

Freeze-induced gelation of dilute alginate suspensions enables encapsulation of suspended microparticles

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Short Report

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Abstract

Freeze-induced concentration is a well-established consequence of ice growth in aqueous systems, yet its ability to trigger gelation from initially subcritical formulations is understudied. Here, we demonstrate that slow freezing of dilute alginate/ Ca^{2+} suspensions increases the local concentrations of polymer and crosslinking ions beyond the gelation threshold, producing an irreversible hydrogel during freezing without additional processing, unlike conventional freeze-casting approaches that typically require subsequent freeze-drying or post-gelation treatments to achieve structural integrity. Polymeric microparticles, used as a model suspended phase, became simultaneously incorporated into the developing hydrogel, with approximately 91% of the particles retained within the gel network. Smaller particles exhibited higher incorporation efficiencies than the larger ones, consistent with their greater mobility within the unfrozen liquid during freezing. Rather than proposing a scalable particle-separation technology, this work establishes a simple physicochemical mechanism coupling freeze-induced gelation and particle encapsulation, providing a basis for future studies on hydrogel fabrication and particle immobilization.

Introduction

Freezing-induced gelation happens when growing ice crystals reject solutes from the advancing solidification front, progressively concentrating the remaining liquid.[1, 2] When the concentration reaches the minimum needed for gelation, gel is formed, and the growing crystals can puncture it, forming scaffolds with several applications. Although freeze-induced concentration has been extensively investigated,[3, 4, 5, 6] its ability to drive chemical or physical transitions by locally exceeding critical concentration thresholds has not, to our knowledge, been exploited to trigger alginate gelation from an initially sub-gelling formulation while simultaneously incorporating suspended particles.

Alginate hydrogels are commonly formed through ionic crosslinking between alginate chains and divalent cations such as Ca^{2+} . [7, 8] Gelation occurs only when the local concentrations of polymer and crosslinking ions become sufficiently high to establish an interconnected network. Our hypothesis here is that freezing alone could progressively concentrate an initially sub-gelling alginate/ Ca^{2+} solution until this threshold is exceeded, producing an irreversible hydrogel during freezing without requiring external gelation or post-processing steps. If suspended particles are small enough to be rejected by the growing ice crystals, they can be encapsulated by the hydrogel formed and become immobilized, producing a composite.

Here, we demonstrate that slow freezing of dilute alginate/ Ca^{2+} suspensions spontaneously triggers hydrogel formation through freeze-induced concentration while simultaneously incorporating suspended polymeric microparticles used as a model particulate phase. Rather than proposing a new particle separation technology, this work establishes a simple physicochemical mechanism that may be exploited for hydrogel fabrication and particle immobilization in future materials-processing applications.

Materials and methods

Sodium alginate (FGP 250), calcium chloride (CaCl_2), Tween 20, and Milli-Q water were used throughout the study. Preliminary experiments identified an initially sub-gelling formulation consisting of 0.04% (w/v) sodium alginate and $0.002 \text{ mol L}^{-1} \text{ CaCl}_2$, which remained fluid before freezing but produced a mechanically stable hydrogel after freeze-induced concentration.

Spray-paint microparticles were produced by spraying commercial blue paint into a clean enclosed room and allowing the aerosol to settle for five days to ensure solvent evaporation (Figures S1 and S2), as thoroughly described in Boeing (2024).[9] The collected particles were dry sieved ($25\text{--}53 \mu\text{m}$) and dispersed in the alginate/ CaCl_2 solution containing 0.4% Tween 20. After resting for 3 h to allow sedimentation of larger particles (Figure S3),[10] 5 mL aliquots were collected from the upper suspension and transferred to polypropylene containers. Particle morphology was characterized by scanning electron microscopy.

Aliquots containing particles and particle-free controls were divided into frozen and unfrozen groups ($n = 3$). Frozen samples were placed inside a styrofoam insulating box and kept in the freezer compartment of a domestic refrigerator for 3 h until complete solidification, while unfrozen controls were stored under refrigeration. After thawing at room temperature in a laminar-flow hood, all suspensions were vacuum-filtered through $0.45 \mu\text{m}$ membranes. Each container was rinsed three times with filtered Milli-Q water to recover residual suspended particles.

