionic transport at the nanoscale is manifested by ionic current rectification. This is characterized by potentials of opposite polarity (and equivalent magnitude) exhibiting different ionic current response. Therefore nanoscale devices exhibit behavior which deviates from Ohm’s law. Nanoscale apertures of various dimensions and composition are described in the literature.\(^1\) Materials include glass (various types such as quartz, lead doped glass, carbon etc) and biological materials such as protein. The direct study of biological apertures is desirable due to the ubiquitous presence of these important transporters in the cell membrane. An example of a commonly used biological aperture is alpha hemolysin. Synthetic nanopores are typically used due to the instability of lipid membranes in which alpha hemolysin has to be contained for experimentation and the ease of manipulation of pore size in synthetic nanopores.
1.1.2 Nanoscale apertures in detection, quantitative analysis and elucidation of properties of analytes

Ease of fabrication, well characterized composition and the ease of functionalization make synthetic nanopores attractive for various quantitative and qualitative studies of analytes. Biological apertures have the advantage over synthetic apertures of being selective for a particular ion or molecule. Functionalization of synthetic nanopores overcomes this problem. Of particular interest has been the extraction of information on the properties of biological macromolecules with significant implications for biotechnology and medicine. The type of information accessible using synthetic nanopores from the study of these macromolecules gives insight into interactions between macromolecules and the environment. Nanopores have been used to distinguish between bare DNA and DNA bound by protein and also the position of the bound protein along the DNA sequence. The unfolding of bovine serum albumin (BSA) by chemical, thermal and electric field and the resulting forms of BSA caused by these external stimuli have been differentiated using nanopores. The adaptability of nanopores to various functionalization is illustrated by Au/Polycarbonate nanopores functionalized with an E. coli receptor protein which changes conformation upon binding of an analyte of interest. This change in conformation results in a change in the conductivity of the device resulting in the detection of the analyte. Nanopores functionalized with aptamers (oligonucleotides) have been used to detect immunoglobulin molecules and the terrorist agent Ricin. In the area of Alzheimer’s research nanopores have been used to study the aggregation of the protein beta-Amyloid peptide which is thought to play a key role in the occurrence of the disease. Alpha-hemolysin pores were used to study the effect of beta-cyclodextrin and congo red on AB42.
Nanopores offer advantages over conventional analytical techniques such as chromatography, assays and electrophoresis\textsuperscript{10}. Advantages such as miniaturization (Lab on chip), smaller samples, less sample preparation time and single molecule detection, are attractive attributes of nanoscale devices.\textsuperscript{10} Single molecule detection of enantiomers has been demonstrated using cyclodextrin in a nanopore.\textsuperscript{2} Label free detection of protein molecules has been demonstrated.\textsuperscript{4} The current fluctuations observed in this investigation has been correlated with the charge and size of the protein.\textsuperscript{4} Label free measurement of the protein concentration is also demonstrated.\textsuperscript{4} This study demonstrates the advantages of using nanoscale devices over conventional methods such as ELSA assays.

1.2 Importance of studying ionic transport in nanoscale devices

Ionic transport through nanoscale devices is not a trivial concern as is demonstrated by the observation of interesting effects such as ion current rectification. The information extracted from the systems discussed above using nanoscale devices is limited by the ionic current which is the background signal that is measured when the analyte of interest is not interacting with the opening of the aperture. Therefore the investigation of the mechanisms of ionic transport through nanodevices is important if those applications are to have any practical relevance. Feng et al\textsuperscript{11} have resolved the geometric contribution and the surface contribution to ion current rectification. Using AC impedance techniques a distinction between the effects contributed by surface charge of amine modified lead glass conical nanopores and the asymmetric conical geometry has been demonstrated.

The study of ionic transport in nanoscale devices also has important applications in fields far removed from some of the more familiar analytical chemistry applications. Simulation stud-
ies have demonstrated that nanoscale apertures can behave as memcapacitors. A memcapacitor is part of a larger family of elements which are analogous to the conventional circuit elements studied in physics with difference being memelements display a ‘memory’\textsuperscript{13}. This effect is observed upon application of a periodic input such as a sinusoidal potential. It is manifested by the appearance of a pinched hysteresis in a plot of two constitutive variables such as current and potential, charge and potential, and flux and current. In the case of the simulation study the physical origin of this effect was due to the slow polarizability of the ions in water at higher frequency of the applied voltage and due to the transport of ions at lower frequencies. A physical description of memory effects can more clearly demonstrate this memory effect. At a particular potential and time the arrangements of ions in solution will be determined by this the externally applied potential. Since the applied potential is sinusoidal in form the slow polarizability of the ions in solution will cause the previous state of the system (the capacitance defined at a particular potential and time) to define the output (in this case charge) of the system at different potentials. The study of memelements has applications in low power computing, learning and DNA translocation in nanopores.

1.3 Crystallization

Voltage controlled nanoprecipitation in conical nanopores has been demonstrated using slightly soluble inorganic salts. The authors demonstrate a threshold concentration for precipitate formation by addition of divalent cation (CaCl\textsubscript{2}) or buffer (Phosphate buffer)\textsuperscript{14}. There is also a threshold potential for precipitate formation. It was found that the divalent inorganic salts used in this experiment precipitated only at negative potentials (positive potentials for this paper). Constant potential measurements monitoring the current response with time illustrated oscillating
ionic current consistent with the formation and dissolution of precipitate. These findings illustrate a potential new methodology for crystallization. Crystallization of inorganic salts along with providing insight into the mechanisms of crystallization also offer a framework on which more complex systems such as protein crystallization can be modeled. Current crystallization methods involve the mixing of protein with precipitant with the correct composition that is suitable for nucleation. The process of nucleation produces the crystal seeds which promote growth of the crystals. This growth occurs in a metastable state where the solution concentration is below saturation, preventing nucleation, but still promotes crystal growth. A methodology can be developed which is based on potential controlled growth of crystals. It has been demonstrated that nanoscale devices enhance the concentration relative to bulk concentrations at high conductivity states. Precipitate formation occurs as a result of rapid attachment of the constituent ions resulting in a disordered formation. The identification potentials at which (1) nucleation occurs and (2) rates of crystal growth which are suitable for crystal formation under metastable conditions would be the goal of any potential controlled crystallization methodology. This may be difficult as these conditions may depend on temperature, pH, surface charge density of the selected nanopore, radius of the pore and other possible considerations.
2. IONIC TRANSPORT IN QUARTZ NANOPIPETTES

2.1 Introduction

The background signal when analyte is not interacting with the smallest aperture in a nanoscale device is a result of ionic transport through the device. The information that can be gained about the properties of analytes and for detection of analytes of interest is limited by this background signal. Resolving the fundamentals of mass transport in nanoscale devices is therefore important for enhanced performance of these devices. Ionic current rectification is a phenomena which is observed in nanoscale devices characterized by a surface charge and asymmetric geometry. Ionic current rectification is defined as different current magnitudes at equivalent potential magnitudes of opposite polarity. Feng et al\textsuperscript{11} have studied amine modified conical nanopores in various concentrations of KCl. The relative contribution of surface effects and geometric effects are investigated and are correlated with the ion current rectification behavior in different solution concentrations of KCl. It was shown that rectification diminishes (approaches ideal Ohm’s law behavior) as the concentration increases. AC impedance techniques were used to probe the relative contribution of surface effects and geometric effects. AC impedance techniques (described in experimental section) use a periodic potential input and the impedance response of the system can be differentiated into a real component and an imaginary component. A plot of imaginary impedance versus real impedance is called a Nyquist plot. The experiments using AC impedance were carried out at different potentials which define the amplitude of the periodic potential input. At a particular concentration two potentials at positive bias were chosen and two at negative bias with equivalent magnitude along with zero potential. In 1M KCl all spectra overlapped for the Nyquist plot. At concentrations 100mM and less some interesting features are observed. At negative potentials a second hemicircle is observed in the first quad-
rant at the low frequency end and at positive potentials an inductive loop is observed in the fourth quadrant at the low frequency end of the Nyquist plot. Considering that in 1M KCl all spectra overlapped this was taken as the geometric limit where the ion transport behavior at 1M concentration is dominated by geometric effects. This is because in 1M KCl the electrostatic interactions between the ions effectively screen them from interaction with the double layer. For lower concentrations the extra loops observed at the low frequency end are indicative of the role of surface charge. At these concentrations the double layer may extend significantly into the pore area.

