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Water Uptake by Mars Salt Analogs: An Investigation of Stable Aqueous Solutions Using Raman Microscopy

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Water Uptake by Mars Salt Analogs: An Investigation of Stable Aqueous Solutions Using Raman Microscopy

by

Danielle L. Nuding

B.A., University of Alabama in Huntsville, 2009
M.S., University of Colorado Boulder, 2012

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This thesis entitled:
Water Uptake by Mars Salt Analogs: An Investigation of Stable Aqueous Solutions Using Raman Microscopy
written by Danielle L. Nuding
has been approved for the Department of Atmospheric and Oceanic Sciences

__________________________
Dr. Margaret Tolbert

__________________________
Dr. Raina Gough

Date ______________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Water uptake by Mars salt analogs: An investigation of stable aqueous solutions using Raman microscopy

Thesis directed by Prof. Dr. Margaret Tolbert

Liquid water processes that may occur on the surface and near-subsurface of Mars have important implications for the present-day water cycle, habitability, and planetary protection policies. The presence of salts on Mars plays a role in surface-atmosphere interactions as salts enhance the soil’s ability to retain water. This thesis explores the phase transitions of water upon interaction with Mars relevant salt analogs. Water uptake and loss properties of a single and complex Mars analog are examined using a Raman microscope equipped with an environmental cell. The effect of the hygroscopic salts on bacterial spores was evaluated with a focus on potential terrestrial contamination on outbound spacecraft and its influence on planetary protection concerns.

Calcium perchlorate (Ca(ClO$_4$)$_2$) is a highly deliquescent salt that may exist on the surface of present-day Mars. Here, we quantify the deliquescent relative humidity (DRH) and efflorescent relative humidity (ERH) of Ca(ClO$_4$)$_2$ as a function of temperature (223 K to 273 K) to elucidate its behavior on the surface of Mars. Mars relevant temperature and relative humidity (RH) conditions were simulated and deliquescence (solid to aqueous) and efflorescence (aqueous to solid) phase transitions of Ca(ClO$_4$)$_2$ were characterized. Experimental DRH values were compared to a thermodynamic model for three hydration states of Ca(ClO$_4$)$_2$. Calcium perchlorate was found to supersaturate, with lower ERH values than DRH values. Additionally, we conducted a 17-hour experiment to simulate a subsurface relative humidity and temperature diurnal cycle. This demonstrated that aqueous Ca(ClO$_4$)$_2$ solutions can persist without efflorescing for the majority of a martian sol, up to 17 hours under Mars temperature heating rates and RH conditions. Applying these experimental re-
results to martian surface and subsurface heat and mass transfer models, we find that aqueous 
Ca(ClO$_4$)$_2$ solutions could persist for most of the martian sol under present-day conditions.

To investigate complex brine mixtures, a salt analog, deemed ‘Instant Mars,’ was devel-
oped to closely match the individual cation and anion concentrations as reported by the Wet 
Chemistry Laboratory instrument at the Phoenix landing site. ‘Instant Mars’ was developed 
to fully encompass and closely replicate correct concentrations of magnesium, calcium, potas-
sium, sodium, perchlorate, chloride, and sulfate ions. Here we use two separate techniques, 
Raman microscopy and particle levitation, to study the water uptake and loss properties of 
individual Instant Mars analog particles. Raman microscope experiments reveal that Instant 
Mars particles can form stable, aqueous solutions at 56 ± 5% RH at 243 K and persist as 
a metastable, aqueous solution down to 13 ± 5% RH. The results presented in this thesis 
demonstrate that a salt analog that closely replicates in-situ measurements from the Phoenix 
landing site can take up water vapor from the surrounding environment and transition into 
a stable, aqueous solution. Furthermore, this aqueous Instant Mars solution can persist as a 
metastable, supersaturated solution in RH conditions much lower than the deliquescent RH.

Finally, laboratory experiments presented here examine the interaction of B. subtilis 
spores (B-168) with liquid water in Mars relevant temperatures and RH conditions. In 
addition, Ca(ClO$_4$)$_2$ was mixed with the B. subtilis spores and exposed to the same diurnal 
cycle conditions to quantify the effects of Ca(ClO$_4$)$_2$ on the spores. A combination of Raman 
microscopy and an environmental cell allows us to visually and spectrally analyze the changes 
of the individual B. subtilis spores and Ca(ClO$_4$)$_2$ mixtures as they experience present-day 
martian diurnal cycle conditions. Results suggest that B-168 spores can survive the arid 
conditions and martian temperatures, even when exposed to Ca(ClO$_4$)$_2$ in the crystalline or 
aqueous phase. The extreme hygroscopic nature of Ca(ClO$_4$)$_2$ allows for direct interaction of 
B-168 spores with liquid water. The results impact the understanding of planetary protection 
and forward contamination concerns for future missions.
Dedication

A world where science and engineering meet enables boundless exploration opportunities.

To the extensive encouraging network of people that has grown throughout my lifetime; all who helped a small town ranch girl achieve her dreams.
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Chapter 1

Introduction

Mars, a planetary destination of over 20 spacecraft, is a terrestrial body that lies on the edge of the habitable zone of the solar system. The Goldilocks zone is often used to describe the habitable zone; not too hot, not too cold, but just right. To put this in a planetary context, the habitable zone describes bodies that are the appropriate distance from the sun for liquid water to be stable; a key requirement for life as we currently understand it. Earth is the primary example for the ‘just right’ terrestrial body, with an atmospheric pressure up to 1013 mb and temperature range of 208-328 K, conditions that encompass the triple point of water thus allowing for liquid water stability. Mars lies on the edge of the ideal habitable zone, with temperatures in the 148-293 K range and an atmospheric pressure approximately one one-hundredth (6-9 mbar) of Earth’s atmospheric pressure. Pure liquid water stability is not favorable on the present-day martian surface due to the low pressure, temperature, and relative humidity conditions [Haberle et al., 2001; Ingersoll, 1970]. However, surface features observed by in-situ and orbiting spacecraft suggest that liquid water processes are occurring on the surface or in the near-subsurface despite the low pressure, arid, and cold conditions observed on Mars today. Recent discoveries increasingly position Mars within the de facto Goldilocks zone, thus expanding the idea that Mars could be habitable.

Understanding liquid water activity on Mars has important implications for the present-day water cycle, habitability, planetary protection concerns and thus, future exploration missions to Mars. The Phoenix Lander was one of the first to observe in-situ evidence of
potential liquid water processes on the present-day surface and subsurface (down to 10 cm). Landing in the northern plains of Mars, one of the main scientific objectives was to search for evidence of the habitable zone and understand past and present water processes. Equipped with a robotic arm to dig into the near-subsurface and a robust instrumentation suite, the Phoenix Lander revealed a near pure water ice table and processes indicating that aqueous solutions could be present in current martian conditions. Examples of such features are possible subsurface thin film liquids [Cull et al., 2010] and spheroids on the Phoenix lander leg [Renno et al., 2009].

Furthermore, evidence for liquid water in the southern mid-latitudes and equatorial regions of Mars has been reported by two current instruments aboard the Mars Reconnaissance Orbiter (MRO); the High Resolution Imaging Science Experiment (HiRISE) [McEwen et al., 2011, 2014] and more recently, the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) [Ojha et al., 2014]. Recurring slope lineae (RSL) are narrow, dark markings found on steep crater slopes that begin to appear in the warm season and disappear in the cold season [McEwen et al., 2011]. RSL are described as flow-like features and are suggested to result from aqueous processes [McEwen et al., 2011, 2014; Chevrier and Rivera-Valentin, 2012]. Figure 1.1 is an example of south-facing RSL located on the steep slopes within a crater in Melas Chasma, with black arrows pointing to the individual lineae features [McEwen et al., 2014]. Briny aqueous solutions are presently the favored explanation for liquid water features observed on present-day Mars due to their stability in low temperature and pressure conditions. Salts are ubiquitous on Mars and allow for a pathway for stable and metastable aqueous solutions to exist in present-day martian conditions.

The presence of salts is important because of their impact on the soils ability to retain water, thus influencing the water cycle and habitability of Mars. Perchlorate and chloride salts are known to be present in the Martian soil [Hecht et al., 2009] and readily absorb water vapor from the atmosphere and deliquesce, or transition from a solid crystalline into an aqueous solution, [Gough et al., 2011; Zorzano et al., 2009] under Mars relevant temperature
Figure 1.1: HiRISE image of south-facing RSL located on steep slopes of the crater floor of Melas Chasma. In the southern spring and summer, the dark flow features, or individual lineae, are observed (highlighted with black arrows). Image credit: McEwen et al. [2014]
and relative humidity (RH) conditions. The Phoenix Lander detected the presence of 0.5% by weight perchlorate (ClO$_4^-$) with the Wet Chemistry Laboratory (WCL) [Hecht et al., 2009]. Figure 1.2 is an image from the trenches from which the samples were excavated and evaluated with the WCL ranging from 0-5 cm in depth. Perchlorate was also identified with the Thermal and Evolved Gas Analyzer (TEGA), which heated Martian soil samples and detected evolved oxygen gas (O$_2$) at the thermal decomposition temperatures expected for alkali or alkaline Earth perchlorates [Hecht et al., 2009]. Reanalysis of the Viking gas chromatography-mass spectrometry suggested that the Viking Lander, located in Chryse Planitia, potentially detected the presence of perchlorate in the local regolith [Navarro-González et al., 2010]. More recently, the Mars Science Laboratory (MSL) rover has reported perchlorate in Gale Crater [Glavin et al., 2013]. The numerous in-situ observations suggest perchlorate is distributed at several landing sites, and is potentially globally distributed via atmospheric processes [Catling et al., 2010].

Highly deliquescent salts can also persist as metastable solutions [Gough et al., 2011, 2014]. More specifically, perchlorates tend to remain in a supersaturated aqueous phase instead of efflorescing back into a solid crystal when RH is lowered [Gough et al., 2011, 2014]. This hysteresis behavior allows liquid brine solutions to exist at low relative humidity (RH) values, relevant to a diurnal cycle experienced on the surface and in the near-subsurface today. Understanding the deliquescent and efflorescent properties of perchlorates under martian temperature and RH conditions can give insights into present-day water activity on Mars. Laboratory studies have also shown bulk briny aqueous solutions can also exist as supercooled solutions at Mars relevant temperatures [Toner et al., 2014]. In this work, several single salt and simple salt mixtures have been characterized under martian environmental conditions in an attempt to quantify the stability of briny aqueous solutions on Mars.

Understanding liquid water stability, specifically at spacecraft landing sites, is important in order to gauge the level of contamination and impact presented by terrestrial microbes aboard the spacecraft. As we continue to send spacecraft to other planetary bodies, the sur-
Figure 1.2: Stereo image of the trenches, ranging from 0-5 cm in depth, at the Phoenix landing site. Rosy Red consisted primarily of surface material while Sorceress 1 and 2 scraped the boundary of the ice table. Samples were delivered to the WCL, revealing the presence of perchlorate. Image credit: [Hecht et al., 2009]
vival of terrestrial microbes should be considered to protect the scientific integrity of future missions. Planetary protection policies are established by the Committee on Space Research (COSPAR) with a key goal of controlling potential biological contamination from inbound and outbound spacecraft and sample return. Bacterial spores sampled from spacecraft surfaces are very resilient; the hygroscopic nature of perchlorates may provide a mechanism for the spores to interact with liquid water in surface conditions observed on Mars today.

1.1 Single Salt Characterization

Sodium perchlorate (NaClO₄) and magnesium perchlorate (Mg(ClO₄)₂) are highly deliquescent salts that form aqueous solutions at humidity values as low as 42% RH, and 51% RH, respectively, at 273 K [Gough et al., 2011]. Deliquescence was observed down to 223 K for all perchlorates studied thus far, with a general trend of increasing RH with decreasing temperature. A significant hysteresis effect is observed during efflorescence of these salt solutions, expected due to the kinetic inhibition of crystal nucleation. The efflorescence of NaClO₄ and Mg(ClO₄)₂ is not dependent on temperature with ERH values of 13% RH and 19% RH, respectively [Gough et al., 2011]. These laboratory studies indicate that perchlorate salts could exist as stable or metastable aqueous solutions over a wide range of martian RH and temperature conditions. When compared to modeled temperature and RH conditions at the Viking 1 landing site as a function of time [Savijarvi, 1995], NaClO₄ can persist as an aqueous solution for two to three hours of the sol as shown in Figure 1.3. Late morning and early evening hours provide the required temperature and RH conditions necessary for stable or metastable aqueous perchlorate solutions to persist.

Although the low temperature deliquescence and efflorescence of Na and Mg perchlorate salts has been characterized, instruments onboard Phoenix and MSL have identified calcium perchlorate (Ca(ClO₄)₂) as the likely parent salt [Cull et al., 2010; Glavin et al., 2013]. Ca(ClO₄)₂ is known for its highly deliquescent properties and low eutectic point [Pestova et al., 2005]; however, the deliquescence and efflorescence behavior of this salt have
Figure 1.3: Modeled RH line (solid) and surface temperature (dashed) as a function of time at the Viking 1 landing site [Savijarvi, 1995]. Gough et al. [2011] compared experimental phase transition points to predict aqueous stability of a NaClO₄ solution. The late morning and early evening (blue shaded region) are the times of sol that support either stable or metastable aqueous perchlorate solutions. Figure credit: [Gough et al., 2011]
not yet been quantified as a function of temperature. In this thesis, chapter 2 reports experimentally determined deliquescence and efflorescence relative humidity values of Ca(ClO$_4$)$_2$ as a function of temperature. The experimental results are compared to thermodynamic models to assess the stability and metastability of an aqueous (ClO$_4$)$_2$ solution in martian surface and subsurface conditions.

1.2 Binary Salt Mixtures

Many individual salts have now been well characterized but understanding the effects of other cations and anions on the stability of aqueous solutions is imperative for increasing the relevance to martian regolith processes. Gough et al. [2014] demonstrated that NaClO$_4$/NaCl and Mg(ClO$_4$)$_2$/MgCl$_2$ mixtures form stable aqueous solutions above 38% and 28% RH, respectively, at temperatures as low as 243 K. These results are reported to be 2-5% lower than the most deliquescent pure component in the mixtures. This work revealed that even if a less deliquescent salt is present in a brine mixture, a stable aqueous phase can form below the full deliquescent RH for the mixture. The hysteresis effect was also observed in these mixtures, allowing for metastable solutions to persist down to 5% RH for Mg(ClO$_4$)$_2$/MgCl$_2$ and 13% for NaClO$_4$/NaCl. The metastability of more complex mixtures, specifically Mars soil analogs, was recently studied by Toner et al. [2014]. They found significant supercooling of bulk Mars salt solutions, in some cases to temperatures as low as the glass transition temperature.

Chapter 3 of this thesis examines multiple species in a Mars salt analog and the phase changes occurring in response to changing the RH environment. We have developed a salt analog so called, ‘Instant Mars’ to closely match the individual cation and anion concentrations as reported by the Wet Chemistry Laboratory aboard the Phoenix Lander [Hecht et al., 2009; Kounaves et al., 2010]. Instant Mars closely replicates the measured relative concentrations of magnesium, calcium, potassium, sodium, perchlorate, chloride, and sulfate ions. Several cases of in-situ and remote sensing detection of salts on Mars [Hecht et al.,
suggests that these ions may coexist. This work investigates the water uptake and loss properties of the Instant Mars salt analog to quantify the effects of complex brine solutions.

1.3 Interaction of Terrestrial Spores, Liquid Water, and Perchlorate

The exploration of other planetary bodies searching for potential habitability and life is a top priority in space sciences. If life exists on other planetary bodies, we need to be able to distinguish between (and understand the potential for) terrestrial life that was carried onboard the outgoing spacecraft and life that could be discovered at the landing site. With the growth of the interest in sample return and human exploration of Mars, potential for contamination must be considered. Terrestrial spores sampled from spacecraft surfaces survive very harsh environmental conditions, such as space based radiation environments and Mars UV radiation \cite{Osman et al., 2008; Vaishampayan et al., 2012}. It is important to understand spores' interaction with their surrounding environments and the conditions in which they survive in order to keep planetary protection policies relevant and updated. Planetary protection policies continue to become more complex and pressing as missions continue to be formulated to land in potentially habitable environments and samples begin to be cached for sample return missions from Mars.

