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Gelation of Vesicles and Nanoparticles Using Water-Soluble Hydrophobically Modified Chitosan

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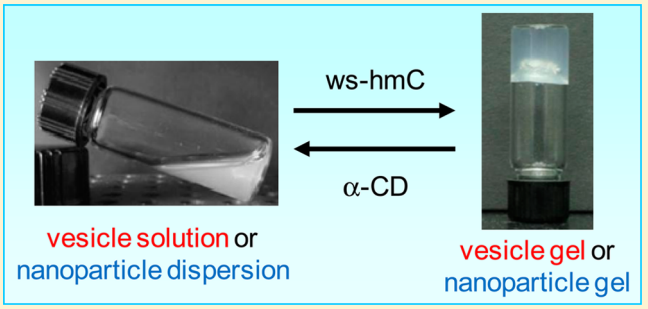
Supporting Information

ABSTRACT: Hydrophobically modified chitosan (hmC) is a self-assembling polymer that has attracted recent attention for many applications, including as a hemostatic agent. One limitation with chitosan and its derivatives like hmC is that these polymers are soluble in water only under acidic conditions (because the pK_a of chitosan is about 6.5), which could be undesirable for biomedical applications. To circumvent this limitation, we have synthesized a derivative of a C_{12}-tailed hmC that is soluble in water at neutral pH. This water-soluble hmC (ws-hmC) is obtained by grafting *O*-carboxymethyl groups onto some of the primary hydroxyls on hmC. The solubility of ws-hmC at neutral pH is shown to be the result of a net anionic character for the polymer due to ionization of the carboxymethyl groups (in comparison, hmC is cationic). We also demonstrate that ws-hmC retains the self-assembling properties of hmC. Specifically, ws-hmC is able to induce gelation at neutral pH in dispersions of anionic surfactant vesicles as well as polymethylmethacrylate latex nanoparticles. Gelation is attributed to hydrophobic interactions between the hydrophobes on ws-hmC with vesicle bilayers and nanoparticle surfaces. In each case, gelation can be reversed by the addition of α-cyclodextrin, a supramolecule with a hydrophobic cavity that sequesters the hydrophobes on the polymer.



INTRODUCTION

Hydrophobically modified chitosan (hmC) has emerged as an interesting and useful polymer in recent years.[1−12] The polymer is a derivative of chitosan, which is a linear aminopolysaccharide obtained by deacetylation of the naturally occurring biopolymer, chitin.[13] The primary amines on chitosan get protonated at low pH, i.e., below the polymer's pK_a of 6.5, and thus allow the polymer to be solubilized in acidic water.[13] Moreover, the reactivity of the primary amines can be used to functionalize chitosan. In particular, hmC can be synthesized by grafting alkyl (e.g., C_{12}) tails onto chitosan by reacting some of the primary amines with a corresponding alkyl aldehyde.[11,14] hmC is thus a polymer with a hydrophilic (cationic) backbone and with hydrophobic grafts sticking out of the backbone. In turn, hmC has an amphiphilic character and it thereby displays associating and self-assembling properties.[14−16]

Several groups have studied the self-assembly of hmC on its own and in conjunction with colloidal structures.[3,4,7−16] In our laboratory, we have investigated mixtures of hmC with vesicles, which are nanoscale containers covered by a bilayer of surfactants or lipids.[7,8,11] We found that the addition of hmC to cationic vesicles converted thin vesicle solutions into elastic gels.[7,11] Gelation was attributed to the bridging of vesicles by hmC chains into a three-dimensional (3-D) network, i.e., the vesicles formed the junction points in this network.[7] The driving force was the propensity of hydrophobes from hmC chains to insert into the hydrophobic bilayers of vesicles. Importantly, native chitosan (having no hydrophobes) did not cause such gelation.[7] We then extended the above results to mixtures of hmC with blood cells.[9] hmC again converted liquid blood into a gel, and this was also attributed to polymer bridging, driven by the interaction of hydrophobes on hmC with blood cell membranes.[9] Such gelation could also be reversed by addition of α-cyclodextrin (α-CD), a supramolecule with a hydrophobic cavity that sequesters the single-tailed hydrophobes on hmC.[9] Lastly, in a recent collaborative study, we have also studied the effect of hmC on dispersions of carbon microspheres.[10] Once again, hmC was shown to gel these dispersions, which was driven by the adsorption of hydrophobic tails on the surfaces of the carbon particles.[10] Native chitosan did not induce such gelation.

The above examples illustrate the remarkable self-assembly of hmC together with vesicles, cells, and colloids. Gels of hmC with vesicles could have applications in site-specific drug delivery.[11] Mixtures of carbon microspheres with hmC could be useful in lubrication[10] or even in environmental remediation.[12] Moreover, the ability of hmC to gel blood indicates its potential utility as a hemostatic agent, i.e., as a material that can stop bleeding and hemorrhage from serious wounds.[9] Tests of hmC in animal injury models have proved successful and have

