Soft Matter

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Cite this: *Soft Matter*, 2011, **7**, 4170

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Carbon microspheres as network nodes in a novel biocompatible gel†

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Received 6th December 2010, Accepted 15th March 2011
DOI: 10.1039/c0sm01430c

The gelation of hydrophobically modified chitosan solutions can be accomplished through the incorporation of uniform carbon microspheres. The carbon particles act as nodes in the gel network where hydrophobic alkyl groups, attached to the polysaccharide backbone, interact with multiple carbon microspheres to form a three-dimensional matrix. Rheological characterizations show significant increases in elastic moduli upon incorporation of the carbon microspheres.

Chitosan is a linear copolymer, composed of glucosamine and *N*-acetylglucosamine residues. It is a derivative of chitin, which is obtained from seafood-processing wastes (crab, shrimp and lobster shells). The production of chitosan is thereby environmentally friendly,[1] and the polymer is considered fully biocompatible with significant applications in drug delivery and hemostasis.[2–5] The amine groups on chitosan also facilitate derivatization. In particular, hydrophobically modified chitosan (hm-chitosan, structure in Fig. 1a) can be synthesized by attaching alkyl (*e.g.*, *n*-dodecyl) tails to some of the amine moieties on the chitosan backbone. It has been shown that hm-chitosan can form gels when contacted with low viscosity solutions of lipid vesicles.[6] Gelation occurs because the polymer chains insert their alkyl hydrophobic moieties into the vesicle bilayers and thereby non-covalently crosslink the vesicles into a network.[7] The vesicles then become nodes in a network where they are connected by multiple hm-chitosan chains. These "vesicle gels" have significant potential in drug delivery systems since they are fully biocompatible.[8] The concept follows earlier work by other researchers on the gelation of vesicles using other types of hydrophobically modified polymers.[9] It is noteworthy that gelation of vesicles is not observed if the added polymer does not have hydrophobic tails.[10]

The above concept is extended here to the use of carbon microspheres as gelation nodes in an hm-chitosan network. We hypothesized that hydrophobic carbon microsphere surfaces may function to anchor the alkyl groups of hm-chitosan through physical adsorption, and varied concentrations of hm-chitosan and carbon microspheres to determine if gelation could be induced. For the experiments, chitosan (85% deacetylated with a molecular weight of 370 000) and *n*-dodecyl aldehyde were purchased from Sigma-Aldrich. α-Cyclodextrin (α-CD) was obtained from Wacker Chemical as CAVAMAX W6. Hm-chitosan was prepared by reacting *n*-dodecyl aldehyde with chitosan using Schiff base chemistries.[11–13] Briefly, in an ethanol and water mixture, *n*-dodecyl aldehyde was reacted with chitosan (0.025 molar ratio of aldehyde to glucosamine residues). Sodium cyanoborohydride was then added to reduce the Schiff base. The hm-chitosan was precipitated by raising the pH, and this precipitate was washed with ethanol and then with deionized water four times.

Monodisperse hydrophobic carbon microspheres (HCS), ranging in size from 0.3 to 1.2 μm, were prepared by hydrothermal dehydration and pyrolysis as reported in the literature.[14,15] Briefly,

