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Communication

Complex coacervate core micelles containing poly(vinyl alcohol) inhibit ice recrystallization

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Complex coacervate core micelles (C3Ms) form upon complexation of oppositely charged copolymers. These co-assembled structures are widely investigated as promising building blocks for encapsulation agents, nanoparticle synthesis, multimodal imaging, and coating technology. Here we investigate the impact on ice growth of C3Ms containing poly(vinyl alcohol), PVA, which is well known for its high ice recrystallization inhibition (IRI) activity. The PVA-based C3Ms are prepared from co-assembly of poly(4-vinyl-*N*-methylpyridinium iodide) and poly(vinyl alcohol)-*block*-poly(acrylic acid). We study their formation conditions, size and performance as ice recrystallization inhibitors. We find that the C3Ms exhibit IRI activity at PVA monomer concentrations as low as 1 mM. The IRI efficacy for PVA-C3Ms is similar to that of linear PVA

and PVA graft polymers, underlining the influence of vinyl alcohol monomer concentration rather than polymer architecture.

1. Introduction

Complex coacervation is the electrostatically driven, liquid-liquid phase separation that occurs when two aqueous solutions of oppositely charged polyelectrolytes are mixed.^[1] Attaching a neutral hydrophilic polymer block to one or both of the polyelectrolytes leads to microphase separation and the formation of micelles, with a complex coacervate core, surrounded by a water-soluble neutral corona.^[2] Hence, complex coacervate core micelles (C3Ms) are structures co-assembled in aqueous solutions through electrostatic interactions. This makes them responsive to changes in their environment such as pH, ionic strength and temperature. These properties make C3Ms ideal systems to use as multimodal imaging contrast agents,^[3] nanogels,^[4] nanoreactors^[5] and encapsulation agents^[6].

C3Ms have advantages when used to produce polymer films due to their ease of preparation, fast adsorption and low desorption.^[7] Furthermore, they readily adsorb onto hydrophilic as well as hydrophobic surfaces by exposing their core to the surface while the corona chains extend out into solution.^[7a,8] The surface chemistry of C3M-coated substrates can easily be tuned for specific applications, such as antifouling,^[7b,9] by changing the corona-forming block. In this work, we use poly(vinyl alcohol), PVA, a well-known ice recrystallization inhibitor,^[10] as our corona-forming block. Recently, it was shown that block copolymerization^[11] and chain architecture^[12] do not affect the ice recrystallization inhibition (IRI) activity of PVA. However the IRI efficacy of self-assembled structures containing PVA remains a question. To address this challenge we study PVA-based C3Ms formed by poly(4-vinyl-*N*-methylpyridinium iodide), P4VMP, a cationic homopolymer, with poly(vinyl alcohol)-*block*-poly(acrylic acid), PVA-*b*-PAA, a neutral-anionic diblock copolymer, in aqueous conditions. We first determine the critical micelle concentration (CMC) and preferred micelle composition (PMC) at pH 10.5 in 10 mM NaNO₃ solution by light

scattering. Next, we check whether PVA-based C3Ms inhibit ice recrystallization. A commonly used method to study IRI is the splat assay.^[13] Alternatively, to simultaneously view multiple samples, a practical capillary method can be adopted.^[14] However, we determine ice growth rate constants (k_d) over a range of polymer concentrations in the more recently presented sucrose sandwich assay.^[15] This method warrants a constant, low ice volume fraction. We find that PVA-based C3Ms effectively inhibit ice recrystallization at vinyl alcohol monomer (VAM) concentrations as low as 1 mM. Their activity is comparable to linear chains and PVA bottlebrushes.^[12] This indicates that the IRI activity of PVA is dependent on the VAM concentration rather than the polymer architecture. These results unveil that PVA can be formulated into polymeric micelles without loss of IRI activity.

2. Experimental section

2.1 Materials

Poly(vinyl alcohol)-*b*-poly(acrylic acid), PVA₂₇₃-*b*-PAA₃₅₃ ($M_n = 37,464$ g/mol), was obtained through complete hydrolysis of poly(acrylonitrile)-*b*-poly(vinyl acetate), PVAc₂₇₃-*b*-PAN₃₅₃ (SEC (DMF): $M_n = 130,700$ g/mol, $D = 1.22$), which was synthesized by cobalt-mediated radical polymerization as described previously.^[16] Poly(4-vinyl-*N*-methylpyridinium iodide), P4VMP₂₃₃ (SEC: $M_n = 28,000$ g/mol, $D = 1.2$, after quaternization), was purchased from Polymer Source Inc. (Montreal, Canada). The degree of quaternization is >98%.