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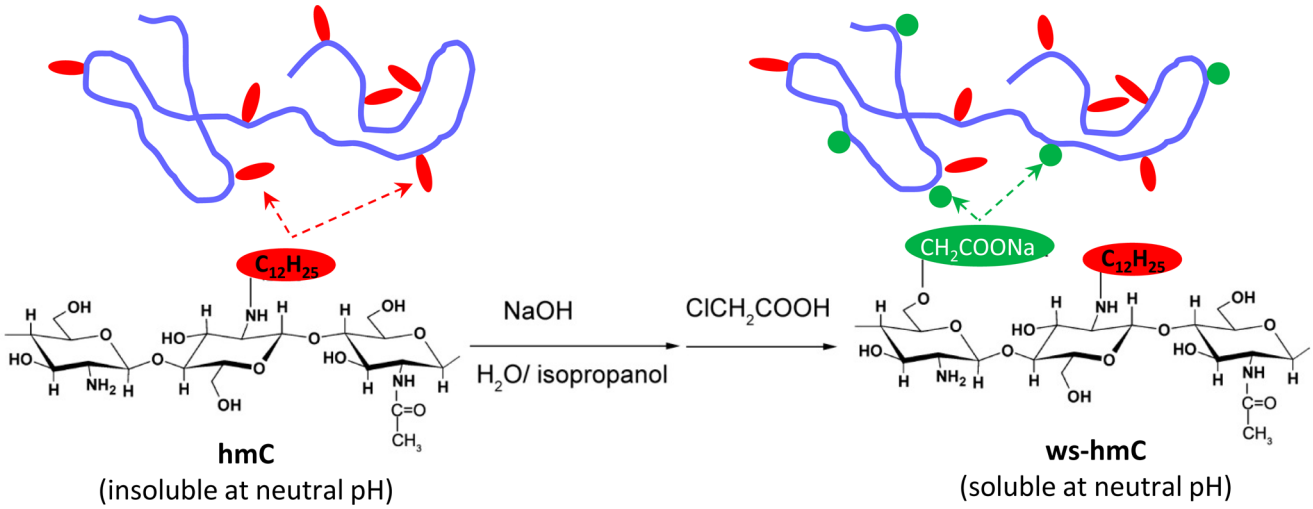

Figure 1. Scheme for synthesis of water-soluble hydrophobically modified chitosan (ws-hmC). hmC with C_{12} hydrophobes (shown in red) is reacted with monochloroacetic acid, which results in some of the primary hydroxyls being replaced by *O*-carboxymethyl groups (shown in green).

confirmed its hemostatic capabilities.[9] In many of the above applications, one limitation is the insolubility of hmC in water at neutral or basic conditions or in aqueous buffers (e.g., phosphate buffered saline at pH 7.4). As noted, this insolubility is due to the pK_a of the parent chitosan being about 6.5, i.e., above this pH, the amines get deprotonated and the polymer loses its charge.[13] If hmC could be rendered soluble at neutral pH, it could substantially enhance its application potential, especially in biology and medicine.

In this study, we attempt to overcome the above limitation by synthesizing a water-soluble hmC (ws-hmC) and we then study the self-assembling properties of this polymer. Attempts to render chitosan water soluble have typically involved introducing hydrophilic or charged moieties such as carboxylates, sulfates, thiols, or glycols into the polymer architecture.[5,6,17−22] In the present case, we incorporate *O*-carboxymethyl groups by reacting hmC with monochloro-acetic acid in the presence of alkali as catalyst.[19−22] In terms of comparable studies, there is one report by Chen et al. who synthesized an *N*-carboxymethylated hmC and used it for drug encapsulation.[22] A schematic of the overall reaction scheme employed here and of the final ws-hmC product are shown in Figure 1. We then study mixtures of this ws-hmC in water at neutral pH with surfactant vesicles and with polymethyl-methacrylate (PMMA) latex nanoparticles. The ws-hmC is able to gel anionic vesicles whereas it forms a precipitate with cationic vesicles. In addition, ws-hmC is able to gel PMMA latex dispersions. Finally, gels with both vesicles and nanoparticles are shown to be reversible by addition of α-CD.

EXPERIMENTAL SECTION

Chitosan and Hydrophobically Modified Chitosan (hmC). All chemicals were purchased from Sigma-Aldrich unless otherwise noted. The parent polymer was a chitosan of medium molecular weight (190−310 K) and a degree of deacetylation of 80%. To make hmC with C_{12} hydrophobes, chitosan was reacted with dodecyl aldehyde following procedures described in the literature and in our earlier studies.[7,9,14] The degree of hydrophobic substitution followed the reaction stoichiometry[7] and here it was fixed at 5 mol % of the available amine groups on chitosan.

Water-Soluble Chitosan (ws-C) and Water-Soluble Hydrophobically Modified Chitosan (ws-hmC). Water-soluble derivatives of chitosan and hmC were prepared by reaction with monochloroacetic acid, as described in the literature[21] with minor modifications. The reaction procedure for ws-hmC is as follows: initially, 0.64 g of sodium hydroxide (NaOH) was added to 8 mL of a 1:4 (v:v) solution of water and propanol at 50 °C. hmC (0.28 g) was then added. After 1 h, chloroacetic acid (0.8 g) was added and the mixture was allowed to react for 3 h at 50 °C followed by 8 h at room temperature. Thereafter, the mixture was filtered, washed five times with 70% ethanol, and dried to obtain ws-hmC (sodium salt). The polymers were characterized by FTIR spectroscopy using a Thermo Nicolet Nexus instrument and by ^{1}H NMR spectroscopy using an INOVA 600 from Varian. The NMR experiments were done at 50 °C using D_2O as the solvent and tetramethylsilane (TMS) as the internal standard. Polymer solutions were prepared by adding the dried polymer to either deionized (DI) water or phosphate buffered saline (PBS) and warming the mixture till a clear solution was obtained. All polymer concentrations are reported as wt %.