Residual particles remaining in the liquid phase after freezing were quantified by optical microscopy (Leica DM3000). Five fields per membrane were imaged and combined into extended-focus composites before analysis (Figure S4). Particle segmentation and area measurements were performed using the Trainable Weka Segmentation plugin in Fiji (ImageJ), followed by manual correction of segmentation artifacts and particle counting using the Analyze Particles routine (Figure S5). Total particle numbers were estimated by extrapolating the counts to the membrane area. Particle incorporation efficiency was calculated from the reduction in residual suspended particles after the freeze–thaw process relative to the unfrozen controls. All sample preparation and filtration procedures were conducted under a laminar-flow hood, and particle-free controls confirmed the absence of detectable contamination throughout the experiments.

Results and Discussion

Freezing of the initially sub-gelling alginate/ Ca^{2+} suspension produced an irreversible hydrogel after thawing, whereas the unfrozen formulation remained entirely liquid. The proposed mechanism is illustrated in Fig. 1. During ice growth, water crystallizes preferentially while dissolved alginate, Ca^{2+} ions, and suspended particles are rejected into the remaining liquid domains.[11, 12] Their progressive concentration eventually exceeds the gelation threshold of the alginate/ Ca^{2+} system, leading to ionic crosslinking through the egg-box mechanism.[13] Because suspended particles are simultaneously

concentrated within the same liquid regions, they become immobilized as the hydrogel network develops. Unlike conventional freeze casting, where structural integrity is commonly obtained after freeze-drying or post-gelation treatments,[4, 14] the present approach produces an irreversible hydrogel directly during freezing through freeze-induced concentration.

The macroscopic behavior of the system agrees with this mechanism (Fig. 2). Before freezing, the suspension remained fluid and part of the suspended particles gradually sedimented to the bottom of the container. After freezing and thawing, an irreversible hydrogel was recovered floating in the suspension, with the blue particles visibly incorporated into its structure. SEM images of the recovered material revealed a porous morphology consistent with the growth of ice crystals during freezing, confirming that the hydrogel retained the characteristic architecture expected from ice-templated materials.

To quantify particle incorporation, the liquid phase remaining after thawing was filtered and the residual suspended particles were counted by optical microscopy. A marked decrease in particle abundance was observed after freezing (Fig. 3), corresponding to an incorporation efficiency of approximately 91%. Since only particles remaining outside the hydrogel were quantified, this reduction provides an indirect estimate of particle immobilization during freeze-induced gelation. These results establish that freeze-induced gelation efficiently transfers suspended particles from the liquid phase into the developing hydrogel network.

Particle size also influenced incorporation efficiency (Fig. 4). Smaller particles consistently exhibited higher incorporation than larger ones, suggesting that they remained suspended within the unfrozen liquid phase for longer as freezing progressed. Larger particles, in contrast, were more likely to sediment before gelation or interact differently with the advancing ice front, reducing their probability of becoming incorporated into the hydrogel (Figure S6).[4] Although the present study was not designed to establish a quantitative transport model, these observations indicate that particle mobility during freezing contributes to the incorporation process.

The phenomenon demonstrated here should therefore be viewed as a physicochemical consequence of freeze-induced concentration rather than as a microplastic-removal technology. The suspended particles merely serve as a model dispersed phase, and the same mechanism is expected to apply to other particulate systems whose dimensions and transport behavior allow their retention within the unfrozen liquid during gel formation. This mechanism may provide a simple route for particle immobilization during hydrogel fabrication, particularly in applications where only small sample volumes require processing.

Conclusion

This study demonstrates that freeze-induced concentration can drive an initially sub-gelling alginate/Ca²⁺ formulation beyond its gelation threshold, producing an irreversible hydrogel during

freezing while simultaneously incorporating suspended microparticles. Approximately 91% of the suspended model particles became immobilized within the hydrogel, with smaller particles showing higher incorporation efficiency than larger ones. Rather than proposing a scalable particle-separation technology, this work establishes a simple physicochemical mechanism that couples freeze-induced gelation and particle encapsulation. The phenomenon may provide a straightforward platform for future studies on hydrogel fabrication, particle immobilization, and microscale materials processing.

