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Published in:
Langmuir

DOI:
10.1021/acs.langmuir.7b03977

Publication date:
2018

Document version:
Accepted manuscript

Citation for published version (APA):

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Langmuir, Just Accepted Manuscript • DOI: 10.1021/acs.langmuir.7b03977 • Publication Date (Web): 06 Mar 2018
Downloaded from http://pubs.acs.org on March 8, 2018

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Manipulating Single Microdroplets of NaCl Solutions: Solvent Dissolution, Microcrystallization, and Crystal Morphology

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Key Words: Micropipette manipulation, microdroplet dissolution, diffusion, dehydration, salt crystallization, water activity, supersaturation, solubility, microdroplet growth, NaCl, octanol, decane, Epstein-Plesset
Abstract
A new ‘three micropipette manipulation technique’ for forming, dehydrating, crystallizing, and re-solvating nano-grams of salt material has been developed to study supersaturated single microdroplets and microcrystals. This is the first report of studies that have measured in situ both supersaturation (as homogeneous nucleation) and saturation (as microcrystal re-dissolution) for single microdroplets of NaCl solution using the micropipette technique. This work reports a measure of the critical supersaturation concentration for homogeneous nucleation of NaCl (10.3 ± 0.3 M) at a supersaturation fraction of S = 1.9, the saturation concentration of NaCl in aqueous solution as measured with nanograms of material (5.5 ± 0.1 M), the diffusion coefficient for water in octanol, $D = 1.96 \pm 0.10 \times 10^{-6} \text{ cm}^2/\text{s}$, and the effect of the solvent’s activity on dissolution kinetics. It is further shown that the same Epstein-Plesset (EP) model, which were originally developed for diffusion-controlled dissolution and uptake of gas, and successfully applied to liquid-in-liquid dissolution, can now also be applied to describe the diffusion-controlled uptake of water from a water-saturated environment using the extended activity-based model of Bitterfield et al. This aspect of the EP model has not previously been tested using single microdroplets. Finally, it is also reported how the water dissolution rate, rate of NaCl concentration change, resulting crystal structure, and the timeframe of initial crystal growth is affected by changing the bathing medium from octanol to decane. A much slower loss of water-solvent and concomitant slower up-concentration of the NaCl solute resulted in a lower tendency to nucleate and slower crystal growth since much less excess material was available at the onset of nucleation in the decane system as compared to the octanol system. Thus, the crystal structure is reported to be dendritic for NaCl solution microdroplets dissolving rapidly and nucleating violently in octanol, while they are formed as single cubic crystals in a gentler way for solution-dissolution in decane. These new techniques and analyses can now also be used for any other system where all relevant parameters are known. Examples of this is control of drug/hydrogel/emulsion particle size change due to solvent uptake.
Introduction and Theory

As reviewed by Löffelmann and Mersmann, supersaturation is the most decisive parameter for crystallization processes including crystal growth, agglomeration and aggregation, and primary and secondary nucleation. It therefore influences crystal-product qualities such as crystal morphology, purity, specific surface area, and crystal size distribution. Löffelmann and Mersmann also point out that existing supersaturation measurement methods have restrictions including certain ranges of temperature, turbidity, viscosity, or suspension density and that each use a physical property (such as density, viscosity or electric conductivity) to obtain the actual level of supersaturation with the help of solubility data. Their main criticism is that "the crystallization process in itself is not considered in the various measurement principles". They introduced a new method to induce crystallization on the surface of a sensor and observed the development in time of the first deposition of solid matter. Similarly, we recognized that a direct measure of supersaturation (and saturation) of simple crystalline materials could also be obtained if single microdroplets of their solutions could be individually-manipulated and directly-observed in media that removed the solubilizing aqueous solvent. We have therefore developed and utilized the micropipette manipulation technique to study supersaturated single microdroplets and microcrystals by removal of the water solvent into a second immiscible solvent (in this case octanol or decane). This paper then reports new studies that have measured in situ both supersaturation (as homogeneous nucleation) and saturation (as crystal re-dissolution) for single microdroplets of NaCl solution using the micropipette technique and has characterized microdroplet growth in saturated solutions as well as crystallization morphology as a function of rate of supersaturation.

This method has the advantage of being able to manipulate individual microdroplets and their eventual microcrystals as single, individual microparticles. As such we have shown that it informs bulk homogenization and microfluidic investigations of APIs and polymer microparticle fabrication. Using such minute nanograms of material, also allows us to make measurements on APIs that are expensive or in short supply. Another advantage of using the micropipette technique comes from the ease of probing the formed microparticles by applying well-defined forces to the microparticle
by adjusting the micropipette pressure. This is extremely useful and important in order to test for single microparticle rigidity, which is not always obvious when forming microparticles of polymers, sugars or proteins and observing them microscopically, simply on a cover slip. Finally, by holding a single microdroplet stationary in a second dissolution medium allows us to maintain diffusion-controlled conditions, and even measure the diffusion coefficients of the dissolving materials. In this sense it is similar to other studies where the Peclet number is smaller than one, as in Saidov et al’s study of sodium sulfate films by air-drying of various porous materials at different temperatures.

Saturation and Supersaturation in Single Microdroplets
As shown previously by Duncan and recently by Bitterfield, when an aqueous NaCl solution microdroplet is introduced into an immiscible medium, such as a long chain alcohol, the water-solvent will leave the microdroplet, dissolving into the alcohol. Correspondingly, the concentration of NaCl inside the microdroplet will increase, leading to the concentration of NaCl passing its solubility limit and moving into the meta-stable supersaturated regime. In the absence of a heterogeneous nucleation site, at some critical point, the solubilized NaCl will undergo homogeneous nucleation. This has a higher chance of happening the higher the supersaturation gets, and, at a specific concentration, homogeneous nucleation will spontaneously occur. This “critical” concentration, commonly referred to as the supersaturation limit, has been a central aspect of the physical chemistry of solutions and crystallization since the 1800s. The supersaturation fraction or dimensionless supersaturation, $S = \frac{C}{C_s}$ also sometimes called $\beta$, where $C$ is the actual concentration at any time and $C_s$ is the solubility of the solute, is the most commonly used term when discussing supersaturation. The term is often used in nucleation equations like Classical Nucleation Theory or more advanced nucleation models, where the critical value is signified by $S_c$.

Supersaturation and its Current Measurements for NaCl
The supersaturation fraction of NaCl in water has been measured for both homogeneous and heterogeneous nucleation. In NaCl- and KCl-H$_2$O systems Tang et al. measured $S = 1.87$ in a microdroplet solution using a levitating micron-sized
electrolyte solution method. For heterogeneous nucleation Grossier et al. \textsuperscript{11} measured $S \sim 1.97$ using a sharp tip microscopy method where single nucleation events were launched as soon as a microtip touched the confined supersaturated metastable solution at $S > 1.97$. Other groups such as Desarnaud et al. \textsuperscript{14} have worked on NaCl nucleation inside micro capillaries of different geometries (square and circular) and chemical surfaces (silanized and non-silanized), by controlling the water evaporation rate. They studied spontaneous nucleation and growth of sodium chloride crystals spanning several orders of magnitude in volume (capillary sizes from 20 – 2000 $\mu$m), finding that nucleation happens reproducibly at a supersaturation of $S \sim 1.6$, irrespective of capillary size, geometry and relative humidity. They further showed from classical nucleation theory that $S \sim 1.6$ corresponded to the point where nucleation first became observable on experimental time scales, confirming that sodium chloride can easily reach high supersaturations. Thus, the supersaturation fractions reported in the literature for both homogeneous and heterogeneous nucleation cover a wide range, between $S = 1.60 – 1.97$. As shown here, we measure a supersaturation fraction of 1.9.

It is interesting to consider the ion-to-water molar ratio at these high supersaturation fractions. With a solubility of 5.4 M, supersaturation fractions of 2.0 will have 10.8 M of both Na$^+$ and Cl$^-$ or 21.6 M ions. Since pure water has a water concentration of 55.5 M, more than a third of a liquid NaCl solution microdroplet at a supersaturation level of 2.0 will consist of solvated ions, i.e., a water-to-ion mole ratio of 2.6. In a paper by Afanasiev \textsuperscript{26} that described the state of hydration shells of sodium chloride in aqueous solutions at and below the complete solvation limit (CSL) of 21.53 wt% (5.4 M), measurement of the solvation number ($h$) gave a value of 11.1 at 4.5 M NaCl. Here, the hydration number is the number of water molecules per molecule of solute whose density and compressibility substantially differ from those of a pure solvent. The values of $h$ gradually decrease with increase of electrolyte concentration. Interestingly, the form of the hydration number dependence on solute concentration is close to exponential and so if we extrapolate this data to 10.8 M, we get a hydration number value of approximately $h = 3.6$. This value is in close agreement with our estimated value and, as also mentioned by Afanasiev \textsuperscript{26}, indicates a strong overlap of hydration shells of ions at high salt concentrations.
The level of supersaturation is also important for the type of crystal that is created. Another interesting feature of crystal nucleation and crystal growth is that the rates of concentration-increase and nucleation influence the crystal morphology of the precipitated crystal. High levels of supersaturation at the time of nucleation have been observed to grow either dendritic crystals or ‘Hopper’ (skeletal) crystals, which grow at a much faster rate than the cubic crystals formed at lower supersaturation concentrations. This phenomenon of different crystal structures was also observed by Duncan in his PhD thesis. Following Su, who showed that water dissolves much faster into long chain alcohols compared to alkanes, Duncan studied dissolution of water solvent from single microdroplets of salt solutions into long chain alcohols and alkanes. Dendritic crystals were formed in decanol at high supersaturation values of ~10 M, while cubic single crystals were formed in decane at supersaturations of ~7 M. These data are now confirmed in this paper together with a possible new hypothesis to be tested regarding interfacial nucleation.