Sodium nitrate >99% (Agros Organics) was purchased from Fischer Scientific. Sodium hydroxide >98% (Emprove®) and hydrogen peroxide (Emprove® 35% H₂O₂) was purchased from Merck Millipore chemicals. Sulfuric acid (Rectapur® 95% H₂SO₄) was purchased from VWR. Sucrose ≥99.5% (BioUltra), silanization solution I (~5% dimethyldichlorosilane in heptane,

Selectophore™), technical grade acetone and isopropanol, and GE Whatman syringe filters of 0.2 µm pore size with PVDF and PTFE membranes were purchased from Sigma Aldrich. All chemicals were used as received unless indicated otherwise.

2.2 Sample preparation

All stock solutions were prepared in ultrapure water with 10 mM NaNO₃. The pH was adjusted to 10.5 in all solutions using NaOH to ensure full deprotonation of PAA. The polymer stock solutions were prepared at 20 mM chargeable monomer concentrations, so that after mixing

$$c_{tot} = c_{+} + c_{-} = c_{4VMP} + c_{AA} \quad (1)$$

and dilutions were done with the 10 mM NaNO₃ solution at pH 10.5. The polymer solutions were mixed at desired mixing ratio which is expressed as the molar ratio of cationic charges (4VMP) to the total number of chargeable monomers

$$f_{+} = \frac{[n_{+}]}{[n_{+}] + [n_{-}]} \quad (2)$$

No change in pH was observed after mixing the polymers to produce micellar solutions. All solvents and solutions, except the polymer solutions, were filtered through 0.2 µm syringe filters to remove trace quantities of dust for light scattering experiments. Experiments and sample storage were done at room temperature.

2.3 Methods

2.3.1 Static and dynamic light scattering

Light scattering experiments were performed on an ALV/CGS-3 MD-4 Goniometer System, equipped with a 50 mW Nd:YAG laser operating at 532 nm. The temperature was regulated at 20.0

± 0.2 °C using a Lauda RM6-S Refrigerated Circulating Bath. The light scattering intensity was recorded at various angles between 30° and 150°. Characteristic decay rates (Γ) were obtained from the cumulant method and used to calculate the translational diffusion coefficient (D_T). We determined the hydrodynamic radius (R_H) of the C3Ms using the Stokes-Einstein relation and the size distribution of the C3Ms using the CONTIN method.

2.3.2 Ice recrystallization inhibition assay

A 2 μ L droplet of sample was sandwiched between two cover slides and placed in a stage attached to a Nikon ECLIPSE Ci-Pol Optical Microscope where the temperature is controlled by a Linkam LTS 420. A thin polycrystalline ice layer was created by rapidly freezing (20 °C/min) to -40 °C. The temperature was then raised (10 °C/min) to -7 °C and kept constant for one hour. Microphotographs were taken every 5 minutes and analyzed with ImageJ, followed by an in-house written Matlab script, to obtain the equivalent radius for each ice crystal (details in Supporting Information). The rate constants, $k_d(c)$, were obtained from the temporal increase in the cube of the number average radius as described by LSW theory,^[15a] according to

$$\langle R \rangle^3(t) = \langle R \rangle^3(0) + \frac{8\gamma c_\infty v^2 D}{9RT} t = \langle R \rangle^3(0) + k_d \cdot t \quad (3)$$

Finally, the inhibitory concentration c_i was obtained from $k_d(c)$ as a function of VAM concentration fit to

$$k_d(c) = k_{d0} - \frac{k_{d0}}{1 + \exp\left(\frac{c_i - c}{s}\right)} \quad (4)$$

where k_{d0} is the growth rate constant at zero inhibitor concentration.^[15]

2.3.3 Ice Nucleation Activity

We used a homemade setup consisting of a Peltier stage to cool an array of 24 droplets in a controlled environment (SI Figure S3). Glass cover slides (32x32 mm) were etched with piranha solution (3:1 mixture of sulfuric acid and hydrogen peroxide). The slides were then rinsed with ultrapure water and acetone consecutively, followed by drying with N₂ flow. Then, a hydrophobic surface was obtained by exposing the slides to a ~5% dimethyldichlorosilane solution for 30 minutes, which ensures reproducible contact area between the slide and droplets. The slides were rinsed with heptane and dried under nitrogen. Droplets of 1 μ L were placed on a slide and cooled at 5 $^{\circ}$ C/min until all were frozen, while recording video with a Lumenera Infinity 1. The freezing of individual droplets is signaled by the sudden increase of scattered light by the crystalline phase. Thus, the temperature at which the greyscale intensity suddenly rises is registered as the nucleation temperature (details in Supporting Information). We performed at least three assays to obtain data for sufficient statistics on the distribution of nucleation temperatures.