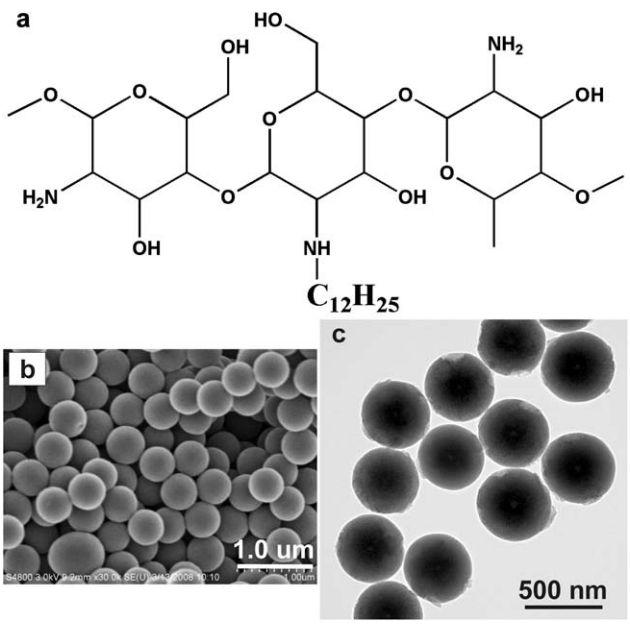

Fig. 1 (a) Chemical structure of hm-chitosan with alkyl hydrophobes. (b) SEM and (c) TEM of carbon microspheres.

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† Electronic supplementary information (ESI) available: Details on cryo scanning electron microscopy and NMR relaxation studies. See DOI: 10.1039/c0sm1430c

saturated solutions of α-CD in water were first prepared by dissolving 129.5 g of α-CD in 1 L of deionized water. These samples were hydrothermally treated by loading pressure cylinders to 90% capacity, sealing and heating them to 190 °C for 6 h. Following the hydrothermal treatment, the suspensions were filtered, oven-dried at 52 °C and then carbonized in a tube furnace under an argon atmosphere with a 25 mL min^{-1} flow rate for 10 h at 1000 °C to activate the resulting carbons. Fig. 1b and c illustrate the dimensions and morphologies of the carbon microspheres used in this work. FTIR measurements show no remnant functional groups on the carbon after carbonization.

To prepare the carbon microsphere/hm-chitosan networks, the microspheres were ultrasonicated in deionized water to minimize aggregation and settling of these hydrophobic particles prior to addition of the polymer. Hm-chitosan was added to the microsphere suspension to the desired composition and held at 50 °C for 2 h. The carbon microsphere/hm-chitosan mixture was observed to gel rapidly, although a 24 h equilibration time was minimally allowed prior to any characterization. The gel was stored at room temperature. Parametric concentration variations were done to determine gelation threshold levels. With an hm-chitosan concentration of 1.1 wt%, we find that firm gelation, as detectable by vial inversion, occurs at a particle concentration of about 4 wt%. Control experiments with unmodified chitosan do not show gelation, with or without carbon microspheres. Fig. 2a shows a photograph of two samples: (i) a control of 1.1 wt% hm-chitosan in solution and (ii) a gel containing 1.1 wt% hm-chitosan and 4 wt% carbon microspheres. We note that the hm-chitosan in water is a moderately viscous solution, whereas with the addition of carbon leads to a gel that is able to hold its weight upon vial inversion with the carbon dispersed throughout the gel phase.

Cryo-SEM imaging of the gel was performed on a field-emission scanning electron microscope (SEM) (Hitachi 4800). Briefly, the procedure involves rapid plunging of the sample into liquid nitrogen, followed by freeze-fracture using the flat edge of a cold (−130 °C) knife and then sublimation for 5 min at −95 °C to etch away surface water and expose internal features. The sample was then sputter-coated with platinum at 10 mA for 88 s and imaged on the SEM at a voltage of 3 kV and at a working distance of ~6 mm. Fig. 2b presents cryo-SEM images of the gel. We observe cell-like structures with tendrils protruding from the cell walls. Interestingly, the carbon microspheres are primarily located at cell junctions with virtually no particles in the interior of the cell. Occasionally, particles are observed along a cell wall rather than at a junction. Our hypothesis is that the cell walls reflect assemblies of hm-chitosan chains. The location of microspheres at junctions may imply that the carbon facilitates physical crosslinking with polymer alkyl groups from multiple chains or chain assemblies adsorbing to the carbon surfaces to create junctions and enhance the crosslink density.