**Mechanisms of Solution Dissolution and NaCl Nucleation**

The two-step mechanism for NaCl nucleation proposed by Chakraborty and Patey described concentration fluctuations followed by spatial ordering. This mechanism supports the idea that the concentration of the precipitating solute is very important for the availability of ions, resulting in the occurrence of dense ion ‘patches’ prior to spatial ordering. While salt-water solutions and evaporative systems have been studied since 1952, a model description of the process of evaporation/dissolution leading to eventual nucleation and crystallization events have only recently been introduced in the literature. The nucleation model of Desarnaud et al. describes the drying of sandstone imbibed with a saturated NaCl solution. They divide the dehydration into three regimes, namely a capillary regime, an exponential regime, and a diffusive regime. Chu and Prosperetti, inspired by Su’s binary microdroplet dissolution model, derived a more complex dissolution model based on Epstein and Plesset’s model (EP model) for dissolving gas bubbles (which was also shown to be valid for pure liquid droplet dissolution), but this time incorporating the UNIQUAC (UNIversal QUAsiChemical) model for more precise prediction of the activity of the microdroplet components. Bitterfield et al. then presented a simplified activity-driven dissolution model validated by experimental
results from NaCl solutions dissolving in octanol and butyl acetate. This model proved to be more practical regarding experimental approaches and was able to precisely predict the total dissolution process of a dissolving NaCl solution microdroplet with changing water activity. Thus, being able to predict parameters such as diffusion coefficients, solubilities and supersaturations governing the dissolution process is important for processes such as nucleation/precipitation \(^7, 41-42\), nano/micro particle/microdroplet formulations \(^12, 42-44\), pharmaceutical formulations \(^12, 44-50\) and ore processing \(^51-53\). Furthermore, crystal morphology and water activity are important in food sciences \(^54\), especially when talking about conservation and longevity of food products, where salted food can take up water from the surrounding environment to reduce longevity.

**The Micropipette Manipulation Technique**

The micropipette manipulation technique has been used extensively since the early 1970s to manipulate and investigate biological cells \(^55-56\), lipid vesicles \(^2\) and the swelling and lipid-coating of microgels \(^57-58\). It has also been adapted to make measurements of surface tensions at air-water and oil-water microscopic interfaces inside the tapered micropipette by Lee et al, including lipid and surfactant adsorption \(^59-60\) and recently for common surfactants \(^61\) and more complex lung surfactants by Parra et al \(^62\). Since the pioneering work of Duncan \(^63\), it has found specific application in making equilibrium and dynamic, real-time measurements of single microdroplet dissolution \(^8-9, 39\), protein Microglassification \(^43, 64\), polymer microspheres \(^65\) and salt microcrystals \(^6-7\). Here, we follow this earlier microdroplet work to expand on the parameters that can be measured using the micropipette manipulation technique. We describe a new “three pipette technique” for forming, dehydrating, crystallizing, and re-solvating nano-grams of salt material. This method allows accurate and reproducible measurements to be made of the critical supersaturation and saturation concentrations of crystals, tested here for NaCl. We also use the “2-pipette technique” \(^3\) to evaluate the effects of slower rates of approach to critical supersaturation crystallization (seconds to form single cubic crystals vs milliseconds to form dendritic crystals) by using NaCl solution microdroplets in n-decane, in which water dissolution was slower. These data suggest a possible new hypothesis for single crystal nucleation and growth that could involve a form of heterogeneous
nucleation at the hydrophobic interface. Furthermore, the work also reports new applications of the EP model applied here for the first time to predict microdroplet growth in saturated solvent solutions.

**The Epstein-Plesset Model**

In order to analyze the microdroplet dissolution data, we use the EP equation in its dimensional and dimensionless forms. Briefly, as presented in several recent papers and reviewed in the Supporting Information (S1), in 1950 Epstein and Plesset derived an equation for the dissolution of a stationary gas bubble undergoing diffusive controlled dissolution in water. The equation was fully tested for the first time on micro-gas bubbles and then on a liquid microdroplet system by Duncan and Needham. In these studies, the original EP equation was expressed in the dimensional form of the laboratory frame (actual radii and time) for direct comparison with experiments carried out using the micropipette manipulation setup. Thus, the dissolution rate of the microdroplet is expressed as the measurable radius as a function of time \( \frac{dR}{dt} \), given by:

\[
\frac{dR}{dt} = -\frac{DC_s}{\rho} (1 - f) \left[ \frac{1}{R} + \frac{1}{\sqrt{\pi Dt}} \right]
\]  

(1)

where, for the water-in-octanol system, \( C_s \) is the solubility of water in octanol, \( D \) is the diffusion coefficient of water throughout the chamber medium (octanol), \( \rho \) is the density of water, and the concentration at \( r = \infty \) is the initial concentration of water in the surrounding medium, \( C_b \). Hence, \( f = C_b / C_s \), the fraction of pre-saturation of the microdroplet material (water) in the bath medium (octanol).

However, microdroplets of different initial sizes can only be compared by converting the size and time axes to dimensionless form. Following Duncan and Needham’s papers on water-dissolving gas bubbles and liquid-liquid systems, Zhu described how the dissolution profiles collapse onto a single dissolution curve, independent of initial size. By setting \( \xi = R/R_0 \) and \( \tau = \frac{\pi D t}{R_0^2} \) eq. 1 can be written in the dimensionless form of eq. 

\[
\frac{d\xi}{d\tau} = -\frac{C_s}{\pi \rho} (1 - f) \left[ \frac{1}{\xi} + \frac{1}{\sqrt{\tau}} \right]
\]  

(2)

Fejl! Henvisningskilde ikke fundet.
In the recent paper of Bitterfield et al.\textsuperscript{7}, the EP equation was modified to account for the kinetic effects of the changing water activity at the microdroplet interface during the dissolution of an aqueous salt solution microdroplet into a second, immiscible, liquid phase. By introducing the term, \( f_i = \frac{C_i}{C_s} \) (where \( C_i \) is the concentration of water in octanol at the microdroplet interface), which represents the saturation fraction of the microdroplet solvent in the surrounding phase, exactly at the microdroplet interface, the assumption of a local thermodynamic equilibrium at the microdroplet boundary was shown to be correct. This led to the activity-based dissolution model\textsuperscript{7}.

\[
\frac{\delta R}{\delta t} = -\frac{DC_s}{\rho}(f_i - f) \left[ \frac{1}{R} + \frac{1}{\sqrt{\pi D t}} \right]
\]  

(3)
The Epstein-Plesset Model for Microdroplet Growth Predictions

Just as the EP model can predict microdroplet dissolution, we hypothesized that it could also represent solution microdroplet growth in a saturated medium. This aspect of the EP model has not been tested before using single microdroplets. Referring now to Figure 1, any microdroplet formed in an environment represented by the top-right part of Figure 1 (i.e. above the equilibrium line) will undergo microdroplet growth by taking up water from the octanol phase until it reaches the conditions symbolized by the equilibrium line (downward arrow signifying growth). A NaCl-containing microdroplet introduced into an environment represented by the bottom-left part of Figure 1 (i.e., below the equilibrium line) will undergo dissolution of water until it reaches the equilibrium conditions symbolized by the equilibrium line (upward arrow signifying microdroplet dissolution). If the water saturation in the chamber is low enough (f < 0.25), microdroplets will undergo water dissolution until they reach the supersaturation limit, where NaCl nucleation will occur.

Figure 1 Boundary Concentration. Correlation between the concentration of NaCl inside a water solution microdroplet, and the saturation fraction of water immediately at the microdroplet interface and in the surrounding octanol phase. The equilibrium curve represents the points where a solution
microdroplet will neither undergo dissolution nor growth (created from literature data represented by  
the open circles 67-68). When a solution microdroplet of low NaCl concentration has a water saturation 
at its interface (fI) higher than that in the bulk octanol, it will undergo dissolution by losing water 
(bottom left). However, if it has a water saturation at its interface lower than that in the bulk octanol, it 
will undergo growth by imbibing water (top right). The green shades indicate the level of water 
saturation (i.e. high water activity in dark green areas).