Quartz nanopipettes are also investigated to determine the relative contributions of surface effects and geometric effects. Quartz is particularly attractive for its use in both detection technologies and studies of the properties of biomolecules due to its inertness and the ease of functionalization. The same experimental techniques are used in the investigation of quartz nanopipettes.

2.2 Experimental Section

2.2.1 Materials

Silver wire (99.95%, 0.203 mm diameter) from Alfa Aesar, Quartz glass capillaries (OD 1.0 mm, ID 0.70 mm, Sutter Instrument), KCl from JT Baker ,LiCl, and FeCl$_3$ from Sigma Alrich. Nanopure water (18.2 Ω·cm) was obtained from a Barnstead Epure- water purification system.

2.2.2 Nanopipette Fabrication

Nanopipette Fabrication Quartz glass capillaries were pulled into nanopipettes using a P-2000 Micropipette Puller (Sutter Instrument). Pulling parameters were based on the procedure used by Karhanek et. al.$^{17}$ A representative list include: Heat at 700; FIL at 4; VEL at 60; DEL at 150;
and PUL ranging from 155-175. Adjustment of the parameter PUL resulted in pores with larger/smaller current response, a direct measure to characterize the size of the nanopipettes widely employed in literature.

2.2.3 Electrochemical Methods and Description of Methods

Cyclic Voltammetry and impedance spectroscopy measurements were carried out using Reference 600 (Gamry). Typical impedance spectra were measured at the frequency range between 10 mHz and 1 MHz and the amplitude of the AC waveform at 10 mV (rms). Ag/AgCl wires were used as electrodes. The nanopipette was filled with the same solution as that of the bulk solution. The reference/counter was connected to the electrode inside the nanopipette while the working electrode was placed into the bulk solution. The polarity of the potential was therefore defined as outside versus inside. **Scheme 2.1** below is a representation of the experimental setup.

**Scheme 2.1.** Illustrates the experimental set up. Solution is both inside and outside the nanopipette and is depicted in blue. The red line represents the reference electrode located inside the nanopipette and the green line represents the outside electrode to which the applied potential is adjusted.
An electronic device called a potentiostat is used to adjust the applied potential between the two electrodes.

**Scheme 2.2** illustrates the setup the applied waveform for a Cyclic Voltammogram (CV).

![Cyclic Voltammetry](image)

**Scheme 2.2.** Applied waveform in cyclic voltammetry.

The important parameters in cyclic voltammetry are the rate at which the potential is scanned (scan rate) and the applied potential amplitude ($V_{amp}$). The scan range is between $+V_{amp}$ and $-V_{amp}$. The potential range is not limited to negative and potential amplitude as depicted above.

In AC impedance the applied waveform is a sinusoidal waveform. For a closed circuit containing only a resistor the current response is in phase with the applied potential. For a capacitor in a closed circuit the voltage between the capacitor plates lags the current by 90°. For an inductor in a closed circuit the voltage across the inductor leads the current by 90°. **Scheme 2.3** illustrates the complex impedance $Z$ as a vector. In a circuit consisting of phase sensitive ele-
ments such as capacitors or inductors the impedance consists entirely of an imaginary component and depends on the frequency of the applied potential (-90° capacitor and +90° inductor phase shift). In a circuit containing a resistor the impedance consists entirely of a real component and is independent of the frequency of the applied potential. In a circuit consisting of a resistor and phase sensitive elements such as capacitors and/or inductors the complex impedance will have a real and imaginary component along with a phase shift (θ).

Scheme 2.3. The complex impedance of a circuit depicted with a real component (Z real) and an imaginary component (Z img). Nyquist plot, of real versus imaginary component, depicted with capacitive behavior in the first quadrant and inductive behavior in the fourth quadrant. Frequency decreases from left to right.

The Nyquist plot illustrates the imaginary component versus the real component of the impedance. Points in the first quadrant represent a combination of capacitive and resistive behavior while points in the fourth quadrant represent a combination of inductive and resistive behavior. The frequency dependence is depicted by the moving from higher frequency at the left to lower frequency at the right.
2.3 Impact of electrolyte concentration on ionic transport

Figure 2.1 shows the normalized CV curves in different KCl solutions measured with a ca. 28-nm-radius nanopipette. The electrolyte concentration was normalized to illustrate the respective contributions of current at positive and negative bias potentials to the overall rectification. The 10mM curve shows significantly higher normalized current at positive potentials, suggesting enhanced conductivity upon the dilution of electrolytes. The trend is less drastic at negative potentials. The negatively charged glass surface at the mass transport limiting opening attracts the cations and repels the anions in solution. At positive potentials, cations will be driven into the pore from the bulk solution while the anions are forced out. This process is facilitated by the negative surface charges. Consequently, high conductivity states are established at positive bias potentials (outside vs inside)\textsuperscript{18}.

![](image)

**Figure 2.1** Normalized conductivity in KCl solutions measured by cyclic voltammetry. The voltammogram at concentrations of 10mM, 100mM and 1M was multiplied by the factor of 100, 10, and 1 respectively. The scan rate was 20mV/s. The radius of the quartz glass nanopipette is ca. 28 nm, calculated using the current data in 1 M KCl at +/- 50 mV.
Due to the asymmetric geometry of entering versus exiting the nanopipette tip from outside, cations function as main charge carrier and dominate the ionic current response of the system. An opposite effect is expected at negative potentials. The flux of both cations and anions in solution driven by the external potential is resisted by the negative surface potential. In other words, the exit of cations is hindered by electrostatic interactions with surface charges. The entrance of anions is geometrically favored due to the asymmetric geometry but limited by the negative surface charges. Low conductivity states are established. It is not clear at this point which type of ions is the main charge carrier as the two effects cancel each other \(^{19}\). It has been demonstrated that cations are the majority charge carriers at high conductivity states at concentrations less than 100 mM KCl in nanopores.\(^{20}\) As the concentration of the solution electrolytes increases, electrostatic interactions with fixed surface charges are more effectively screened and the contributions from both cations and anions at approach parity at both high conductivity and low conductivity.\(^{20}\) Consequently the rectification effect is attenuated and the conductivity curves approach linearity.

While the qualitative phenomenon is consistent with those reported in the literature, it is interesting to discover the differences at positive and negative potentials. The relative consistent normalized conductivity at negative potentials at different electrolyte concentration indicates that the overall current rectification effect is mainly originated from the high conductivity states at positive bias potentials. The apparent hysteresis and higher noise in 10 mM curve were resulted from the normalization factor (100) instead of ionic transport processes during the measurements.
Figure 2.2 CV response of a nanopipette in 10mM, 100 mM and 1 M KCl. Normalized conductivity in KCl solutions measured by cyclic voltammetry. The voltammogram at concentrations of 10mM, 100mM and 1M was multiplied by the factor of 100, 10, and 1 respectively. The scan rate was 100mV/s. The radius of this nanopipette was 8 nm.