Surfactant Vesicles. The cationic surfactant cetyl trimethyl-ammonium tosylate (CTAT) and the anionic surfactant sodium dodecyl benzenesulfonate (SDBS) were used to create catanionic vesicles.[7] CTAT and SDBS were separately dissolved in deionized water at a concentration of 1.25 wt % to form stock solutions. The two stock solutions were then mixed in a weight ratio of CTAT:SDBS = 30:70 to obtain anionic vesicles and in a weight ratio of CTAT:SDBS = 70:30 to obtain cationic vesicles.[7] Mixtures of vesicles with polymer were prepared by combining appropriate weights of their respective solutions, followed by vortex mixing.

PMMA Nanoparticles. Emulsion polymerization was used to synthesize PMMA latex nanoparticles.[23] Five grams of methyl methacrylate (MMA) monomer, 0.2 g of the surfactant sodium dodecyl sulfate (SDS), and 15 g of deionized water were added to a flask, and the mixture was emulsified for 20 min at room temperature. Then, 0.02 g of the initiator ammonium persulfate (APS) was introduced and sodium bicarbonate ($NaHCO_3$) was added to adjust the pH of the mixture to 7. The mixture was then heated for 4 h to 65 °C under a nitrogen atmosphere. Following the reaction, the particles were dialyzed for 5 days against deionized water, which was refreshed at 8 h intervals. The particles were finally stored as a 25 wt % dispersion in deionized water.[24] Mixtures of particles with polymer were prepared by combining appropriate weights of the particle dispersion and polymer solution, followed by vortex mixing.

Cyclodextrin Gel Reversal. The supramolecule, α-cyclodextrin (α-CD), was purchased from TCI America. A concentrated stock solution (13 wt %) of α-CD was prepared in DI water, and a small volume (200 μL) of this concentrated solution was added to approximately 1.5 mL of the ws-hmC containing gel, followed by vortex mixing. Adding just a small volume of the α-CD solution ensured that the gel was negligibly diluted in the process.

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Dynamic Light Scattering (DLS). Samples of vesicles and PMMA particles were characterized at room temperature using DLS. A Photocor FC instrument with a 5 mW laser at 633 nm was used at a 90° scattering angle. The autocorrelation function was measured by a logarithmic correlator and was converted to a size distribution using the Dynals software package.

Zeta Potential Measurements. Zeta potentials of dilute polymer solutions were measured by a Malvern Zetasizer Nano ZS90 at a 90° scattering angle. Samples were measured in disposable polystyrene cuvettes. All measurements were conducted in triplicate.

Rheological Studies. An AR2000 stress-controlled rheometer (TA Instruments) was used to perform steady and dynamic rheology experiments. All experiments were done at 25 °C using a cone-and-plate geometry (40 mm diameter and 2° cone angle). A solvent trap was used to minimize drying of the sample during measurements. Dynamic frequency spectra were conducted in the linear viscoelastic regime of the samples, as determined by dynamic strain sweep experiments.

RESULTS AND DISCUSSION

Properties of ws-hmC. We synthesized ws-hmC, as described in the Experimental Section, by reacting hmC with monochloroacetic acid in the presence of alkali as a catalyst. As shown by Figure 1, ws-hmC has carboxymethyl ($-CH_2COO^-$) groups replacing some of the primary hydroxyls along the polymer chain.[21] FTIR and NMR were used to confirm the presence of carboxymethyl groups and quantify their extent (Figure S1, Supporting Information). From these studies, we estimate the degree of carboxymethylation to be about 75%. Note that ws-hmC also has 5% of its amines substituted by C_{12} hydrophobes. The hydrophobes provide self-assembling character whereas the carboxymethyl groups impart water-solubility. In addition to ws-hmC, we also carboxy-methylated the native chitosan to the same degree and that polymer is designated as ws-C.

Preliminary tests were performed on ws-hmC and ws-C to test their aqueous solubility. Both polymers were found to be soluble in water over a wide range of pH (~3 to 13). Specifically, both are soluble in deionized water at neutral pH and also in aqueous buffers such as PBS (pH 7.4). In comparison, native chitosan and hmC (both lacking carboxymethyl groups) were insoluble in these media. We measured the zeta potential of ws-hmC chains in water at pH 7, and this was found to be −74.7 mV. Note that the pK_a of chitosan (and by extension, hmC) is about 6.5. Thus, hmC in the absence of carboxymethyls is expected to be weakly charged at pH 7. In the case of ws-hmC though, the carboxymethyls are expected to ionize and become anionic at pH 7.[21] The large number of these anionic groups ensures a strong anionic character for ws-hmC, and in turn allows the polymer to be solubilized at neutral pH.