Calcium perchlorate has been reported at both the Phoenix \cite{Hecht et al., 2009; Kounaves et al., 2014} and the Mars Science Laboratory (MSL) \cite{Glavin et al., 2013} landing sites; an investigation of spore interaction with this salt is warranted. Chapter 4 explores the interaction of B. subtilis spores (B-168) with liquid water in Mars relevant temperatures and RH conditions. In addition, \( \text{Ca(ClO}_4\text{)}_2 \) was mixed with the B. subtilis spores and exposed to the same diurnal cycle conditions in order to quantify the effects of the salt on the spores and their surrounding environment.
Chapter 2

Deliquescence and Efflorescence of Calcium Perchlorate: An Investigation of Stable Aqueous Solutions Relevant to Mars

2.1 Introduction

One of the most unexpected discoveries made by the Phoenix Lander in the Martian arctic plains was the presence of 0.5% by weight perchlorate ($\text{ClO}_4^-$) with the Wet Chemistry Laboratory (WCL) \cite{Cull2010, Hecht2009}. Perchlorate was also confirmed with the Thermal and Evolved Gas Analyzer (TEGA), which heated Martian soil samples and detected evolved oxygen gas ($\text{O}_2$) at the thermal decomposition temperatures expected for alkali or alkaline Earth perchlorates \cite{Hecht2009}. Reanalysis of the Viking gas chromatography-mass spectrometry results also suggest perchlorates were present in the soil \cite{Navarro-Gonzalez2010}. Additionally, the Mars Science Laboratory (MSL) rover has potentially found perchlorate at Gale Crater \cite{Glavin2013}.

The presence of perchlorate is important because of its impact on the soil’s ability to retain water, thus influencing the water cycle and potential habitability of Mars. Perchlorate salts are known to readily absorb water vapor from the atmosphere and deliquesce (crystalline solid to aqueous solution) into aqueous solutions \cite{Chevrier2009, Gough2011, Zorzano2009}. Perchlorates, like most salts, also tend to remain in a supersaturated aqueous phase instead of efflorescing (aqueous solution to crystalline solid) back into a solid crystal \cite{Gough2011, Wills2009}. This hysteresis behavior allows liquid brine solutions to exist at low relative humidity (RH) values, relevant to the martian day.
Understanding the deliquescent and efflorescent properties of perchlorates under martian temperature and RH conditions can give insights into present-day water activity on Mars.

Previously, we performed laboratory studies to investigate the deliquescence and efflorescence of two perchlorate salts at low temperatures. These studies showed that sodium perchlorate (NaClO$_4$) and magnesium perchlorate (Mg(ClO$_4$)$_2$) are highly deliquescent, forming aqueous solutions at humidity values as low as 40% RH at temperatures as low as 223 K [Gough et al., 2011], which were conditions observed at the Phoenix landing site [Zent et al., 2010]. A significant hysteresis was observed during efflorescence of these salt solutions, as expected, due to the kinetic inhibition of crystal nucleation. The efflorescence RH values of sodium and magnesium perchlorate solutions were found to be 13% RH and 19% RH, respectively, indicating that perchlorate salts could exist as stable or metastable aqueous solutions over a wide range of martian RH and temperature conditions [Gough et al., 2011].

Although the low temperature deliquescence and efflorescence of Na and Mg perchlorate salts has been characterized, instruments onboard Phoenix and MSL have identified calcium perchlorate (Ca(ClO$_4$)$_2$) as the likely parent salt [Cull et al., 2010; Glavin et al., 2013]. Ca(ClO$_4$)$_2$ is known for its highly deliquescent properties and low eutectic point [Pestova et al., 2005]; however, the deliquescence and efflorescence behavior of this salt have not yet been quantified as a function of temperature. Here, we report experimentally determined deliquescence and efflorescence relative humidity values of Ca(ClO$_4$)$_2$ as a function of temperature and apply the results to predicted martian surface and subsurface conditions.

### 2.2 Experimental Setup

To characterize the deliquescence and efflorescence of Ca(ClO$_4$)$_2$, changes in salt phase and hydration state were studied using a combination of Raman and optical microscopy. The instrument setup used in this study is described in detail in [Baustian et al., 2010; Gough et al., 2011] and shown in Figure 2.1. A Raman microscope equipped with an environmental cell was used to determine the deliquescence relative humidity (DRH) and efflorescence
relative humidity (ERH) as a function of temperature (223 K to 273 K). Specifically, a Nicolet Almega XR Dispersive Raman spectrometer was outfitted with a Linkam THMS600 environmental cell, a Linkam automated temperature controller, and a Buck Research chilled-mirror hygrometer.

\( \text{Ca(ClO}_4\text{)}_2 \cdot 4\text{H}_2\text{O} \) was purchased from Sigma Aldrich (99% purity). A calcium perchlorate solution (1% by weight) was nebulized using high purity nitrogen and droplets were deposited onto a hydrophobic quartz disk that was placed directly on a silver block in the environmental cell. The temperature of the silver block was controlled by a combination of cooling using a continuous flow of liquid nitrogen and resistive heating. Particle size diameters ranged from 1-40 \( \mu \text{m} \). Initially, high purity dry \( \text{N}_2 \) continuously flowed through the cell to ensure a dry environment with RH < 1%. To control RH in the cell, a separate stream of \( \text{N}_2 \) passed through a \( \text{H}_2\text{O} \) vapor bubbler before combining with the dry flow. After passing through the cell, the flow was directed to a frost point hygrometer for measurement of water vapor. Frost point measurements, specifically dewpoint, from the hygrometer and sample temperature measurements from the platinum resistance sensor allow us to determine the RH with respect to the sample. A diaphragm pump pulling at a rate of 1 L/min was attached to the outlet of the hygrometer to ensure a constant airflow through the cell regardless of any variability in flow rate through the \( \text{H}_2\text{O} \) vapor bubbler. For experiments above 253 K, \( \text{H}_2\text{O} \) vapor was increased or decreased stepwise (at intervals less than or equal to 1% RH) while temperature was held constant. For experiments below 253 K, temperature was varied while holding \( \text{H}_2\text{O} \) partial pressure constant. This process allowed for fine tuned RH control at lower temperatures. RH was varied slowly (≤ 1% RH/min) and then held at each value for several minutes until the vapor flow through the cell was constant (for step-wise experiments) and the Raman spectra of the individual particles did not change. The estimated uncertainty in the DRH and ERH values due to the accuracy of the instrumental apparatus is ± 1% RH. Uncertainty in the experimental data due to reproducibility of results is larger (± 1-5% RH). Error is reported as the standard deviation of multiple measurements with at
Figure 2.1: A schematic of the Raman microscope equipped with an environmental sample cell.
least three experiments per data point.

To probe the phase state and morphology of individual Ca(ClO₄)₂ droplets free from any substrate, droplets were levitated in an optical trap recently developed in our laboratory. Figure 3.4 shows a schematic of the experimental setup. The concept of optical levitation has been well established since the early 1970s [Ashkin, 1970] and is addressed in detail elsewhere [Dholakia and Zemánek, 2010]. In brief, two vertically counter-propagating laser beams generated from the second harmonic output (532 nm) of individual continuous-wave Nd:YAG lasers are focused into the center of a custom made aluminum flow tube (12 mm x 110 mm) to form the trapping site. Droplets are generated from an aqueous solution using a piezo-driven glass capillary device (not shown) with a 15 micron orifice (Microfab, MJ-APB-015) mounted horizontally above the trapping site. Once a droplet enters the trapping site, the radiation pressure from the lasers, as well as an upward-directed humidity controlled nitrogen gas flow, balance the force of gravity acting on the droplet, resulting in levitation. Laser power must be regulated to maintain the proper balance of forces (10-50 mW typical). Using Mie theory, the angular variation in scattered light intensity is used to provide sensitive measurements of the size of levitated calcium perchlorate particles as well as the particle phase and morphology.
Figure 2.2: A schematic of the experimental setup used for optical levitation and characterization. Lenses L1 and L2 focus the output of the diode-pumped solid-state (DPSS) Nd:YAG lasers. Relative humidity is monitored with probes RH1 and RH2. Images are collected with a microscope objective (MO), optical filter (OF) and CCD camera. For far-field images OF is a polarization filter. For bright-field images a white LED is focused by L3 onto the droplet and OF is a notch filter to remove the 532 nm laser scatter.
2.3 Results and Discussion

2.3.1 Typical Experiments

The DRH and ERH of Ca(ClO$_4$)$_2$ were measured under relevant martian temperature conditions (223-273 K). Figure 2.3 shows optical images and corresponding Raman spectra for an anhydrous Ca(ClO$_4$)$_2$ particle undergoing deliquescence at 273 K. Initially, the particle is dried at room temperature in the environmental cell and the anhydrous state is confirmed by the absence of features in the O-H stretching region (3550 cm$^{-1}$) of the Raman spectrum. As soon as the particle is cooled to 273 K, the RH increases to an immeasurable RH less than 1%, but high enough to observe small changes in the O-H stretching region. Immediately after water vapor is added to reach 1% RH, the particle begins to take up water and a solid-solid hydration phase transition is observed. The growth of the O-H stretch (3550 cm$^{-1}$), the shift in the ClO$_4^-$ stretch (990 cm$^{-1}$ to 946 cm$^{-1}$), and the change in the optical images indicate a phase change occurred. Due to the absence of Raman spectra in the literature for hydrated Ca(ClO$_4$)$_2$, we are not able to determine the exact hydration state. However, the Raman spectra confirms crystalline water of hydration is present. By 13% RH, both the Raman spectra and optical images suggest deliquescence is complete. Once deliquesced, the O-H stretch at 3550 cm$^{-1}$ broadens and the ClO$_4^-$ stretch shifts from 946 cm$^{-1}$ to 938 cm$^{-1}$. The shift to a lower wavenumber of the ClO$_4^-$ stretch upon deliquescence has been documented for other perchlorates [Gough et al., 2011; Miller and Macklin, 1985]; we observe the same trend. As the RH is increased above the DRH value, the O-H stretch increases in height and width as the particle absorbs additional liquid water, as demonstrated by the spectra in Figure 2.3. The ClO$_4^-$ stretch remains at 938 cm$^{-1}$, suggesting that deliquescence is complete. After deliquescence, the optical images show the particle increases in volume as RH is increased.

Efflorescence of Ca(ClO$_4$)$_2$ droplets was also studied at 273 K and an example is shown in Figure 2.4. As the RH is lowered from 71% RH, the optical images show that the particle
decreases in volume, but remains spherical in shape. In addition, the Raman spectra show the perchlorate peaks do not shift to the frequencies characteristic of a dry particle, even at 16% RH. When the RH is lowered to 1%, the ClO$_4^-$ stretch finally shifts from 938 cm$^{-1}$ to 946 cm$^{-1}$ signifying efflorescence. The crystalline phase formed is consistent with the hydrate observed in the early stages of the deliquescence experiments. In addition, the O-H stretch at 3550 cm$^{-1}$ loses the shoulder signature, indicating the particle is no longer aqueous. Thus, in this experiment at 273 K, efflorescence was observed at 1% RH.
Figure 2.3: Optical images and Raman spectra of a typical anhydrous Ca(ClO$_4$)$_2$ particle undergoing deliquescence at 273 K. This deliquescence experiment starts with a dry anhydrous Ca(ClO$_4$)$_2$ particle (black). As RH is increased to 1% RH (pink), water uptake is confirmed by the appearance of a large peak in the O-H stretching region and the shift of the ClO$_4^-$ peak from 990 cm$^{-1}$ to 946 cm$^{-1}$. Deliquescence occurs by 13% RH (blue), apparent by the broadening of the O-H stretch and shift of the ClO$_4^-$ peak from 946 cm$^{-1}$ to 938 cm$^{-1}$. As RH is increased to 50% RH (purple), no further shifts in the ClO$_4^-$ stretch are observed and the fully deliquesced particle continues to increase in volume.
Figure 2.4: Optical images and Raman spectra of a typical anhydrous Ca(ClO$_4$)$_2$ efflorescence experiment at 273 K. This efflorescence experiment starts with a deliquesced Ca(ClO$_4$)$_2$ particle at 71% RH (purple). At 16% RH (blue) the particle is smaller in volume, but still visually appears to be liquid. The shoulder in the O-H stretch at 16% is still present, with a slight shift in the ClO$_4^-$ stretch remaining in the liquid regime. By 1% RH (black), no visual changes are observed but a shift in the ClO$_4^-$ stretch to 946 cm$^{-1}$ and the disappearing shoulder in the O-H stretch region indicates the particle has effloresced.
To further investigate the low efflorescence RH of Ca(ClO$_4$)$_2$, efflorescence experiments were conducted by optically levitating aqueous droplets of Ca(ClO$_4$)$_2$, as well as Mg(ClO$_4$)$_2$ for comparison. The droplets were optically levitated in a humidity controlled flow cell and monitored continuously in the far-field while lowering the relative humidity at 298 K. A sequence of far-field images for a droplet Mg(ClO$_4$)$_2$ and a droplet of Ca(ClO$_4$)$_2$ are shown in Figures 2.5 and 2.6, respectively. In the far-field images, the elastically scattered laser light from a levitated particle will take the form of a series of linear horizontal interference fringes if the particle is homogenous (i.e. liquid), but will be chaotic and irregular if the particle is heterogenous (i.e. crystalline). For a process such as efflorescence, the transition from spherical liquid droplet to crystalline solid particle is quite distinct, as can be seen with Mg(ClO$_4$)$_2$ in Figure 2.5. The particle is clearly liquid (Figure 2.5a,b) until 15% RH at which point efflorescence occurs (Figure 2.5c). This value, at 298 K, is in close agreement with Gough et al. [2011] who reported an efflorescence RH of 19% in the 223-273 K range for Mg(ClO$_4$)$_2$ deposited on a plate. No such efflorescence transition is observed for the optically levitated Ca(ClO$_4$)$_2$ droplets as shown in Figure 2.6. Even when exposed to RH levels less than 1% for over one hour, the particle remains homogeneous and spherical. This is evident by the persistence of the linear interference fringes characteristic of a liquid particle. Therefore, optically levitated Ca(ClO$_4$)$_2$ droplets can persist in the liquid phase, even under extremely dry conditions.
Figure 2.5: Images of the far-field elastically scattered laser light from a single optically levitated 10 µm Mg(ClO$_4$)$_2$ microparticle at 298 K. This efflorescence experiment demonstrates the significant changes that occur when salt efflorescence occurs. a) The aqueous particle at 36% RH. b) The aqueous particle at 15% RH. c) The effloresced particle at 15% RH. Efflorescence occurred between images b and c (<0.04 seconds) at 15% RH. Note that the imaged laser scatter takes on a circular shape due to the back aperture of the camera collection optics and is not indicative of the particle morphology.
Figure 2.6: Images of far-field elastically scattered laser light from a single optically levitated aqueous Ca(ClO$_4$)$_2$ microdroplet at 298 K. The particle is 10 µm in size. The images show crystallization of the Ca(ClO$_4$)$_2$ particle did not occur and the particle remained aqueous at extremely low RH. Time at RH $<2\%$ was two hours with a total observation time of six hours.
Deliquescence and efflorescence of Ca(ClO$_4$)$_2$ were also studied at low temperatures. Figure 2.7 demonstrates a typical deliquescence experiment at 253 K. This experiment began with an anhydrous Ca(ClO$_4$)$_2$ crystal particle at 253 K. The optical image shows a salt crystal and the Raman spectrum indicates that no H$_2$O is present. As RH is increased, spectral and visual changes suggest that a hydrated salt forms by 9% RH. In contrast to cases at 273 K, different sharp features indicative of crystalline water in a hydrate appear in the Raman spectra in the O-H stretch region (3500 cm$^{-1}$). There is also a peak shift from 990 cm$^{-1}$ to 958 cm$^{-1}$ in the ClO$_4^-$ stretch region. The difference in the spectral features may suggest a different hydrate formed. Lower temperatures typically correlate to the formation of a more hydrated crystalline particle and a similar trend is observed with other salts [Wise et al., 2012]. In addition, the optical microscope images show a highly scattering crystalline particle not seen at 273 K. Alternatively, the hydrate may be the same as the 273 K cases with the difference in spectral and optical features explained by temperature dependence. By 26% RH, optical images suggest deliquescence into an aqueous solution. The O-H stretching feature broadens and the ClO$_4^-$ stretch shifts to 938 cm$^{-1}$, confirming the deliquescence phase change. The same deliquescence behavior is observed at temperatures down to 223 K.