As previously shown by Epstein and Plesset for expanding gas bubbles 40, an 
oversaturation of gas in the surrounding medium relative to the bubble will cause the 
bubble to grow. Similarly, a microdroplet containing a solute or a second solvent, 
which lowers the water activity inside the microdroplet to a value lower than the 
activity of water dissolved in the surrounding phase, will cause the microdroplet to 
widen and grow 38 by diffusion controlled growth (by imbibing water from the 
surroundings). We therefore hypothesized that the extended EP model would also be 
able to predict diffusive growth of a microdroplet containing relatively high 
concentrations of NaCl. Literature data already available for the water-NaCl-octanol 
system 25, 67-68 was used to construct a polynomial function for fI(C), Figure 1. The 
dashed line (obtained by combining data from Lenzi and Segatin 67-68, open circles) in 
Figure 1 is an equilibrium line representing systems where the activity of water inside 
the microdroplet is at the same value as that of the water in the surrounding octanol 
phase. This indicates that all water in the system is in thermodynamic equilibrium and 
no net diffusion of water will happen, thus leaving the microdroplet at the same size 
indefinitely.

Experimental Section

Research Design

The goal of the study was to first establish and use the three-pipette technique to 
simultaneously measure the supersaturation concentration and the solubility of NaCl 
in water, using only nanograms of material. Furthermore, it was to test the 
applicability of the activity-based Epstein-Plesset model to diffusion-driven 
microdroplet growth in a water-saturated medium such as octanol, by measuring the 
size of a growing NaCl solution microdroplet with time. Finally, it was to investigate 
the difference in nucleation concentration and crystal morphology by changing the
external medium from octanol to decane, thus changing the dissolution rate from fast to slow, thus increasing the induction time for nucleation to occur.

Expanding on the single micropipette technique used in the earlier study by Bitterfield, it is possible to use the two-pipette technique developed by Kinoshita et al to ensure fast microdroplet formation and eliminate the need for ‘time of formation’ correction as well as correction for micropipette coverage, since the ‘catching’ pipette will contain the chamber medium (see supporting information S2).

Using the approach of Bitterfield, by introducing an aqueous solution microdroplet of NaCl with a known concentration into a surrounding octanol medium it is possible to get data of size and concentration vs time for measuring the supersaturation and nucleation concentrations. By introducing a source of pure water close to the nucleated NaCl crystal (with a known weight), the difference in chemical potential of the pure water source and water dissolved in the octanol medium, will create a water gradient into the octanol medium. Water will adsorb onto the NaCl crystal surface, thus initiating re-solubilization and creating a new NaCl-saturated solution with low water activity. When enough water is present around the NaCl crystal it will again become a spherical microdroplet where concentration can be measured as a function of time for the microdroplet until the entire NaCl crystal re-dissolves in the new water phase, thus giving the solubility of NaCl in water.

Placing a concentrated NaCl solution microdroplet into a water-saturated octanol system will, in the same way as above, result in the microdroplet imbibing water from the surrounding phase based on the difference in chemical potential. Microdroplet growth of size and decrease in NaCl concentration vs time can then be measured and compared with the activity-based model predictions.

It is hypothesized that the induction time of a NaCl solution microdroplet in the supersaturated area can affect the concentration reached at the time of nucleation because of the stochastic nature of nucleation events. Thus, by changing the microchamber medium from octanol to decane, in which water dissolves more slowly, it will be possible to measure the NaCl concentrations reached at longer experimental times. It will furthermore be possible to observe the effect on crystal morphology.
Materials
Sodium chloride (NaCl) (≥99.8%; Sigma-Aldrich, Brøndby, Denmark; Lot: SZBB215BV) was used as received. 1-octanol (≥99%; Sigma-Aldrich, Brøndby, Denmark; Lot: SHBD9399V) (≥99.5%; Fluka, Switzerland; Lot: 422511/2 54201) and decane (≥99%; Sigma-Aldrich, Brøndby, Denmark; Lot: MKBP6360V) were stored over molecular sieves (4 Å; Sigma-Aldrich, Brøndby, Denmark; Batch: 034K0229) to keep them dry. Hexamethyldisilazane (HMDS) (≥99%; Sigma-Aldrich, Brøndby, Denmark; Lot: SHBB7795V) was used as received. Milli-Q water was prepared by a Millipore Integral 5 ultrapure water system (>18 MΩ·cm). Hexadecane (≥99%; Sigma-Aldrich, Brøndby, Denmark; Lot: STBD2011V) was used as received as a solvent plug. All experiments were carried out at a temperature of 20 – 22 °C.

NaCl Solubility in Octanol
NaCl is, in fact, somewhat soluble in the octanol phase. Its solubility in octanol was predicted to be 2.26 mg/kg\textsuperscript{69-70}, i.e., 32.8 µM (supporting information, S4) and so, although relatively small compared to water in the microdroplet experiment, NaCl will dissolve into the octanol phase if exposed for an extended period, thus reducing the NaCl concentration inside the microdroplet and increasing the water activity. This will result in the movement of water out of the microdroplet until the system returns to the equilibrium state. This process will continue until the entire microdroplet is dissolved, controlled entirely by the rate of NaCl dissolution into the octanol phase. In the time frame of our experiments, the NaCl dissolution into the octanol phase is negligible (due to its very low solubility) and we can therefore assume the system to be in a state of quasi-equilibrium.

Methods
For detailed information about making, filling, mounting, and manipulating the micropipettes, details of microscope chambers, pressure regulation, temperature and time measurements the reader is referred to \textsuperscript{3, 7-8, 43, 63-64, 71-75}. In the micropipette experiments it is common to use either a single micropipette to form and manipulate the microdroplets \textsuperscript{8-9, 39, 43, 63}, two micropipettes, including a forming and ‘catching’ pipette, developed by Kinoshita et al \textsuperscript{61}, or three micropipettes, bringing the third pipette to change the local environment of the formed microdroplet or microparticle.
(e.g., a micro crystal) \(^{76}\). Thus, in this study three micropipette techniques were available for microdroplet dissolution experiments. The ‘single’- and ‘two’- pipette methods have been presented previously \(^3, 61, 63\) and so are briefly described in supporting information S2. The ‘three-pipette’ method presented here is a new development and so will be described in more detail.

**Three-Pipette Method**

In the ‘three-pipette method’, as shown in Figure 2a, two pipettes are introduced into the microchamber (2.8 mm x 5.0 mm rectangular capillary from CM Scientific) from opposite sides. The right hand (RH) micropipette contains a NaCl solution and the left hand (LH) micropipette contains the octanol that is already filling the chamber. A microdroplet is gently blown out of the RH pipette containing the NaCl solution (Figure 2a-b) until it just contacts the opposite, LH pipette (Figure 2b). This is referred to as the ‘Microdroplet Catching Method’ \(^61\). As the microdroplet emerges from the RH pipette, the LH “catching” pipette uses a low suction pressure to hold onto the microdroplet Figure 2b, and the RH pipette is withdrawn from the NaCl solution microdroplet, leaving it isolated, bathed in octanol, on the tip of the second pipette Figure 2c. In the dissolution experiment, water then leaves the microdroplet into the octanol phase, eventually precipitating a NaCl crystal Figure 2d. By measuring the microdroplet volume change, and hence the concentration from a known initial concentration, the nucleation point gives the critical supersaturation concentration of NaCl at its precipitation (Figure 2d). A source of pure water is then introduced close to the NaCl crystal from a third water-containing pipette (colored in dark blue) coming in from an angle (Figure 2e). There is a meniscus of pure water right at the micropipette tip and this acts as the exposing-source of pure water. The water diffuses from this meniscus interface through the octanol and re-solubilizes the NaCl crystal (Figure 2f), leaving a small concentrated NaCl solution as a newly formed microdroplet, from which the solubility of NaCl in water can similarly be determined by noting the exact volume at which the crystal completely dissolves. The microcrystal dissolution in the newly formed aqueous phase microdroplet is very rapid and is thus not the limiting step during re-solubilization.
Figure 2 The ‘Three-pipette’ technique. The ‘three-pipette’ technique uses a chamber, which is open in both ends. **a)** Two pipettes are introduced, one from each end; one contains a solution of concentrated NaCl, RH; the other contains the octanol filling the chamber, LH. **b)** The RH pipette containing the NaCl solution blows out a microdroplet until it just touches the LH pipette. **c)** The LH pipette uses a low suction pressure to hold onto the microdroplet, while the RH pipette is retracted away from the microdroplet, leaving an isolated NaCl solution microdroplet in octanol. **d)** Water leaves into the octanol phase and the NaCl crystal nucleates and the critical supersaturation concentration of NaCl in water can be determined. **e)** A source of pure water is introduced close to the NaCl crystal from a third pipette coming from an angle. **f)** The water diffuses through the octanol and re-solubilizes the NaCl crystal, leaving a small concentrated NaCl solution, from which the solubility of NaCl in water can be determined. For images of the experiment, refer to Figure 3.