Figure 2.2 illustrates normalized curves for a second nanopipette of radius 8 nm in the same concentrations as the 28 nm nanopipette. Interestingly the 1 M curve illustrates current rectification. This was not the case for the nanopipette estimated at 28 nm which gave a linear cyclic voltammogram curve in 1 M KCl. Similar to the 28 nm nanopipette the extent of rectification approaches is seen to decrease as the concentration increases. Due to higher surface charge and/or smaller radius the double layer extends significantly even in 1 M KCl. At that concentration there are not enough ions to effectively screen the surface charge. The trend of the normalized current at both high and low conductivity states are different from the 28 nm nanopipette. The normalized current increases at both high and low conductivity. The rectification of the ion
current in 1 M KCl indicates that this nanopipette has a smaller radius and/or higher surface charge than the previously described nanopipette.

Figure 2.3 illustrates the CV curve from Figure 2.2 in 10 mM KCl. The arrows indicate the direction of the applied potentials. Arrows indicating that the direction of scan is towards positive potentials are defined as forward scans. Arrows indicating that the scan direction is towards negative potentials are defined as backward scans. The letter labeling indicates the current values recorded within a certain potential range and a certain scan direction. A indicates current values between 0.1 V to -0.4 V forward scanning, B indicates current values 0.1 V to 0.4 V forward scanning, C indicates current values 0.1 V to 0.4 V backward scanning and D indicates current values 0 V to -0.4 V backward scanning. The letter labeling also corresponds to the calculation of the current ratio values in Figures 2.4 and 2.5. More will be said below. The potential at which forward and backward scans have the same current is defined as the cross potential or cross point. Current values of the forward scan at positive potential magnitudes, B, are found to be lower than those of the potential range C. At negative potentials the backward scan (D) has a higher current value than the forward scan at negative potentials (A). These observations have been reported previously and are due to the fact that the resistance and capacitance of nanoscale systems made of SiO₂ substrate are time dependent unlike classical resistors and capacitors.²¹ This time dependency arises because of the polarization of the double layer within the sensing zone of the nanopipette. This polarization is time dependent since there is a lag in the response of the double layer to a change in potential. This lag causes the nanopore or pipette to ‘remember’ the previous polarization state of the double layer.
Figure 2.3. CV response for a nanopipette in 10 mM KCl. The arrows indicate the direction of the applied potential. The letter labeling denotes current values selected from scans from a given potential window and scan direction used for calculation of the current ratio in Figures 2.4 and 2.5. A denotes current values recorded within a potential range of 0V to -0.4 V scanning towards positive potentials. For B a potential range of 0 V to 0.4 V scanning towards positive potentials, C 0 V to 0.4 V to away from positive potentials and D 0 V to -0.4 V scanning away from positive potentials.

Figure 2.4 illustrate current ratio plots versus the applied potential for CV measurements in 10 mM KCl and 100 mM KCl. The current ratio is calculated by taking the ratio of the current in 100 mM KCl to 10 mM KCl. Panel A corresponds to the ratio of the current values at forward scan from -0.1 V to -0.4 V. Panel B corresponds to the current ratio at forward scan from 0.1 V to 0.4 V. Panel C corresponds to the current ratio at backward scan from 0.1 V to 0.4 V and panel D corresponds to backward scan from -0.1 V to -0.4 V. A tenfold increase in concentration is
expected to generate a corresponding tenfold increase in current at equivalent potentials. This is not the case. The current ratio values in panels A, B, C and D do not surpass a value of 4.

**Figure 2.4.** Illustrates the current ratio of 100 mM KCl to 10 mM KCl. Panel A corresponds to the current ratio at forward scan at negative potentials. Panel B corresponds to the current ratio for forward scan at positive potentials. Panel C corresponds to current ratio for backward scan at positive potential. Panel D corresponds to current ratio for backward scan at negative potential.

**Figure 2.5** illustrates the concentration ratio versus potential for 100 mM KCl and 1 M KCl. Panel A illustrates the current ratio for forward scan at positive potentials. Panel B illustrates current ratio at forward scan at positive potentials. Panel C illustrates current ratio at backward scan at positive potential and panel D at backward scan at negative potentials. In contrast to the current ratio plots in **Figure 2.4** all current ratio plots in **Figure 2.5** have a value above 4. In panel A the ration decreases from approximately 7 at -0.1 V to around 6.6 at -0.4 V. In panel B the current ratio decreases from approximately 5.75 at 0.1 V to approximately 5 at 0.4 V. In panel C has the same current ratio dependence described in panel B and panel D has a current ratio value of
approximately 6.5 at most potentials. Therefore with respect to 100 mM KCl the value of the current ratio increases when comparing current measurements in 10 mM KCl to 1 M KCl. This is due to the increased screening of the surface charge as the concentration of the electrolyte increases. In the higher 1 M concentration the debye length, which indicates the length of the diffuse layer, would be smaller compared to the lower 10 mM concentration. Therefore the diffuse layer has more of an effect on the sensing region of the nanopipette at lower concentration than at higher concentration. Figures 2.4 and 2.5 illustrate that a tenfold increase in concentration does not lead to a corresponding increase in the current. This is observed in nanopipettes and it is not clear at present what causes this effect.

![Graphs of current ratio vs. potential for different concentrations and scan directions.](image)

**Figure 2.5.** Illustrates the current ratio of 1 M KCl to 100 mM KCl. Panel A corresponds to the current ratio at forward scan at negative potentials. Panel B corresponds to the current ratio for forward scan at positive potentials. Panel C corresponds to current ratio for backward scan at positive potential. Panel D corresponds to current ratio for backward scan at negative potential.
Corresponding impedance spectra are presented in Nyquist format in Figure 2.6 for the 28 nm nanopipette, which plots the imaginary components (with phase shift) against the resistive component (no phase shift) in the two dimensional impedance data. The spectra are directly correlated with the conductivity obtained for each concentration (normalized curves in Figure 2.1). In the spectra measured in 10 mM KCl solution, the impedance at + 0.4 V is lower relative to those at zero and - 0.4 V potentials, which is in agreement with the rectified DC conductivity (i/E, the reciprocal of resistance) shown in Figure 2.1. By increasing electrolyte concentration, the differences of the spectra at different bias potentials decrease. The spectra in 1M KCl solution at different DC potentials approximately overlap. The trend correlates with the increasing linearity or loss of rectification of the conductivity curves with increasing concentration. The DC current and AC impedance features illustrate significant impacts on ionic transport by surface effects. The electrostatic interactions between the ions and interfacial charges are screened increasingly as the concentration increases. There is no significant rectification at high concentration of electrolytes, which indicates that the contribution of surface effects to the mass transport behavior is negligible at 1M KCl concentration. The current is dominated by geometric effects, which allows the convenient characterization of nanopipette radius.
Figure 2.6 Impedance spectra in 10 mM, 100 mM and 1 M KCl solutions measured with the same 28-nm-radius nanopipette. The DC bias potentials of the spectra in each panel were held at +0.4V, 0V and -0.4V respectively. The frequency range and amplitude of the AC waveform were between 10 mHz and 1MHz and 10 mV rms.