At temperatures below 263 K, three different efflorescence regimes were observed. Table 2.1 summarizes the change in observed Raman ClO$_4^-$ shift in different temperature regimes, indicating different hydration states (or temperature dependence) observed in the efflorescence experiments. As mentioned previously, further research needs to be done to identify the hydration states that are formed in each temperature regime.

Table 2.1: ClO$_4^-$ shift

<table>
<thead>
<tr>
<th>Temperature</th>
<th>ClO$_4^-$ Shift Upon Efflorescence</th>
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<tbody>
<tr>
<td>263 K - 273 K</td>
<td>938 cm$^{-1}$ to 946 cm$^{-1}$</td>
</tr>
<tr>
<td>253 K - 243 K</td>
<td>938 cm$^{-1}$ to 958 cm$^{-1}$</td>
</tr>
<tr>
<td>233 K - 222 K</td>
<td>No shift observed</td>
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Figure 2.7: Optical images and Raman spectra of Ca(ClO$_4$)$_2$ deliquescence at 253 K. The spectra are offset for clarity. Starting with anhydrous salt (black), formation of a hydrated salt by 9% RH (green) is observed. The deliquescence of this hydrate occurs by 26% RH (blue). At 50% RH, a broad O-H stretch is observed in the spectra while a visual increase in particle size (purple).
In the temperature range of 273 K to 243 K, efflorescence was observed at extremely low RH (2-10% RH). At the lower end of this temperature range, efflorescence starts to occur at higher RH value of 13% ± 1%. In all cases, efflorescence to a hydrated salt occurs.

In the lowest temperature range of our experimental results (223 K - 233 K), the Raman signatures of efflorescence are not observed. Rather, as RH is lowered, the Raman spectra indicate a decrease in the amount of water as the shoulder in the O-H stretch decreases in width. In addition, at 14% RH, visual images indicate that precipitates are forming in the particle. This is shown in Figure 2.8 by the optical image (top) and the 3D representation of pixel brightness (bottom). As the precipitates start to form at 14% RH, there is a change in the central brightness peak in the 3D image. The heterogeneous distribution of pixel brightness in the 3D image verifies the formation of precipitates. However, the ClO$_4^-$ peak (938 cm$^{-1}$) does not shift. To further investigate the efflorescence of Ca(ClO$_4$)$_2$ at these low temperatures, the particles containing precipitates were exposed to increasing RH again and the particles demonstrated uptake of water at an RH 15% lower than the deliquescence observed with the hydrated crystalline particle. This result indicates that the particles with precipitates had not fully effloresced. We believe the particles started to crystallize, but the low temperature conditions increased the viscosity of the solution and slowed water diffusion; therefore, full efflorescence did not occur. To test this hypothesis, we held the particle with precipitates under constant low temperature and RH conditions for 30 minutes and no change in the Raman spectra or optical image was observed. Kinetic inhibition may play a significant role at these low temperatures.
Figure 2.8: Representative example of a Ca(ClO$_4$)$_2$ 20 µm particle efflorescing at 14% at 253 K. At 50% RH, the particle is fully deliquesced. As RH is lowered, the particle visually decreases in size at 26%, but remains in the liquid phase. At 14%, the images show the formation of precipitates in the particle but complete efflorescence did not occur. The bottom image set is a 3D representation of pixel brightness. As the precipitates start to form at 14%, there is a change in the central brightness peak and the formation of precipitates is verified by the heterogenous distribution of pixel brightness.
2.3.2 Compilation of Experiments

Experiments on the deliquescence, efflorescence, and solid-solid phase transitions were performed for the temperature range 223 K to 273 K. The deliquescence and efflorescence results are summarized in Figure 2.9. A calculated thermodynamic stability diagram for the Ca(ClO$_4$)$_2$ + H$_2$O system is also included in this figure and suggests there are three hydration states of Ca(ClO$_4$)$_2$: the anhydrous, tetrahydrate and octahydrate forms [Dobrynina et al., 1984; Pestova et al., 2005]. To arrive at these results, we first determined the brine-ice equilibrium line using previously published Pitzer parameters [Marion et al., 2010]. Although this step is not absolutely necessary, we use this approach to verify that the line (and thus Pitzer parameters) fit previous experimental data. In a temperature verse water activity environment, the ice line is constant and not dependent on the type of salt used. For the ice line we used the data from Pestova et al. [2005] because the eutectic from Dobrynina et al. [1984] reports a eutectic of 212 K; a result that is too high compared to the ice line and thus leads to abnormal Pitzer parameters. We use a combination of both datasets for the salts’ hydrates and brine equilibria as they both converge to the same values. We first use a second order polynomial fit of the data and convert the concentration of salt (in weight percent) into water activity using the Pitzer model. Then, the temperature verse concentration fit curve is plotted in a temperature verse water relative humidity space.

In Figure 2.9, the black line represents the ice line calculated from the Pitzer parameters. The calculated phase transition boundaries are shown for the three expected hydration states, anhydrous Ca(ClO$_4$)$_2$, Ca(ClO$_4$)$_2$•4H$_2$O and Ca(ClO$_4$)$_2$•8H$_2$O, in orange, green, and blue lines, respectively. The solid lines represent where the hydrates are predicted to deliquesce and the dashed lines represent metastable deliquescence of the hydrate. The model indicates higher hydration states are expected to be less deliquescent. The symbols represent average experimental DRH and ERH values of at least three experiments with error bars representing the standard deviation of the dataset.
Figure 2.9: The calculated phase transition boundaries are plotted on a stability diagram for the Ca(ClO$_4$)$_2$ + H$_2$O system for the expected hydration states, anhydrous Ca(ClO$_4$)$_2$ (orange), Ca(ClO$_4$)$_2$$\cdot$4H$_2$O (green) and Ca(ClO$_4$)$_2$$\cdot$8H$_2$O (blue). The solid lines represent thermodynamically predicted stable phase transitions with dashed lines representing the metastable transitions. Experimental DRH (circles) and ERH values (triangles) are plotted with the green shaded region indicating where hydrate formation (between 1-10%) was observed during deliquescence experiments. The size of the triangle is representative of the number of times it was observed. The red lines indicate the efflorescence range observed in the 243 K to 253 K range. The dark blue shaded region represents the stable aqueous phase of Ca(ClO$_4$)$_2$, while the light blue shaded region expands to the metastable aqueous phase of Ca(ClO$_4$)$_2$. 
At all temperatures, we observed a solid-solid phase transition to form a hydrate prior to deliquescence. The green shaded region in Figure 2.9 indicates hydrate formation was observed at all temperatures studied. Hydrates formed in very low RH conditions, but additional experiments need to be performed to accurately quantify the relationship between temperature, RH, and hydrate formation. Hydrated Ca(ClO$_4$)$_2$ DRH values increase from an average of 10% ± 4% RH at 273 K to 55 ± 4% RH at 223 K. The shaded dark blue region represents the stable aqueous Ca(ClO$_4$)$_2$ phase region based on the model and experimental results. Experimental results of deliquescence are generally in agreement with the model. Deliquescence of the hydrate occurs at higher RH as temperature decreases and follows the general model trend; however, below 233 K there is a discrepancy between the predicted liquid-phase transition line for Ca(ClO$_4$)$_2$•8H$_2$O and the experimental results. It’s not clear if this difference is due to model or experimental error, but could be explained by a few experimental conditions. If the particles viscosity increases as temperature decreases, the water vapor diffusion rate would decrease requiring more time to fully deliquesce. In addition, large particle sizes might bias the results requiring more time to reach thermodynamic equilibrium. Both cases would suggest the experimental results are an upper DRH limit.

Figure 2.9 also shows Ca(ClO$_4$)$_2$ efflorescence RH (triangles) as a function of temperature. It can be seen that under all temperature conditions, efflorescence is found to occur at RH values well below the DRH. The measured ERH values do not have a clear temperature dependence, with an average value of 15± 4% RH from 223 K to 263 K; however, at temperatures above 243 K, the ERH was found to be less than 1% RH. This is in agreement with our levitated droplet experiments at 298 K showing no efflorescence at <1% RH over several hours of observation time. At temperatures of 243 K and above, there are two efflorescence outcomes and the size of the symbol in Figure 2.9 represents the relative number of times each case was observed. The extended light blue shading represents the metastable aqueous region based on the upper limit of the experimental data.

The efflorescence results confirm that all perchlorate salts studied, thus far, exhibit a
significant hysteresis effect during crystallization; thus, Ca(ClO$_4$)$_2$ readily forms supersaturated, metastable aqueous solutions. We find that Ca(ClO$_4$)$_2$ is the most deliquescent salt under martian conditions studied to date with extremely low ERH values. Such low ERH values have important implications for the martian water cycle and surface-atmosphere interactions on present-day Mars. Because perchlorates have been detected at most landing sites, this further supports the idea that aqueous briny solutions are capable of existing at current Mars relevant temperatures and relative humidities.

2.4 Martian Implications

We investigate the stability of briny solutions on Mars by using a combination of surface simulations, subsurface simulations, and our experimental DRH and ERH values for Ca(ClO$_4$)$_2$. Previous investigations quantitatively showed perchlorate salts could allow for several hours of briny solutions under current martian atmospheric conditions [Gough et al., 2011; Chevrier and Rivera-Valentin, 2012]. Our experimental results allow us to expand these predictions based on the phase transition behavior of Ca(ClO$_4$)$_2$ at relevant martian surface temperatures. Given there are areas on Mars where the surface RH varies from 0-100% throughout a diurnal cycle [Savijarvi, 1995; Zent et al., 2010], it is likely that Ca(ClO$_4$)$_2$ salts could deliquesce, providing a mechanism for aqueous solution formation under present-day martian conditions. The blue shaded region in Figure 2.9 suggests that at temperatures below 273 K, the extreme hygroscopic nature of the anhydrous Ca(ClO$_4$)$_2$ allows for stable solutions between 5-55% and metastable solutions to persist down to 1% RH. This indicates that brine solutions could be stable on the martian surface for a longer period of time than described in previous literature [Gough et al., 2011].

To investigate the formation and stability of Ca(ClO$_4$)$_2$ brines on Mars, we compare our experimental results to simulated temperature and RH conditions for the martian surface and subsurface in Figure 2.10. Simulations of surface ground temperature and RH based on model results (blue line) from Savijarvi [1995] were compared to our experimental data. The diurnal
surface temperature and RH profile (1.6 m above the surface) are representative of a sol in the summer season at the Viking 1 landing site. In this example, the temperature and RH conditions for the stable aqueous phase of anhydrous or hydrated Ca(ClO$_4$)$_2$ particle could occur in the early morning hours. Once the aqueous solution is present, a Ca(ClO$_4$)$_2$ liquid solution could persist for a few hours and would likely effloresce into a crystalline hydrate solid by 09:00. In the late evening hours, 21:00, the stable liquid phase is favored for a few hours until H$_2$O ice is formed; the evening curve is at temperatures below our experimental limits. The extremely low ERH of Ca(ClO$_4$)$_2$ at warmer temperatures indicates this briny solution could persist during the warmest and driest period of the Martian sol, but it is more likely that the particle will effloresce into a crystalline hydrate before it reaches warmer temperatures where low ERH values are favored. When compared to NaClO$_4$, presented in Gough et al. [2011], a Ca(ClO$_4$)$_2$ brine may provide an additional two hours of aqueous stability for martian surface temperature and RH conditions.

Perchlorates are also reported down to 5 cm in depth at the Phoenix landing site [Cull et al., 2010; Hecht et al., 2009] so Ca(ClO$_4$)$_2$ solution stability was also examined for the martian subsurface environment. Atmosphere-regolith transport processes were simulated by coupling a heat and mass transfer model for the regolith [Chevrier and Rivera-Valentin, 2012; Kereszturi and Rivera-Valentin, 2012; Rivera-Valentin, 2012] and the Planetary Boundary Layer (PBL) [Rivera-Valentin, 2012], which follows the construct of Savijarvi [1999]. Conditions are modeled for the Phoenix landing site (68.2N, 234.3E) throughout the year and sols 8, 31, and 44 of the mission are specifically analyzed since droplets were observed on the lander legs during these sols [Renno et al., 2009]. The Phoenix lander found a shallow ice table whose depth ranged between 5 and 18 cm [Hecht et al., 2009; Smith et al., 2009] with typical depths near 5 cm [Hecht et al., 2009; Mellon et al., 2009]; here we assume an ice table depth of 10 cm. Soil thermal properties are derived from Zent et al. [2010] and assumed constant with temperature. Mass transfer in the regolith column is simulated following diffusion-advection [Urbich, 2009] assuming a porosity of 0.5 [Zent et al., 2010] and
tortuosity of 2 [Hudson and Aharonson, 2008; Sizemore and Mellon, 2008]. The PBL is modeled as a 1-D atmospheric slab 1 km thick with a mass conservation boundary condition at the atmosphere-regolith interface.

The pink line in Figure 2.10 presents a diurnal temperature and RH profile for sol 31 at 3 cm depth. The other simulated sols (8, 44) produced similar results. To begin, the model results from the surface are compared to Phoenix humidity measurements [Smith et al., 2009]. The surface model RH results are in good agreement, indicating an average RH during the day of 5% while reaching 100% in the evening and temperature measurements indicating variation from 200 to 255 K. Figure 2.10 demonstrates that deliquescence would occur at approximately the same time as we observe with surface model results, but the warmer temperature and higher relative humidity conditions in the subsurface allow additional metastability for Ca(ClO$_4$)$_2$ aqueous solutions. These conditions are expected due to the lower thermal and vapor diffusion in the subsurface environment. In contrast to the results from the surface simulations [Chevrier et al., 2009; Gough et al., 2011; Savijarvi, 1995; Zorzano et al., 2009], conditions in the subsurface suggest that metastability could persist for up to 8 hours of the martian sol.

Based on the subsurface model results, we conducted a martian diurnal cycle experiment to better predict the stability of Ca(ClO$_4$)$_2$ aqueous solutions in the martian subsurface. We chose to simulate the subsurface environmental conditions as opposed to the surface conditions, based on the extended regions of aqueous phase stability. The subsurface model predictions span a temperature and RH regime that includes stable liquid, metastable liquid, and crystalline states of Ca(ClO$_4$)$_2$. Table 2.2 provides the heating rate calculated based on the hourly subsurface temperature changes simulated for sol 31 at a 3 cm depth for the Phoenix landing site. The laboratory experiment was performed over a 17-hour period, stopping briefly at each hour time step to collect Raman spectra and a microscopic image. The experiment began with a deliquesced Ca(ClO$_4$)$_2$ particle at 222 K and 57% RH, representative of the subsurface environment at 09:00. The H$_2$O vapor flow was held constant
Figure 2.10: Stability diagram for the Ca(ClO$_4$)$_2$ + H$_2$O system with a simulated surface diurnal profile (dark blue line) [Savijarvi, 1995] and subsurface diurnal profile (pink line) where temperature and RH data are based on simulated results at the Phoenix landing site (depth=3 cm). The solid lines represent thermodynamically predicted stable phase transitions with dashed lines representing metastable phase transition (orange anhydrous Ca(ClO$_4$)$_2$, green Ca(ClO$_4$)$_2$•4H$_2$O and light blue Ca(ClO$_4$)$_2$•8H$_2$O). The blue shaded region represents the upper limit of the stable and metastable aqueous phase of Ca(ClO$_4$)$_2$. This plot suggests that Ca(ClO$_4$)$_2$ can remain in the liquid phase for a few hours in the morning and evening hours on the surface. In the simulated subsurface environment, Ca(ClO$_4$)$_2$ may remain in the liquid phase for up to 8 hours of the martian sol.
while the temperature was increased at the rates indicated in Table 2 (supplemental). At 16:00, the temperature was held constant for one hour at the peak predicted temperature (lowest RH) and then the sample was cooled starting at 17:00. The experiment ended at 01:00 with a temperature of 220 K and 67% RH, conditions that predict ice formation based on the theoretical results shown in Figure 2.10.