Thus, since pure water by definition has an activity of 1, it dissolves into the surrounding octanol phase and moves away from the pure water source via diffusion and establishes a water concentration gradient. The concentration of water in this gradient is sufficient to re-solubilize a NaCl crystal when the water source is held in close proximity (<10 µm meniscus to crystal distance). Thus, as in Figure 1, the high concentration of water in the octanol (on the order of its saturated concentration, 2.4 M) envelopes the NaCl crystal, forms a new aqueous solution microdroplet around the microcrystal and begins to dissolve the NaCl crystal. The water activity in the immediately surrounding octanol phase is ‘controlled’ by changing the distance between the crystal, or the newly formed microdroplet and the pure water source micropipette. Upon removing the water source, the microdroplet undergoes another round of dissolution and re-crystallization. This sequence of re-solubilization in water from an enveloping water-saturated octanol phase, and re-crystallization in water-
depleted octanol, was repeated up to four times. Since the initial volume and concentration of the formed microdroplet of NaCl solution is known, and its size can be accurately measured, this experiment provides a measure of the supersaturation concentration for the initial crystallization as well as the saturation concentration (solubility) of NaCl in water, and a second check on both when repeated. The only caveat is that during this process, and especially for the isolated crystal, there is some dissolution of the NaCl into the surrounding water-saturated octanol. So practically, this experiment can only be repeated 2 times to 15% accuracy assuming the initial amount of NaCl in, for example, a 48 µm radius microdroplet of a 0.1 M NaCl solution.

Considerations in the Measurement of Relative Solubility
The solubility of NaCl in water is 315 g/L or 5.4 M at ambient temperature \(^{11, 14, 70}\) (see supporting information S3). The solubility of NaCl in dry octanol, predicted from the solubility in the C\(_1\) – C\(_5\) n-alcohols \(^{69-70}\), is 2.26 mg/kg or 32.8 µM (see supporting information S4), but is expected to be higher in water-saturated octanol. Therefore, if all the solvent (water) is lost from a 50-µm radius microdroplet of 5 M NaCl solution, and there is no loss of NaCl into the octanol phase during the time of the experiment, a 153-ng microcrystal should be formed. The size of the microchamber filled with octanol used for this work was 0.16 mL. Approximately 300 ng of NaCl was therefore needed to reach saturation of the entire chamber. Figure S2b from the supporting information of Bitterfield \(^7\) shows how, after the dissolution of the aqueous phase from a NaCl solution microdroplet into pure octanol, which took about 5 minutes, a NaCl microcrystal was formed and then slowly dissolved in pure octanol over the following 32 hours. Since the crystal was not of symmetric geometry it could not be measured exactly, but the dissolution rate of a ~4.9 ng crystal of NaCl in pure octanol was estimated to be 2.3 pg/min. The much larger 153 ng NaCl crystal mentioned above would thus take ~1108 hours (or 46 days) to dissolve. This is a very long time when compared to dissolution experiments, which are usually less than 30 min. The NaCl is therefore assumed kinetically stable during experiments in pure octanol, even though it is thermodynamically unstable and will dissolve if left for extended periods \(^7\).
Results and Discussion

Results are first presented for measurements of both the critical supersaturation and saturation of NaCl in water by using the ‘Three-Pipette’ method, previously developed together with Kinoshita. In that case, experiments were for hydrophobic microparticles precipitated into water from an immiscible solvent (chloroform) and are now adapted here for this study. Then, the extended Epstein Plesset model of Bitterfield was used to predict microdroplet growth (see Figure 1). This required the development of another new experimental protocol that tested the EP equation by exposing microdroplets of highly concentrated salt solution to a water-saturated octanol medium. Finally, the external chamber medium was changed from octanol to decane to test the effect of microdroplet dissolution rate (slower in decane) on crystal formation; the decane-water interface also presented a more hydrophobic interface than for octanol. Note: Control experiments and data are presented in Supporting Information, S5 + S6.

For experiments with NaCl solution, we anticipated that we would have to produce large droplets to produce large crystals for sufficient microscopic resolution observation and measurement. Thus, having had to use water droplets that were larger in diameter (160 µm) by about a factor of 3-4 compared to those used by previous workers (Duncan, 50 µm and Su, 40 µm) the negative controls are still important to show (Supporting Information, S5). Also, we used the relatively new two-pipette technique to form larger droplets in the same amount of time as Duncan, Su and Bitterfield in order to lessen the significance of formation-losses of material. We therefore tested this for measurements of diffusion coefficient against the corrections made by Su. Finally, as controls for saturation, supersaturation and crystallization in decane, Supporting Information S6 contains similar NaCl experiments to the crystallization experiments carried out by Bitterfield, but this time using larger microdroplets and none of the equation corrections described in Supporting Information, S1. These experiments include measurement of the supersaturation limit where homogeneous nucleation occurred at 10.3 ± 0.3 M, which is a supersaturation fraction of S = 1.9. These values are in great agreement with Bitterfield’s results and slightly higher than the values of Desarnaud et al. When doing experiments with NaCl solutions it is important to keep in mind that the
rearrangement of the solvated ions need to happen faster than the movement of the receding microdroplet boundary. This was confirmed previously for Na\(^+\) and Cl\(^-\) ions in water \(^7\). If this rearrangement does not happen fast enough, a more concentrated boundary layer will build-up and affect the nucleation kinetics \(^77\) or form surface structures around the microdroplet \(^4\).

**Supersaturation and Saturation Measurements**

Previously, Kinoshita et al. \(^76\) observed and measured the diameter of a microdroplet solution of a series of hydrophobic materials in chloroform dissolving in an aqueous solvent as the hydrophobic material was precipitated into water. It was found that, for a known initial concentration of solute in the microdroplet, the supersaturation concentration, \(C_{ss}\), of the material was measured when enough solvent had been removed to induce solute precipitation. Then, after precipitation, if the precipitated crystal was brought up to the same solvent source (e.g., exposed to a flow of saturated solvent solution from a third pipette), the particulate crystal imbibed the solvent and eventually re-dissolved. At this point its solubility, \(C_s\), was measured. Thus, this unique micropipette experiment could measure both the critical supersaturation and the solubility limit for a solute in any solvent that was immiscible with the surrounding dispersion medium. While Duncan \(^6\) and Bitterfield et al. \(^7\) had measured the supersaturation limits for NaCl in water (and we confirmed these in Supporting Information, S6), the re-dissolution experiment was never carried out. Preliminary experiments were carried out on hydrophilic crystals \(^78\), and these laid the foundation for the present study \(^4\). Additionally, Desarnaud and Bonn \(^79\) used a similar approach of deliquescence/crystallization cycling of NaCl crystals using relative humidity control to resolubilize the crystal. They found that repeated cycles produced increased supersaturation and formed large single cubic crystals with increased purity.

As shown in Figure 3, \((t = 0 – 190 \text{ s})\), the ‘three-pipette technique’ (Figure 2) was used to form and dissolve the 0.1 M NaCl microdroplet solution. Water solvent dissolved out of a water or solution microdroplet into the surrounding octanol. For the NaCl solution microdroplet, it eventually precipitated a NaCl crystal from the aqueous solution. For this 96 \(\mu\)m diameter microdroplet the time to reach precipitation took
180 s and the microcrystal had a calculated mass of 2.7 ng. After having formed the 
NaCl crystal, the third, relatively large pipette containing pure water \((a_w = 1)\) was 
introduced at \(t = 210\) s as shown in Figure 3. The interface of the water meniscus 
was not blown out of the micropipette tip but was kept at the tip of the pipette. This 
water-octanol interface was then moved close to (<10 µm) the NaCl crystal. Due to 
the water activity difference between the pure water inside the large pipette and the 
water in the octanol phase immediately outside the interface, water dissolved into 
the octanol phase and the NaCl crystal started to imbibe water. Water first started to 
appear on the crystal surface. It then formed as a microdroplet around the crystal, 
Figure 3, \(t = 240\) s. The NaCl crystal continued to imbibe water, until it was finally re-
solubilized in the form of a new aqueous-phase microdroplet, as shown in Figure 3, \(t 
= 252\) s.

![NaCl solution microdroplet dissolution and NaCl crystallization](image)

**Figure 3. Critical Supersaturation and Saturation Measurement. Top Row.** Dissolution of a 0.1 M NaCl solution microdroplet followed by NaCl nucleation and drying to a 2.7 ng crystal \((t = 0 – 190\) s). **Bottom Row.** A third large micropipette containing pure water was then introduced in close proximity to the NaCl crystal \((210\) s). Water now diffused from the pure water meniscus, through the octanol phase and onto the crystal surface, and started re-solubilizing the crystal \((t = 240\) s). This continued until the point of total re-solubilization \((t = 252\) s), from which the solubility of NaCl in water could be calculated. Once dissolved, the microdroplet continued to imbibe water as it diluted the NaCl solution \((t = 262\) s). As soon as the third pipette was removed, the solution microdroplet dissolved and the NaCl re-crystallized \((t = 302\) s).