Declarations

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Author contributions: CRediT

Rafael Vinícius de Oliveira: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, review & editing. **Walter Ruggeri Waldman:** Conceptualization, Funding acquisition, Methodology, Project administration, Validation, Writing – original draft, review & editing.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used a Large Language Model (ChatGPT) to proofread and correct grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Data availability

The data that support the findings of this study are openly available in Zenodo at: <https://doi.org/10.5281/zenodo.21811681>

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Figures

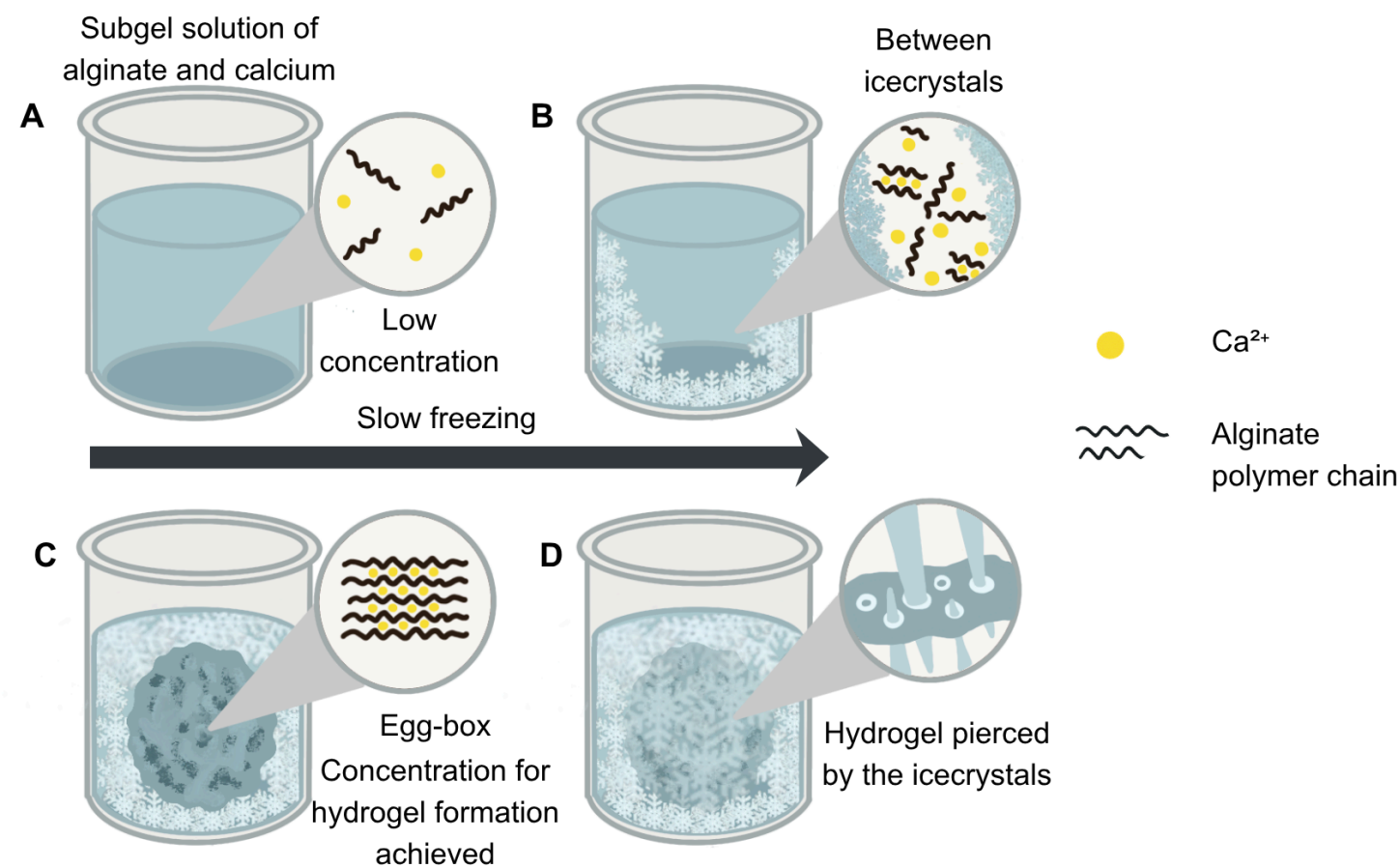


Figure 1

Proposed mechanism of freeze-induced gelation and particle incorporation. Water crystallization progressively excludes alginate, calcium ions, and suspended particles from the growing ice front, increasing their local concentrations (A and B). Once the gelation threshold is exceeded (C), an irreversible hydrogel forms and simultaneously immobilizes the suspended particles (D).