In general, a hemicircle in the first quadrant in Nyquist plot is modeled as a resistor in parallel with a capacitor. The distortion of the hemicircles in the spectra in Figure 2.6 diminishes from low to high concentration. Note at the low frequency range the data are widely scattered that prevent quantitative fitting as previously reported. Qualitatively, the additional well-defined low frequency loops discovered in conical nanopores was not observed, though the rectification effects observed by CV are similar. The discrepancy suggests the impedance technique
being much powerful in resolving the finite geometric differences in the nanostructures employed. The high noise at low frequency ranges could not be simply assigned to instrumental or measurement factors, as comparable impedance magnitude can be accurately measured when conical nanopores were used. The low frequency response is postulated to be affected by the long stem next to the tip region of the pulled nanopipettes that could lead to different osmotic flow, which is under ongoing investigation. 22 23

2.4 Effect of Size

To confirm the observed ionic transport features and the proposed interpretations, similar investigation were performed using larger nanopipettes. The results from a ca. 500-nm-radius pipette in 10 mM LiCl are presented in Figure 2.5. The voltammogram display a linear conductivity response in the concentration range from 1M down to 1 mM. The impedance spectrum at different DC bias potentials overlaps over wide frequency ranges. As the nanopipette radius is significantly larger, the impacts by the immobilized charges on the substrate-solution interface are attenuated. The linear voltammogram and overlapped impedance spectra support the proposed interpretation, as electrostatic interaction is inversely dependent on the distance based on Coulomb’s law. At low frequency range (less than ca. 1 Hz), a slight resistive change was observed, especially at negative potential. The features are more apparent in 1 mM but the noise level is generally higher. The weak low frequency deviations are attributed to the surface charge effects that are not as evident at higher frequencies, indicating that at lower frequencies surface charge effects are more detectable.
Figure 2.7 Impedance and CV results from a ca. 500 nm radius nanopipette measured in 10 mM LiCl. The DC bias potentials of the spectra were held at +0.4V, 0V and -0.4V respectively. The frequency range and amplitude of the AC waveform were between 10 mHz and 1MHz and 10 mV rms. The scan rate in CV measurement was at 20mV/s.

2.5 Ion current transport behavior of monovalent chloride salts

As the bias potential is applied between two macroscopic Ag/AgCl wires, Cl⁻ anion is necessary for the electron transfer process at the electrode surface and to maintain the potential drop at the nanopipette orifice region. Cations of different mobility are therefore the candidates to investigate the respective contributions of cations and anions to the overall current rectification effect. To further test the hypothesis that cations are the main charge carrier of the current rectification of negatively charged nanopipettes, and that the contribution is more significant at positive over negative bias potentials (outside vs. inside), the CV results in LiCl and KCl at the same concentration were presented in Figure 2.5. Excitingly, the two curves display drastic differences in the positive potential ranges. The ionic mobility of K⁺ and Li⁺ is 7.619 X 10⁻⁴ and 4.010 X 10⁻⁴ respectively, which reasonably reflect the current differences observed in the positive bias potential range. The conductivity at negative potentials is almost identical, suggesting
that differences in cation mobility are negligible to the overall current measured. This can be explained by the opposing surface charge effects and asymmetric geometric impacts described earlier. Again, the direction of the ionic transport driven by the surface potential is against that of applied negative potentials, which establishes the low conductivity states due to the fixed negative charges at the substrate-solution interface. As a consequence, the ion flux experiences the leveling effects (minimized number of mobile ions as charge carriers) under those conditions. The differences in individual ion mobility could only be directly related to the ion flux when the concentration is comparable. The argument is supported by our current simulation studies using Comsol Multiphysics that will be reported separately.

Figure 2.8 Comparison of cation effect on the CVs from a ca. 28 nm radius nanopipette. The data were measured in 100 mM KCl and LiCl solution. The scan rate was at 100mV/s.

2.6 Time dependent surface charge and the effects on ion current

To quantify the ionic transport features affected by the fixed charges at the substrate-solution interface, the amount of effective interface charges, or surface potential profiles, is required. Unfortunately it is a challenge to quantify the surface charge density at substrate-solution
interface. SiO$_2$ based materials are known to hydrate and form a gel layer in aqueous environments$^{24, 25}$. Furthermore, the surface charge density depends on the measurement condition, which is attested by the wide scope of values reported in the literatures ranging from a few mC/m$^2$ up to tens even hundreds. The transition of current rectification from the pulled nanopipettes was studied. Since the fabrication of the nanopipettes employs a heating-pulling procedure that does not involve aqueous environments, the pre-existing hydration is believed negligible in the results presented in Figure 2.6.

![Graph](image)

**Figure 2.9.** The scan rate in CV measurement was at 20mV/s.

The nanopipette used was soaked in aqueous solution continuously during the measurements. The ionic transport current is found to increase gradually over time at positive bias potentials. At negative potentials, the current decreases to a threshold low value. The observation is actually consistent with those results presented in Figure 2.1 and Figure 2.8, and can be explained with the same rationale qualitatively. The finding is significant considering the wide applications of the nanopipettes in sensing and delivery, as the efficacy would change during the continuous usage in aqueous environments. An independent measure of surface charge density is required to
further quantify the features observed. This is being pursued by the simulation using classic Poisson and Nernst-Planck equations.

In summary, time domain voltammetry and frequency domain impedance techniques are employed to characterize the ionic transport behaviors at single quartz nanopipettes. The well known current rectification in DC conductivity is correlated with the two dimensional impedance spectra for method validation. The phenomena are interpreted with the geometric and fixed charges at the quartz substrate-solution interface.

2.7 Effect of varying pH on ionic current

The previous section indicated that surface charge plays a significant role in the origin of the rectification effect. The origin of surface charge density at the smaller opening of the nanopipette arises due to deprotonation of silanol groups on the glass surface. The pKa of the silanol groups is approximately around pH 4 but this value is subject to variation due to the effect of surface curvature on the pKa value of the silanol groups. Figure 2.10 indicates cyclic voltammograms taken in 100mM KCl at different pH values. The current at positive potentials are affected more than those at negative potentials and indicates that the extent of the rectification diminishes as the pH increases. A threshold conductance is observed at negative potentials. Clearly the system conductivity decreases as the pH decreases at positive potentials. The silanol groups at lower pH will be protonated and will not be able to attract potassium ions near the surface as much as higher pH values where there is more deprotonation. This decrease in cation concentration accounts for the decrease in system conductivity. The conductivity at negative potentials are not affected by the change in pH at negative potentials. This indicates that geometric effects may be more significant at negative potentials.
2.8 Conclusion

The rectification in quartz nanopipettes is affected by both geometric and surface charge effects. The system conductivity in a given concentration is higher at positive potentials compared to negative potentials. This effect was explained by the higher concentration of cations due to the negative surface charge and the asymmetry of entry and exit in the nanopipette. With these observations the discernment of surface charge and geometric effects was carried out with experiments which primarily tested how much of a role surface charge plays at positive and negative potentials. The results indicated that surface charge plays a more significant role at positive potentials while at negative potentials geometric effects are more significant. The hydration effect illustrates that surface charge of nanopipettes increase over time and this is significant if nanopipettes are used in any sensing applications.
3. NANOPRECIPITATION OF INORGANIC SALTS