We then studied the rheology of ws-C and ws-hmC solutions in water at neutral pH. From visual observations, it was evident that the ws-hmC solutions were much more viscous than those of ws-C at equivalent concentrations. This is anticipated from the polymer architectures, i.e., the hydrophobes on ws-hmC are expected to associate and form transient cross-links between the polymer chains, leading to the higher viscosity.[14−16] Figure 2 compares the rheology of ws-C and ws-hmC solutions at a concentration of 1.5%. The dynamic rheological data (Figure 2a) show the elastic (G') and viscous (G'') moduli as functions of angular frequency ω. The ws-hmC sample shows the rheological signature of a weak gel,[25] with both moduli being weak functions of frequency and with $G' > G''$ over the frequency range. On the other hand, the ws-C sample shows a viscous response with both moduli showing a strong dependence on frequency and with $G'' > G'$ over the frequency range.[25] Figure 2b shows the corresponding data from steady-shear rheology on the two samples. The ws-C sample shows a constant viscosity around 10 mPa·s over the range of shear stresses (Newtonian behavior). In contrast, the viscosity of the ws-hmC sample is higher by ~5 orders of magnitude at low shear stresses and decreases at higher shear stresses (shear-thinning). Similar rheological differences are seen between chitosan and hmC solutions at acidic pH.[14−16] Overall, our data show that ws-hmC, much like hmC, has the ability to form viscous solutions by self-assembly of its hydrophobic parts. The difference is that ws-hmC functions at neutral and basic pH whereas hmC does so only at acidic pH.

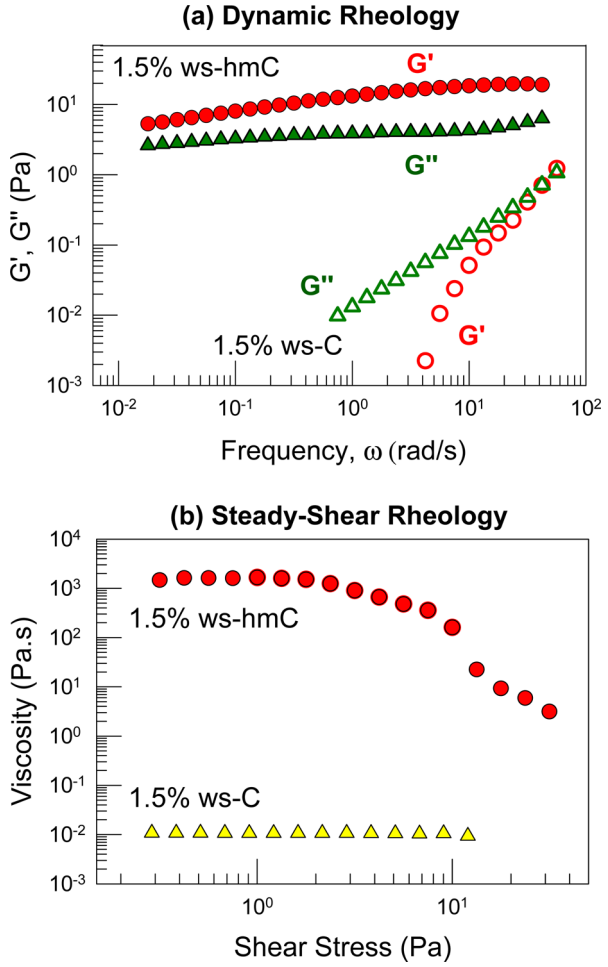

Figure 2. Solution rheology of ws-hmC and ws-C. (a) Data from dynamic rheology for the elastic modulus G' (red circles) and the viscous modulus G'' (green triangles) as functions of frequency ω are shown for solutions of 1.5 wt % ws-hmC and ws-C in water at neutral pH. (b) Data from steady-shear rheology for the apparent viscosity as a function of shear stress for the same solutions.

Gelation of Vesicles with ws-hmC. Next, we proceeded to examine ws-hmC together with vesicles. Catanionic surfactant vesicles were prepared in deionized water by combining the cationic surfactant CTAT and the anionic surfactant SDBS. A weight ratio of 70:30 CTAT:SDBS gave vesicles with a net cationic nature whereas anionic vesicles corresponded to a weight ratio of 30:70 CTAT:SDBS. Both sets of vesicles had average diameters around 104 nm. In the

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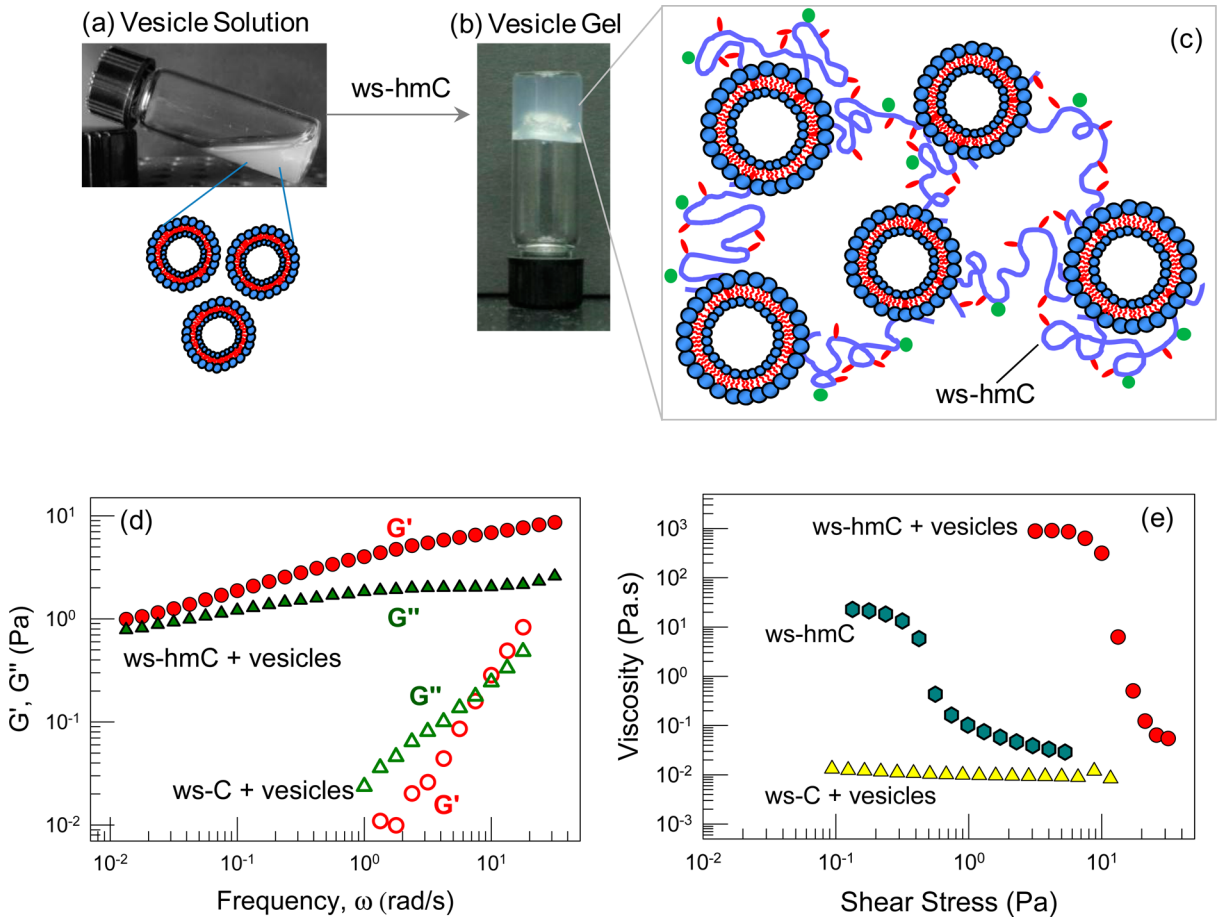