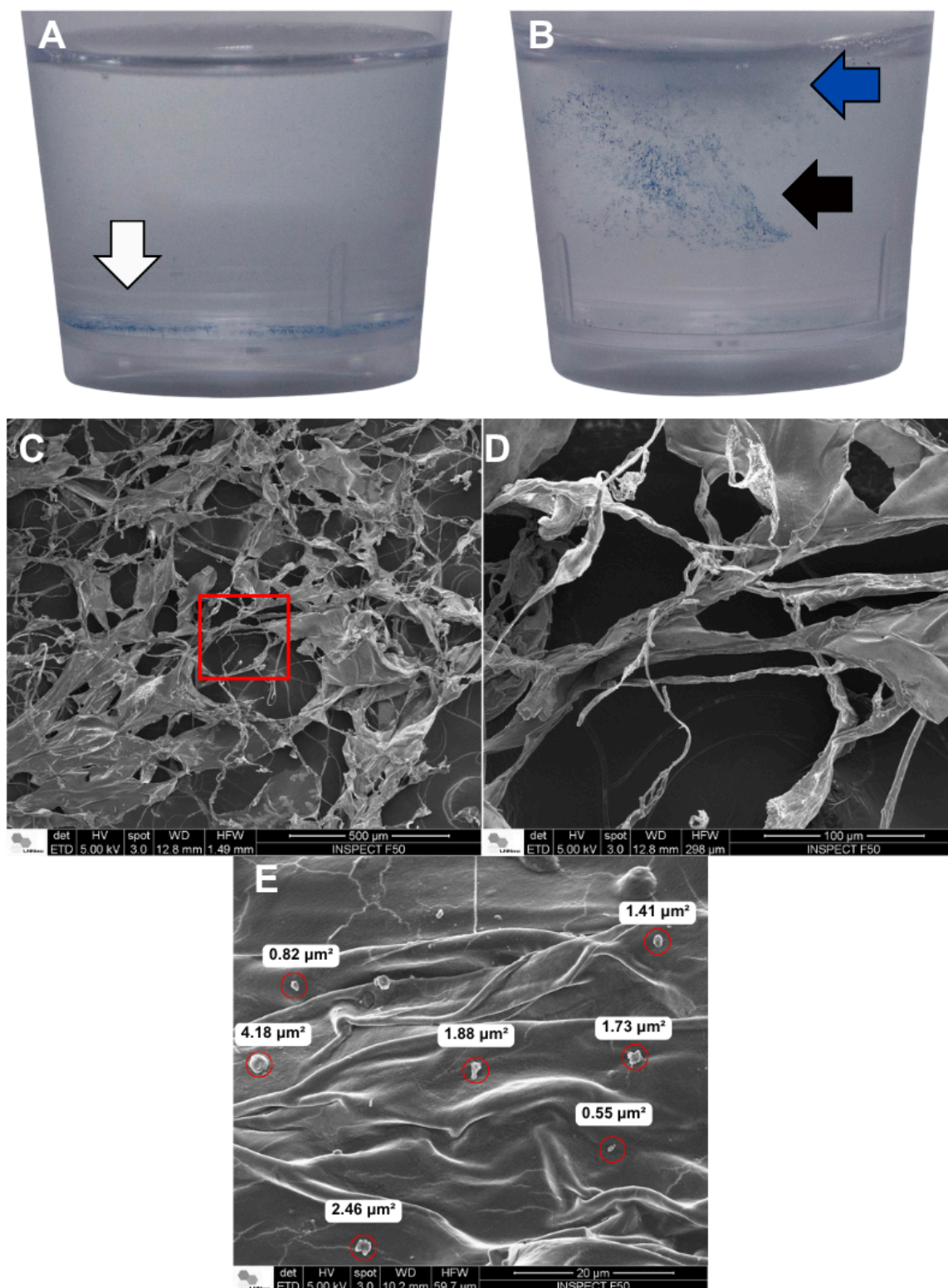


Figure 2

Macroscopic evidence of freeze-induced gelation and particle incorporation. (A) Initially sub-gelling suspension before freezing, showing sedimentation of suspended particles. (B) Irreversible hydrogel recovered after freezing and thawing with visibly incorporated particles. (C–D) Representative SEM images of the recovered hydrogel showing the porous morphology generated during freezing, D is the magnification of the red square in C. (E) SEM images of the particulates entrapped in the gel.