Fig. 3a provides a schematic illustration of the proposed mechanism of carbon microsphere-induced gelation. We believe the ribbon-like cell wall structure in Fig. 2b is an assembly of hm-chitosan

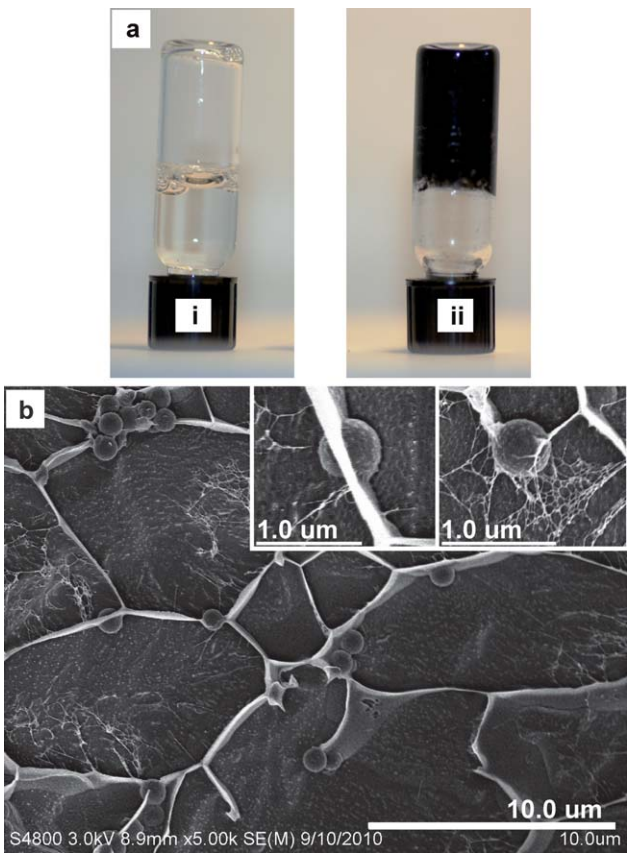

Fig. 2 (a) Photograph of samples: (i) 1.1 wt% hm-chitosan and (ii) 1.1 wt % hm-chitosan and 4 wt% carbon microspheres. (b) Cryo-SEM of 1.1 wt % hm-chitosan and 4 wt% carbon microspheres shows the microspheres located primarily at junctions.

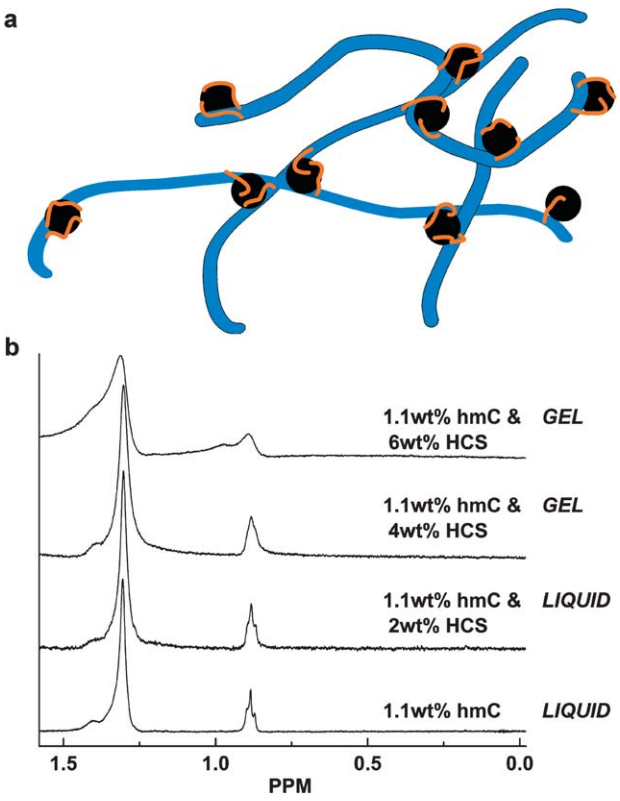

Fig. 3 (a) Schematic of gel formation: hydrophobic tails attached to chitosan backbones interact with the hydrophobic HCS. Not to scale. (b) 1H NMR of 1.1 wt% hm-chitosan samples with increasing carbon microsphere concentration.

molecules where hydrophobic interactions between the alkyl side chains bind the polymers into thick fibrillar bundles. The carbon microspheres may provide further functionality by facilitating network nodes through adsorption of multiple bundles on the surface.