Figure 3. Gelation of anionic vesicles by ws-hmC. (a) Photograph of a 0.625% CTAT/SDBS (30:70) vesicle sample showing that it flows freely. (b) Photograph of the gel formed upon addition of 0.75% ws-hmC to the vesicles. The gel holds its weight in the inverted vial. (c) Schematic of the ws-hmC-vesicle gel network. Hydrophobes on ws-hmC are shown to be embedded into vesicle bilayers to form a connected network of vesicles. (d) Dynamic frequency sweeps (elastic modulus G' and viscous modulus G'' as functions of frequency ω) comparing the rheology of samples with 0.625% vesicles and 0.75% ws-hmC or 0.75% ws-C. (e) Steady-shear rheological data (viscosity vs shear-stress) for the samples in panel d. In addition, data are also shown for a sample of 0.75% ws-hmC with no vesicles.

case of cationic vesicles, their mixture with ws-hmC resulted in an inhomogeneous coacervate. This is to be expected given the fact that ws-hmC is anionic in neutral water and will thus bind electrostatically to cationic structures. Similar coacervation has been noted in the case of hmC under acidic conditions when combined with anionic vesicles.[7]

In contrast, when the anionic ws-hmC is combined with anionic vesicles at neutral pH, the polymer transforms the vesicle solution into a self-supporting gel. Figure 3 (top) provides visual evidence for such gelation. The CTAT/SDBS vesicle solution (0.625%) is a thin liquid with a viscosity close to that of water, i.e., 1 mPa·s (Figure 3a). When 0.75% of ws-hmC is added, the sample is converted into a gel that supports its weight in the inverted vial (Figure 3b). Dynamic rheological data (Figure 3d) confirm that this sample shows the response of a weak gel. Here again, both the elastic (G') and viscous (G'') moduli are weak functions of frequency, and $G' > G''$ over the frequency range. For comparison, we also examined the addition of 0.75% of ws-C (i.e., the polymer without hydrophobes) to the same vesicle solution. In this case, no gelation was observed, and the rheological data for this mixture (Figure 3d) show frequency-dependent moduli and $G'' > G'$, indicating viscous behavior. Corresponding steady-shear data (Figure 3e) reveal that the ws-C + vesicle sample is a Newtonian, low-viscosity liquid whereas the ws-hmC + vesicle sample has a much higher viscosity at low shear stresses followed by shear-thinning. Moreover, the ws-hmC sample shows a sharp drop in viscosity around a stress of 10 Pa, which is then the yield stress of the sample. Note also that ws-hmC alone (without vesicles) at the concentration of 0.75% is not a gel (Figure 3e); rather it is a viscous liquid with moderate shear-thinning. Thus, the gel is not one of ws-hmC alone but it is formed synergistically when ws-hmC is combined with vesicles.

Gelation of anionic vesicles by ws-hmC at neutral pH is similar to gelation of cationic vesicles by hmC at acidic pH.[7,8,11] In each case, the polymeric counterpart without hydrophobes does not induce gelation. Thus, the likely mechanism for gelation in both cases is by the hydrophobes inserting into vesicle bilayers and thereby connecting the vesicles via anchored polymer chains into a 3-D network.[7] Support for this mechanism from small-angle neutron scattering (SANS) and cryo-transmission electron microscopy (cryo-TEM) has been previously published.[7,8,11] A schematic depiction of this mechanism is shown in Figure 3c. Note that, although the polymer and the vesicles have similar charge, the hydrophobic associations seem to be favorable enough to overcome the electrostatic repulsions.[7] A similar associating polymer-induced