Leaving the newly formed aqueous microdroplet in close proximity to the water 
interface, the solution microdroplet continued to imbibe water and grow Figure 3, \(t =
This growing process continued until the third pipette was removed allowing pure octanol to again bathe the microdroplet, at which point the microdroplet again lost water into the octanol and the NaCl re-crystallized Figure 3, \( t = 302 \) s. Although not known specifically, the water saturation and the water activity in the surrounding octanol was controlled simply by controlling the proximity and placement of the water source in the third pipette in relation to the crystal (Bitterfield has previously calculated that the water-saturation level drops from >90% saturated at a distance of 10% radius from the pure water source’s interface, to <50% at a distance of 100% of the radius\(^8\)). Such small-displacement manipulation is a feature of the micropipette technique that allows precise movement and placement of all pipettes in 3 dimensions using the stage-micrometers and a custom built pneumatic joystick controller. It was therefore possible to control the rate of water diffusing to the NaCl crystal or solution microdroplet by such precise and controlled manipulation of the water-filled micropipette. With a diffusion coefficient of water in octanol of \(2.0 \times 10^{-6}\) cm\(^2\)/s as measured by Su et al.\(^9\) (and confirmed by us in Supporting Information, S5), one molecule of water would travel a mean distance of 20 µm in one second. Therefore, simply moving the pure water interface at the tip of the large pipette several 100 µm away from the re-solubilized NaCl microdroplet resulted in the rapid isolation of the microdroplet in pure octanol again, and allowed the water to re-dissolve and the NaCl to re-nucleate and precipitate as a crystal once more, Figure 3, \( t = 302 \) s. These images were analyzed to provide quantitative data of the radius of the solution microdroplet versus time. Since the initial concentration was known, the NaCl concentration in the microdroplet was therefore also known as a function of time. Thus, in Figure 4, plots were created of the radius of the microdroplet from start to finish, along with the calculated concentrations. As shown in Figure 4A, the radius of the NaCl solution microdroplet decreased as water solvent was lost to the octanol phase. The points of nucleation are indicated by a star symbol. The gap in the time course simply indicated that the crystal was present as dehydrated material surrounded by ‘dry’ octanol until the third pipette was brought into position (corresponding to Figure 3, \( t = 210 \) s) and it started to imbibe water (\( t \sim 230 \) s not shown). This imbibement of water caused an increase in the size of the particle and a new microdroplet was formed. At this point, the spherical radius was again
measurable (corresponding to Figure 3, t = 240 s), but still contained crystalline material, as indicated by the filled circles in Figure 4A. An increase in radius of about 8 µm resulted in the entire crystal being re-dissolved (filled triangles). When the third water-pipette was removed, the radius of the microdroplet, as shown by the open circles, again decreased until re-crystallization, indicated by the second star. This sequence was repeated two more times, as shown in Figure 4A. The system was setup with a new chamber and micropipettes and measured a total of 4 times, showing good reproducibility.

Figure 4 Microdroplet size and concentration for Supersaturation and Saturation Measurements. A Radius as a function of time for the initial 0.1 M NaCl solution microdroplet dissolving in pure octanol, followed by re-solubilization and re-crystallization (3 cycles). B Corresponding concentration changes as a function of time for the same NaCl solution microdroplet. Concentrations are calculated as total amount of NaCl (solution + crystal) inside a confined area (microdroplet boundary). Open circles represent NaCl solutions. Filled circles represent time points where a crystal is present inside the microdroplet. Triangles represent points of total re-solubilization.
and stars represent points of nucleation. For each cycle, the nucleation and re-solubilization concentrations measured increased, indicating that NaCl mass was not constant during repeated cycles. Reference values of NaCl solubility (5.4 M, dotted line) and average supersaturation limit (10.3 M, dashed line) are presented giving a relative supersaturation $S$ of 1.9.

The corresponding concentration changes (Figure 4B) gave the concentration of the solution at any time point represented by an open red circle. Filled red circles represent time points during re-solubilization, where a solid NaCl crystal was present inside the microdroplet boundary. The ‘concentration’ at these time points was calculated as the total NaCl amount (solution + crystal) present inside the microdroplet boundary. As mentioned earlier, keeping the pure water interface close to the re-solubilized microdroplet after the point of total re-solubilization resulted in the microdroplet taking up water beyond that which was necessary to reach the solubility limit of NaCl. This meant that, as seen in Figure 4B, $t = 262$ s, the concentration went lower than the saturation limit (in this case to approximately 5.0 M), showing that the chemical potential of water in the new salt solution microdroplet had not yet equilibrated with the pure water in the micropipette.

Thus, by knowing the initial concentration of NaCl in the microdroplet, (0.1 M in a 465 pL microdroplet, NaCl mass = 2.7 ng) and measuring the radius, and therefore volume, of the initial microdroplet solution, the loss of water and first crystallization (star symbol at 180 s) gave the supersaturation value, in this case 11 M in Figure 4B. Similarly, for the new growing solution microdroplet (decreasing concentration filled circles) the exact moment of crystal re-solubilization was obtained (first filled triangle), giving the solubility limit of NaCl in water, calculated here to be $5.5 \pm 0.1$ M. This value for the solubility limit of NaCl in water is in good agreement with our own gravimetric measurements of NaCl solubility in water of $5.42 \pm 0.06$ M (see supporting information, S3). Figure 4B shows three repeated cycles of re-solubilization and re-nucleation, all producing a single crystal as in the work by Desarnaud and Bonn $^{79}$, but here from the first cycle due to the relative small microdroplet size and lack of surface impurities. On average from the first re-solubilization points, the values of NaCl solubility were $5.5 \pm 0.1$ M (micropipette) and $5.4 \pm 0.06$ M (gravimetric) and the supersaturation limit was $10.3 \pm 0.3$ M, giving a relative supersaturation $S$ of 1.9.
Comments on the new technique: limitations and system requirements

This technique has thus been shown to successfully measure solubility using very small amounts of material. Since the density of NaCl is 2.16 g/cm$^3$, a relatively large microdroplet, like the one shown in Figure 3, formed a ~2.16 ng NaCl crystal. It is important to note that this technique has an obvious limit to how small a crystal it can handle. This limit is dependent on two criteria: the optical resolution limit (which for this objective and numerical aperture was 620 nm); and the practical size of the micropipette tips used to handle the microdroplets and microcrystals, where the smallest tip size we make and can visualize is 1-2 µm.

For each material investigated it is always important to design the experiment with optimal solvents and solutes, considering the solubility of each chemical in each other. In this case, we would want to maximize the water dissolution into the solvent (i.e. into-octanol) and minimize the solvent dissolution into the aqueous microdroplet solution (i.e. octanol-into-water), as well as optimize the solvents so that the solubility of the salt, (NaCl) is maximized in the aqueous phase to ensure sufficient material to be observable when crystallized and minimized in the solvent phase to reduce or eliminate losses. Clearly, both water-into-oil and oil-into-water systems can be studied. Thus, there are at least four important criteria associated with the chemistry of the materials themselves in order to use the technique and perform a successful experiment. These include:

- The two solvents must be immiscible to form a microdroplet, but the microdroplet material should still be soluble in the surrounding medium (40 mg/mL for water in octanol).

- Also, it is preferable for the surrounding medium to have a lower solubility into the microdroplet material if possible (0.46 mg/mL for octanol in water), to avoid the medium diffusing into the microdroplet to an extent that can influence the size $^8$.

- The solute (in this case NaCl) should have a low solubility in the surrounding dissolution-medium compared to the microdroplet medium (NaCl in water, 315 mg/mL, and in octanol, 1.9 µg/mL) to fulfill the assumption of constant solute mass.
Finally, the amount of nucleated material to be investigated must be visible with a light microscope, and the crystal must have dimensions at least as big as the micropipette tip to be handled properly (at least >2 µm, but preferably ≥10 µm). This can, of course, be controlled by changing the initial microdroplet concentration or size, e.g. a 50 µm radius microdroplet with 0.1 M NaCl, as in Figure 3, produced a ~10 µm length crystal, while an 80 µm radius microdroplet with 5.0 M NaCl, as in Figure S4D, produced a ~100 µm length crystal. However, with increasing microdroplet size comes much longer dissolution times (50 µm radius microdroplet of water in octanol dissolves in ~150 s, while a 100 µm radius microdroplet dissolves in ~600 s) and thus more experimental uncertainty when it comes to model assumptions such as constant solute mass during experiments.

NaCl Solution Microdroplet Growth in a Water-Saturated System

While the loss of material in a gas-liquid and liquid-liquid system has been tested and explored in several studies, the actual growth of liquid microdroplets has not so far been experimentally quantified and tested by the EP model. The observation of water uptake continuing past the saturation limit, shown in Figure 3 and Figure 4, thus inspired a new experimental setup where the octanol phase was initially completely saturated with water and thus $a_w \approx 1$. It thus grew a concentrated NaCl solution microdroplet, (initial NaCl concentration of 5.0 M) to a larger volume because of the gradient in water chemical potential.