Fig. 3b provides indirect evidence of the carbon/hm-chitosan interaction through ^{1}H nuclear magnetic resonance (NMR) spectroscopy of the alkyl groups. The experiments were conducted in deuterium oxide using a Bruker Avance 500 MHz NMR spectrometer. The spectra indicate subtle but observable changes in the hm-chitosan alkyl group resonances with increasing concentration of the carbon microspheres. A clear line broadening of the CH_2 and CH_3 proton resonances is observed at gel formation conditions. The CH_2 and CH_3 peaks of the carbon microsphere/hm-chitosan samples with 6 wt% of carbon microspheres and 1.1 wt% hm-chitosan [δ_H (500 MHz, D_2O) 1.3 (CH_2, 138.0 Hz) and δ_H (500 MHz, D_2O) 0.8 (CH_3, 81.0 Hz)] are nearly twice as broad as those of the control sample with only 1.1 wt% hm-chitosan [δ_H (500 MHz, D_2O) 1.3 (CH_2, 79.0 Hz) and δ_H (500 MHz, D_2O) 0.8 (CH_3, 23.2 Hz)]. The line broadening indicates motional restrictions of the alkyl side chains upon introduction of the particles. Indeed at particle levels beyond the gelation threshold (3 wt%), the two methyl group ^{1}H resonance bands indicate two distinct populations of methyl groups. ^{1}H spin–lattice relaxation experiments (ESI†) indicate that the relaxation times (T_1) for the methyl and methylene groups increase upon gel formation through carbon microsphere addition. Additionally, T_1 for the water also increases due to sequestration of water in the confined volume of the gel and interaction with the polymer chains.

Conclusive evidence for gel formation is provided through rheological studies. The experiments were done at 25 °C on a TA Instruments AR 2000 rheometer using a 25 mm diameter parallel-plate set-up. Fig. 4 shows the dynamic frequency response of samples containing hm-chitosan, both with and without carbon microspheres. A solution of hm-chitosan at a low concentration of 0.55 wt% exhibits a viscous response typical of weakly entangled polymer solutions: here, the elastic modulus (G') is lower than the viscous modulus (G'') over the range of frequencies, and both moduli show a strong frequency-dependence.[16] At a higher concentration of hm-chitosan (1.1 wt%), the sample behaves like a weak transient gel: in this case, G' is higher than G'' over the range of frequencies, although there is a crossover with $G'' > G'$ at low frequencies.[16] At a higher concentration of hm-chitosan (1.1 wt%), the sample behaves like a viscoelastic, transient network: in this case, G' is higher than G'' over most of the frequency ranges, although there is a crossover with $G'' > G'$ at low frequencies.[16] The same sample under steady-shear rheology (Fig. 4b) shows a moderately high viscosity with a plateau in the viscosity being observed at low shear rates (note from Fig. 2a that this sample does flow down an inverted vial). This viscoelastic response likely reflects the formation of hydrophobic crosslinks between the hm-chitosan chains.

Upon addition of 4 wt% carbon microspheres to the hm-chitosan solution, the dynamic response becomes gel-like, with no dependence of the moduli on frequency in the range studied. Note also that the moduli are 10-fold higher in the presence of the microspheres, and correspondingly from Fig. 4b, the low-shear viscosities are 1000-fold higher. Furthermore, note that the viscosity curve tends to a slope close to −1 on the log–log plot, which is indicative of a yield stress in the material.[17,18] The gel-like behavior and the yield stress correlate with the ability of the sample to hold its weight under tube inversion.

