HYDROGELS FORMED BY ENDLINKING PEG TO DENDRIMER CROSSLINK AGENTS

Brent D. Viers, Barry J. Bauer, Yvonne Akpalu, Franziska Gröhn, Dawei Liu, Ginam Kim

National Institute of Standards and Technology, Polymer Blends and Processing Group, Mail Location 8542 Gaithersburg, MD 20899-8542

Introduction

There has been an interest in using poly(ethylene glycol) (PEG) as a biocompatible matrix.[1,2] PEG is unique among the polyethers for being water soluble, yet will dissolve in other organic solvents such as benzene and methylene chloride. Furthermore, PEG surfaces are non-immunogenic and generally are not subject to protein adsorption or blood clot formation. A crosslinked PEG network could conceivably be loaded with a variety of hydrophilic and/or hydrophobic drugs for in vivo delivery. However most crosslinking mechanisms suggested for PEG (e.g. radiation crosslinking, or use of non-selective isocyanates) are poorly controlled.

We have thus attempted to form "model" PEG networks by endlinking telechelic vinyl sulfone endfunctionalized PEG with the terminal amines on commercially available dendrimers. These dendrimers are novel crosslinking agents in that they have multiple reactive amines (and thus would likely tether PEG chains into the network at both ends) as well as being of sufficient size (ca. 5 nm. to 10 nm.) such that they might be conveniently seen in a microscopy and/or scattering experiment. Thus these dendrimer based networks might be good models for the effects of crosslink functionality and chain length effects on the network properties.

Experimental

(Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental protocol. In no case does such identification imply recommendation by the National Institute of Standards and Technology, nor does it imply that such material of equipment identified is necessarily the best available. ISO has replaced the term molecular weight with the term relative molecular mass and the symbol M_r. We have used the older nomenclature to better conform with the previous literature.)

All α,ω endfunctionalized poly(ethylene glycol) was used as received from from Shearwater Polymers. Isocyante, Acrylate, and Epoxide terminated PEG had nominal M_n = 3,400 g mol^{-1} and $M_w/M_n < 1.1$ Vinyl Sulfone (VS) terminated PEG with M_n = 3516 g mol^{-1}, M_w/M_n = 1.023 and a high molecular mass VS terminated PEG with M_n = 22171 g mol^{-1}, M_w/M_n = 1.05 was custom synthesized by Shearwater Polymers and used as received. Poly(propylene imine) dendrimers (DAB-PA-x, where x is the number of terminal primary amine groups) were used as received from Aldrich. Poly(amido amine) (PAMAM) dendrimers were used as solutions in methanol. Generations 1-4 were used as received from Aldrich, while higher generation dendrimers (G5-G10) were donated by Dendritech.

Hydrolytically stable gels were formed by dissolving accurately weighed portions of the PEG-VS_2 and dendrimer into polar solutions of water, methanol, or DMSO at mass fraction 10 % solids content. The quiescent mixture cured over the course of several hours in glass vials. The extractable soluble fraction was calculated in the standard manner, and was noted to be approximately a mass fraction (2.9 ± 2.7) % which we took as evidence of well made gels. The ratios of dendrimer to PEG are based on mass ratios in the text.

Equilibrium swelling measurements were made by immersing the gels in an excess of a good solvent, generally water. The pH was adjusted by addition of concentrated HCl, and accurately measured to ± 0.1 pH with a pH meter. The swelling ratio was determined as the mass ratio between the swollen and dry state, and multiple measurements of the swollen mass vary by less than 10 %. The error listed was the standard deviation based on at least 3 mass measurements on a sample that had sensibly come to swelling equilibrium.

SAXS data were collected at the Advanced Polymer Beamline at Brookhaven National Laboratory, X27C. Instrumental details can be found on the X27C website, http://bh03.chem.sunysb.edu/X27C. The usable span of scattering vector magnitudes ($q = (4\pi/\lambda)\sin(\theta)$ and 2θ the scattering angle) was in the range 0.007 Å^{-1} < q < 0.3 Å^{-1}. Scattering patterns from silver behenate and Lupolen were used for angular and intensity calibration of the detectors. The experimental intensities were corrected for background scattering from the camera, empty cell and incident intensity. Errors are listed or plotted as the standard deviation of the averaged intensity measurements.

Results and Discussion

Dendrimers are imminently suitable as crosslinking agents for PEG, typified by the wide variety of endlinking chemistries that can form gels. For example, an acrylate terminated PEG can react via a Michael addition similar to what is used to "grow" successive generations of the dendrimer. Gelation via this Michael addition occurred relatively slowly (hours) to form monolithic, mechanically robust gels. These gels were reasonably elastic, as indicated by the ability to sorb non-protic solvents and swell to a reproducible, equilibrium value. Unfortunately, these gels were not hydrolytically stable, and would break apart in water or methanol. We attribute this behavior as resulting from hydrolysis of the ester linkage in the acrylate moiety. Conversely, we were able to form gels from the reaction of an isocyanate terminated poly(ethylene glycol), forming an urea linkage. This linkage, as expected, was hydrolytically stable. However, the gelation reaction was so fast as to be almost instantaneous and as a result gel tended to be more friable. Epoxide terminated PEG also gels, but with a soluble fraction that is larger than average. We chose to use a vinyl sulfone terminated PEG, which had the advantages of reacting relatively slowly yet fairly completely, as well as having hydrolytically stable linkages.