Figure 2.11 shows visual images and Raman spectra of a Ca(ClO$_4$)$_2$ particle at the simulated 09:00, 17:00, 20:00, 23:00, and 01:00 timestamps. The black line at 57% is the starting point of the experiment and shows a broad O-H stretch and a ClO$_4^-$ centered at 938 cm$^{-1}$. As the temperature is increased and 8% RH (orange) is reached at 20:00, the O-H stretch is not as broad indicating a decrease in liquid H$_2$O, but the ClO$_4^-$ stretch remains at 938 cm$^{-1}$. The signature split O-H peak, typically observed in this temperature range for a hydrate, is also not present in the Raman spectra. As the particle is cooled and reaches 13% RH (teal) simulating 20:00, the O-H stretch starts to broaden, indicating uptake of liquid water before the predicted DRH. The shoulder in the O-H stretch continues to grow as RH is increased to 40% (pink). At 01:00, 67% RH (purple) is reached and the O-H stretch continues to broaden while the ClO$_4^-$ stretch remains at 938 cm$^{-1}$. Figure 2.12 summarizes the liquid stability region as observed in the diurnal experiment over a martian sol, with the simulated subsurface temperature (dashed) and RH (solid) profiles. This experiment demonstrates Ca(ClO$_4$)$_2$ can remain aqueous up to 17 hours when exposed to Mars subsurface temperature and relative humidity conditions over a martian diurnal cycle.

The presence of liquid spheroids on the Phoenix lander strut [Renno et al., 2009] and Recurring Slope Lineae (RSL) [McEwen et al., 2011, 2014] may be at least partially explained by aqueous solutions that contain Ca(ClO$_4$)$_2$. A better understanding of the RH and temperature conditions in the martian surface and subsurface at different times of day or year and at different geographic locations are needed to accurately predict the duration of these aqueous solutions in order to apply this work to RSL.
### Table 2.2: Experimental Diurnal Cycle Heating Rates

<table>
<thead>
<tr>
<th>Martian Time (Hr)</th>
<th>Rate (K/min)</th>
<th>Temperature (K)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>–</td>
<td>222</td>
<td>57</td>
</tr>
<tr>
<td>10:00</td>
<td>0.07</td>
<td>226</td>
<td>38</td>
</tr>
<tr>
<td>11:00</td>
<td>0.07</td>
<td>230</td>
<td>26</td>
</tr>
<tr>
<td>12:00</td>
<td>0.08</td>
<td>235</td>
<td>16</td>
</tr>
<tr>
<td>13:00</td>
<td>0.03</td>
<td>237</td>
<td>13</td>
</tr>
<tr>
<td>14:00</td>
<td>0.05</td>
<td>240</td>
<td>10</td>
</tr>
<tr>
<td>15:00</td>
<td>0.02</td>
<td>241</td>
<td>9</td>
</tr>
<tr>
<td>16:00</td>
<td>0.02</td>
<td>242</td>
<td>8</td>
</tr>
<tr>
<td>17:00</td>
<td>0.00</td>
<td>242</td>
<td>8</td>
</tr>
<tr>
<td>18:00</td>
<td>-0.02</td>
<td>241</td>
<td>9</td>
</tr>
<tr>
<td>19:00</td>
<td>-0.03</td>
<td>239</td>
<td>11</td>
</tr>
<tr>
<td>20:00</td>
<td>-0.05</td>
<td>236</td>
<td>13</td>
</tr>
<tr>
<td>21:00</td>
<td>-0.07</td>
<td>233</td>
<td>18</td>
</tr>
<tr>
<td>22:00</td>
<td>-0.07</td>
<td>229</td>
<td>26</td>
</tr>
<tr>
<td>23:00</td>
<td>-0.07</td>
<td>225</td>
<td>40</td>
</tr>
<tr>
<td>24:00</td>
<td>-0.07</td>
<td>222</td>
<td>54</td>
</tr>
<tr>
<td>01:00</td>
<td>-0.07</td>
<td>220</td>
<td>67</td>
</tr>
</tbody>
</table>
Figure 2.11: Optical images and Raman spectra (offset for clarity) of a Ca(ClO$_4$)$_2$ particle throughout the 17 hour subsurface diurnal cycle experiment with a 15 µm particle. The black line indicates the starting point at 09:00 of the experiment at 57% where a broad O-H stretch is observed with a ClO$_4^-$ peak centered at 938 cm$^{-1}$. At 8% RH (orange, 17:00), the particle visually decreases in size and the O-H stretch narrows while the ClO$_4^-$ peak does not shift. By 13% RH (teal, 20:00), the O-H stretch begins to broaden. As RH continues to increase to 40% RH (pink, 23:00), the O-H stretch continues to grow. At the end point of the experiment, 01:00 (67%, purple), the optical image shows particle growth and a broad O-H stretch. In addition, the particle’s ClO$_4^-$ stretch did not shift for the entirety of the experiment, suggesting the particle remained in the liquid phase for 17 hours in martian subsurface temperature and RH conditions.
Figure 2.12: Subsurface (depth=3 cm) temperature (dashed line) and RH (solid line) plotted over a diurnal cycle. Based on experimental results, the subsurface environment at the Phoenix landing site could allow aqueous Ca(ClO$_4$)$_2$ solutions to be stable for several hours; up to 17 hrs.
2.5 Conclusion

In an effort to move beyond model dependent interpretation of briny aqueous solutions, this work evaluates phase change processes in a simulated Mars environment. We have experimentally examined the deliquescence and efflorescence properties of Ca(ClO$_4$)$_2$ under relevant martian temperatures (223 to 273 K). A very low DRH of 5% for Ca(ClO$_4$)$_2$ at 273 K could allow for formation of aqueous solutions under very dry environmental conditions. In addition, over the temperature range studied, all hydration states of Ca(ClO$_4$)$_2$ deliquesce at $\leq$55% RH.

We also found that the solutions remain liquid until very low RH values. Optical levitation experiments at 298 K demonstrate Ca(ClO$_4$)$_2$ particles do not effloresce at $<2\%$ RH, even over the timescale of two hours. At 273 K, the ERH is $3 \pm 2\%$ and is, on average, less than $15 \pm 4\%$ for all temperatures studied. The experimental results were compared to a thermodynamic model for three hydration states of Ca(ClO$_4$)$_2$. As predicted, the higher hydration states were less deliquescent and the experimental results are generally in good agreement with the model results. When applied to RH models, Ca(ClO$_4$)$_2$ aqueous solutions could persist for 3-4 hours on the surface and up to 8 hours in the subsurface. The subsurface diurnal cycle experiment demonstrated Ca(ClO$_4$)$_2$ aqueous solutions can persist for a majority of the martian sol; 17 hours under Mars-relevant diurnal temperature and RH cycles. The detection of perchlorates, specifically the potential for Ca(ClO$_4$)$_2$ at the MSL landing site, impacts surface-atmosphere interactions and the water cycle on Mars. Ca(ClO$_4$)$_2$ provides an avenue for the stable and metastable aqueous solutions to exist in present-day martian temperature and relative humidity conditions.
Chapter 3

Water Uptake by Mars Salt Analogs: An Investigation of Stable Aqueous Solutions Using Raman Microscopy

3.1 Introduction

Briny aqueous solutions are the favored explanation for possible liquid water features observed on present-day Mars due to the stability of brines in low temperature conditions. Examples of such features are subsurface thin film liquids [Cull et al., 2010], spheroids on the Phoenix lander leg [Renno et al., 2009] and recurring slope lineae (RSL) [McEwen et al., 2011, 2014; Ojha et al., 2014]). Understanding liquid water stability on Mars has important implications for the present-day water cycle, astrobiology, planetary protection concerns and thus, future exploration missions to Mars. However, given the low pressure, temperature, and relative humidity conditions on Mars, pure liquid water is not stable [Haberle et al., 2001; Ingersoll, 1970]. Salts allow for a pathway for stable and metastable aqueous solutions to exist in martian conditions. Perchlorate and chloride salts are known to be present in the martian soil [Hecht et al., 2009]. These salts readily absorb water vapor from the atmosphere and deliquesce (transition from a solid crystalline into an aqueous solution) under Mars relevant temperature and relative humidity (RH) conditions [Chevrier et al., 2009; Gough et al., 2011; Nuding et al., 2014]. Perchlorates and chloride salts are not only interesting for their highly deliquescent properties, but also for their ability to persist as supersaturated, metastable solutions [Gough et al., 2011, 2014; Nuding et al., 2014].

Past work has shown that Ca(ClO₄), NaClO₄ and Mg(ClO₄)₂ are highly deliquescent
salts that form aqueous solutions at humidity values as low as 13% RH, 42% RH, and 51% RH, respectively, at 273 K [Gough et al., 2011; Nuding et al., 2014]. Deliquescence was observed to the instrumental lower temperature limit of 223 K for all perchlorates studied thus far, with a general trend of increasing deliquescent RH with decreasing temperature. A significant hysteresis effect was observed during efflorescence (transition from aqueous solution to solid crystalline) of these salt solutions, expected due to the kinetic inhibition of crystal nucleation. The efflorescence relative humidity is salt dependent. Metastable Ca(ClO$_4$)$_2$ solutions persist down to 1% RH at temperatures as low as 243 K [Nuding et al., 2014]. Efflorescence of NaClO$_4$ and Mg(ClO$_4$)$_2$ is less dependent on temperature, with ERH values of 13% RH and 19% RH, respectively [Gough et al., 2011]. These laboratory studies indicate that perchlorate salts could exist as stable or metastable aqueous solutions over a wide range of martian RH and temperature conditions.

Although many individual salts have now been well characterized, it is important to quantify the effects of other cations and anions on the stability of aqueous solutions. Gough et al. [2014] demonstrated that NaClO$_4$/NaCl and Mg(ClO$_4$)$_2$/MgCl$_2$ mixtures form stable aqueous solutions above 38% and 28% RH, respectively, at temperatures as low as 243 K. These results are independent of composition (perchlorate to chloride ratio) and reported to be 2-5% lower than the most deliquescent pure component in the mixtures. This work revealed that even if a less deliquescent salt is present in a brine mixture, a stable aqueous phase can form below the full deliquescent RH for the mixture. The hysteresis effect was also observed in these mixtures, allowing for metastable solutions to persist down to 5% RH for Mg(ClO$_4$)$_2$/MgCl$_2$ and 13% for NaClO$_4$/NaCl. The metastability of more complex mixtures, specifically Mars soil analogs, was recently studied by Toner et al. [2014]. They found significant supercooling of bulk Mars salt solutions, in some cases to temperatures as low as the glass transition temperature.

In this present work, we examine how multiple species in a Mars salt analog respond to changes in RH. We have developed a salt analog so called, ‘Instant Mars’ to closely match the
individual cation and anion concentrations as reported by the Wet Chemistry Laboratory aboard the Phoenix Lander [Hecht et al., 2009; Kounaves et al., 2010]. ‘Instant Mars closely replicates the measured relative concentrations of magnesium, calcium, potassium, sodium, perchlorate, chloride, and sulfate ions. Several cases of in-situ and remote sensing detection of salts on Mars [Hecht et al., 2009; Kounaves et al., 2010; Langevin, 2005; Murchie et al., 2009; Osterloo and Anderson, 2010; Ruesch et al., 2012], suggests that these ions may coexist. This work investigates the water uptake and loss properties of the Instant Mars salt analog to quantify the effects of complex brine solutions.

3.2 Experimental Setup

3.2.1 Instant Mars Solution

The Instant Mars solution was created based on the reported averages of soluble salt ion concentrations at the Phoenix landing site as measured by the Wet Chemistry Laboratory from Hecht et al. [2009] and Kounaves et al. [2010]. The composition of our Instant Mars analog and results from the Phoenix landing site are compared in Figure 3.1. NaSO₄, KCl, Ca(ClO₄)₂, MgSO₄, and MgCl₂ were purchased from Sigma Aldrich at 99% purity. The compounds were combined such that the cations and anions closely matched the reported relative concentrations (mole %). Carbonate was not added when formulating the Instant Mars analog due to its lower solubility in water. To generate particles that are compositionally consistent, the initial solution must be homogenous. If carbonate was left undissolved in the solution, each particle produced with the droplet generator would not be identical in composition. The common carbonate salts reported on Mars (Ca, Mg, Na, and K) [Bandfield, 2003; Ehlmann et al., 2008; Morris et al., 2010] have relatively high eutectic temperatures and would likely not contribute to the formation of brines. However, it is still important to include salts with high eutectic temperatures that are not known to be highly deliquescent to investigate their impact on highly deliquescent salts with low eutectic tem-
peratures (ClO$_4^-$). MgSO$_4$ acts as this representative salt without introducing experimental uncertainty due to undissolved solids.

3.2.2 Raman Microscope and Optical Trap

The impact of relative humidity on the size, spectral characteristics, and phase of Instant Mars particles was studied using a Raman microscope equipped with an environmental cell. The experimental setup is shown in Figure 3.2 and described in detail in Baustian et al. [2010]. Briefly, a Nicolet Almega XR Dispersive Raman spectrometer was outfitted with a Linkam THMS600 environmental cell, a Linkam automated temperature controller, and a Buck Research chilled-mirror hygrometer. Samples were produced using a MicroJet droplet generator (40-50 µm diameter particles) or a nebulizer with high purity nitrogen (10-30 µm diameter particles). The mixed salt particles were deposited onto a hydrophobic quartz disc. The sample was placed directly on a silver block in the environmental cell and cooled to 253 K. The temperature of the silver block was controlled by a combination of cooling using a continuous flow of liquid nitrogen and resistive heating. Initially, high purity dry N$_2$ continuously flowed through the cell to ensure a dry environment with RH < 1%. To control RH in the cell, a separate stream of N$_2$ passed through a H$_2$O vapor bubbler before combining with the dry flow. After passing through the cell, the flow was directed to a hygrometer for measurement of water vapor. The dewpoint measurement from the hygrometer and sample temperature measurements from the platinum resistance sensor allow determination of the RH at the sample. A diaphragm pump pulling at a rate of 1 L/min was attached to the outlet of the hygrometer to ensure a constant airflow through the cell regardless of any variability in flow rate through the H$_2$O vapor bubbler. For experiments above 253 K, H$_2$O vapor was increased or decreased stepwise (at intervals less than or equal to 1% RH) while temperature was held constant. For experiments below 253 K, temperature was varied while holding H$_2$O partial pressure constant. This process allowed for fine tuned RH control at lower temperatures. RH was varied slowly (≤ 1% RH/min) and then held at each value for several
Figure 3.1: Instant Mars solution developed to closely match the soluble component of the martian regolith as measured by the Wet Chemistry Laboratory instrument on board the Phoenix Lander.
minutes until the vapor flow through the cell was constant. The estimated uncertainty of the RH values due to the accuracy of the instrumental apparatus is ±1% RH. Uncertainty in the experimental data due to reproducibility of results is larger. Error is reported as the standard deviation of multiple measurements with at least three experiments per data point.

The spatial resolution of the Raman microscope is one micrometer and thus different portions of the same particle can be sampled. Figure 3.3 is a 2D Raman map of an Instant Mars particle at 298 K and 0% RH. Each panel represents a different Raman wavenumber region, with warm colors representing high Raman intensities. Figure 3.3 clearly demonstrates that the particles contain multiple species and have a heterogeneous distribution of \( \text{ClO}_4^- \) and \( \text{SO}_4^{2-} \) throughout. The distribution of \( \text{Cl}^- \) is unknown, as it is not detectible with Raman spectroscopy due to the lack of covalent bonds. Instant Mars starts out as a well-mixed solution free of precipitates assuring the overall ion concentration is the same in each particle generated. As the particle dries on the quartz disc, local inhomogeneities develop into heterogenous particles. The heterogenous distribution of salts throughout each particle resulted in a different spectral signature depending on exact location probed within the particle. Although each particle contained all ions in the mixture, the particles were not identical in their physical recrystallization. At the beginning of liquid water uptake, this heterogeneity became especially apparent as different regions of the particle absorbed water as the individual salts changed phase. To overcome these inconsistencies, overall particle size quantification became the main analysis technique for determining water uptake.