3.1 Introduction

Nanoprecipitation of divalent salts has been reported using polyethylene terephthalate pores.\textsuperscript{14, 26} The surface of the pores has a carboxylic acid functional group. The pores therefore had a negative charge in solution due to deprotonation of the carboxylic acid group. These experiments were carried out at and approaching the Ksp of CaHPO\textsubscript{4} and CoHPO\textsubscript{4}. At high conductivity states (where cations are driven into the pore) the current was unstable and decreased as the magnitude of the potential increased. Ion current oscillations were observed in the constant potential current time series measurements (current vs time at constant potential). The frequency of oscillations were shown to be dependent on ion concentration of cation and anion, and also potential dependent. It was also shown that the frequency of ion current oscillations could be affected by the salt type. These results demonstrated that these ion current oscillations were due to nanoprecipitation of these inorganic salts at the opening of the nanopores. Nanoprecipitation has also been demonstrated using nanopipettes.\textsuperscript{27} Nanoprecipitation of ZnHPO\textsubscript{4} at concentrations near or at Ksp has been demonstrated. The dynamics of nanoprecipitation were investigated and it was demonstrated that negatively charged ZnHPO\textsubscript{4} was responsible for blockage of the nanopipette. The pore could be reopened by changing the polarity which could be used to shuttle the precipitate towards and away from the opening of the nanopipette.

Chapter 2 demonstrated that nanopipettes display ion current rectification in electrolyte concentrations of 1 M or less. It was also found that a decrease in radius and/or an increase in surface charge could significantly enhance this rectification effect. This chapter will consist of the study of nanoprecipitation of salts consisting of monovalent and divalent cations.
3.2 Experimental

3.2.1 Materials

Na$_2$SO$_4$ and CaCl$_2$ from Sigma Aldrich. Other chemicals mentioned in experimental of chapter 2.

3.2.2 Electrochemical Methods and Description of Methods

Constant potential current time series (current versus time) measurements and cyclic voltammery of LiCl and KCl electrolytes were taken using a Gamry reference 600 at 260 Hz in 10 mM, 100 mM and 1 M LiCl. Constant potential current time series measurements involving calcium sulfate studies used a Axon CNS Axopatch 2000B with a sampling rate 10 Hz.

![Constant Potential Current Time Series](image)

Scheme 3.1. Constant applied potential over time.
3.3 Nanoprecipitation of monovalent salts in nanopipettes

3.3.1 Nanoprecipitation of LiCl

Figure 3.1 One panel A illustrates a cyclic voltammogram between -1 V to 1 V at 100 mV/s in 1 M LiCl. The colored lines and corresponding numbering indicate the sequence in which the data was recorded. The red curve illustrates the first sequence starting from 0 V and ending at 1 V. The black curve starts at 1 V and ends at 0 V. The third sequence is the green curve which starts at 0 V and ends at -1 V. The final sequence is the blue curve starts at -1 V and ends at 0 V. The cyclic voltammogram illustrates sharp transitions in the current at points in the selected potential range. The red sequence, starting from 0 V and ending at 1 V, indicates that the current transitions sharply at around 0.5 V. The black sequence from 1 V to 0 V indicates that the current is higher from about 0.5 V to 0 V compared to the initial red sequence. Transitions are observed in the green sequence from 0 V to -1 V and the blue sequence -1 V to 0 V. Rectification ratio which is simply the ratio of the currents at potentials of equivalent magnitude and opposite polarity gives a measure of the degree of rectification. A comparison of the current response of magnitude 1 V at negative and positive potential indicates a rectification ratio of approximately 1.2. This is surprising since at lower concentrations nanopipettes show a higher degree of rectification. Panel B indicates constant potential current time series measurements at -0.1 V, -0.3 V, -0.5 V, -0.7 V and -0.9 V. Oscillations in the current signal are observed at each potential. At each potential the current is characterized by a high conductivity state and a low conductivity state. The nanopipette spontaneously switches between both states during these constant potential measurements. The frequency of these oscillations increases with the magnitude of the applied potential. This is consistent with the CV for the green and blue sequences where some current transitions were observed at negative potentials. Panel C illustrates meas-
urements carried out at positive potentials at 0.1 V, 0.3 V, 0.5 V, 0.7 V, 0.8 V and 0.9 V. Current oscillations are also observed 0.7 V, 0.8 V and 0.9 V.

**Figure 3.1.** Panel A illustrates a Cyclic Voltammogram in 1 M LiCl the colors indicate the sequence in which the measurements were taken. Red indicates the first sequence from 0 to 1 V, Black from 1 V to 0 V, blue from 0 V to -1 V and green from -1 V to 0 V. Panel B indicates current time series measurements taken in 1 M LiCl at negative potentials. Panel C illustrates current time series measurements in 1 M LiCl at positive potentials.
This is consistent with the red and black sequences in panel A. The oscillations observed at positive potentials do not display a regular repeating pattern like that observed in panel B at negative potentials.

**Figure 3.2** Panel A indicates the CV in 10 mM LiCl from -1 V to 1 V at a scan rate of 100 mV/s. The arrows indicate the sequence in which the data was recorded. The current is higher when the potential is scanned from 0 V to 1 V compared to scans from 1 V to 0 V. From 0 V to -1 V the current is higher compared to the current from -1 V to 0 V. The observation that currents from 0 V to 1 V are higher than from 1 V to 0 V is not typically observed in nanopipettes. The normal expected behavior was demonstrated in chapter 2. Typically scanning the potential from zero potential to higher magnitudes has lower current values than from higher magnitudes to zero potential and this behavior has been previously described for conical nanopores. Similar to what was observed in the 1 M measurements the CV does not display significant ion current rectification and the rectification ratio approaches unity. Panel B displays chronoamperometric measurements carried out at potentials 0.1 V, 0.2 V, 0.3 V, 0.4 V, 0.5 V, 0.6 V, 0.7 V, 0.8 V and 0.9 V. Each segment was 200 seconds long. The red curve indicates measurements at 0.1 V, 0.2 V and 0.3 V. The first measurement at 0.1 V began at –200 seconds and ended at 0 seconds. The second segment at 0.2 V began at zero seconds and ended at 200 seconds. The third segment at 0.3 V began at 200 seconds and ended at 400 seconds. The transition in potential at 0 seconds and 200 seconds are marked by a sharp increase in current. The same sequence was carried out for the green curve (0.4, 0.5 and 0.6 V) and black curve (0.7 V, 0.8 V and 0.9 V). Oscillations do not begin to appear until at 0.5 V. Similar to what has been observed for 1 M LiCl measurements as the magnitude of the potential is increased the frequency of these oscillations increases.
Figure 3.2. Panel A illustrates the Cyclic Voltammogram in 10 mM LiCl, the arrows indicate the direction of the applied potential. Panel B indicates current time series measurements taken at positive potentials. Red curves from the left are 0.1 V, 0.2 V and 0.3 V. The ordering is the same for the green and black curves. Panel C indicates current time series measurements taken at negative potentials. The red curve from the left indicates -0.3 V, -0.2 V and -0.1 V. The ordering is the same for the green and black curves.
Panel C illustrates chronoamperometric measurements at negative potentials. As described for the 10 mM LiCl measurements the sequence begins at -200 seconds for each colored curve. For example the red curves indicates measurements in the sequence -0.3 V, -0.2 V and -0.1 V. Oscillations are also observed which begin at around -0.5 V. The waveforms of these oscillations are more irregular compared to those at positive potentials.