Prima facie, all of the terminal amines of the dendrimer are identical. If one assumes that the reactivity of these amines are identical, then it is possible to form networks of varying functionality for a given dendrimer by varying the stoichiometry of the polymer endgroups to the dendrimer amines. (We note that there is a definite change in reactivity of the dendrimer amines as the reaction progresses. This is a likely result of steric barriers to addition of the chains limiting complete conversion. It is still possible to react a majority of the arms and thus change the functionality. See also Bauer, B.J.; Viers, B.D. in this volume for details). The swelling from gels of various (nominal) functionality formed from PEG-VS_2 (M_n = 22,171 g mol^{-1}) and a DSM DAB-PA-64 dendrimer is shown in Figure 1. The swelling behavior can be rationalized by using the phantom theory for swelling in the rubric of Flory-Rehner theory. The operative equation is[3]

$$Q \sim 1/v_2 \sim \left(\frac{M_c(1/2 - \chi)}{(1 - 2/\varphi)V_1 \rho} \right)^{3/5}$$

where Q is the swelling ratio, v_2 is the volume fraction of polymer in the swollen system, φ is the crosslink functionality, M_c is the average molecular weight between crosslinks, V_1 is the molar volume of the swelling solvent, ρ is the polymer density, and χ is the polymer-solvent interaction parameter. We assume that M_c is a constant equal to the value of the molecular weight of the precursor PEG chains. Networks formed with an average functionality of less than 3 are not stable gels, and dissolve in solution-in accord with the classical Flory-Stockmayer prediction of formation of soluble branched species and not a macroscopic gel.[3] Networks with low functionality (ca. 4) swell the most, likely because the 2/φ term is not negligible. The networks with the highest functionality are firmly embedded in the network, and thus the swelling ratio does not vary much. We note that the higher functionality gels do not appreciably overswell when immersed in an excess of methanol (the swelling ratio remains 1 e.g. the swelling ratio at the state of preparation). We view this as evidence of making the desired "endlinked" gels that do not have a preponderance of dangling chains or intermolecular cyclics. These defects do not carry load and thus would not provide an elastic counteraction to overswelling. Similar behavior is seen for the swelling in water, viz: an excess swelling at low crosslink functionality which then has a swelling response that is invariant at higher functionality. The swelling ratio in all cases is higher in water than the equivalent gel in methanol, reflecting that water is a better solvent for the PEG. Most interestingly of all, the equilibrium swelling is significantly larger in an acidic solution of median pH, and the swelling profile has a significantly different shape (does not asymptote at higher functionality). The maximum swelling is seen to occur at a pH ≅ 4. This corresponds to the pK_a of the primary amines. These amines would protonate and hence the dendrimer crosslink centers are repulsive.

Conversely, when the pH is further reduced to 2, there is a net excess of positively charged protons. These unattached charges would screen the repulsive interactions, and thus the elasticity of the PEG chains would decrease the dendrimer distance, and decreases the observed swelling. This behavior is schematically depicted in Figure 2. Thus, these networks are pH sensitive and might fall in the general class of the critical volume phase transitions often seen in "smart gels." [4]

The SAXS of similar hydrogels reveals the fine detail of the dendrimer interactions, as shown in Figure 3. The sample is formed from a mass fraction 10 % PAMAM G8 dendrimer endlinked to mass fraction 90 % of a PEG VS_2 having M_n = 22,171 g mol^{-1}. The sample was further swollen (so that the approximate PAMAM G8 volume fraction was ≅ 1 %) in an aqueous solution of mass fraction 0.5% sodium phosphotungstate salt. This stain associates very strongly with dendrimers[5]and thus provides significant heavy metal contrast for a SAXS experiment. The curve shows a noticeable maximum at low q, and then a transition to a concave downward portion, followed by smaller peaks at higher q. These higher order peaks are indicative of the monodisperse, spherical character of the dendrimers.[6] This portion of the scattering profile agrees with previous results on dilute solutions of PAMAM G8 dendrimers-and thus the dendrimers don't appear to be affected by the crosslinking. In these conditions, there does not appear to be inter-dendrimer interactions which cause the dendrimers to shrink, rather than interpenetrate. We should note that in these conditions, we assume that the swollen sample is "dilute" in dendrimer. The peak at low q thus shows the spacing of dendrimer centers. If one assumes that the dendrimers pack on a cubic lattice, the spacing d between the dendrimer centers would be:

$$d = 1.23 * 2\pi / q = 1.23 * 2\pi / 0.017\,\mathring{A}^{-1} = (450 \pm 50)\mathring{A}$$

a value that is in good agreement with the spacing assuming volume fraction 1 % of dendrimer. It is likely that this correlation peak would be sensitive to the changes in swelling.