To probe the phase state and morphology of individual Instant Mars droplets free from any substrate, droplets were levitated in an optical trap at 298 K. Figure 3.4 shows a schematic of the experimental setup. The concept of optical levitation has been well established since the early 1970s [Ashkin, 1970] and is addressed in detail elsewhere [Dholakia and Zemánek, 2010]. In brief, two vertically counter-propagating laser beams generated from the second harmonic output (532 nm) of individual continuous-wave Nd:YAG lasers are focused into the center of a custom made aluminum flow tube (12 mm x 110 mm) to form
Figure 3.2: A schematic of the Raman microscope equipped with an environmental sample cell.
Figure 3.3: Two-dimensional Raman map of a 10 μm Instant Mars particle at 298 K and 0% RH. Each color map represents the intensity of the Raman peak at the labeled location, with warmer colors indicating higher Raman intensities and thus higher concentrations of that species. The high intensities over a majority of the particle in the O-H region and ClO$_4^-$ stretch centered at 961 cm$^{-1}$ indicates the particle is a crystalline hydrate. The ClO$_4^-$ map indicates the perchlorate is detected in the same regions as the O-H stretch. The SO$_4^{2-}$ stretch demonstrates a clear distribution throughout the particle.
the trapping site. Droplets are generated from an aqueous salt solution using a piezo-driven glass capillary device (not shown) with a 15 micron orifice (Microfab, MJ-APB-015) mounted horizontally above the trapping site. Once a droplet enters the trapping site, the radiation pressure from the lasers, as well as an upward-directed humidity controlled nitrogen gas flow, balance the force of gravity acting on the droplet, resulting in levitation. Laser power must be regulated to maintain the proper balance of forces (10-50 mW typical). Using Mie theory, the angular variation in scattered light intensity is used to provide sensitive measurements of the size of levitated Instant Mars particles as well as the particle phase and morphology.

By combining Raman microscopy and particle levitation techniques, we can characterize the phase transitions of the complex salt mixture we denote as Instant Mars. This work quantifies the RH at which liquid water uptake begins to occur when crystalline Instant Mars particles are exposed to increasing RH conditions. In addition, the RH at which liquid water is lost from aqueous Instant Mars particles is identified as RH is decreased. This allows us to report the conditions in which a supersaturated, metastable Instant Mars solution exists.
Figure 3.4: A schematic of the experimental setup used for optical levitation and subsequent phase/morphology characterization. Lenses L1 and L2 focus the output of the diode-pumped solid-state (DPSS) Nd:YAG lasers. Relative humidity is monitored with probes RH1 and RH2. Images are collected with a microscope objective (MO), optical filter (OF) and CCD camera. For far-field images OF is a polarization filter. For bright-field images a white LED is focused by L3 onto the droplet and OF is a notch filter to remove the 532 nm laser scatter.
3.2.3 Data Analysis Techniques

3.2.3.1 Instant Mars Water Uptake

The deliquescence, or phase transition from a solid crystalline to an aqueous phase, of perchlorates and binary chloride/perchlorate mixtures has previously been quantified in the laboratory [Gough et al., 2011, 2014; Nuding et al., 2014]. When the relative humidity is increased around a solid crystalline phase, the salts in the particle will deliquesce when the phase transition is thermodynamically favored. If the relative humidity is increased further, the particle’s liquid water content will increase, the particle will grow in size, and become more spherical in shape. The growth of deliquesced particles can be detected in the optical images and used to quantify the RH values at which water uptake by Instant Mars particles began.

The size of Instant Mars particles was determined using image processing techniques, and subsequently used to evaluate initial water uptake. As mentioned previously, the physical heterogeneity in the Instant Mars particles made it difficult to utilize Raman spectroscopy to analyze initial water uptake. Optical images of each particle, taken at each RH step, were processed in a three-step analysis (Figure 3.5). First, to mitigate the effects of noise and internal particle structure, all images were smoothed using a Gaussian filter (Figure 3.5B) that convolves a kernel of a given pixel radius with the raw image. Second, the smoothed images were thresholded to highlight and identify the particles while simultaneously eliminating background (non-particle) details. A histogram of pixel values for each image was used to determine the best threshold value for each dataset. The third step converted the thresholded image from RGB to binary, to further isolate the area of interest (Figure 3.5C). Finally, particle detection utilized a function that selects circular components in the image based on a given size criteria. Figure 3.5D shows the final output of the particles selected with their pixel size and location identified. This output provided the method for quantifying particle growth at each RH step thus identifying the RH where initial water uptake
occurred.

Once the image taken at each RH step underwent the image processing, the dataset had to be reviewed to assure the particles incorporated into the final initial water uptake average were spherical. The image processing technique described above was developed specifically to parameterize circular features and is therefore optimized for spherical, or nearly spherical, particles. Irregular or non-spherical particles were excluded from the analysis since their characterization leads to unknown systematic error. The average initial water uptake is based on more than ten experiments with at least three particles per experiment analyzed. Overall, initial water uptake was observed at the same RH for all particles within each individual experiment. However, in two cases a difference in initial water uptake of 8% RH was observed for individual particles within an individual experiment in two cases.

3.2.3.2 Instant Mars Water Loss

A unique property of inorganic salts that is often observed is the hysteresis effect that occurs with the efflorescence phase transition from aqueous to solid particles. When RH is decreased, the aqueous salt solution does not transition back to a solid at the deliquescence RH value. Instead, salt crystallization is kinetically hindered allowing for a metastable, supersaturated solution to persist down to a lower RH than where deliquescence occurred. This has important implications for the existence and persistence of aqueous brine solutions in the present-day Mars environment.

To effectively quantify water loss characteristics of Instant Mars particles, two experimental techniques were used to define the range of initial water loss RH and final water loss RH. We were unable to utilize the automatic particle detection technique because the particles typically recrystallize into non-spherical shapes. The larger particles (40 µm) were large enough to visually identify initial salt recrystallization. For each experiment, we quantified the fraction of particles in the image that undergo visual precipitation of a solid phase.

In addition to the visual analysis, smaller Instant Mars particles (∼20 µm) were an-
Figure 3.5: The image processing technique used for Instant Mars water uptake analysis is shown from left to right (A-D). Starting with the original image (A) from the microscope, the noise effects and internal particle structure were minimized using a Gaussian filter (B). A histogram of pixel values for each image was used to identify the best threshold to eliminate background while simultaneously identifying the particle. The third step (C) converted the thresholded image from RGB to binary and removed the edges to avoid identifying particles out of the field of view in the dataset. The final step utilized the particle detection function based on circular component identification based on a size criteria. The final output is shown (D), highlighting the particles identified providing particle size and location in each image processed.
alyzed using a combination of Raman spectroscopy and optical images. A representative example of this water loss experiment at 243 K is shown in Figure 3.6. In this example an aqueous Instant Mars particle is exposed to high RH (greater than 95%). As RH is decreased, the particle decreases in size visually but the particle remains spherical until 64% RH. This is consistent with recrystallization RH results from the particle size analysis technique previously discussed. However, the ClO$_4^-$ stretch does not shift from the aqueous ClO$_4^-$ 938 cm$^{-1}$ peak location [Nuding et al., 2014], likely indicating the presence of aqueous perchlorate salts. As RH continues to decrease, the particle is no longer spherically shaped at 22% RH but the ClO$_4^-$ stretch remains at 938 cm$^{-1}$, suggesting the presence of liquid H$_2$O. In this example, the ClO$_4^-$ stretch shifts to 961 cm$^{-1}$ at 9% RH, indicating the particle is no longer aqueous. As the RH is lowered to 3% RH, the ClO$_4^-$ remains at 961 cm$^{-1}$ and confirms that the particle is completely crystallized. By combining the particle size analysis, optical images, and Raman spectra we can characterize the beginning of Instant Mars recrystallization and can also detect when the particle has fully transitioned into the solid phase.
Figure 3.6: A representative example of a water loss experiment at 243 K used to quantify the lower limit of metastable, supersaturated Instant Mars solutions. In this example an aqueous Instant Mars particle is first exposed to high RH (greater than 95%). The optical images show Instant Mars particles as RH is decreased with the colored boxes matching the Raman spectra lines. At 75% RH (black), the optical image shows an aqueous Instant Mars particle. The Raman spectra shows a peak centered at 938 cm$^{-1}$, the aqueous ClO$_4^-$ stretch. As RH is decreased, the particle visually decreases in sizes but the particle remains spherical until 64% RH. The ClO$_4^-$ stretch, however, does not shift. As RH continues to decrease, the particle is no longer spherical by 22% RH, but the location of the ClO$_4^-$ stretch remains at 938 cm$^{-1}$. In this example the ClO$_4^-$ stretch shifts to 961 cm$^{-1}$ at 9% RH, indicating the particle is no longer aqueous (reference!). As the RH is lowered to 3% RH, the ClO$_4^-$ remains at 961 cm$^{-1}$ and confirms that the particle is completely crystallized.
3.3 Results and Discussion

3.3.1 Raman Microscope

The water uptake properties of Instant Mars particles were characterized using particle size analysis. Figure 3.7 is an example of the particle growth as a function of increasing RH. As RH is increased from 1% to 56%, the particle size has no detectable change. After 64% RH is reached, a significant increase in particle size is observed for both particles in the image. This is characteristic of liquid water uptake, and the growth continues as RH increases. In this example, 64% RH represents the initial liquid water uptake point for the two particles shown. This is the highest RH value observed out of the 28 particles analyzed. Initial water uptake occurred at $56 \pm 5\%$ RH (1 SD) a value obtained by averaging 28 particles in the temperature range of 235-243 K. After this RH point, the amount of aqueous solution continued to increase but in a majority of the cases the particles never fully deliquesced, even at relative humidities as high as 95% RH. Regardless of the solid precipitates, Instant Mars particles displayed a rapid increase in size and a spherical particle shape; known characteristics of aqueous solutions. These experiments demonstrate that liquid water uptake is possible at relative humidities as low as many of the highly deliquescent single salts or binary salt mixtures previously examined [Chevrier et al., 2009; Gough et al., 2011, 2014; Nuding et al., 2014].

To understand the activity of metastable, supersaturated solution of Instant Mars particles that form when RH is lowered, a combination of optical and Raman microscopy were utilized. Figure 3.8 represents the percentage of observed initial recrystallization and the RH it occurred for the 56 particles analyzed by visual inspection. A majority of the particles (52%) exhibited initial recrystallization at an average RH of $64 \pm 3\%$. This initial recrystallization does not demonstrate a significant hysteresis effect, likely explained by a seed crystal initiating recrystallization hence not allowing a metastable, supersaturated solution to persist. Heterogeneous nucleation diminishes the energy barrier required for the solution to
Figure 3.7: A representative example of the particle growth as a function of increasing RH. As RH is increased from 1% to 56%, there is no detectable change in particle size. After 64% RH is reached, a drastic increase in particle size is observed for both particles. This is characteristic of liquid water uptake, and the growth continues as RH increases to 84% RH. In this example, 64% RH represents the initial water uptake point for the two particles shown and is thus used in the average RH water uptake point.
transition into the crystalline phase. However, this higher RH phase transition did not occur in all cases. Additionally, Raman spectra indicates that an aqueous phase is still present when partial visual recrystallization is identified. Partial recrystallization at $36 \pm 6\%$ RH was observed for $46\%$ of the $40 \mu m$ Instant Mars particles. More variability was observed in this fraction of particles, but is still within $\pm 6\%$ RH. Particles that underwent recrystallization at a lower RH can be explained by the hysteresis effect reported for several single salts \cite{Gough2011, Nuding2014} and two salt mixtures \cite{Gough2014}. One outlier was observed in the dataset, with an initial phase transition observed at $82\%$. Note in all cases, values reported with this technique are a measure of the upper limit of recrystallization humidity as particles still exhibit visual liquid characteristics until lower RH. Even after initial recrystallization, the particles continue to decrease in size and morphologically change as RH is decreased. We can characterize the lower limit of recrystallization by monitoring the Raman ClO$_4^-$ stretch. Although the particle clearly loses liquid water as RH is decreased, examination of the ClO$_4^-$ peak suggests the Instant Mars particle contains liquid water until the humidity is lowered to $13\pm 5\%$ between 235-243 K. This dramatic hysteresis effect indicates that Instant Mars particles can persist as metastable solutions at very low relative humidity values.
Figure 3.8: Larger particles (40 μm) were analyzed via visual inspection. The figure represents observed initial recrystallization percentage of particles observed and the RH it occurred for the 56 particles analyzed. A majority of the particles exhibited initial recrystallization at an average RH of 64 ± 3%. Initial recrystallization at 36 ± 6% RH was observed for slightly less than half of the Instant Mars particles. One outlier was observed in the dataset, with an initial phase transition observed at 82%.
3.3.2 Optical Levitation

To further investigate the interaction of water vapor with Instant Mars particles, we conducted optical levitation water uptake experiments at 298 K. This experimental setup allows for precise determination of initial water loss and complete phase transitions within the particle. Figure 3.9 is a representative experiment that began with an Instant Mars particle levitated at 79% RH. Solid particulate is evident by the slightly distorted interference fringes in the far-field although the droplet remains spherical and predominately liquid. As RH is decreased, the droplet begins to decrease in size suggesting liquid water loss but it remains in the liquid phase. By 27% RH, the particle starts to lose water and crystallize, apparent by the distinct waves in the far-field fringe lines. Further decreasing RH results in gradual loss of residual water, and complete recrystallization is observed at 19% RH. Little change is observed when RH is decreased to 8% RH. On average, Instant Mars particles effloresced at 18 ± 7% RH using the optical levitation technique. Figure 3.9B shows a representative experiment as RH increased. Water uptake was not observed until 41%, where the fringe lines start to take on the linear feature of an aqueous Instant Mars particle. By 48% RH, the particle is mostly aqueous and the particle continues to grow as the RH is raised to 81%. After initial water uptake, a portion of the particle remains solid. As RH continues to increase, the particle continues to gradually uptake water with solid particulate remaining up to 85%. The average initial water uptake for optically levitated particles at 298 K is 42 ± 8% RH based on three experiments. In addition to observations of interference fringes in the far-field, data is available on particle size as a function of RH in the near-field. Figure 3.9C represents the particle size analysis results from the near-field images in Figure 3.9B. The Instant Mars particle shows rapid growth between 41-48% RH, which is consistent with the changes we observed in the far-field, confirming a phase-change is occurring around 42% RH.
Figure 3.9: The Instant Mars droplet was initially levitated at 79% RH at 293 K (A). At 79% RH, some solid particulate is evident by the distorted interference fringes in the far-field image (green) but the droplet is predominately liquid. As RH is decreased, the droplet decreases in size but liquid water is still present. At 27%, the particle loses water and crystalizes but an aqueous component is still present. As RH is lowered to 19%, complete efflorescence is observed. As RH is increased (B), water uptake does not begin until 41% RH and a portion of the particle remains solid. Further increasing RH to 48% RH results in more linear fringe lines in the far-field image where the particle indicating the particle is mostly aqueous. As the RH is increased to 81%, RH, the particle undergoes continual uptake of liquid water even though solid particulates still remain. The particle size analysis results (C) show rapid growth between 41-48% RH, indicating the particle is behaving as an aqueous solution and matches the phase changes observed in the far-field images.
3.4 Mars Implications

By combining our experimental results with results presented in literature, a broader picture of the stability and metastability of brine solutions in a Mars RH and temperature environment emerges. Given the highly hygroscopic nature of most ClO$_4^-$ salts, the stability and metastability of pure salts were initially examined. The deliquescence and efflorescence of pure NaClO$_4$, Mg(ClO$_4$)$_2$, and Ca(ClO$_4$)$_2$ [Gough et al., 2011; Nuding et al., 2014] were quantified; forming aqueous solutions that persisted in the low temperature and RH conditions reported on Mars today. Gough et al. [2014] increased the complexity of the system by examining the deliquescence and efflorescence of binary ClO$_4^-$/Cl$^-$ salt systems. The binary salt systems are reported to be more deliquescent (2-5% RH) than the most deliquescent salt in the mixture. Once an aqueous solution is present in the binary mixtures, specifically Mg(ClO$_4$)$_2$/MgCl$_2$, a metastable, supersaturated solution can persist down to 5% RH at 243 K in some cases (dependent on mole ratio). Here we quantified the liquid water uptake and loss by a complex mixture that is representative of the soluble salts that have been reported in the martian surface and subsurface. Instant Mars is presently the most Mars relevant salt mixture studied to date as it closely replicates in-situ measurements from the Mars Phoenix lander.