**Figure 3.3** panel A indicates a CV measurement in 100 mM LiCl of potential width -1 V to 1V at a scan rate of 100 mV/s. The colors indicate the sequence in which the measurements were carried out. The red curve indicates scanning the potential from 0 V to 1 V and the black curve from 1 V to 0 V. The green curve illustrates scanning from 0 V to -1 V and the blue curve from 1 V to 0 V. Panel B illustrates chronoamperometric measurements carried out in the same way as previously described. For example the red curve begins measurement at 0.3 V at -200 seconds then proceeds to 0.2 V at 0 seconds and 0.1 V at 200 seconds. Each segment lasts 200 seconds. In these measurements current oscillations begin at 0.3 V and increases in frequency with the magnitude of the applied potential. Panel C indicates measurements at negative potentials. These measurements do not display any recognizable current oscillations compared to those at positive potentials. These observations are similar to those observed in 1 M LiCl and 10 mM LiCl. The CV measurement is not significantly rectified compared to what was previously observed for nanopipettes. The current response in 100 mM is comparable to that in 10 mM LiCl even though the concentration has increased tenfold. The current at 1 V in 10 mM LiCl is approximately 100 pA while that at 1 V in 100 mM LiCl is 150 pA. The current in 1 M LiCl is significantly higher at approximately 6 nA. This is more than what would be expected for a tenfold increase in concentration.
Figure 3.3. Panel A demonstrates the cyclic voltammogram in 100mM LiCl. Arrows indicate the direction of the applied potential. Panel B demonstrates the current time series measurements in 100 mM LiCl at different positive potentials. The red curves from the left represent 0.3 V, 0.2 V and 0.1 V. Potential was changed in the same way for black and green curves. Panel C represents the current time series at negative potentials. The potentials measured in the red curves were taken in the sequence -0.3 V, -0.2 V and -0.1 V. The ordering is the same for the green and black curves.
Figure 3.4 indicates CV and constant potential time series measurements at -0.1 V, -0.3 V, -0.5 V, -0.7 V and -0.9 V carried out in 5 M LiCl. The CV measurement indicates that forward scans have a higher current than backward scans. Nanopipettes usually display lower current at forward scans compared to backward scans. This change in behavior can be explained using the polarizable double layer model or memory effect.\textsuperscript{21} The observation that currents are lower at forward scans compared to backwards scans is due to the lag in the potential dependent conformation of the double layer. At very high concentrations such as 5 M LiCl the extent of the double layer would be significantly reduced. Such high concentrations would reduce the memory effect and the nanopipette approaches the behavior expected without the memory effect. At negative potentials conductivity state switching is observed. Constant potential current time series measurements also illustrate current oscillations which begin at 0.5 V and increase in frequency with the potential magnitude. Interestingly the CV has lower current compared to the measurement in 1 M LiCl. At 1 V the current is 6 nA in 1 M LiCl and 2 nA in 5 M LiCl.

![5 M LiCl](image)

**Figure 3.4.** Illustrates the cyclic voltammogram of in 5 M LiCl. The arrows indicate the direction of the applied potential. The current time series measurements in 5 M LiCl were carried out at positive potentials.
Different conductivity states are observed in the constant potential measurements in LiCl concentrations of 10 mM, 100 mM, 1 M and 5 M. The two parameters that were varied in these experiments were the potential and the concentration of the electrolyte. At all concentrations the frequency of the oscillations increased with increasing potential magnitude. Current oscillations were observed at both potential polarities. There was no consistent change in the nanopipette behavior when concentration was adjusted. An explanation of the previously described phenomena cannot rely on the variations of these two parameters. In chapter two nanopipettes were studied which did not demonstrate the behaviors observed in the LiCl measurements. The nanopipettes have an estimated radius of 8 nm and 28 nm. It is important to note that these nanopipettes have a single conductivity state at a particular applied potential. For the nanopipette tested in the LiCl solutions there a different conductivity states at a given potential. This is due to a smaller radius which causes precipitation of LiCl. Simulation of ion transport through negatively charged nanopores demonstrated that the ionic concentrations at the tip (100 nm from the opening) are higher than the bulk concentration of the solution. Nanopipettes also display rectification in 1 M KCl while nanopores typical of the size used in the simulations display ohmic behavior in these concentrations. The negative surface charge in a smaller aperture would result in a higher concentration of cations in the tip due to the higher electric field experienced. Therefore nanopipettes will have a higher concentration enrichment effect than the nanopores due to smaller radius which causes the negatively charged surface to have a more significant effect on ion transport. It is likely that these observations occur due to the concentration enhancement effect which causes the salt concentration in the tip of the nanopipette to exceed the saturation point for LiCl of approximately 15 M.
3.3.2 Nanoprecipitation of KCl

Ion current oscillations have been observed in systems with negatively charged nanoscale devices. These involve a sparingly soluble salt containing a divalent cation such as zinc or calcium with a buffer and salt concentration close to the $K_{sp}$ value of the salt.\textsuperscript{14, 26-27} In these systems highly soluble salts such KCl are used as a background electrolyte. It is interesting that in these systems with nanodevices less than 10 nm in radius no current fluctuations are observed in the CV when concentrations such as 1 M KCl are tested using these nanoscale devices.

Figure 3.5. Panel A illustrates a cyclic voltammogram between -0.4 V-0.4 V in 1M KCl for a nanopipette approximately 8 nm in radius. Panel B illustrates scans between 0 V-1 V in 1 M KCl for a nanopipette approximately 14 nm in radius. The arrows indicate the direction of the current. Panel C illustrates a cyclic voltammogram between 0 V -1 V for the 14 nm nanopipette. The arrows indicate the direction of the current. Panel D indicates cyclic voltammograms for the 14 nm nanopipette illustrating different potential ranges by varying the initial potential and keeping the end potential constant at 1 V. The dotted line illustrates where the low conductivity state would be.
**Figure 3.5** panel A illustrates the CV response of a nanopipette in 1 M KCl with a radius of approximately 23 nm. The potential is scanned between -0.4 V and 0.4 V. The nanopipette displays ion current rectification. At each potential the current is constant over several scans. Panel B illustrates the current response of a nanopipette in 1 M KCl with a radius of approximately 14 nm. Panel B illustrates positive only scans between 0 V - 1 V. The arrows indicate the direction of the current. It can be seen that there are two conductivity states associated with the pore, one at a higher potential and another at a lower potential. As the applied potential is increased from 0 V to higher magnitudes the lower conductance state is predominant until at a high enough potential (approximately 860 mV) the current transitions sharply to the higher conductance state. This transition is indicated by the upward pointing arrow. The nanopipette remains in this high conductivity state until at low enough applied potential (approximately 200 mV) it transitions back to the low conductivity state. This transition is indicated by the downward pointing arrow. Similar behavior is observed at negative potentials in panel C with a potential window from 0 - -1 V. Transition from a lower conductivity state to a higher conductivity state (at -0.576 V) as the magnitude of the potential is increased and transition back from the higher conductivity state to the lower conductivity state (approximately -0.4 V) as the potential magnitude is decreased. Panel D indicates CVs taken at the same end potential of 1 V and different initial potentials. The dashed line indicates the low conductivity state which was superimposed on the graphic and was not observed during these measurements. As illustrated in panel B the transition back to low conductivity occurs at approximately 200 mV. By narrowing the potential range and avoiding the transition at 200 mV to low conductivity the pore was maintained in the high conductivity state. Measurements at an initial potential of 0.4 V, 0.5 V, 0.6 V, 0.8 V and 0.9 V all overlapped. The behaviors observed for the 14 nm nanopipette differ significantly from that ob-
served for the 8 nm nanopipette in panel B. The 8 nm nanopipette displays a single conductance state at each potential and this state is independent of the selected potential range. The 14 nm nanopipette illustrates different conductance states which are dependent on the selected potential range. These observations may be explained by nanoprecipitation of KCl salt. The 8 nm nanopipette does not display different conductivity states at a given potential. They key difference higher surface charge density. Simulation studies have demonstrated increase in the concentration of electrolyte compared to bulk concentration in conical nanopores. It may be that the 14 nm nanopipette has a higher surface charge density which increases the concentration above the bulk Ksp value of KCl.