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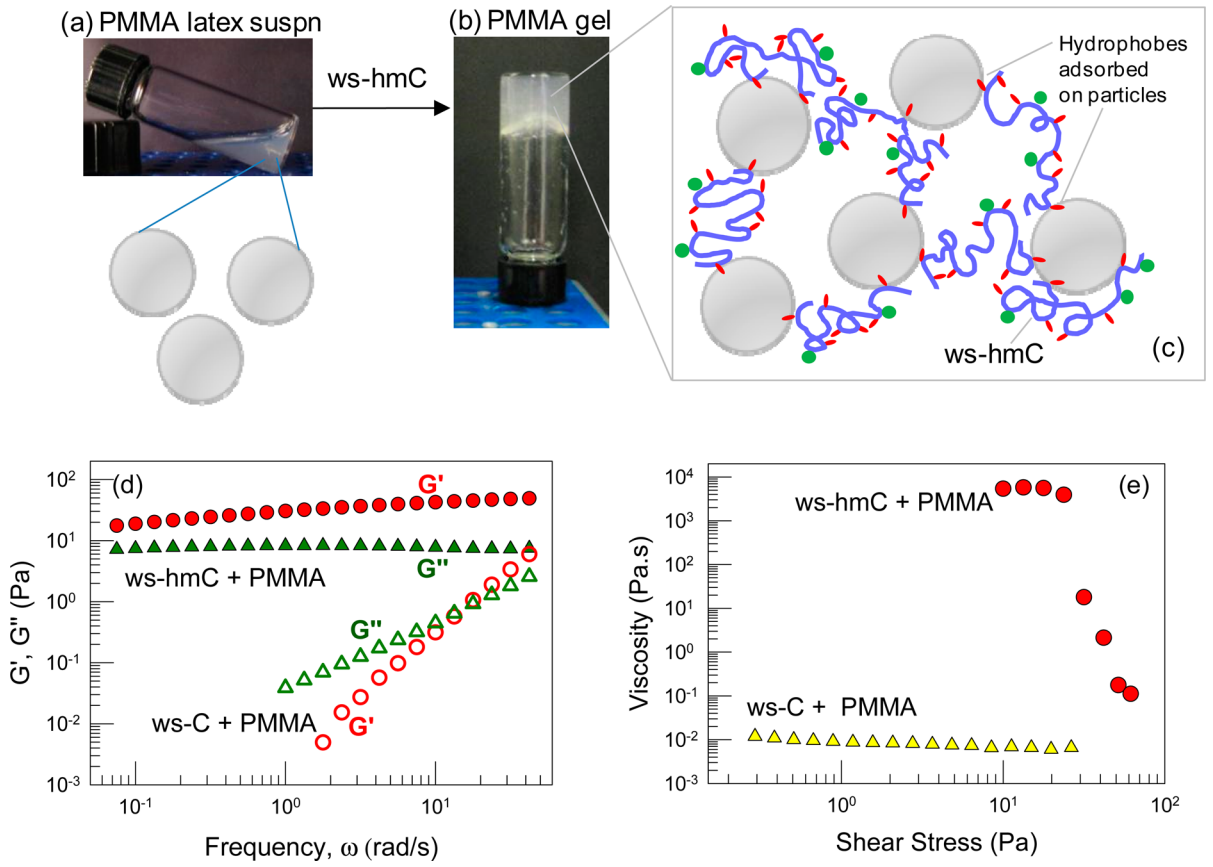

Figure 4. Gelation of PMMA latex particles by ws-hmC. (a) Photograph of a vial containing a freely flowing suspension of 4% PMMA latex particles. (b) Photograph of the gel formed upon addition of 0.75% ws-hmC to the above vial. (c) Schematic of the proposed network formed by ws-hmC and PMMA particles. Hydrophobes on ws-hmC are shown to be adsorbed onto PMMA particle surfaces and thereby the particles are connected by polymer chains into a network. (d) Dynamic frequency sweeps (elastic modulus G' and viscous modulus G'' as functions of frequency ω) comparing the rheology of samples with 4% PMMA particles and 0.75% ws-hmC or 0.75% ws-C. (e) Steady-shear rheological data (viscosity vs shear-stress) for the samples in panel d.

interconnection of vesicles has also been noted in other studies.[26,27] Also, as discussed in our earlier studies, a percolating (volume-filling) network requires a sufficient concentration of both vesicles and polymer.[7,11] Beyond this threshold, the gel modulus is expected to increase steadily with increasing vesicle and polymer concentrations.[7] Overall, the ability to gel vesicles by ws-hmC at neutral pH is significant because it enhances the applicability of the polymer in biomedical and pharmaceutical applications. Similar gelation by ws-hmC is also observed for vesicles formulated in PBS and other buffers.

Gelation of PMMA Nanoparticles with ws-hmC. Next, we proceeded to test ws-hmC with PMMA latex particles. The particles had an average diameter of 60 nm (from DLS), and they are expected to have a negative surface charge at neutral pH.[28] The particles are thus electrostatically stabilized against flocculation. Note that when the particles were combined with hmC under acidic conditions, precipitation occurred due to the unlike charges between the cationic polymer and the anionic particles. In contrast, when the particles are combined with ws-C or ws-hmC under neutral conditions, the anionic nature of the polymers ensures a lack of precipitation. Instead, we find that ws-hmC (but not ws-C) transforms the nanoparticle dispersion into a gel. Figure 4 presents visual and rheological results for a mixture of 4% PMMA particles with 0.75% of ws-hmC or ws-C. The particle dispersion is a thin flowing liquid (Figure 4a) whereas the mixture with ws-hmC is a gel that holds its weight in the inverted vial (Figure 4b). Dynamic rheological data (Figure 4d) confirm the gel-like rheology: the moduli are nearly independent of frequency, and $G' > G''$. In comparison, ws-C does not induce gelation and a sample with 0.75% ws-C + 4% PMMA particles shows a viscous response ($G'' > G'$, both moduli are frequency dependent). Steady-shear rheological data (Figure 4e) also further confirm that the sample of ws-C + particles is a low-viscosity liquid whereas the sample of ws-hmC + particles shows yield stress behavior.