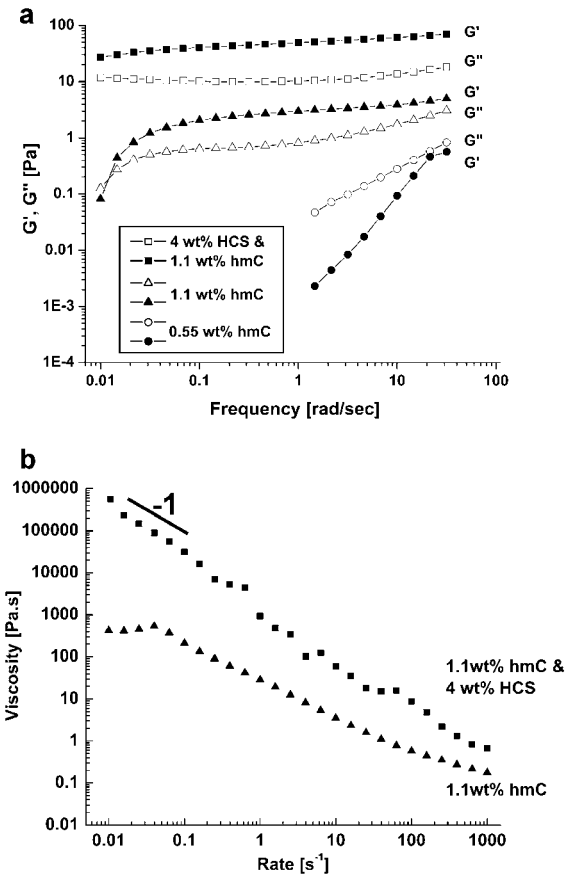

Fig. 4 (a) Loss and storage moduli of 1.1 wt% hm-chitosan with carbon microspheres, 1.1 wt% hm-chitosan without carbon microspheres, and 0.55 wt% hm-chitosan. (b) Steady-shear measurements of 1.1 wt% hm-chitosan samples with and without carbon microspheres.

Taken together, the rheology data confirm the presence of a volume-filling network in the sample when the microspheres are added. The measurements reinforce the hypothesis that the carbon microspheres enhance the crosslink density within the network. While a 1.1 wt% hm-chitosan system exhibits weak gel-like properties, we believe the >10-fold increase in dynamic moduli upon addition of carbon microspheres is a consequence of enhancement of crosslink density. There are similarities with earlier studies of wormlike micelles, which show increases in viscosity and dynamic moduli upon the addition of inorganic nanoparticles which become incorporated into the micellar network.[19–21] Here, we exploit the hydrophobic effect to enhance gelation in biocompatible polymeric systems using well-defined monodisperse carbon microspheres. The phenomenon is closely aligned with the gelation of polymer solutions upon contact with vesicles through insertion of the alkyl side groups into vesicle bilayers.[1,6–10]

Conclusions

We report a new system to gel a biocompatible polymer solution with monodisperse carbon microspheres that can be easily synthesized from inexpensive precursors. The potential applications of these gels are significant due to the biocompatibility of hm-chitosan, the strong adsorption capacity of the carbon microspheres and their relatively

monodisperse size distribution. Such uniform carbon microspheres have found use in electrode fabrication,[22] tissue engineering[23] and in environmental remediation.[24,25] Incorporation of these materials into biocompatible gel structures may enhance application potential in drug delivery, biolubrication and tissue engineering platforms, and facilitate processing.

Acknowledgements

Funding from the National Science Foundation (Grant 1034175) and the Department of Energy (DOE-DE-FG02-05ER46243) is gratefully acknowledged for this work. Additional funding was provided by the Advanced Materials Research Institute of the University of New Orleans through the PKFSI program. J. St. Dennis acknowledges a Graduate Fellowship from NASA and the Louisiana Board of Regents, BoRSF, under agreement NASA/LEQSF (2005–2010)-LaSPACE and NASA/LaSPACE.

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Supporting Information

Cryogenic Scanning Electron Microscopy

Figures 1a and 1b show cryo-SEM images of 1.1 wt% hm-chitosan without carbon microspheres and 1.1wt% unmodified, native chitosan respectively.