**Figure 3.6** illustrates CV measurements at scan rates of 20 mV/s, 60 mV/s, 100 mV/s and 300 mV/s. The direction of the current is the same as that described in **Figure 3.5** panel B. As the scan rate is increased the transition point from low to high conductivity shifts to higher potential magnitudes. This also means that the low conductivity state is more stable over a wider potential range with increasing scan rate and conversely the high conductivity state becomes less stable.

![Figure 3.6](image)

**Figure 3.6.** Different CV in 1 M KCl at different scan rates for a 14 nm nanopipette.
**Figure 3.7** panels A and B illustrate constant potential current time series measurements carried out at positive potentials for the 14 nm nanopipette. Panel A illustrates measurements carried out at 0.266 V which is approximately the value where transitions from the high conductivity to low conductivity states are observed for CV measurements between 0 V and 1 V. The time series indicates the switching of the current between a high conductivity to low conductivity state. Panel B illustrates time series measurements carried out at 0.8 V. A comparison with the CV measurements at this potential indicates that the pore remains in the high conductivity state. The measurement at 0.8 V is carried out away from the transition point from high to low conductivity at 0.266 V. At 0.8 V this measurement is carried out at a higher potential and it would be expected that the pore should remain predominantly in the high conductivity state. This finding is also consistent with experiments in **Figure 3.5** panel D which indicated that scanning the potential range away from the transition point from high to low conductivity resulted in a predominantly high conductivity state. Panel C indicates a constant potential measurement carried out at a potential of -0.576 V. This represents the transition point from a lower conductivity state to a higher conductivity state in the CV measurement from 0 V - 1 V in **Figure 3.5** panel C. The current switches between a high and low conductivity state in this measurement. Panel C indicates a constant potential measurement at -0.9 V. This potential was away from the transition point at a higher potential magnitude. The constant potential measurement at -0.9 V indicates that the current increased from the lower conductivity state to a higher one at around 10 seconds and decayed back to the lower conductivity state. These measurements indicate some interesting differences between the measurements at positive polarity and negative polarity. The measurements at 0.266 V and 0.8 V represent measurements at the transition point from high to low conductivity and away from the transition point towards higher potentials respectively. Both negative poten-
tial measurements at -0.576 V and -0.9 V represent measurements away from the transition point state at around -0.4 V. The observation is that switching between conductivity state occurs in these constant potential measurements. This was not observed in the positive potential measurement at 0.8 V. A single high conductivity state was observed. There is an approximately 500 mV difference between the transition point from high to low conductivity and the constant potential measurement at 0.8 V. This is also the case for the measurement at -0.9 V and the transition point from high to low conductivity at negative polarity measurements. As mentioned the conductivity state switches at -0.9 V measurements. This was not observed for positive potential measurements. This indicates that the polarity of the applied potential has an impact on the nanopipette behavior. At positive potentials measurements at potential magnitudes higher than the transition point results in the high conductivity state predominating. At negative potentials it is possible to observe high and low conductivity states at higher potential magnitudes than the transition point. It is not clear which would predominate. Asymmetric geometry at the opening of the nanopipette may offer an explanation for this observed difference in behavior which is dependent on potential polarity. Imaging studies are under way for the nanopipettes.
Figure 3.7. Panel A illustrates constant potential measurements at 0.266 V. Panel B illustrates constant potential measurements at 0.8 V. Panel C illustrates constant potential measurements at -0.576 V. Panel D illustrates constant potential measurements at -0.9 V.
3.4 Nanoprecipitation of divalent salts in nanopipettes

3.4.1 Nanoprecipitation of calcium sulfate

**Figure 3.8** panel A demonstrates the setup used in this study with CaCl₂ outside of the nanopipette and Na₂SO₄ inside the nanopipette. This asymmetric setup is desirable because mixing of these solutions directly would produce precipitation of calcium sulfate. This setup also allows control of the type of ions which would be driven towards the opening of the nanopipette based on the polarity of the applied potential. At positive potentials calcium and sulfate ions are driven towards the entrance of the nanopipette while sodium ions and chloride ions are driven away from the opening. At negative potentials sodium and chloride ions are driven towards the opening of the nanopipette while calcium and sulfate ions are driven away from the nanopipette. It has been demonstrated that precipitation of CaSO₄ proceeds from the more soluble bassanite form (approximately 40 mM at 25 °C) to the less soluble gypsum form (20 mM at 25 °C). Studying precipitation of CaSO₄ using nanopipettes has advantages over other methods. Using constant potential time series measurements high temporal resolution can be obtained. This precipitation mechanism can be studied with millisecond resolution. The second is that this reaction can be carried out in a localized area of nanometer dimension. This can give insight into the early stages of precipitation or crystallization which has important practical aspects for more complex systems such as protein crystallization.
Figure 3.8. Panel A illustrates the experimental set up with 50 mM Na$_2$SO$_4$ inside the nanopipette and 50 mM CaCl$_2$ outside of the nanopipette. The red line illustrates the reference electrode and the green line illustrates the working electrode. Panel B illustrates measurements carried out at a potential magnitude of 500 mV. The green arrows indicate switching from a potential of 500 mV to -500 mV. The blue arrow indicates switching from -500 mV to 500 mV. Red arrows indicate spontaneous partial blocking of the nanopipette. Panel C illustrates measurements at magnitude 900 mV and panel D magnitude 300 mV.
Panel B illustrates results of measurements using this setup at a potential magnitude of 500 mV. The green arrow indicates that the potential was switched to negative polarity and the blue arrow indicates that the potential was switched to positive polarity. At positive polarities the red arrows indicate that the current decreases suddenly with a difference of approximately 2.7 nA. This behavior was absent at negative potential polarity. At potential magnitude 900 mV this behavior is again observed at positive polarity (difference of approximately 6 nA) and at negative polarity this is not observed. This behavior is not observed at 300 mV at either positive or negative polarity. This series of experiments supports the conclusion that this partial blockage of the nanopipette is due to precipitation of calcium sulfate. This would be expected because calcium ions and sulfate ions are driven towards the opening of the nanopipette at positive potential polarity. This behavior is absent at negative polarity which is also consistent with what would be expected as calcium and sulfate ions are driven away from the opening of the nanopipette. At 300 mV this behavior was not observed at either potential polarity. This is due to the lower ionic flux at this potential which would not increase the concentration enough to observe precipitation.