The above finding is similar to that found in our earlier study with carbon microparticles and hmC.[10] As in that case, we observe gelation only with the polymer having hydrophobes, which means that hydrophobes have a crucial role to play. The likely mechanism involves the hydrophobe-mediated adsorption of ws-hmC chains onto the PMMA nanoparticle surfaces. It is well-known that the adsorption of hydrophilic polymers onto particles is enhanced by the presence of hydrophobic grafts.[10] This is especially so when the polymer and the particles are like-charged because the polymer would have to overcome electrostatic repulsions in order to adsorb. Thus, we believe that the hydrophobes on ws-hmC greatly enhance its adsorption over that of ws-C. Figure 4c shows a schematic of the nanostructure in a ws-hmC/PMMA gel: ws-hmC chains are shown to be adsorbed via their hydrophobic portions onto the

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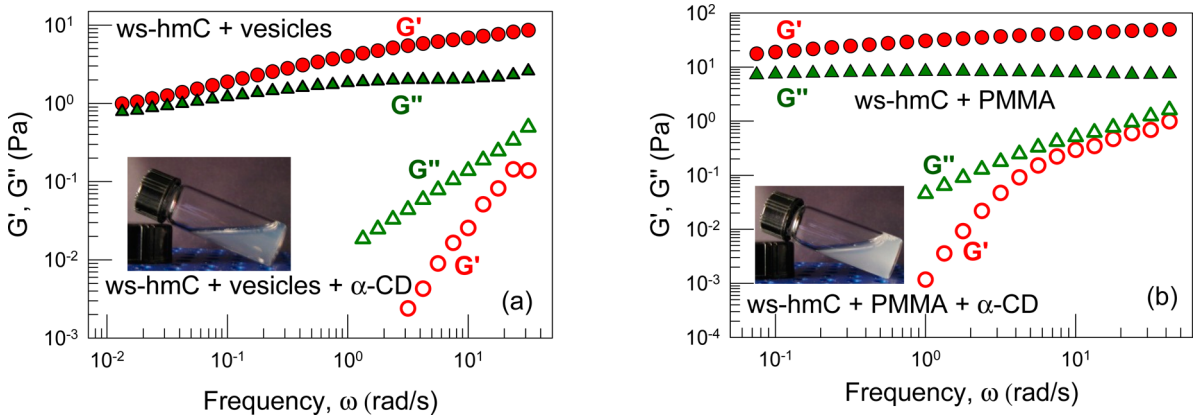

Figure 5. Reversal of gelation of vesicles and nanoparticles. Dynamic frequency sweeps (elastic modulus G' and viscous modulus G'' as functions of frequency ω) are shown. (a) A gel formed by 0.75% ws-hmC + 0.625% vesicles and the same sample after addition of 1.5% α-CD. The α-CD reverses the gelation, as shown also by the photograph in the inset. (b) A gel formed by 0.75% ws-hmC + 4% PMMA latex particles and the same sample after addition of 1.5% α-CD. The α-CD reverses the gelation, as shown also by the photograph in the inset.

PMMA particles and thereby they bridge the particles into a volume-filling 3-D network.

Reversal of Gelation of Vesicles and Nanoparticles. The gels of vesicles and nanoparticles formed with ws-hmC are both networks of weak, physical bonds, i.e., hydrophobic interactions. The weak bonds act co-operatively to build a sufficiently strong and dense network.[7,11] But because each individual bond is weak, it is possible to undo the gelation by disengaging these bonds. This can be done by introducing a competing species with a strong affinity for hydrophobes.[29−32] We previously demonstrated this aspect in the context of blood gelled with hmC. In particular, the addition of α-cyclodextrin (α-CD), a supramolecule with an inner cavity that can bind hydrophobes, was shown to liquefy the blood gel.[9]

Here, we explore the addition of α-CD to gels of ws-hmC with both vesicles and nanoparticles. Specifically, Figure 5a shows the results of adding 1.5% α-CD to a gel formed by 0.625% anionic vesicles + 0.75% ws-hmC. The gel is seen to be transformed into a thin, flowing solution by the α-CD. Dynamic rheology confirms the viscous nature of this sample, i.e., $G'' > G'$ and both moduli are strongly dependent on frequency. Note the contrast with the original gel-like rheology. A similar result is also found upon adding 1.5% α-CD to a gel formed by 4% PMMA nanoparticles + 0.75% ws-hmC (Figure 5b). In this case also, the sample is converted from a gel to a viscous liquid, as seen from the photograph. Again, dynamic rheology confirms the viscous nature of the ungelled sample because both moduli are again frequency dependent, especially at low frequencies. Thus, α-CD is able to reverse both types of gels formed by ws-hmC.