Experiments characterizing phase transitions in bulk supercooled briny solutions further contribute to the understanding of brine stability on Mars. Toner et al. [2014] demonstrated that sulfates, chlorides, and perchlorate solutions can persist as stable, supercooled bulk liquid well below their predicted eutectic temperatures. Ca(ClO$_4$) and Mg(ClO$_4$)$_2$ remained as supercooled liquids down to 153 K at which point they transitioned into glassy, amorphous solids [Toner et al., 2014]. When mixed with the Mars soil simulant, JSC-Mars1, the supercooling of the brine solutions studied was not affected. We observe a similar result; the less soluble salt, sulfate, in Instant Mars did not consistently impact the ability of the more soluble salts, perchlorates and chlorides, to remain in the aqueous phase. The behav-
ior of a complex mixture such as Instant Mars mixed with a Mars soil simulant would be interesting, and merits future investigation.

Our experimental results allow us to expand our knowledge and understanding of the behavior of complex brine solutions in present-day Mars conditions. Although our data is limited to a temperature range of 235-243 K, we have determined the phases of the first complex Mars salt analog, Instant Mars, as a function of RH from 1-100%. Figure 3.10 summarizes deliquescence results for single, binary, and complex salt systems as a function of RH. The binary salt systems reported are 80% ClO$_4^-$/20% Cl$^-$ (mole %), the most Mars relevant when compared to the Phoenix results [Hecht et al., 2009]. The orange bar represents RH for which the salt exists as a solid with the beginning of the blue bar indicating the deliquescence point for single and binary salt systems and initial water uptake for the Instant Mars systems. Initial water uptake observed for Instant Mars falls in the upper end of the range of the single salt ClO$_4^-$ systems (36-52% RH) at 243 K. When compared to the binary ClO$_4^-$/Cl$^-$ salt systems, Instant Mars deliquesces in a RH range similar to the Mg(ClO$_4$)$_2$/MgCl$_2$ mixture and differs only a few percent RH from the NaClO$_4$/NaCl mixture. Figure 3.10 indicates that Instant Mars particles form a stable, aqueous solution that forms at 56± 5% RH at 243 K, similar to some single and binary salt systems previously examined.

Figure 3.11 summarizes the experimentally identified phases of single, binary, and complex supersaturated brine solutions as the RH environment is decreased around an aqueous solution. The orange bar represents the crystalline phase with the dark blue bar indicating the stable or metastable aqueous phase. The ClO$_4^-$ salt systems show a significant hysteresis effect, allowing for supersaturated ClO$_4^-$ to persist as low as 6% RH at 243 K. The binary salt systems exhibited a two step efflorescence process. In the case of the 0.8/0.2 NaClO$_4$/NaCl mixture, the first appearance of a solid phase was observed at 23% RH with a supersaturated, aqueous solution persisting down to 16% RH. This region is labeled as the 'mixed phase' in Figure 3.11. A similar behavior is detected for the Instant Mars mixture.
A majority of the Instant Mars particles exhibited initial formation of the solid phase at 65% RH, but the aqueous phase persisted in the Raman spectra down to 13% RH. The Instant Mars results presented demonstrate that complex metastable supersaturated aqueous solutions can persist in martian RH and temperature environment.
Figure 3.10: Phases observed when RH is increased around single, binary, and complex salt systems. The binary salt system values reported are 80% ClO$_4^-$/20% Cl$^-$ (mole %), as this ratio is the most Mars relevant according to the Phoenix Wet Chemistry Lab results. The orange bar represents when the salt will exist as a solid. The beginning of the blue bar indicates the deliquescence point for single and binary salt systems and initial water uptake for the Instant Mars systems. Initial water uptake observed for Instant Mars falls in the upper limit of the range of the single salt ClO$_4^-$ systems (36-52% RH) at 243 K. When compared to the binary ClO$_4^-$/Cl$^-$ salt systems, the water uptake of Instant Mars correlates well with the deliquescence of the Mg(ClO$_4$)$_2$/MgCl$_2$ mixture and is only a few percent above the NaClO$_4$/NaCl mixture.
Figure 3.11: Phases observed when RH is decreased around a single salt, binary salt mixture, and complex brine solutions. The orange bar represents the solid phase and the dark blue bar indicates the stable or metastable aqueous phase. The ClO$_4^-$ salt systems show a significant hysteresis effect, allowing for supersaturated ClO$_4^-$ to persist as low as 6% RH at 243 K. As we examine the binary salt systems, we observe a two step efflorescence process. In the case of the 0.8/0.2 NaClO$_4$/NaCl mixture, the first appearance of a solid phase was observed at 23% RH with a supersaturated, aqueous solution persisting down to 16% RH. This region is labeled as the ‘mixed phase’ in Figure 3.11. A similar behavior is detected for the Instant Mars mixture. A majority of the Instant Mars particles exhibited initial formation of the solid phase at 65% RH, but the aqueous phase appeared in the Raman spectra down to 13% RH. Comparison of this figure with Figure 3.10 illustrates the tendency of all perchlorate salts and mixtures to supersaturate and exist in a metastable state under low RH conditions.
Chapter 4

Assessing the Impact of Calcium Perchlorate on Bacillus subtilis Spores in Martian Environmental Conditions using Raman Spectroscopy

4.1 Introduction

The exploration of other planetary bodies searching for their potential for habitability and life is a top priority in space sciences. As we continue to send spacecraft to other planetary bodies, it is important to understand and consider the survival of terrestrial microbes to protect the scientific integrity of future missions. Planetary protection policies are established by the Committee on Space Research (COSPAR) with a key goal of controlling potential biological contamination from inbound and outbound spacecraft and sample return. Bacterial spores sampled from spacecraft associated surfaces have been shown to be resilient, surviving very harsh environmental conditions such as space based radiation environments and Mars UV radiation [Osman et al., 2008; Vaishampayan et al., 2012]. In addition to being radiation tolerant, spores could interact with their surrounding environment and their survival may depend on the specific soils present, the temperature and relative humidity environment. As more is learned about specific planetary conditions, planetary protection policies must be updated to maintain relevance. Specifically, planetary protection policies for Mars continue to become more complex and pressing as missions are formulated to land in potentially habitable environments and samples begin to be cached for sample return.

The case for current habitability on Mars is a challenging topic to address due to the cold temperatures and dry environmental conditions. However, salts discovered on the Mars
surface and in the subsurface provide a pathway for the formation and stability of liquid water in present environmental conditions. Liquid water is essential to life as we know it and thus water cycle processes on Mars today must be better understood to determine the potential for habitability. The discovery of perchlorates at the Phoenix landing site and potentially in the Gale Crater region at the Mars Science Laboratory (MSL) landing site may impact localized liquid water stability [Glavin et al., 2013; Leshin et al., 2013; Ming et al., 2014]. Perchlorates could provide a mechanism for water vapor to interact with spores as perchlorates have been shown to be extremely hygroscopic in present-day martian temperature and RH conditions [Chevrier et al., 2009; Gough et al., 2011, 2014; Nuding et al., 2014]. If liquid water is present at landing sites it is important to probe the interaction of liquid brines with bacterial spores.

Laboratory investigations can serve as a proxy for in-situ studies on Mars. Magnesium perchlorate does not influence spore growth under present-day Mars atmospheric conditions [Schuerger et al., 2012]. Because spore growth is not inhibited, high-confidence regarding contamination of Mars via spores found on spacecraft cannot be guaranteed (at least not by this mechanism). Calcium perchlorate has been reported at both the Phoenix [Hecht et al., 2009; Kounaves et al., 2014], and the Mars Science Laboratory (MSL) [Glavin et al., 2013] landing sites; an investigation of spore interaction with this salt is thus warranted. Recent investigations of Ca(ClO$_4$)$_2$ is extremely hygroscopic and able to persist as a metastable liquid down to 1% RH at Mars relevant temperatures [Nuding et al., 2014].

This work explores the interaction of Bacillus subtilis spores (B168 strain) with water vapor in Mars relevant temperatures and relative humidity (RH) conditions. In addition, Ca(ClO$_4$)$_2$ was mixed with the B. subtilis spores and exposed to the same diurnal cycle conditions in order to quantify the effects of the ClO$_4^-$ on the spores. A combination of Raman microscopy and an environmental cell allows us to visually and spectrally analyze the changes of the individual B. subtilis spores and Ca(ClO$_4$)$_2$ mixtures as they experience present-day martian diurnal cycle conditions.
4.2 Method

4.2.1 Sporulation of Bacillus subtilis

The Jet Propulsion Laboratory (JPL) provided the B. subtilis sample. A detailed description is reported elsewhere Osman et al. [2008], but the process is briefly described here. A nutrient broth sporulation medium was used to induce sporulation from the various isolates [P Schaeffer, 1965; Nicholson and Setlow, 1990]. A single purified colony of the strain to be sporulated was inoculated into liquid nutrient broth sporulation medium. After 2 to 3 days of incubation at 305 K, the cultures were examined in wet mounts on an Olympus BX-60 phase-contrast microscope to determine the level of sporulation. Spores appeared as phase-bright bodies when viewed with the phase-contrast microscope. Once the number of free spores in the culture was 90% greater than the number of vegetative cells, the culture was harvested, and the spores were purified. Spores were purified by treatment with lysozyme and washing with salt and detergent [Nicholson and Setlow, 1990]. Cultures were harvested by centrifugation (5,000 rpm, 10 min, 277 K). The pellets were washed with 1 M KCl/0.5 M NaCl and incubated for 60 min at 310 K in Tris-HCl (50 mM, pH 7.5) containing lysozyme (50 µg/ml) to degrade immature spores, any remaining vegetative cells, and cell-wall debris. The spores were cleaned by additional centrifugation (10,000 rpm, 10 min, 277 K) and washing sequentially with NaCl (1 M), deionized water, sodium dodecyl sulfate (0.05%), TEP buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride), and finally three washes with deionized water. The purified spores were resuspended in sterile deionized water, heat-shocked (353 K for 15 min), and stored at 277 K in glass tubes.

4.2.2 Preparation of B. subtilis and Ca(ClO$_4$)$_2$ Solutions

The B. subtilis sample prepared had an initial concentration of $1 \times 10^7$ spores per mL of sterile water. Ca(ClO$_4$)$_2$·4H$_2$O was purchased from Sigma Aldrich (99% purity). To investigate the impact of Ca(ClO$_4$)$_2$, 100 µL of the spore solution ($1 \times 10^7$) was mixed with
3 mL of a 1% Ca(ClO$_4)_2$ solution. The control solution that did not contain Ca(ClO$_4)_2$ contained 100 µL of the spore solution ($1 \times 10^7$) diluted in 3 mL of sterile water. The test solutions were nebulized individually at room temperature using high purity nitrogen and droplets were deposited onto a quartz disc.

4.2.3 Experimental Setup

To characterize the impact of the Martian temperature and RH environment on B-168 spores with and without Ca(ClO$_4)_2$, the spores were exposed to martian temperature and RH conditions while being probed using a combination of Raman and optical microscopy. The instrument setup used in this study is described in detail in Baustian et al. [2010] and Gough et al. [2011] and is shown in Figure 4.1. A Raman microscope equipped with an environmental cell was used to visualize B-168 spores and detect liquid water uptake by the spore and/ or perchlorate when exposed to Mars relevant temperature and RH conditions. Specifically, a Nicolet Almega XR Dispersive Raman spectrometer was outfitted with a Linkam THMS600 environmental cell, a Linkam automated temperature controller, and a Buck Research chilled-mirror hygrometer. Raman spectra and line maps were generated with a double frequency Nd:YAG laser at 532 nm (1 µm spectral resolution).

Each quartz disc sample was placed directly on the silver block where the temperature was controlled by a combination of cooling using a continuous flow of liquid nitrogen and resistive heating. Initially, high purity dry N$_2$ continuously flowed through the cell to ensure a dry environment with RH < 1%. To control RH in the cell, a separate stream of N$_2$ passed through a H$_2$O vapor bubbler before combining with the dry flow. After passing through the cell, the flow was directed to a frost point hygrometer for measurement of water vapor. Frost point measurements, specifically dewpoint, from the hygrometer and sample temperature measurements from the platinum resistance sensor allow determination of the RH at the sample. A diaphragm pump pulling at a rate of 1 L/min was attached to the outlet of the hygrometer to ensure a constant airflow through the cell regardless of any variability in
Figure 4.1: A schematic of the Raman microscope equipped with an environmental sample cell.
flow rate through the H$_2$O vapor bubbler. Each experiment simulated martian diurnal cycle temperature (223-273 K) and RH (0-100%) conditions with a heating/cooling rate of 1.7 degrees (K)/minute. Mars subsurface models report a range of heating/cooling from 0.02 degrees (K)/minute to 0.08 degrees (K)/minute [Nuding et al., 2014]. In all experiments temperature was varied while holding H$_2$O partial pressure constant. This process allowed for fine tuned RH control at lower temperatures. The estimated uncertainty in the RH values reported due to the accuracy of the instrumental apparatus is ± 1% RH.

Once the solutions were nebulized, the samples were placed in the environmental cell and analyzed visually and spectrally. Figure 4.2 shows microscopic images (50X objective) with correlating Raman spectra at 60% RH for the pure spore and spore/perchlorate samples, respectively. The pure spore sample (Figure 4.2, top) shows 1 µm B-168 spores. The spores are spectrally fluorescent, but a signature peak is detectable at 1085 cm$^{-1}$ [Hutsebaut et al., 2006]. No O-H stretch is detected, indicating the spores are not taking up a measurable amount of H$_2$O. Although no visual or spectral evidence of liquid water uptake is present, the resolution of the signal is impacted due to the small spot size (1 µm) of the spores. The B-168 mixed with 1% Ca(ClO$_4$)$_2$ sample (Figure 4.2, bottom) resulted in several spores conglomerating to form a cluster in the center of the large perchlorate crystal. Overall, particles were typically 50 µm, but particles were as large as 150 µm in diameter were observed in some cases. The larger concentration of spores in the spore/perchlorate sample still showed the 1085 cm$^{-1}$ spore peak, but allowed for identification of additional spore peaks centered at 2908 cm$^{-1}$, 1665 cm$^{-1}$, 1434 cm$^{-1}$, and 1365 cm$^{-1}$. Ca(ClO$_4$)$_2$ in the aqueous phase is identified by the position of the perchlorate peak at 936 cm$^{-1}$ [Nuding et al., 2014]. In addition, water is clearly associated with the perchlorate as evident by the O-H stretch (3500-3635 cm$^{-1}$).
Figure 4.2: (Top) B-168 spore sample nebulized and exposed to Mars surface conditions; shown is a snapshot of the cycle at 61% RH and 233 K. Spores are spectrally fluorescent, but a signature peak centered at 1085 cm$^{-1}$ is detected. (Bottom) B-168 spores mixed with 1% Ca(ClO$_4$)$_2$ solution nebulized and exposed to Mars surface conditions; shown is a snapshot of the cycle at 62% RH and 238 K. The aqueous Ca(ClO$_4$)$_2$ solution surrounds the spores. The larger concentration of spores in the particle show additional spectral features. The presence of the O-H and ClO$_4^-$ stretches indicate and aqueous Ca(ClO$_4$)$_2$ solution surrounds the B-168 spores and is confirmed visually in the microscopic image.
Table 4.1: B. subtilis and Ca(ClO$_4$)$_2$ Experiments

<table>
<thead>
<tr>
<th>Solution</th>
<th>Control</th>
<th>Single Sol Diurnal Cycle</th>
<th>Six Sol Diurnal Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>B. subtilis + 1% Ca(ClO$_4$)$_2$</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

A systematic approach was designed to quantify the effect of Mars temperature and RH environment on B. subtilis spores and how the hygroscopic behavior of Ca(ClO$_4$)$_2$ might impact spore growth. Table 4.1 summarizes the scenarios that were investigated in this work. A new sample was made for each case; no diurnal temperature and RH cycle (control), a single diurnal temperature and RH cycle, and six diurnal temperature and RH cycles. First, B. subtilis samples are exposed to a Mars temperature and RH environment to quantify the effect of a single diurnal cycle. To investigate exposure of multiple diurnal cycles on the spore, six martian diurnal cycles were simulated on a new sample. Finally, to understand the impact of Ca(ClO$_4$)$_2$, these experiments were repeated for the samples of B. subtilis mixed with Ca(ClO$_4$)$_2$ samples.