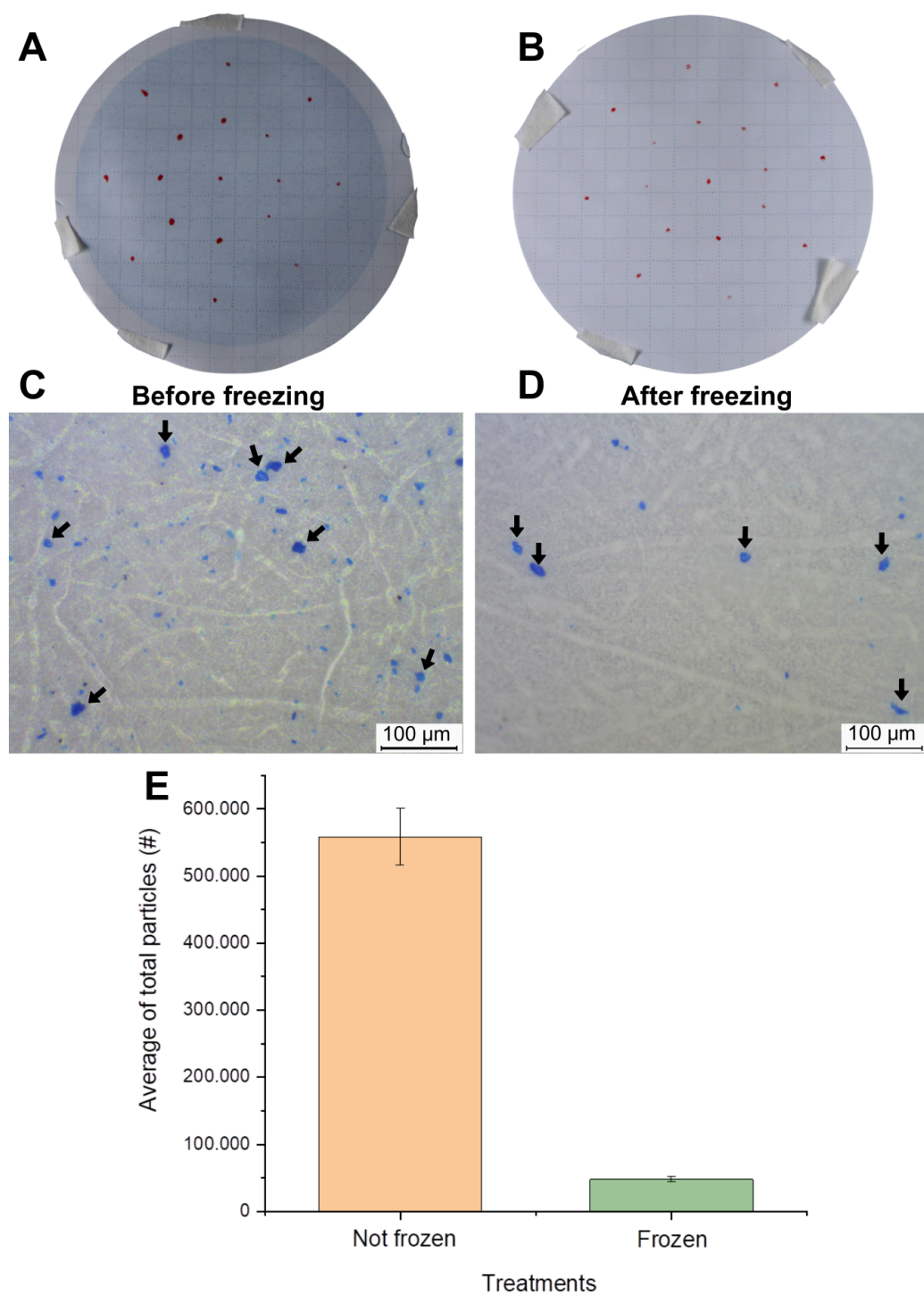


Figure 3

Quantification of particle incorporation. (A and B) Representative filtration membranes before and after freezing, respectively. (C and D) Representative microscopic images of the membranes before and after freezing, respectively. (E) Residual suspended particles