Conclusions

Hydrolytically stable gels can be formed from VS terminated PEG endlinked to dendrimer crosslinking molecules. The swelling of gels of variable functionality qualitatively agrees with the classical rubberlike elasticity theories. Furthermore, The equilibrium swelling of these gels can be changed significantly by changing the pH of an aqueous swelling solvent. This behavior is attributable to repulsive and/or screened interactions of the dendrimer crosslink sites. Small Angle X-ray scattering on these gels appears to be very sensitive to the dendrimer crosslink sites, exhibiting correlation peaks. SAXS can prove to be an unique way of measuring the changes that occur in a network.

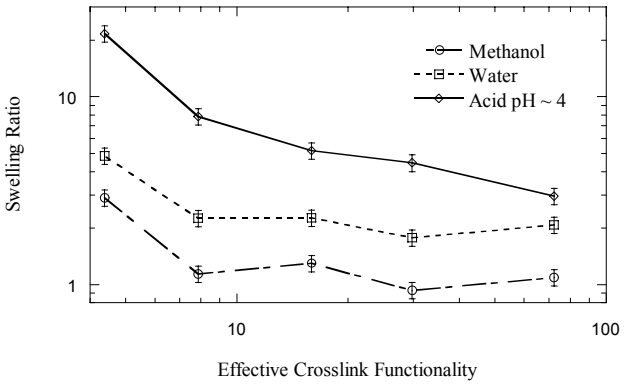

Figure1: Equilibrium Swelling of DSM DAB-PA-64 dendrimer based gels endlinked to PEG-VS_2 M_n = 22,171 g mol^{-1}

.

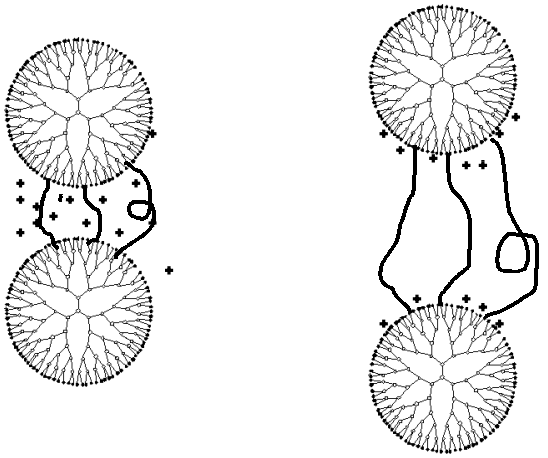

Figure 2. Schematic illustration of screened interactions at low pH (left) and repulsive interactions at pH ≅ pK_a of the amines.

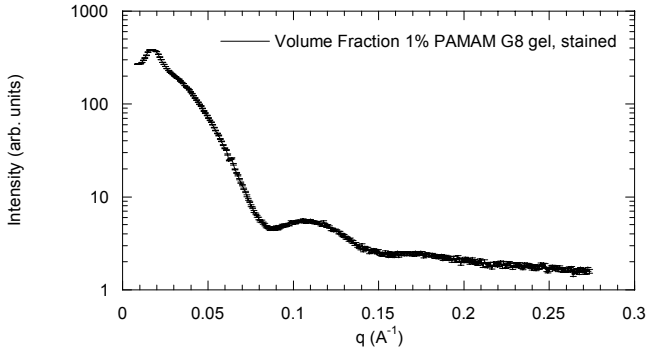

Figure 3. SAXS of a mass fraction 10 % PAMAM G8/90 % PEG VS_2 22,171 g mol^{-1} gel stained and swollen to v_2 ≅ 0.1

Acknowledgement. This work was funded in part by the U.S. Army Research Office under contract number 35109-CH. We thank Donald Tomalia of Dendritech for his generous donation of PAMAM dendrimers.

References

(1) *Poly(ethylene glycol) chemistry : biotechnical and biomedical applications*; Plenum Press: New York, 1992.
(2) Zalipsky, S.; Harris, J. M. *Poly(Ethylene Glycol)* **1997**, *680*, 1-13.
(3) Flory, P. J. *Principles of polymer chemistry*; Cornell University Press: Ithaca, 1953.
(4) Tanaka, T. *Acs Symposium Series* **1992**, *480*, 1-21.
(5) Jackson, C. L.; Chanzy, H. D.; Booy, F. P.; Drake, B. J.; Tomalia, D. A.; Bauer, B. J.; Amis, E. J. *Macromolecules* **1998**, *31*, 6259-6265.
(6) Prosa, T. J.; Bauer, B. J.; Amis, E. J.; Tomalia, D. A.; Scherrenberg, R. *Journal of Polymer Science Part B-Polymer Physics* **1997**, *35*, 2913-2924.