3. Results and discussion

Micelles form above a critical polymer concentration known as the critical micelle concentration, CMC. To determine the CMC, we perform static light scattering (SLS) experiments at a fixed ionic strength, pH and mixing ratio while varying c_{tot} from 0.01 mM to 20 mM. We prepare C3Ms at charge stoichiometry by mixing aqueous solutions of PVA₂₇₃-*b*-PAA₃₅₃ and P4VMP₂₃₃ at pH 10.5 in 10 mM NaNO₃ and record the SLS intensity at a fixed scattering angle of 90 $^{\circ}$. We find a gradual increase in SLS intensity with increasing chargeable monomer concentration until it exhibits a sudden jump in between concentrations of 1 mM and 2 mM (**Figure 1A**). We attribute this sudden increase in SLS intensity to the transition from small, charged, soluble complexes to C3Ms in

solution. Thus, we consider a 1 mM chargeable monomer concentration as the CMC for this system.

Next, we prepare C3M solutions with $c_{\text{tot}} = 10$ mM, well above the CMC, at various mixing ratios and a fixed pH = 10.5 and salt concentration of 10 mM NaNO₃. To confirm the presence of micelles and to determine the PMC, we again perform SLS experiments. Upon increasing P4VMP₂₃₃ in solution, we observe an increase in SLS intensity which indicates the presence of complexes formed in solution due to electrostatic interactions (**Figure 1B**). The intensity reaches a maximum around charge stoichiometry where saturation of binding occurs. For this system, we obtain the maximum intensity corresponding to the PMC at $f_+ = 0.52$. Subsequently, we perform dynamic light scattering (DLS) experiments to determine the hydrodynamic radius (R_H) of the C3Ms. We obtain the area normalized size distribution by CONTIN analysis and find a unimodal distribution (**Figure 1C**). Using the Cumulant method we obtain a hydrodynamic radius of $R_H = 47.8 \pm 1.1$ nm. The micelles are furthermore observed to maintain this size for a period of more than ten weeks (Supporting Figure S1).

To reliably test whether PVA-containing C3Ms impact ice growth, we measure ice growth rates at low ice volume fractions in 30 wt% sucrose. This high sucrose concentration utilized in a so-called sucrose sandwich assay ensures that individual grains of ice are distinguishable during the entire assay.^[15a] Therefore, we first check if sucrose addition influences the size distribution and the hydrodynamic radius of C3Ms by DLS. Figure 1C shows the micellar size distributions determined in both the presence and absence of 30 wt% sucrose at the PMC. We observe no significant changes in the micellar size distributions and the R_H of the C3Ms upon addition of sucrose.

To quantify IRI activity, we perform IRI assays over a range of VAM concentrations from 0.2 to 7.5 mM. Ice growth rates are drastically decreased when VAM concentrations exceed 0.3 mM. Full

inhibition of ice recrystallization occurs at concentrations above 1 mM (or 0.044 mg/mL PVA), which corresponds to 2.7 mM chargeable monomer concentration (**Figure 2A**). This concentration is in the range of those reported earlier for PVAs at comparable polymerization degrees: PVA₂₄₆ (0.1 mg/mL) and PVA₃₅₁ (0.05 mg/mL).^[17] The IRI efficacy (c_i) is determined from the inflection point of the curve where the ice growth rate is decreased to half of its value at zero inhibitor concentration.^[10e,15b] PVA-based C3Ms exhibit $c_i = 0.33$ mM, which is similar to linear PVA ($c_i = 0.38$ mM) and grafted bottlebrushes ($c_i = 0.31$ mM)^[12] and is slightly lower than PVA_{273-*b*}-PAA₃₅₃ ($c_i = 0.47$ mM) (**Figure 2B**). This result implies that the VAM concentration is the defining factor for IRI activity instead of the architecture of the polymer chain and/or its association state. However, the block copolymer itself exhibits a somewhat lower IRI activity, which might be due to repulsive interactions between anionic polymer chains (see Supporting Information for representative micrographs).