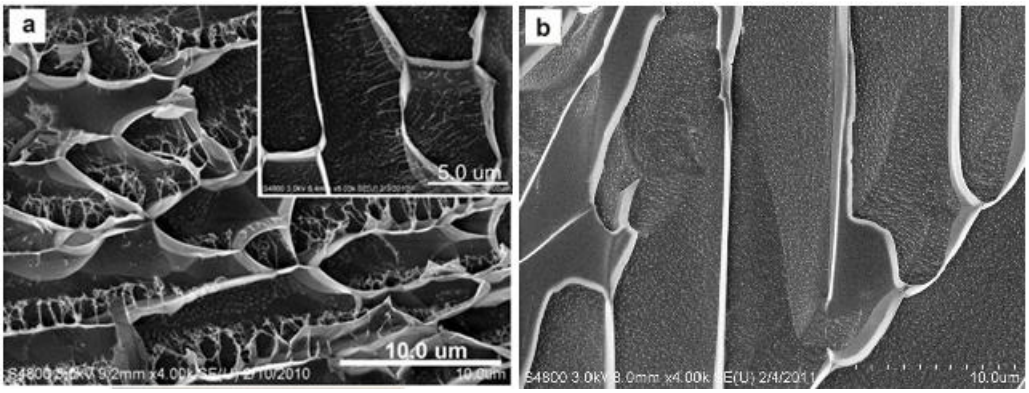

Figure 1: Cryo-SEMs of (a) 1.1wt% hm-chitosan; (b) 1.1wt% native chitosan.

At these concentrations neither of these systems forms a gel.[1-5] Both systems show a cell-like structure, but the distinctive aspect in the hm-chitosan system are the tendrils protruding from the cell walls. It is difficult to attribute the tendrils to the hydrophobic groups, but we have consistently observed these structures in hm-chitosan systems. The cell-like structures are not artifacts but are representative of the fact that chitosan and hm-chitosan form loose aggregates in solution, even without the addition of the gelling agent (carbons, liposomes).

^{1}H NMR Relaxation Experiments

^{1}H spin-lattice relaxation measurements were conducted by the usual inversion-recovery method, employing π-τ-$\pi/2$ pulse sequences with ten scans each and with a relaxation delay of at least 5 times the maximum T_1 estimated in the spin set. Samples were not degassed before the experiment, and therefore the T_1 values reported here cannot be taken to be absolute. Instead, they provide information about the relative relaxation times. Table I below lists the T_1 values for the methyl and methylene groups on the alkyl tails together with the corresponding values for water.

Peak Position [ppm]	1.1wt% hm-chitosan (solution)	1.1wt% hm-chitosan with carbon microspheres (gel)
.758 (-CH3)	2.064 sec	3.566 sec
1.174 (-CH2-CH2-CH2-)	0.759 sec	1.502 sec
4.8 (H2O in D2O)	6.399 sec	10.52 sec

Table 1: ^{1}H NMR Relaxation Times.

As intuitively expected, the T_1 values of the alkyl groups increase upon the addition of carbon and the formation of the gel, due to the binding of these groups to the carbon surfaces through hydrophobic interactions. Recent studies have revealed that the chemical interactions of water molecules with surrounding groups of macromolecules in water-based gels lead to a larger T_1 indicating the presence of bound water through interaction with the polymer network[6-9]. This observation is consistent with the hm-chitosan and carbon system here, where the formation of the gel framework leads to confined volumes for the solvent (water).

The NMR spectra presented in the manuscript indicate subtle but observable changes in the hm-chitosan alkyl group resonances with increasing concentration of the carbon microspheres. A clear line broadening of the CH_2 and CH_3 proton resonances is observed at gel formation conditions. This broadening indicates motional restrictions of the alkyl side chains upon introduction of the particles, and at particle levels beyond the gelation threshold of 3 wt%, the two methyl group ^{1}H resonance bands indicate two distinct populations of methyl groups. It is possible that the two observably different bands represent two populations of methyl groups: those adsorbed onto the surfaces of the carbon microspheres and those interacting with other dodecyl groups attached to other hm-chitosan backbones. The distinction between these two populations is difficult to separate out.

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