Growth in Terms of Diameter

The microchamber was filled with water-saturated octanol. As presented in methods experimentally, this was accomplished by leaving a 50/50% V/V water/octanol mixture overnight to allow the system to reach equilibrium and for each phase to be saturated with the other, i.e., for the water to dissolve in the octanol to its saturation limit of 40 mg/mL and for the octanol to dissolve in the water to 0.46 mg/mL. The water-saturated octanol phase should then have a water activity, $a_w$, that is identical to the mainly aqueous, (strictly, octanol-in-water) phase. For the entire experiment to proceed with a water activity in the octanol phase as close to unity as possible, the plug system described in Supporting Information S2 was used. In this experiment,
the plugs consisted of pure water, which acted as a continuous water reservoir during the experiment, prevented evaporation, and kept the water activity as close to unity as possible.

A 5.0 M NaCl solution microdroplet was formed in the same way as described earlier in the ‘three-pipette’ method. Because of the saturation of water in the chamber compared to the highly concentrated NaCl solution microdroplet, water diffused from the octanol into the microdroplet. Microdroplet growth in terms of diameter occurred as shown in Figure 5. There was more rapid increase in diameter over the first 200 s and a slowing down over the next 800 s.

By measuring the microdroplet diameter, this growth vs time could be matched with the EP model as shown in Figure 6A. It is plotted as microdroplet radius as a function of time for three independent ~30 µm radius microdroplets (as in Figure 5) of initial NaCl concentration of 5.0 M. As can be seen, these microdroplets grew in size in water-saturated octanol, i.e., in octanol at a water-saturation fraction of $f = 1$. 

**Figure 5 NaCl Solution Microdroplet Growth.** Video micrographs of an aqueous, initially 5 M NaCl solution microdroplet formed in a chamber with a pre-saturation fraction of water of $f = 1$, with water plugs at both ends of the chamber. The microdroplet initially imbibed water from the surrounding saturated octanol phase at a high rate, but as the microdroplet increased in size, the NaCl concentration inside the microdroplet decreased, thus slowing down the process.
As before for dissolution, the data were converted to dimensionless radius as a function of dimensionless time for the same three 5.0 M NaCl solution microdroplets in Figure 6B.

![Figure 6. NaCl Solution Microdroplet Growth, Size vs Time. A Radius as a function of time for three 5.0 M initial NaCl solution microdroplets growing in octanol pre-saturated to a water saturation fraction of f = 1, having water reservoir plugs. B Dimensionless radius as a function of dimensionless time for the same three 5.0 M NaCl solution microdroplets growing in octanol pre-saturated to a saturation fraction of f = 1 and having water reservoir plugs. Dashed curves are for the EP model and show good agreement with experimental data.]

Thus, we could show that the experimental data were in good agreement with both the extended dimensional EP model and the extended dimensionless EP model. Interestingly, the data started to deviate from the predicted EP model after about 600 s (10 minutes) of growth and is discussed in the context of volumes and
concentrations in the next section. Thus, the EP models, originally developed for
diffusion controlled dissolution and uptake of gas 40, 63, can now also be applied to
describe the diffusion-controlled uptake of water from a water-saturated environment
using the activity-based model of Bitterfield et al. 7. This technique and analysis can
now also be used for any other system where all relevant parameters are known. An
example of this is the control of drug/hydrogel/emulsion particle size due to solvent
uptake 81-83.

**Growth in Terms of Volume and Concentration**

When plotted in terms of microdroplet volume as shown in Figure 7A, the data can
readily be converted to NaCl concentration as shown in Figure 7B. Quantitatively
then, the corresponding concentration of NaCl in the microdroplet solution decreased
from 5.0 M to less than 1.0 M over 1200 s as the microdroplet imbibed water.
Figure 7. NaCl Solution Microdroplet Growth, and Concentration vs Time. A Microdroplet volume as a function of time, and B Concentration as a function of time for three 5.0 M initial NaCl solution microdroplets growing in octanol pre-saturated to a water saturation fraction of f = 1, having water reservoir plugs. Dashed curves are for the EP model and show good agreement with data.

Because of this, the gradient of water chemical potential (from the fully water-saturated bathing solution to the NaCl solution microdroplet) was lowered. That is, as the experiment proceeded the water uptake happened at a slower rate with increasing microdroplet volume and decreasing microdroplet NaCl concentration. Theoretically, the microdroplet should keep increasing in size indefinitely, since, no matter how much water was taken up, some NaCl would still be present inside the microdroplet to lower the water activity from its unity value. It is also assumed that the amount of NaCl in the solution microdroplet does not change, and this would certainly be valid for early times (<10 minutes). What we found experimentally was that, after the first 10 – 20 minutes, the increase in size was so slow that the assumption that the NaCl amount in the microdroplet was constant started to become
invalid. NaCl’s solubility in pure octanol (2.26 mg/kg or 32.8 µM) would be expected to be higher in water-saturated octanol, thus dissolving the microdroplet into the water-saturated octanol over prolonged periods of exposure. This also explained why all experimental microdroplet radii in Figure 6 and volumes in Figure 7A were slightly lower than that predicted by the EP model at long experimental time points, and why deviation from the model becomes larger and larger as experimental time increases. Nevertheless, the microdroplet size and concentration data were within 5% of the predicted values out to 600 s, when a significant 80% of the volume change had occurred.

Nucleation and Growth of NaCl Crystals in Different Dehydrating Solvents: Octanol vs Decane

Here, we were interested in the question, “How can the rate of water loss be slowed down in order to slow the rate at which supersaturation is attained?” Mechanistically, this is important because, giving longer times for the stochastic process of reaching a critical nucleus size could provide a means to change the crystal morphology and reveal crystalline stages that were not exposed during high-concentration, rapid nucleation and growth. While NaCl is a simple ionic system, there also could be instructive value in this for peptide and protein crystallization, where times for more macromolecular rearrangement could impact whether an amorphous glass is formed, as in our earlier Microglassification studies 43, 64, 84 or an ordered crystal. Su et al. 9 has shown that water dissolves in alkanes much slower than in alcohols. We therefore repeated the dissolution experiments with decane as the dehydrating medium to decrease the dehydration rate by approximately two orders of magnitude.

Water Microdroplet Dissolution in Decane: Baseline Measurement

Pure water microdroplets were dissolved in pure n-decane. This gave a baseline dissolution curve and a check on the measured diffusion coefficient of water in decane for our system. As shown in Figure 8, a ~40 µm radius microdroplet dissolved in decane in ~4000 s, whereas the dissolution of an 80 µm radius water microdroplet in octanol happened in ~400 s, as shown in Figure S3. These dissolution curves in decane were again analyzed by the EP model, and the diffusion coefficient of water in decane was measured to be $D = 3.3 \pm 0.2 \times 10^{-5} \text{cm}^2/\text{s}$. This
value was in close agreement with the value obtained by Su et al.\textsuperscript{9}, which was 4.06 ± 0.43 \times 10^{-5} \text{cm}^2/\text{s}.

As noted by Su et al., even though the diffusion coefficient of water in decane (4 \times 10^{-5} \text{cm}^2/\text{s}) is twenty times that of water in octanol (0.20 \times 10^{-5} \text{cm}^2/\text{s})\textsuperscript{9}, the much lower solubility of water in decane (0.05 mg/mL in decane vs 40 mg/mL in octanol) results in the overall microdroplet dissolution process being much slower. Su et al.

Figure 8 Dissolution of Water into Decane. A Radius as a function of time for pure water microdroplets in pure decane. B Dimensionless radius as a function of dimensionless time for pure water microdroplets in pure decane. The curves were calculated according to the normal and dimensionless EP model, and it was clearly shown how data from different microdroplet sizes collapse onto a single curve. From these curves, the diffusion coefficient of water in n-decane was measured to be $D = 3.3 \pm 0.2 \times 10^{-5} \text{cm}^2/\text{s}$.
postulated that this slower diffusion coefficient in the alcohols (vs alkanes) was due to retardation of water transport because of local hydrogen bonding with the OH group of the alcohol. This gave us a way to control the rate of water loss and observe its effects on the kind of crystal morphology that could be obtained. Thus, to expand on these observations and examine further if and to what extent the dissolution rate of water had an influence on the nucleation of NaCl from supersaturated water solutions, we carried out a more in-depth study comparing 1.0 M and 2.0 M NaCl solution microdroplets in decane with the results in octanol.

**Nucleation and Growth in Octanol Bathing Solvent**

As shown earlier by Bitterfield and presented as controls in Figure S5, the radii of the microdroplets decreased as a function of time for NaCl solution microdroplets in pure octanol and, as shown in Figure S4 formed poly-microcrystals. In Figure 9, looking at this process at a higher time resolution using stopped-frame images, NaCl nucleation and growth in the octanol system happened in less than one second in a very ‘violent’ shock-like process from a supersaturation degree of $S = 1.87$. In this system, where nucleation appeared to be homogeneous, the crystal transitioned from an 8-fold symmetric nucleate (Figure 9 at 0.04 s) into a multi-faceted dendritic form in less than one second from initial nucleation. It rapidly moved from its nucleation point to the microdroplet boundary and eventually came to rest at the bottom of the microdroplet and grew as NaCl was deposited from the microdroplet solution forming the larger crystal shown at 2.6 s.
Figure 9 NaCl Nucleation in Octanol. Video micrographs of an initial 2.0 M NaCl aqueous solution microdroplet undergoing NaCl nucleation in pure octanol surroundings. The nucleation and growth in this system happened in less than one second, and after 2 - 3 seconds the crystal had already come to rest. The degree of NaCl supersaturation at the point of nucleation was in this case measured to be 10.1 M, S = 1.87.