**Figure 3.9** panel A illustrates the inverse set up of **Figure 3.8** panel A. At positive potentials calcium and sulfate ions move away from the opening of the nanopipette while sodium and chloride ions move towards the entrance. At negative potentials calcium and sulfate ions move towards the opening of the nanopipette while sodium and chloride ions move away from the opening. **Figure 3.9** panel B illustrates measurements taken at a potential magnitude of 500 mV. At positive potentials with calcium and sulfate ions moving away from the entrance of the nanopipette no significant decrease in current is observed compared to the set up in **Figure 3.8**. At negative potential no significant transition is observed. In panel C the measurements at 900 mV magnitude demonstrate that when potential polarity is switched from positive to negative an in-
crease in current at positive potentials is observed as highlighted by the red circle. It is not clear how calcium sulfate precipitation may have caused the manifestation of this behavior. This series of experiments does further bolster the claim that the large transitions in current observed in Figure 3.8 are due to precipitation of calcium sulfate. At positive potentials in Figure 3.9 at 500 mV and 900 mV there are no observations of the large current transitions observed for Figure 3.8 at the same potential magnitude and polarity. This would be expected because calcium and sulfate ions move away from the entrance of the nanopipette. At negative potential polarity these transitions would be expected since calcium and sulfate ions move towards the entrance. This was not demonstrated at negative potential polarity and may be explained by the concentration depletion which has been demonstrated by simulation studies of rectifying nanoscale systems.
Figure 3.9. Panel A indicates the setup with 50 mM CaCl$_2$ inside the nanopipette and 50 mM Na$_2$SO$_4$ outside of the nanopipette. Panel B indicates measurements at potential magnitude 500 mV. Panel C illustrates measurements at potential magnitude 900 mV and panel D 300 mV.
**Figure 3.10.** Panel A illustrates a cyclic voltammogram potential window -1 V to 1 V with the same set up as that in **Figure 3.8.** Panel B illustrates a cyclic voltammogram potential window 0.7 V to 1 V with the same set up as that in **Figure 3.8.** Panel C illustrates a cyclic voltammogram potential window -1 V to 1 V with the same set up as that in **Figure 3.9.** Panel C illustrates a cyclic voltammogram potential window -0.7 V to -1 V with the same set up as that in **Figure 3.9.**

These observations may also be confirmed using cyclic voltammetry. **Figure 3.10** panel A demonstrates the cyclic voltammogram for a potential range of -1 V to 1 V. The experimental set up for this measurement is the same as that in **Figure 3.8.** This displays a rectified curve. When the potential range is narrowed from 0.7 V to 1 V in panel B the nanopipette changes from the open state current value as obtained in the -1 V to 1 V scan to a current value near zero. This
is consistent with calcium sulfate precipitation as calcium and sulfate ions move towards the entrance of the nanopipette at this positive potential polarity. The cyclic voltammogram for the same experimental set up as Figure 3.9 is illustrated in panel C. Current time series measurements in Figure 3.9 did not clearly demonstrate if nanoprecipitation could be observed at negative potential polarity due to the depletion effect at of rectifying systems. Panel D negative polarity scans between -0.7 V and -1 V. It can be seen that overtime the current decreases. This is consistent with calcium sulfate precipitation as calcium and sulfate ions move towards the entrance of the nanopipette at this potential polarity. In panel E the measurement at positive potential demonstrates that over time the current increases from a lower current state to a higher current state. This is expected as calcium and sulfate ions are moved away from the entrance of the nanopipette by the applied potential. These observations taken together indicate that the observations in Figure 3.8 panel B and C are due to precipitation of calcium sulfate. Further experiments in Figure 3.11 which demonstrates measurements in Na₂SO₄ only and Figure 3.12 in CaCl₂ only demonstrate that no significant transitions comparable to that in Figure 3.8 panels B and C are observed.
Figure 3.11. Panel A illustrates the experimental set up with 50 mM Na$_2$SO$_4$ both inside the nanopipette and outside the nanopipette. Panel B illustrates measurements at 500 mV potential magnitude. Panel C illustrates measurements at 900 mV potential magnitude. Panel D illustrates measurements at 300 mV potential magnitude.
**Figure 3.12.** Panel A illustrates the experimental setup with 50 mM CaCl$_2$ inside and outside of the nanopipette. Panel B illustrates measurements at potential magnitude 500 mV. Panel C indicates measurements at potential magnitude 900 mV. Panel D illustrates measurements at potential magnitude 300 mV.
3.5 Crystallization of Inorganic Salts

3.5.1 Future Approach

Crystallization whether of proteins or inorganic salts is a balancing act. The required conditions should be just right in order to develop crystals and not precipitation. Supersaturating conditions may be appealing because seeds are generated in large amount but the disorderly aggregation gives rise to precipitate instead of ordered crystals. Crystallization of proteins can involve several samples with different concentrations of protein and precipitant in order to find this ‘just right’ zone. Even though efficient sample use means samples down to a few microliters can be used there are still many different samples which would have to be monitored. Crystallization using nanopipettes can solve many of the difficulties involved in conventional crystallization. In section 3.2.1 calcium sulfate precipitation was demonstrated by using calcium chloride solution outside of the nanopipette and sodium sulfate inside the nanopipette and using a potential to drive the ions towards the opening of the nanopipette. This demonstrated that nanopipettes can be used as nanoreactors with the localization of the desired reaction in dimensions of nanometers. The location of the necessary materials for the reaction can be controlled and by tuning the potential magnitude the rate of mixing can also be controlled. These are important considerations for crystallization. Finding the ‘just right’ zone is also much easier by controlling the potential range. Instead of different concentrations of proteins and precipitant requiring different individual setups a variety of conditions can be scanned at the click of a button using the same set up.

A recurring theme in chapter 3 has been that the conductivity state of nanopipettes can be controlled by selecting a certain potential or potential range. In crystallization the desire would be to select a potential range which would generate crystal seeds. Figure 3.13 demonstrates this
concept by narrowing the potential range the transition to the higher conductivity state is eliminated and the nanopipette is maintained in the low conductivity state. Panel B demonstrates the experimental setup for crystallization of inorganic slats. It is hoped that after generating a seed it can be moved away from the nanopipette into the saturating solution where the growth into a crystal will be favorable.

![Figure 3.13](image.png)

**Figure 3.13.** Panel A illustrates Cyclic Voltammograms in 2 M KCl at various potential windows at constant initial potential of 0 V and different end potentials. Panel B illustrates the set up for crystallization of inorganic salts.
3.6 Conclusion

Nanoprecipitation of monovalent and divalent slats has been demonstrated in this chapter. Nanoprecipitation of KCl and LiCl is especially significant since this nanoprecipitation occurs far below the Ksp value for LiCl which is approximately 15 M and approximately 4 M for KCl. Nanoprecipitation of calcium sulfate has been observed and studies of the mechanism behind calcium sulfate nanoprecipitation will proceed. The fact that the conductivity states can be generated and controlled means selecting a conductivity state where crystal seed generation might occur may be possible.
4. CONCLUSIONS

The nanoscale apertures have a high surface to volume ratio which causes these devices to differ markedly in behavior compared to larger sized openings. In chapter 2 it was demonstrated that this high surface impact can significantly affect the mass transport. Surface impacts can be so significant that effects such as concentration enhancement and depletion of solution can happen at the opening of a nanopipette relative to bulk. These novel interactions of ions in solution on the nano-dimensions have opened up new applications for nanoscale devices. Specifically it has been demonstrated that the enhancement effect can be used to generate nanoprecipitation. In fact in the case of LiCl it has been shown that nanopipettes can generate an enhancement of the concentration by atleast 3 orders of magnitude. It has also been demonstrated that nanopipettes can serve as nanoreactors where reactions of interests can be studied. The nanoscale dimensions can offer insights into mechanisms of precipitation which are simply inaccessible at bulk scales. The most exciting application of these novel properties of nanopipettes is in the crystallization of inorganic salts. These simple inorganic salts can serve as model systems for more complex endeavors such as protein crystallization.
REFERENCES