Polymers that inhibit ice growth may also affect ice nucleation. PVA, among other macromolecules, has been reported to promote^[10c,10f,18] or reduce^[19] ice nucleation, depending on the concentration and presence of other compounds like silica nanoparticles. To determine the effect of PVA-based C3Ms on the nucleation of ice crystals, we record the ice nucleation temperatures for aqueous solutions with 7 mM PVA-C3Ms. For comparison, we also assess 10 mM PVA_{273-*b*}-PAA₃₅₃ and P4VMP₂₃₃ solutions and a reference solution at pH = 10.5 and 10 mM NaNO₃. We obtain distributions of nucleation temperatures by normalization, calculating the fraction of frozen droplets (f_F) for recorded temperatures (**Figure 3A**). A comparison of the temperatures at which half of the droplets are frozen, does not reveal significant differences between the three solutions (**Figure 3B**). Statistical tests were performed to further quantify this

finding (Table S1, Supporting Information). A Shapiro-Wilk test shows that the C3M and P4VMP data are not normally distributed, while the two other sets follow a normal distribution. Hence, we adopt a nonparametric test to compare all four distributions (Table S2, Supporting Information). This Mann-Whitney-Wilcoxon test confirms that there is no significant difference between the distributions from the solutions, except for the PVA₂₇₃-*b*-PAA₃₅₃ and P4VMP₂₃₃ solutions. Nevertheless, we can conclude that neither the block copolymer nor the C3Ms reduce or promote the nucleation of ice crystals in aqueous solutions under the investigated experimental conditions.

4. Conclusion

Poly(vinyl alcohol)-bearing C3Ms have been prepared upon electrostatically driven co-assembly of PVA₂₇₃-*b*-PAA₃₅₃ and P4VMP₂₃₃ in aqueous solutions at pH = 10.5 and 10 mM NaNO₃. C3M characterization by light scattering yields a CMC of approximately 1 mM – 2mM chargeable monomer concentration and a PMC of $f_+ = 0.52$. A sucrose sandwich assay at $f_+ = 0.52$ reveals that the PVA-based C3Ms effectively inhibit ice recrystallization even at low VA monomer concentrations of 1 mM. We find the inhibitory concentration of the PVA-C3Ms at $c_i = 0.33$ mM, which is comparable to the c_i values for linear PVA and PVA bottlebrushes studied previously. This suggests that the VAM concentration is more influential for IRI activity than polymer architecture and association state. Furthermore, we observe that ice nucleation is neither promoted nor reduced by PVA-based C3Ms or by the PVA-*b*-PAA diblock copolymer. These results shed new light on the IRI activity of man-made materials and hold promise for the preparation of C3M-based coatings to control ice adhesion and growth on solid surfaces.

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Conflict of Interest: The authors declare no conflict of interest.

Supporting Information is available online from the Wiley Online Library or from the author.

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Figure 1. Light scattering intensity of complexes formed in aqueous condition of pH = 10.5 with 10 mM NaNO₃ by PVA₂₇₃-b-PAA₃₅₃ with P4VMP₂₃₃ as function of (A) chargeable monomer concentration; arrow indicates the intensity jump attributed to CMC and (B) mixing ratio (f_+); dashed line indicates PMC ($f_+ = 0.52$) where saturation of binding occurs; (C) area normalized size distribution of C3Ms and C3Ms with 30 wt% sucrose at PMC and 10 mM NaNO₃.

Figure 2. Normalized ice growth rate constant as a function of VAM concentration of C3Ms (A) and PVA₂₇₃-b-PAA₃₅₃ (B). A curve is fitted through the data points according to Equation (4). The dotted lines indicate IRI efficacy (c_i) with exact values at the x-axes.

Figure 3. Frozen fraction as a function of temperature for solutions of C3Ms (7 mM), PVA₂₇₃-b-PAA₃₅₃ (10 mM), P4VMP₂₃₃ (10 mM, negative control) and 10 mM NaNO₃ as reference. Number of data point reduced for visibility (A); Temperatures at which 50% of droplets is frozen for the four solutions, error bars indicating Median Absolute Deviation (B).