Nucleation and Growth in Decane Bathing Solvent
This process of water-dissolution from the microdroplets containing salt solution was then studied in decane. As shown in Figure 10, water was lost from the salt solution microdroplets over a much longer period of time than we saw in Figure S5. Consistent with the measurements of Su for pure water dissolution into alkanes, and Duncan for salt solutions into decane, these dissolution times reflected the much lower water solubility of water in decane, as predicted by the EP model. Crystals eventually nucleated (shown as the star symbols in Figure 10) and the dissolution speed of water increased slightly due to the NaCl concentration dropping back down to the saturation value.
Figure 10 NaCl Solution Dissolution into Decane. Radius as a function of time for 1.0 M and 2.0 M NaCl solution microdroplets in pure decane. All microdroplets dissolve slowly into the decane phase (2.0 M NaCl microdroplets at a slower rate than 1.0 M NaCl microdroplets as expected) until nucleation occurs (star). After nucleation dissolution speed increased (grey circles) because the NaCl concentration dropped down to the saturation value.

As shown in Figure 11, the nucleation and subsequent growth of the NaCl crystal in the decane system was observed to be much slower and happened during a much longer time frame. Nucleating, again presumably homogeneously (i.e., not at the pipette tip), the initial nucleate was a single crystal formed from a supersaturation degree of $S = 1.13$. The images start at 0 s at the first signs of nucleation. The microcrystal of NaCl grew during the first seconds at a rate of approximately 1 µm side-length per second until, as seen in Figure 11, at 70 s, it reached a projected size of 14 µm x 14 µm producing what appeared to be a perfect cubic crystal. Thus, this nucleation and growth essentially took up the excess supersaturation amount of NaCl and returned the microdroplet solution back to the saturation value of 5.4 M. With continued water-loss from the NaCl-saturated solution microdroplet, the NaCl crystal continued to grow at a reduced rate, as water continued to dissolve into the decane medium, until only the crystal was left on the end of the pipette in decane, example shown in Figure 13A.
Figure 11 NaCl Nucleation in Decane. Video micrographs of an initial 5.0 M NaCl aqueous solution microdroplet undergoing NaCl nucleation in pure decane surroundings. The nucleation and growth rate in this case was much slower than in the octanol system. The degree of NaCl supersaturation at the point of nucleation was in this case $S = 1.13$.

To provide a quantitative measure of initial and subsequent crystal growth rate, the one-dimensional side-length of the crystal was measured as a function of time from the onset of nucleation. As shown in Figure 12, this graph clearly shows the fast initial formation and growth of the single NaCl crystal and the slower growth of the large single crystal with a side-length of about 16 µm at the saturation concentration of NaCl in the microdroplet, as water was continually lost over a period of 5855 s (1.63 hrs).
After the crystal nucleated at time = 855 s, this graph shows the measured size (side-length) of the NaCl crystal in Figure 11. The initial relatively fast growth represents the NaCl coming out of solution, thus depleting the NaCl back down to the saturation concentration of NaCl in water. Then it continued growing at a reduced rate as water continued to dissolve into the surrounding decane medium. The slower loss of water from a microdroplet into decane thereby allowed for the study of salt nucleation and growth over longer time periods than possible in octanol.

This was important because, as is well known, and discussed by Di Profio et al, reducing the work of nucleation directly affects the critical nucleus size, and the relative growth/dissolution velocities of the new phase that, in turn, affect the polymorphic form that will be effectively obtained. That is, given a thermodynamic tendency to reach a degree of supersaturation, the polymorphic form that will actually be obtained “depends on the rate at which a certain level of supersaturation is generated with respect to the relative nucleation and growth/dissolution rates.”

Thus, by using decane as the much slower dehydrating medium (than octanol) the tendency to nucleate is ramped up more slowly than in octanol. In his PhD thesis, Duncan studied crystallization in three solvents: decanol, ethyl hexane-diol and decane. In decanol, Duncan measured the supersaturation of NaCl microdroplets at crystallization as ~10 M, at which point they gave a ‘shock’ crystallization as we confirmed in octanol, (Figure S4). Duncan also saw that changing the solvent from
decanol to ethyl hexane-diol gave a slightly slower dissolution of water microdroplets, but the NaCl solution microdroplets still reached a critical supersaturation value of \( \sim 10 \text{ M} \), again producing the ‘shock’ crystallization. In stark contrast, for an initial NaCl concentration of 5.0 M NaCl (just below its aqueous saturation concentration), the rate of water loss into decane was much slower, and supersaturation only reached values between 7 M and 8 M before a perfect cubic crystal nucleated and more slowly grew. These data are now confirmed here for decane compared to octanol in the next section.

**NaCl Supersaturation and crystal morphologies in the Octanol and Decane Systems**

Comparing the crystals formed due to rapid and slower water loss into the octanol and decane systems respectively, it is clear from Figure 13A, that the structure of the crystal formed with octanol (left) as the dehydrating solvent was different from that in the decane system (right). The octanol system produced a very rapidly-growing dendritic crystal, Figure 13A (left), that was similar to the crystals described by Desarnaud et al.\textsuperscript{14}, whereas the decane system produced a perfect square crystal, Figure 11 at 70 s, and Figure 13A (right). Interestingly, in the Desarnaud study, they were focused on NaCl and its crystallization in confined conditions as one of the major causes of physical weathering and disintegration of rocks, stones, and building materials. They therefore examined nucleation by controlled evaporation for different degrees of confinement in 20 - 2000 \( \mu \text{m} \) capillaries in which the salt solution was trapped by capillary forces. They obtained a value for the critical supersaturation fraction of \( \text{S} \sim 1.6 \), a consequence of which was the very rapid growth of a single skeletal (Hopper) crystal. In contrast, the data we obtained in Figure S6 shows an even higher \( \text{S} \) value of 1.9, possibly reflecting the more free state of the solution to reach and nucleate at a higher supersaturation since the only ‘confinement’ was the water-octanol interface. In this study we did not observe the Hopper crystal growth where only the edges of the NaCl crystal was grown, thus leaving a hollow interior, as in the Desarnaud study. Only dendritic crystal growth, where the entire crystal face is rapidly grown, was observed. This dendritic growth has been observed and explained in papers where gel matrices have been used to control this dendritic NaCl
growth \textsuperscript{29-30}. The role of the interface will be discussed later in the context of the results from the water-in-decane system.

Taking the R vs t plots from Figure 10, and converting them to droplet volumes and then to NaCl concentrations, as shown in Figure 13B, it was observed that NaCl consistently nucleated at a lower supersaturation concentration in the decane system than in the octanol system (Figure S6). Observed values for the critical supersaturation concentration ranged between 6 M and 9 M NaCl with an average from 7 measurements of 7.2 ± 1.3 M. The results showed no correlation with initial microdroplet size and only a slight correlation with initial NaCl concentration, since microdroplets with a lower initial NaCl concentration reached a much smaller size before they reached the supersaturated regime. As described by the papers of Desarnaud and Fontana \textsuperscript{14, 27-28}, the supersaturation fraction is important for the type of crystal created. High levels of supersaturation at the time of nucleation tend to grow dendritic crystals, which grow at a much faster rate than the ‘cubic crystals’ formed at lower supersaturations, and this is exactly what was observed here. The work of Peters \textsuperscript{86} presents expressions for different supersaturation schedules, i.e. different rates of supersaturation change. The rate at which the driving force is increased in the work of Peters is expressed as the factor $R = \frac{d\ln S}{dt}$. This factor is approx. 20 times lower for the 1 M and 2 M NaCl solutions in decane than in the octanol experiments. We did however use the nucleation rate of NaCl as described by Desarnaud et al. \textsuperscript{14} to verify the occurrence of a single nucleation event in the observed volume. The nucleation rate at a supersaturation of $S = 1.8 - 1.9$ is approx. $10^{12} - 10^{16}$ m\textsuperscript{3}s\textsuperscript{-1} = $10^9 - 10^{13}$ L\textsuperscript{-1}s\textsuperscript{-1}. The volume observed at the time of nucleation is app. 100 pL = 10\textsuperscript{-10} L. This would give us approximately 0.1 - 1000 nucleation events per second. This estimated the number of nucleations and explained very well why we have not observed anything above this level of supersaturation.
Figure 13 NaCl Crystal Morphology and Supersaturation in Decane. A Comparison between a crystal formed in the octanol system (left) and a crystal formed in the decane system (right). The cubic crystals of the decane system were observed no matter the supersaturation at which it was formed, and the growth process was always observed to be slower than in the octanol system. B The concentration development during dissolution of seven NaCl solution microdroplets in decane. Several experiments (15+) showed that the concentration at the time of nucleation was stochastic in nature, thus showing no correlation with initial microdroplet size and only slight correlation with NaCl concentration. Dotted line is the saturation limit of 5.4 M and the dashed line is the previous measurements in the octanol system, where the critical supersaturation was 10.3 M.