4.2.4 Quantification of Bacterial Growth

Following exposure to the martian diurnal temperature and RH cycles, each quartz disc was placed in a sterile tube. To analyze the impact of each environmental scenario on spore survival, the samples were returned to JPL for analysis where the surviving spore population was estimated by standard plate assay [Osman et al., 2008]. The quartz discs were placed into 5 mL of sterile water and sonicated for 2 min to dissociate the attached spores from the substrate. After sonication, sample aliquots were then transferred into petri dishes, and cultivable spores were enumerated by pour plate technique using trypticase soy agar (TSA) as the growth medium (305 K for 3-7 days) [Osman et al., 2008].
4.3 Results and Discussion

4.3.1 Impact of Martian Temperature and Relative Humidity Environment on B. Subtilis

B. subtilis spores are not known to be hygroscopic, but to fully understand their interaction with water vapor and potentially with liquid water, the spores were cycled through a Mars diurnal temperature and RH environment. B-168 spores were exposed to one and six diurnal temperature and RH cycles. Raman spectra and optical images were obtained and examined at each step of the cycle. Figure 4.3 shows optical images that are representative of a B-168 cooling cycle experiment. Each image is a snapshot of the cycle, as temperature started at 273 K and 0% RH and cooled at a rate of 1.2 K/min. The first image shows the small, 1 µm B-168 spores with a few larger cluster of spores apparent. As temperature is cooled and RH is increased, there is no evidence of water vapor condensation. Condensation was not observed throughout the entirety of all cycles performed on the B-168 spores confirmed by both Raman spectroscopy and visual observation. As previously shown in Figure 4.2, no O-H stretch or any other spectral change is observed in the B-168 spore spectra as temperature decreases and RH is increased, even after multiple cycles. This work confirms that the spores alone are not hygroscopic under martian conditions.

4.3.2 Influence of Ca(ClO$_4$)$_2$ on B. subtilis Liquid Water Interaction

Perchlorates absorb water vapor from the surrounding environment and deliquesce, or transition from a solid crystalline phase into a stable, aqueous solution [Chevrier et al., 2009; Gough et al., 2011, 2014; Nuding et al., 2014]. Ca(ClO$_4$)$_2$ is highly deliquescent; experimental results report the deliquescence relative humidity (DRH) at 10% and the efflorescence (transition from aqueous solution to solid crystalline) relative humidity (ERH) as low as 1% RH at 273 K [Nuding et al., 2014]. When Ca(ClO$_4$)$_2$ is mixed with the B-168 spores, the hygroscopic salt can provide a pathway for liquid water interaction that is not observed
Figure 4.3: Microscopic images of a cycle experiment for B-168 spores. The first image shown shows spores at 4\% RH and 253 K. The spores are only a few microns in size. Temperature is decreased at 1.2 K/min to reach 228 K and 42\% RH, where no visual change is observed. As RH continues to increase to 75\% RH, there is no evidence of condensation on the spores. At 82\% RH and 222 K, no change is observed.
with the spore only samples. Figure 4.4 shows a series of images providing a representative example of snapshots throughout a diurnal cycle experiment for the mixed Ca(ClO$_4$)$_2$/ B-168 spore particles. This experiment starts at 1% RH and 273 K, with two particles in the field of view. For the cooling cycle (images in blue boxes), the temperature is decreased at 1.2 K/min. The larger particle on the left side of the image show three dark regions that consists of a cluster of B-168 spores surrounded by crystalline Ca(ClO$_4$)$_2$. The smaller 10 µm particle on the right of the image also shows the dark region of spores in the center of the particle surrounded by Ca(ClO$_4$)$_2$ in the crystalline phase. As RH is increased (by decreasing the temperature), the Ca(ClO$_4$)$_2$ deliquesces, surrounding the spores in a saturated aqueous salt solution. As RH continues to increase, ice formation is observed once $S_{ice}$=1.8 at a temperature of 223 K.

To confirm the phase of the Ca(ClO$_4$)$_2$ surrounding the B-168 spores, we utilize Raman spectroscopy. Figure 4.5 is a Raman line map (1 µm resolution) of a Ca(ClO$_4$)$_2$ and B-168 spore particle at 223 K with $S_{ice}$=1.8. Each tick mark on the red line in the microscope image represents the 1 µm step size, thus corresponding to the location of the spectrum obtained for the particle. A smoothing function is used to represent the spectra in between each tick mark, shown as a color map where warmer colors represent higher Raman intensity. The line map shows the Ca(ClO$_4$)$_2$ stretch (938 cm$^{-1}$) is present throughout the entire particle. There is an evident liquid water signature shown by the O-H stretch (centered at 3500 cm$^{-1}$) detected throughout the entire particle with the B-168 spore stretch (1085 cm$^{-1}$) is detected in the two darker regions of the particle in the microscopic image. Although this peak center is different than that reported in Hutsebaut et al. [2006] our use of a higher energy laser (532 nm vs 785 nm) and difference in temperature (of at least 30 K) likely accounts for the 70 cm$^{-1}$ difference. The line map confirms the dark regions in the image represent the B-168 spores surrounded by a Ca(ClO$_4$)$_2$ aqueous solution.

To investigate possible changes of the Ca(ClO$_4$)$_2$ and B-168 spore particles throughout the cooling cycle, Raman spectra were evaluated at each temperature and RH step.
Figure 4.4: Microscopic images of a cycle experiment for a mixture of Ca(ClO$_4$)$_2$ and B-168 spores. The experiment starts at 1% RH and 273 K. The crystalline Ca(ClO$_4$)$_2$ surrounds the spores. Temperature is decreased at 1.2 K/min to reach 233 K (45% RH), where the Ca(ClO$_4$)$_2$ has deliquesced and surrounds the spores in an aqueous Ca(ClO$_4$)$_2$ solution. Once 223 K ($S_{\text{Ice}} = 1.8$) is reached, the particles are heated at 1.2 K/min. The Ca(ClO$_4$)$_2$ aqueous solution effloresces at 8% RH and 253 K, reforming a Ca(ClO$_4$)$_2$ crystalline structure around the spores. No more visual changes are observed as the sample continues to be heated to 273 K, 0% RH.
Figure 4.5: A Raman line map of a B. subtilis and aqueous Ca(ClO$_4$)$_2$ particle at 223 K and $S_{ice}=1.8$. The particle is 80 µ in the y-direction, with optical image shown on the left and the Raman line map on the right. The optical image shows the dark spore regions surrounded by aqueous Ca(ClO$_4$)$_2$, with each tick mark in the y-direction representing 1 µ. The fluorescent features of the spore are apparent between 3700-4000 cm$^{-1}$, correlating with the location in the optical image. A peak at 1085 cm$^{-1}$ are also present in the line map in the spatial location of the spore. The O-H stretch (centered at 3500 cm$^{-1}$) is present throughout the length of the entire particle and the solvated ClO$_4^-$ stretch (938 cm$^{-1}$) indicates an aqueous perchlorate solution surrounds the spores.
Figure 4.6: The experiment is a cooling cycle that starts at 0% RH at 273 K (red line); the spectra is very fluorescent because it is centered on a spore/perchlorate region of the particle. The O-H stretch is masked by the fluorescence at the lower RH, the experiment begins with hydrated crystalline Ca(ClO$_4$)$_2$ as indicated by peak centered at 956 cm$^{-1}$. The B-168 spore peak is centered at 1085 cm$^{-1}$ and does not shift throughout the entirety of the experiment. As RH begins to increase, an immediate shift of the ClO$_4^-$ peak is observed (green line) to 946 cm$^{-1}$ indicating a change in hydration state. As RH continues to increase, the fluorescence decreases and by 24% RH (blue line) the ClO$_4^-$ peak shifts to the aqueous peak centered at 938 cm$^{-1}$. The O-H stretch broadens and becomes more prominent as the fluorescence decreases, showing a broad shoulder between 3200-3600 cm$^{-1}$. Once the ClO$_4^-$ deliquesces, no shift is observed up to an $S_{ice}$=1.8.
Spectra were obtained for each single cycle and each six cycle experiment. Figure 4.6 is a representative example of an increasing RH/cooling cycle. The experiment starts at 0% RH at 273 K (red line); the spore particles are very fluorescent. Because the spectrum is centered on a spore/perchlorate region of the particle, a large background is observed at higher wavenumbers. Although the O-H stretch is masked by the fluorescence at the lower RH, the experiment begins with hydrated Ca(ClO$_4$)$_2$ as indicated by the peak centered at 956 cm$^{-1}$ [Nuding et al., 2014]. The B-168 spore peak is centered at 1085 cm$^{-1}$ and does not shift throughout the entirety of the experiment. As RH begins to increase, an immediate shift of the ClO$_4^-$ peak is observed (green line) to 946 cm$^{-1}$ indicating a change in hydration state. As RH continues to increase, the fluorescence decreases and by 24% RH (blue line) the ClO$_4^-$ peak shifts to the wavenumber characteristic of an aqueous perchlorate (938 cm$^{-1}$). The O-H stretch broadens and becomes more prominent as the fluorescence decreases, showing a broad shoulder between 3200-3600 cm$^{-1}$. Once the ClO$_4^-$ deliquesces, no further ClO$_4^-$ peak shift occurs in the spectra. The Raman spectra confirm the visual observations from the optical images; the aqueous Ca(ClO$_4$)$_2$ solution surrounds the spores once the environmental conditions favor the aqueous phase.

The warming cycle begins by increasing the sample temperature at 1.2 K/min thus decreasing the RH. Figure 4.4 indicates at 50% RH and 233 K, an aqueous Ca(ClO$_4$)$_2$ solution is still present and persists until 8% RH at 253 K, where the Ca(ClO$_4$)$_2$ effloresces by visual inspection. Efflorescence is apparent by the crystal structure surrounding the spores. As temperature continues to increase to 273 K with a dry RH environment, no more visual changes are observed. Figure 4.7 is a representative example of Raman spectra obtained for a Ca(ClO$_4$)$_2$ and B-168 spore particle throughout a warming cycle (increasing RH). Beginning at 100% RH, the sample was heated at a rate of 1.2 K/min. The purple line in Figure 4.7 is at 69% RH with the O-H stretch, spore peak, and aqueous ClO$_4^-$ peak. As RH continues to decrease to 20% RH (teal) the shoulder on the O-H stretch begins to shrink but no shift in the ClO$_4^-$ peak is observed, indicating an aqueous solution is still
present. At 5% RH (orange), the ClO$_4^-$ peak shifts to 946 cm$^{-1}$ indicating a phase change has occurred. Finally, as the temperature is increased to 273 K at 1% RH the ClO$_4^-$ peak shifts to 956 cm$^{-1}$ forming a hydrated crystalline solid at the same peak location reported at the beginning of the cycle. These results indicate that the B-168 spores are surrounded in an aqueous Ca(ClO$_4$)$_2$ solution to extremely low RH values at Mars relevant temperatures. The Ca(ClO$_4$)$_2$ behaves as reported in Nuding et al. [2014] and enables a pathway for spores to interact with liquid water in their surrounding environment. This same process is observed in multiple experiments and is characteristic of the behavior in up to six diurnal cycles.

4.3.3 B. subtilis Growth

To understand the impact of exposure to Mars temperature and RH conditions and the effect of Ca(ClO$_4$)$_2$ on survival of the spores, a control sample for each solution was analyzed. The control samples were nebulized onto the quartz disc, as previously described, but were immediately placed in a sterile tube and stored at 275 K. These results were used as the baseline for comparison to samples exposed to single diurnal cycles and six diurnal cycles. The controls were repeated for the B-168/Ca(ClO$_4$)$_2$ experiments.

To quantify the B-168 survival and thus ability to grow into viable cells, the number of cells per sample were compared for each case. Figure 4.8 shows a cell growth comparison between B-168 and B-168/Ca(ClO$_4$)$_2$ in the most Mars relevant case; exposure to six martian diurnal cycles. Figure 4.8 represents the average number of colony forming units (CFU) per µL for each case. Spores were exposed to temperatures as low as 223 K and an RH environment that varied between 0-100% RH. The B-168 spore growth, reported at 215 ± 4 CFU/µL (1 SD), did not change (within uncertainty) when mixed with Ca(ClO$_4$)$_2$. The B-168/Ca(ClO$_4$)$_2$ mixture contained 243 ± 34 CFU/µL (1 SD). The B-168 and Ca(ClO$_4$)$_2$ mixture sample results demonstrate that exposure to Ca(ClO$_4$)$_2$ had little to no effect on future B-168 growth. The B-168 spores were tolerant to the Ca(ClO$_4$)$_2$ solution, even after exposure to an aqueous Ca(ClO$_4$)$_2$ solution in a martian temperature and RH environment.
Figure 4.7: Warming cycle (increasing RH) of a Ca(ClO$_4$)$_2$ and B-168 spore particle. Beginning at 100% RH, the sample was heated at a rate of 1.2 K/min. The purple line represents 69% RH with the O-H stretch, spore peak, and aqueous ClO$_4^-$ peak. As RH continues to increase to 20% RH (teal) the shoulder on the O-H stretch begins to shrink but no shift in the ClO$_4^-$ peak is observed, indicating an aqueous solution is still present. At 5% RH (orange), the ClO$_4^-$ peak shifts to 946 cm$^{-1}$ indicating a phase change has occurred. The temperature is increased to 273 K at 1% RH the ClO$_4^-$ peak shifts to 956 cm$^{-1}$ forming a hydrated crystalline solid.
(Figure 4.2 B) for single and multiple diurnal cycles. This is representative of all cases examined; no significant change in growth was detected. Detecting no change in growth is surprising, as Schuerger et al. [2012] demonstrated high salt analogs resulted in a 2.5 fold decrease in spore survival.

The survival rates were not impacted by exposure to Mars relevant temperature and RH conditions, even in multiple cycle conditions. This result is not surprising because no direct interaction with water vapor was observed in the optical images or Raman spectra (Figure 4.2, top). Additionally, previous experiments demonstrate the extreme resilience of B-168 spores when exposed to Mars UV, atmospheric composition, pressure, and temperature conditions for multiple days [Schuerger et al., 2012]. However, it was important to conduct the spore only experiments to fully understand the impact of Ca(ClO$_4$)$_2$ in similar conditions.

4.4 Conclusion

Observations and model simulations suggest liquid processes persist on present-day Mars on the surface or in the near subsurface [Renno et al., 2009; McEwen et al., 2011, 2014; Chevrier and Rivera-Valentin, 2012] and are likely explained by aqueous brine solutions. Ca(ClO$_4$)$_2$ is likely the most relevant, hygroscopic salt discovered at the two most recent landing sites (Phoenix and MSL). It is known that such missions transport terrestrial microorganisms via the spacecraft surfaces, regardless of landing techniques [Berry, 2008; Horneck et al., 2010; Schuerger et al., 2003]. The presence of liquid water is an important mechanism in understanding the transport of the spores from the spacecraft surfaces and their survivability to the harsh environmental conditions on the surface of Mars. This work indicates that Ca(ClO$_4$)$_2$ provides a pathway for spores to interact with liquid water in the martian environment.