The above results provide indirect evidence for the mechanism of gelation by ws-hmC. We have stated that the hydrophobes (alkyl tails) on ws-hmC are crucial for gelation: they are expected to either embed in vesicle bilayers (Figure 3c) or anchor onto particle surfaces by adsorption (Figure 4c). The binding pocket in α-CD is just the right size for capturing single alkyl tails.[29,32] In turn, we expect α-CD to bind and sequester the hydrophobes on ws-hmC, preventing their interaction with vesicles or particles and thus explaining the ungelling. In other words, the ungelling ability of α-CD shows that the gelation must have occurred via hydrophobic interactions.[9] Note that the binding pocket in α-CD is not large enough to fit two alkyl tails.[29,32] For this reason, α-CD does not disrupt the catanionic surfactant vesicles because these molecules are usually paired up by virtue of the electrostatic interactions between their headgroups.[32] Thus, α-CD is able to selectively bind to the hydrophobic tails attached to ws-hmC chains rather than the tails of surfactant molecules that constitute the vesicles.

CONCLUSIONS

We have demonstrated that carboxymethylation of hmC gives a polymer (ws-hmC) that is soluble at neutral pH while retaining the associating properties of hmC. Vesicles and PMMA nanoparticles can be gelled at neutral pH by addition of ws-hmC. Gelation occurs due to hydrophobic interactions between the hydrophobic tails on ws-hmC with the bilayers of vesicles or the surfaces of nanoparticles. This gelation can be reversed by addition of a supramolecule α-CD, which has a binding pocket that sequesters the hydrophobic tails. We believe the capability of ws-hmC to transform vesicles and nanoparticles into (reversible) gels at physiological pH will make it a useful material in biomedical, pharmaceutical, and cosmetic applications.

ASSOCIATED CONTENT

Supporting Information

Data from FTIR and ^{1}H NMR on the polymers studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Supporting Information

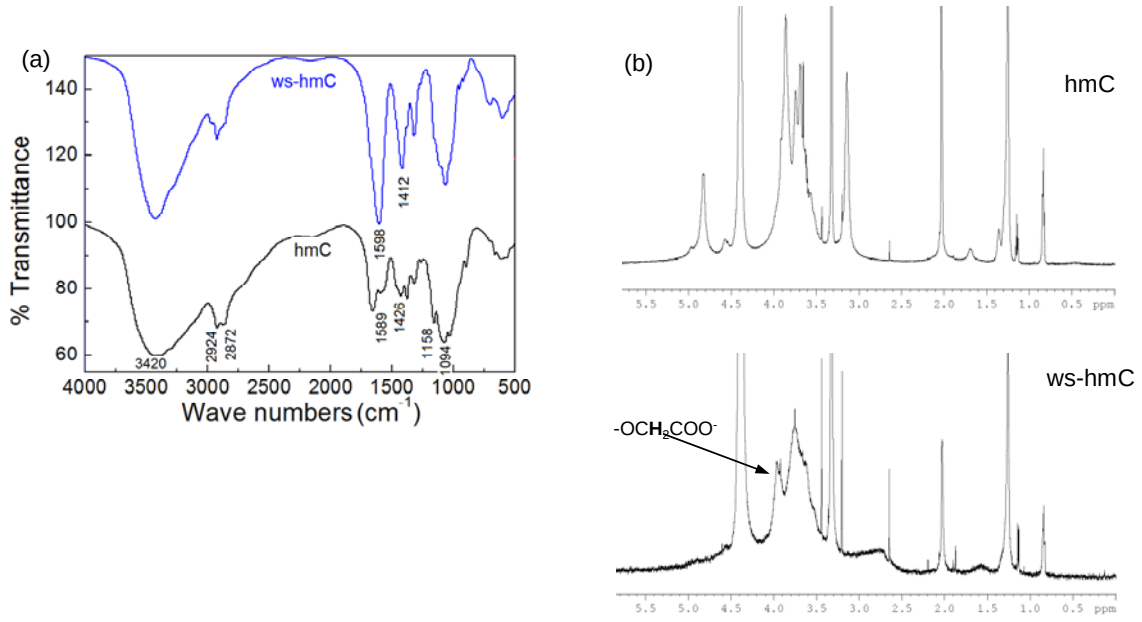

Figure S1. (a) FT/IR spectra of ws-hmC and hmC. (b) ^{1}H NMR of hmC and ws-hmC

The IR spectra of the sodium salt of ws-hmC and hmC are compared in Figure S1a. hmC spectra show characteristic peaks at 3420 cm^{-1} (O–H stretch), 2924-2872 cm^{-1} (C–H stretch), 1589 cm^{-1} (N–H bend), 1158 cm^{-1} (bridge–O stretch), and 1094 cm^{-1} (C–O stretch).[1] In the IR spectra of ws-hmC, the new absorption peaks at 1598 cm^{-1} and 1412 cm^{-1} correspond to carboxy groups.[1] The absence of C-O stretching band at 1030 cm^{-1} which corresponds to the primary hydroxyl group of chitosan indicates high carboxymethylation in ws-hmC.[1] This observation is further strengthened by appearance of a new peak in ^{1}H NMR spectra (as shown in Figure S1b) of ws-hmC between 3.92 and 3.96 ppm which is likely to be due to the chemical shift of –O–CH_2COONa.[2] The degree of carboxymethylation was calculated using the ratio of peak areas of the carboxymethyl group and the –CH_3 group and it was estimated to be about 75%. Other researchers who have used a similar reaction scheme have reported similar degrees of carboxymethylation.[1,2]

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