The level of supersaturation also controls the amount of potential energy released in the nucleation event and the following growth process. When nucleation occurred inside the microdroplet in the octanol system, the formation of the new microcrystal was so ‘violent’ that the microdroplet was often detached from the micropipette tip (even with a considerable negative micropipette suction pressure to hold it in place).
Interestingly, Desarnaud et al. \textsuperscript{14} showed that the effect of high crystallization pressures (160 MPa from a S = 1.6 solution) resulted in destruction of porous material since the crystallization induced pressure can be higher than a material’s tensile strength, such as the case for sandstone. In contrast, nucleation and growth of the NaCl crystal shown in Figure 11 for the solution-decane system formed gently and grew more slowly, as represented in Figure 12, where the time from 0 to 15 \( \mu \text{m} \) crystal size was \( \sim 100 \) s. Thus, the lower supersaturation levels at the time of nucleation observed for dissolution of NaCl solutions into decane is consistent with the process being much less ‘violent’. That is, less material is converted into a new solid phase at the time immediately after nucleation because the supersaturation was not as high, thus lowering the crystallization induced pressure and releasing much less energy. This lower ‘shock’ dissipation at the point of nucleation was also evident since microdroplets in the decane experiments were much easier to control during nucleation and were never lost from the micropipette tip.

As presented above, and also shown by Bitterfield et al. \textsuperscript{7}, the dissolution times and critical supersaturation concentrations for NaCl solution microdroplets dissolving in octanol were achieved within 100s of seconds, while our data above in agreement with Duncan showed that the microdroplet solution dissolution into decane was much slower, Figure 13B, and so from the start of water dissolution from the microdroplet, through passage into the supersaturated state and eventual nucleation took 1000s of seconds. Figure 14 is a composite plot of our data for concentration vs time for our repeats of Bitterfield’s experiments into octanol (Figure S6), and those into decane (Figure 13B). This new plot conveniently shows the differences for these rates and the levels of supersaturation achieved at nucleation of the two different kinds of NaCl crystals, the dendritic crystals formed by the faster dissolution into octanol, and the single square crystals formed by the slower dissolution into decane. The NaCl solution microdroplets in decane thus existed for a longer time above the saturation point (dotted line) and within the supersaturated region shown in Figure 14.
Figure 14 NaCl Nucleation Concentration Comparison. Comparison between the NaCl concentration vs time for dissolution of NaCl microdroplets into octanol and decane. It was evident that in the octanol experiments, concentration increased rapidly when compared to the decane experiments (even for half initial microdroplet size). The relatively long induction time in the decane experiments resulted in nucleation at lower NaCl concentrations.

What is the difference between the two crystal morphologies formed by dissolution into octanol and decane?

These data then present a question that could have an obvious answer or could suggest a role for the aqueous-oil interface. The obvious answer is that as described in the literature, the slower, less violent nucleation and single crystal growth in the solution-decane system was simply due to a much slower loss of solvent and concomitant slower concentrating of the solution resulting in a lower nucleation tendency and growth with much less material available in the decane-system than in the octanol system. If we assume homogeneous nucleation then yes, the increased induction time by dissolution into decane can result in more opportunities for the supersaturated NaCl to overcome the energy barrier and nucleate in this stochastic process. At these lower concentrations the reduced energy advantage and lower availability of material generates a slower growing single crystal.
However, while speculative, there could be another new explanation for the different supersaturation concentrations achieved in the two systems, one that invokes heterogenous nucleation at the aqueous-decane interface. As observed by Trudeau et al.\textsuperscript{88}, extremely hydrophobic (solid) surfaces that display high water-surface contact angles show spectroscopic signatures of uncoupled OH stretching modes. "In this non-wetting regime, water, immediately adjacent to the interface, is oriented such that water-hydrogens are directed toward the surface. In contrast, for more polar surfaces with lower contact angles such semi-wetting regimes have water oriented in the plane of the interface." Applying this information and speculating to analogize with our pure alkane and pure octanol interfaces, the alkane would provide a non-wetting interface and the alcohol with its hydrated OH groups facing the aqueous phase would be the semi- or relatively-wetted surface. Data on polymers show that the contact angle on paraffins is $\sim110^\circ$, while that on polyalcohols is $54^\circ$\textsuperscript{89}, and so we might expect a similar situation for decane vs octanol. This is backed by our observation of the nucleation almost exclusively happening close to, or at the microdroplet boundary, as in Figure 11. Similar supportive findings have been presented by Desarnaud and Bonn\textsuperscript{79} where they describe a high tendency of NaCl nucleation and single cubic crystal growth near the hydrophobized glass surface as opposed to experiments with untreated glass surfaces.

The importance of this can be now seen by inferring the hydrophobic effect and considering the structure of water at these two disparate-wetted surfaces, such that the hydrophobic surface may serve as a heterogenous nucleation site. Molecular dynamics simulations of hydration behavior of a single paraffin-like plate, including the single particle density distribution of water molecules around the plate\textsuperscript{90}, show that well-defined solvation shells exist around each of the paraffin-like plates. Other simulations even show solid-like water structure, as described by Bresme et al.,\textsuperscript{91} "at 300 K the intrinsic width of the gap separating the oil and water phases spans little more than one water molecule diameter, and undergoes very weak short-ranged fluctuations, indicating that the water-oil interface is a rigid molecular structure at ambient temperature".

Our new hypothesis then is that at the highly hydrophobic alkane surface, there is more structured water, almost ice-like, and this can provide a heterogeneous
nucleation site for NaCl. In contrast, at the octanol interface, water is well hydrogen-bonded, the alcohol is well-solvated, and the NaCl concentration reaches a much higher critical supersaturation and nucleates homogeneously. Heterogeneous nucleation has a lower energy barrier for nucleation and growth than for homogeneous nucleation and thus a lower critical supersaturation. Recent simulations by Zimmermann et al., found that the rate-determining step limiting resistance to attachment to a heterogeneous surface is actually ion desolvation. In fact, it is well known that experimentally, salt addition to water typically increases the air-solution surface tension. This implies that there is a negative surface excess (i.e. depletion) in the sum of the density profiles of anions and cations at the interfacial plane, but could they be interacting in the hydration layer? There may even be a differential adsorption of Na\(^+\) ions over Cl\(^-\), because of the influence of water of hydration of the ions. In any event, further experimentation on NaCl and other nucleation, crystallization, supersaturation and growth is amenable by this micropipette technique, and will form the basis for new experiments that would look to test these homogeneous vs heterogeneous nucleation phenomena at other hydrophobic microscopic interfaces in well-controlled microdroplet systems.

**Summary and Conclusion**

A novel ‘three-pipette method’ has been developed and was shown to have great possibilities for investigating solubility of solid NaCl crystals, and potentially any other liquid or solid material, in a second liquid while using very low amounts of material. This novel technique combines the measurement of supersaturation and saturation of NaCl in water dissolving into an octanol system, using ng of material, in one single experimental setup. The critical supersaturation concentration for homogeneous nucleation of NaCl (10.3 ± 0.3 M), the saturation concentration of NaCl in aqueous solution (5.5 ± 0.1 M), the diffusion coefficient for water in octanol, \( D = 1.96 ± 0.10 \times 10^{-6} \text{cm}^2/\text{s} \), and the effect of the solvent’s activity on dissolution kinetics were all reported.

It was also shown that the extended EP model of Bitterfield et al. was successfully applied to diffusion-controlled microdroplet growth. When the water activity in a NaCl
microdroplet was lower than the water activity in the surrounding water-saturated octanol environment, the microdroplet underwent diffusion-controlled growth by imbibing water.

Finally, we have shown the effect on water dissolution rate, rate of NaCl in the decane-system than in the octanol system. Thus, the crystal structure concentration change, resulting crystal structure, and the timeframe of initial crystal growth by changing the bathing medium from octanol to decane. The much slower loss of solvent and concomitant slower concentrating of the solution resulted in a lower supersaturation at nucleation and slower crystal growth was dendritic for NaCl solution microdroplets dissolving rapidly and nucleating violently in octanol and formed single crystals in a gentler way for solution dissolution in decane. These data could be consistent with the rate of ramping up the supersaturation and the stochastic nature of the critical supersaturation and nucleation event, or, in speculation, the possible role of the ice-like hydration layer at the more hydrophobic solution-decane interface providing a heterogeneous nucleation site for NaCl crystallization.

**Acknowledgements**

We gratefully acknowledge the generous support of the Danish National Research Foundation through a Niels Bohr Professorship Award to David Needham, Center for Single Particle Science and Engineering, grant project no. 232149.

**Supporting Information**

Further clarification on the EP model (S1), micropipette methods (S2), gravimetric NaCl solubility measurement (S3), estimations of NaCl solubility in n-alcohols (S4), water microdroplets in octanol (S5) and NaCl solution microdroplet in octanol (S6).
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TOC Graphic
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