Here we designed an experimental technique to quantify B-168 spore behavior in Mars relevant RH and temperature conditions. This technique was expanded to generate particles containing Ca(ClO$_4$)$_2$ and B-168 spore (ranging from 15-100 µm in diameter) to fully
Figure 4.8: The average CFU/µm of B-168 and B-168/Ca(ClO$_4$)$_2$ exposed to six martian diurnal cycles. The B-168 spore growth, reported at 215 ± 4 CFU/µL (1 SD) while the B-168/Ca(ClO$_4$)$_2$ mixture contained 243 ± 34 CFU/µL (1 SD).
characterize the interaction with liquid water during a present-day Mars diurnal cycle. Raman spectroscopy was used to confirm the presence of both Ca(ClO$_4$)$_2$ and B-168 spore in the particle and track the phase changes in the Ca(ClO$_4$)$_2$ as temperature and RH changed throughout the experiment. Water vapor did not condense on the individual B-168 spores when exposed to high RH values (up to 100% RH) at low temperatures (as low as 215 K). No change was observed in spore growth between the control (no cycle), a single diurnal cycle, and six diurnal cycle experiments. When B-168 spores were mixed with Ca(ClO$_4$)$_2$, the spores were surrounded by a solid crystalline phase at low RH values. As RH was increased and then decreased, Ca(ClO$_4$)$_2$ deliquesced and effloresced, respectively at the same RH values reported in Nuding et al. [2014]. Ca(ClO$_4$)$_2$ is extremely deliquescent and can persist as a metastable, supersaturated solution to extremely low RH values allowing for an aqueous solution to potentially be present in the very arid conditions observed on Mars today. The Ca(ClO$_4$)$_2$ surrounded the B-168 spores in each particle, meaning the spores were contained in an aqueous brine solution when the environmental conditions favored the liquid phase (in the stable and metastable regimes). No change in survivability or growth was observed with the addition of Ca(ClO$_4$)$_2$ when compared to the control, even for samples undergoing multiple diurnal cycles. Results from the current study suggest that B-168 spores can survive the arid conditions and martian temperatures, even when exposed to Ca(ClO$_4$)$_2$ in the crystalline or aqueous phase. The extreme hygroscopic nature of Ca(ClO$_4$)$_2$ allows for direct interaction of B-168 spores with liquid water. Additional experiments need to be performed to understand the impacts of other environmental conditions (low pressure, atmospheric composition, and UV).
Chapter 5

Conclusion and Future Directions

The experimental work presented in this thesis provides insight into the formation, stability, and metastability of potential aqueous solutions reported on Mars today. Recent spacecraft observations suggest liquid water may sporadically exist in the present arid, cold conditions of the surface and near-subsurface. Habitability on Mars is supported by the possible existence of liquid water, thus providing an avenue to expand the habitable zone beyond our current understanding.

In an effort to move beyond model dependent interpretation of briny aqueous solutions, this work evaluated the water uptake and loss properties of Mars salt analogs, specifically their phase change processes, in a simulated martian temperature and relative humidity environment. The deliquescence (solid to aqueous) and efflorescence (aqueous to solid) phase transitions were quantified for Ca(ClO$_4$)$_2$ and the complex salt mixture, Instant Mars. The laboratory results were compared to modeled martian surface and subsurface conditions; revealing that liquid water stability and metastability is possible for several hours throughout the martian sol.

The brine solutions examined in this work, specifically Ca(ClO$_4$)$_2$, provide a pathway for liquid water to interact with terrestrial spores. Without the presence of perchlorate, no liquid water was observed on the spores. However, when hygroscopic salt surrounded the spores, liquid water was abundant. The perchlorate surrounded the spores with ultimately no negative impact on their survival. It is imperative for planetary protection policies to
consider liquid water interaction in the presence of perchlorates to fully understand the potential for terrestrial contamination of Mars and any impact it may have on scientific integrity.

5.1 Deliquescence and Efflorescence of Calcium Perchlorate

Calcium perchlorate (Ca(ClO$_4$)$_2$) is a highly deliquescent salt that may exist on the surface and in the near-subsurface of present-day Mars. In chapter 2, laboratory experiments quantified the deliquescent relative humidity (DRH) and efflorescent relative humidity (ERH) of Ca(ClO$_4$)$_2$ as a function of temperature (223 K to 273 K) to elucidate its behavior on the surface of Mars.

A Raman microscope equipped with an environmental cell was used to simulate Mars relevant temperature and relative humidity conditions and monitor deliquescence and efflorescence phase transitions of Ca(ClO$_4$)$_2$. We find that there is a wide range of deliquescence RH values between 5% and 55% RH. This range is due to the formation of hydrates in different temperatures regimes, with the higher DRH values occurring at the lowest temperatures. A very low DRH of 5% for Ca(ClO$_4$)$_2$ at 273 K could allow for the formation of stable aqueous solutions under very dry environmental conditions. Experimental deliquescence results were compared to a thermodynamic model for three hydration states of Ca(ClO$_4$)$_2$. As predicted, the higher hydration states are less deliquescent and the experimental results are generally in good agreement with the model.

Calcium perchlorate solutions were found to supersaturate, with lower ERH values than DRH values, indicating the solutions remained liquid until very low RH values. The ERH results are less dependent on temperature with an average $15 \pm 4\%$, but values as low as $3 \pm 2\%$ are measured at 273 K. Optical levitation experiments aided in the interpretation of the results, supporting the low ERH ($< 1\%$ RH) at 300 K.

Additionally, we conducted a 17-hour experiment to simulate a subsurface diurnal cycle. This demonstrated Ca(ClO$_4$)$_2$ aqueous solutions can persist without efflorescing for
the majority of a martian sol, up to 17 hours under Mars temperature heating rates and RH conditions. Applying these experimental results to martian surface and subsurface heat and mass transfer models, we find that Ca(ClO$_4$)$_2$ aqueous solutions could persist for most of the martian sol under present-day conditions. The aqueous phase stability and metastability quantified for Ca(ClO$_4$)$_2$ under Mars relevant temperature and relative humidity conditions has important implications for the water cycle and the stability of liquid water on present day Mars.

5.2 The Aqueous Stability of Complex Mars Salt Analogs

In an attempt to further understand the stability of brine solutions on Mars, a salt analog was developed to closely match the individual cation and anion concentrations as reported by the Wet Chemistry Laboratory instrument at the Phoenix landing site. Chapter 3 introduces ‘Instant Mars,’ a salt analog developed to fully encompass and closely replicate correct concentrations of magnesium, calcium, potassium, sodium, perchlorate, chloride, and sulfate ions.

Here we use two separate techniques to study the liquid water uptake and loss properties of individual Instant Mars analog particles. A Raman microscope equipped with an environmental cell probed water uptake and loss in a Mars relevant temperature and relative humidity (RH) environment. Particle levitation using an optical trap examined the phase state and morphology of freely floating Instant Mars particles exposed to changing RH conditions at room temperature. We have quantified the phases of Instant Mars in the temperature range of 235-243 K and at 293 K over an RH range of 0-100%. Raman microscope experiments reveal that Instant Mars particles can form stable, aqueous solutions at 56 ± 5% RH at 243 K and persist as a metastable, aqueous solution down to 13 ± 5% RH. Levitation experiments indicate that water uptake began at 42 ± 8% RH for Instant Mars particles at 293 K. As RH is decreased at 293 K, the aqueous Instant Mars particles transition completely into a crystalline solid at 18 ± 7% RH. These combined results demonstrate
that a salt analog that closely replicates in-situ measurements from the Phoenix landing site can take up water vapor from the surrounding environment and transition into a stable, aqueous solution.

Our experimental results allow us to expand our knowledge and understanding of complex brine solutions in present-day Mars conditions. Instant Mars particles form a stable, aqueous solution that persists at $56 \pm 5\%$ RH. This falls within the upper range (36-52\% RH) and within a few percent RH of initial water uptake reported for single and binary salt systems, respectively. Instant Mars solutions also exhibited metastability, remaining aqueous down to 13\% RH at Mars relevant temperatures. The results are similar to experimental values reported for other perchlorate and perchlorate/chloride mixtures. The Instant Mars results presented demonstrate that complex stable and metastable aqueous solutions can persist in Martian RH and temperature environments.

5.3 Impact of Calcium Perchlorate on Liquid Water Interaction with B. Subtilis Spores

Chapter 4 explores the interaction of B. subtilis spores (B-168) with liquid water in Mars relevant temperatures and relative humidity (RH) conditions. The effects of perchlorate, specifically Ca(ClO$_4$)$_2$, on the B. subtilis spores in martian diurnal cycle conditions were quantified with laboratory experiments.

An experimental technique was developed to quantify B-168 spore behavior in Mars relevant RH and temperature conditions. Particles containing Ca(ClO$_4$)$_2$ and B-168 spore were used to fully characterize the interaction with liquid water during a present-day Mars diurnal cycle. Raman spectroscopy was used to confirm the presence of both Ca(ClO$_4$)$_2$ and B-168 spore in the particle and track the phase changes in the Ca(ClO$_4$)$_2$ as temperature and RH changed throughout the experiment. Individual B-168 spores did not interact with liquid water when exposed to high RH values (up to 100\% RH) at low temperatures (as low as 215 K). When B-168 spores were mixed with Ca(ClO$_4$)$_2$, the spores were surrounded
by a solid crystalline phase at low RH values. The deliquescence of Ca(ClO$_4$)$_2$ allows a metastable, supersaturated solution to persist at extremely low RH values. The Ca(ClO$_4$)$_2$ surrounded the B-168 spores in each particle, meaning the spores were contained in an aqueous brine solution when the environmental conditions favored the liquid phase (in the stable and metastable regimes). Spore growth was not affected by diurnal temperature and RH cycles or with the addition of Ca(ClO$_4$)$_2$. Results reported in Chapter 4 suggest that B-168 spores can survive the arid conditions martian temperatures, even when exposed to Ca(ClO$_4$)$_2$ in the crystalline or aqueous phase. The extreme hygroscopic nature of Ca(ClO$_4$)$_2$ allows for direct interaction of B-168 spores with liquid water and additional experiments need to be performed to understand the impacts of other environmental conditions (low pressure, atmospheric composition, and UV).

5.4 Future Directions

To continue to enhance our understanding of aqueous brine stability on the surface of Mars today, laboratory experiments need to continue to increase in complexity. As the system examined in the laboratory becomes more complex, it increases in relevance to Mars. The results in this thesis increased the complexity of the salt mixtures, starting with Mars relevant single salt systems increasing to mixed salt systems similar to in-situ measurements reported on the surface today. An obvious next step is to incorporate non-soluble species, such as regolith. Recent laboratory experiments performed on bulk perchlorate salt-regolith mixtures reported an increased supercooling effect [Toner et al., 2014]. The results presented in Chapter 3 indicate the presence of less soluble salts (such as sulfate) had little to no impact on deliquescence compared to the pure salt or binary salt mixtures. It would be worthwhile to incorporate Mars soil simulants, such as JSC Mars-1 and montmorillonite. These two soil simulants are on the opposite end of the spectrum; JSC Mars-1 can generally be described as hydrophobic basalt while montmorillonite is extremely hygroscopic. The investigation of the two soil regimes mixed with single salt, binary, and complex salt mixtures on a micron
scale would build on the work presented in this thesis. It would enable further contribution in understanding brine aqueous processes observed by past, present, and future spacecraft on Mars.

Another advantage of laboratory experiments is their ability to enhance larger scale model simulations. The work presented in this thesis can not stand alone in explaining large-scale aqueous features, such as RSL; however, when incorporated with thermodynamical models of the martian surface and subsurface we can begin to expand the micro-scale laboratory results to larger scale processes observed on Mars today. The laboratory results presented in this thesis can not replicate full temperature ranges or diffusion processes of the martian surface and subsurface. By integrating the deliquescent and efflorescent results of single, binary, and complex salt systems into heat and mass transfer models that incorporate in-situ relative humidity and temperature results from Phoenix and MSL, we can begin to predict when, where, and how long aqueous solutions can persist on Mars (e.g. Rivera-Valentin and Nuding [2014]) Model results that can predict the deliquescence and efflorescence of salts at a local landing site would aid in the overall understanding of the localized near-surface water exchange.

The survival of Bacillus pumilus SAFR-032 after exposure to martian diurnal cycles and perchlorates should also be explored. B. pumilus is another organism commonly found on spacecraft surfaces [Kempf et al., 2005] and has demonstrated extreme resistance to prolonged space environments [Vaishampayan et al., 2012] and martian UV environments [Link et al., 2004; Kempf et al., 2005]. We performed preliminary experiments that replicated the work explained in Chapter 4 with B. pumilus spores, but results on spore survival were inconclusive. Repeating these experiments would provide valuable results in understanding the probability of forward contamination, specifically on Mars, and help protect the scientific integrity of the missions.
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Appendix A

Particle Size Detection Code

Code used for Instant Mars particle size detection in Chapter 3 was written in Mathematica. To use, see commented code in the following pages. Briefly, optical images are imported into Mathematica and output files are named. Once the images are loaded, a histogram is generated to determine the best threshold to use based on varying background and particle brightness. The kernel radius must also be set and while likely vary between each dataset (but should remain the same for each experiment) and is used to determine how much smoothing is applied with the Gaussian filter command. Particles can also be filtered out by setting a min and max area.

The next step converts the image to grayscale and applying the gaussian filter to reduce background interference and rugged particle edges. The final processing technique converts the image to a binary color scale and removes the edges of the image (to avoid detecting particles that may not be present in all of the images). Finally, the particle detection command is applied to find the circular components within the image.

Output files generated consist of a .csv file that contains filename, particle number, pixel size, and location within the image and images that show particles detected (shown below). Each image should be evaluated individually to assure the circles consistently line up with the edge of the particles detected.
(*Insert directory and filename*)
filename = FileNames["/Users/daniellenuding/RamanData/122513/EXP2/*.*.BMP"]

(*Determine Number of files*)
x = Length[filename];

(*FileOutput is the .csv file generated with particle size and location for every particle detected in each image*)
(*ImageOutput is the directory location for the images showing particles detected*)
FileOutput = "/Users/daniellenuding/RamanData/122513/122513_EXP2_ImageAnalyze.csv"
ImageOutput = "/Users/daniellenuding/RamanData/122513/EXP2/";

(*Define array for particle size*)
RadiusOutput = Array[0 &, {1, 5}];

(*Imports and analyzes all files at once, for testing change n to 1*)
For[n = 1, n <= x, n++,

(*i-Import["/Users/nuding/Desktop/1.BMP"]*)
i = Import[filename[[n]]];
Print["File ", n, " opened"];

(*Use ImageHistogram to determine best threshold value; different background brightnesses and particle brightness will require different threshold values*)

(*ImageHistogram[ColorConvert[i,"Grayscale"],Axes->True,FrameTicks->True]*)
threshold = 0.43; (*Set threshold*)
kernelRadius = 1; (*Set KR, used in Gaussian Filter and determines how much smoothing is applied*)
minBlobArea = 300; (*Used to only detect particles of a certain size range*)
maxBlobArea = 100000;

(*Convert image to Grayscale and apply Gaussian Filter*)
iGrayscale = GaussianFilter[ColorSeparate[i][[1]], kernelRadius];
(*iGrayscale=GaussianFilter[ColorConvert[i,"Grayscale"],kernelRadius];*)

(*Convert to binary color and remove edges of image to avoid partial particle detection*)
iBinary = ColorNegate[Binarize[iGrayscale, threshold]]; cells = SelectComponents[DeleteBorderComponents[iBinary],
{"Area"}, (minBlobArea < #1 < maxBlobArea) &];

(*Report size of particles detected*)
circles = ComponentMeasurements[ImageMultiply[iBinary, cells],
{"Centroid", "EquivalentDiskRadius"}][All, 2];

Print["***** ", Length[circles], " Blobs Identified,*****"]
For[y = 1, y <= Length[circles], y++,
Print["Blob ", y, 
  " Equivalent Disk Radius: ", circles[y][2], circles[[y]][[1]]];
(*If [Length[RadiusOutput]=1, RadiusOutput=}
```mathematica
{filename[[n]], y, circles[[y]][[2]], Flatten[circles[[y]][[1]]], ";"};
RadiusOutput = Append[RadiusOutput, {filename[[n]], y, circles[[y]][[2]],
circles[[y]][[1]][[1]], circles[[y]][[1]][[2]], ""}];
(*);(*
(*Print[Flatten[RadiusOutput]];*)
); (*Show[i,Graphics[{Red,Thick,Circle@@#/@@circles}]]*)
Temp = ToString[n];
tempImageFileName = FileNameJoin @ {ImageOutput, "Circles" <> Temp <> ".png"};
Imagetemp = Show[i, Graphics[{Red, Thick, Circle@@#/@@circles}]]
Export[tempImageFileName, Imagetemp];
]
FinalOutput = Drop[RadiusOutput, 1];
Export[FileOutput, FinalOutput];
Show[i, Graphics[{Red, Thick, Circle@@#/@@circles}]]
```

RadiusOutput = Drop[RadiusOutput, 1]
RadiusOutput[[1]]
{0, 0, 0, 0}