quantified after filtration of not frozen and frozen suspensions.

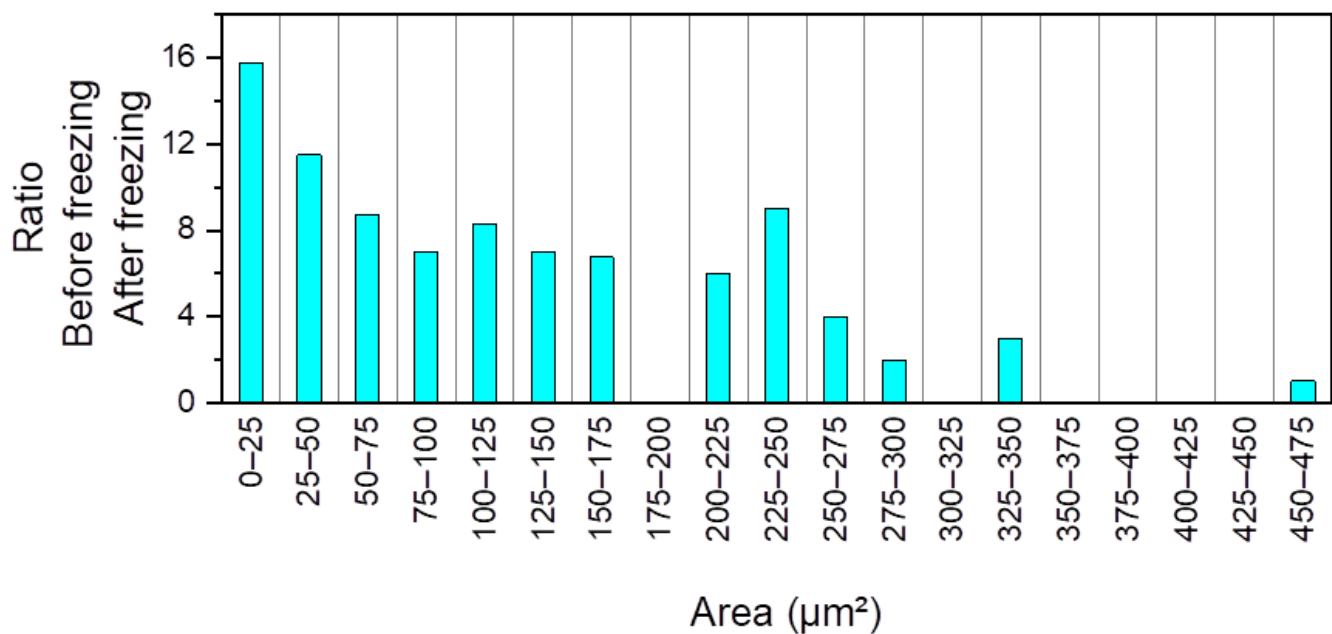


Figure 4

Incorporation efficiency as a function of particle size, showing preferential incorporation of the smaller particles.

Supplementary Files

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