STRUCTURE-PROPERTY RELATIONSHIPS OF PHOTOPOLYMERIZABLE PEGDM HYDROGELS

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Introduction

Hydrogels produced by photo-polymerization have been investigated extensively as biomaterials in applications such as scaffolds for tissue engineering, drug delivery carriers, in the prevention of thrombosis, post-operative adhesion formation, and coatings for biosensors.[1] The photo-polymerization process allows the hydrogel to be generated *in vitro* or *in vivo* from a low viscosity solution of monomer, oligomer, or low molecular mass polymer (macromer) by a free radical pathway in a minimally invasive manner.

We have prepared a series of controlled molecular mass (MM) poly(ethylene glycol) dimethacrylates (PEGDMs) of high purity and low polydispersity by both conventional solution chemistry as well as by a microwave assisted route.[2] Combined proton nuclear magnetic resonance (^{1}H NMR) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) demonstrated that high degrees of conversion from PEG-hydroxyl endgroups to methacrylate endgroups were achieved. PEGDMs were photo-polymerized in water or growth medium (in the presence of cells) to form hydrogels. Preliminary studies showed varied mechanical response but the encapsulated cells are completely viable in hydrogels (PEG precursor molecular mass ranging from 2000 g/mol and 8000 g/mol and mass fractions of 10 % and 20 %) after two weeks.

The present study aims to probe the structure of PEGDM semidilute solutions and corresponding hydrogels using small-angle neutron scattering (SANS). Small-angle neutron scattering, along with other characterization techniques, have been used extensively to characterize the structure of gels.[3] The effects of oligomer molecular mass and concentration on gel structures are examined. The structures of the hydrogels are correlated to the mechanical properties, specifically the shear modulus (G), obtained using uniaxial compression testing. To our knowledge, no scattering measurements have been used to characterize photo-polymerizable end-functionalized crosslinked hydrogels.

Experimental

Materials. Polyethylene glycol (PEG: MM ≈ 2000 g/mol (2k), 4000 g/mol (4k), and 8000 g/mol (8k)), methacrylic anhydride, ethyl ether, triethylamine, and D_2O were purchased from Sigma-Aldrich* and used as received. Dichloromethane was also purchased from Sigma-Aldrich and dried over activated molecular sieves (4 Å) prior to use. Photoinitiator Irgacure 2959 (I2959) was obtained from Ciba Specialty Chemicals and used as received.

Synthesis of PEGDMs and their hydrogels. PEGDMs (2k, 4k, and 8k) were prepared by reacting PEGs with methacrylic anhydride.[2] An example of the synthesis of a 4k-PEGDM is as follows. PEG (5 g), a 2.2 equivalence of methacrylic anhydride, and triethylamine (0.2 mL) was reacted in ≈ 15 mL dichloromethane over freshly activated molecular sieves for 4 d at room temperature. The solution was filtered and precipitated into ethyl ether. The product was collected by filtration, and then dried in a vacuum oven prior to use. The molecular mass and molecular mass distribution were determined using a combination of ^{1}H NMR and MALDI-TOF MS. The relative uncertainty associated with ^{1}H NMR and MALDI-TOF MS are 8 % and 5 %, respectively.

Photo-polymerized hydrogels were prepared according to a previously described procedure.[4] PEGDM (10 %, 20 %, or 30 % by mass fraction) and aqueous I2959 solution (0.05 % by mass fraction) were mixed in distilled deionized water for mechanical testing or in D_2O for the SANS measurements. Photo-polymerization was achieved using a long wavelength UV source (365 nm, 300 μW/cm^2) for 10 min to obtain hydrogels.

Small-angle neutron scattering measurements. PEGDMs and the I2959 initiator were dissolved in D_2O. Samples were measured in solutions cells with 2 quartz windows and a path length of 1 mm. PEGDM solutions (10 %, 20 %, and 30 % by mass fraction) were measured as prepared and subsequently exposed to UV irradiation to form hydrogels in side of the sample cells.

SANS measurements were performed on the NG-7 30 m SANS instrument at the NIST Center for Neutron Research (NCNR) using a 10-position sample block. Sample-to-detector distances were 15 m, 2 m, and in some measurements 1 m, and an incident wavelength (λ) of 8.44 Å for the 15 m and 2 m configuration and 5 Å for the 1 m configuration were used to give q ranges ($q = 4\pi/\lambda \sin(\theta/2)$) of 0.0016 Å^{-1} < q < 0.0.0164 Å^{-1}, 0.0119 Å^{-1} < q < 0.1992 Å^{-1}, and 0.0382 Å^{-1} < q < 0.5968 Å^{-1}, respectively. Data were reduced by established methods with the software provided by the Center for Neutron Research at NIST and the incoherent background was subtracted. The relatively standard uncertainly for SANS measurement is 5 %.

Uniaxial compression testing on hydrogels. The shear modulus was also determined using uniaxial compression measurements performed using a TA.XT2I HR Texture Analyser (Stable Micro Systems, UK). This apparatus measures the deformation (± 0.001 mm) as a function of an applied force (± 0.01 N). Cylindrical hydrogels (height 3 mm, diameter 6 mm) were deformed (at constant volume) between two parallel glass plates. The shear modulus, G, was calculated from the nominal stress, σ (force per unit undeformed cross-section), using the equation[5] $\sigma = G(\Lambda - \Lambda^{-2})$ where Λ is the macroscopic deformation ratio ($\Lambda = L/L_o$, L and L_o is the length of the deformed and undeformed specimen, respectively). Measurements were carried out in triplets at deformation ratios $0.7 < \Lambda < 1$. No volume change or barrel distortion was detected.

Results and Discussion

SANS measurements were performed on semidilute PEGDM solutions and corresponding hydrogels of different molecular masses and varied oligomer mass fractions. **Figure 1** shows the 2-dimensional SANS patterns for the 4k-PEGDM solutions and gels obtained at the 2 m detector distance corresponding to intermediate q scattering. A marked difference is evident as the solutions photo-crosslink to form hydrogels. At high PEGDM mass fractions, a ring develops in the scattering pattern indicating the presence of a well-defined structural length scale (correlation length ξ).

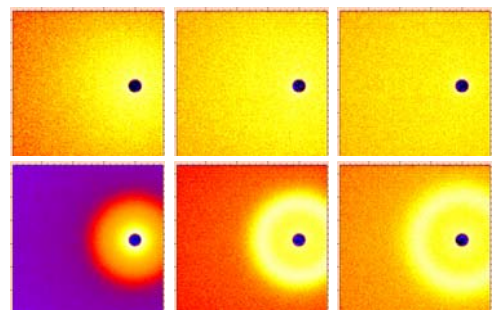

Figure 1. Two-dimensional scattering patterns of 4k-PEGDM solutions (top row) and corresponding hydrogels (bottom row) at various PEGDM mass fractions (10 %, 20 %, and 30 % from left to right)

Figure 2 shows the scattering intensity vs. q for 10 % PEGDM hydrogels prepared from 2k, 4k, and 8k oligomers. Upon photo-crosslinking, the scattering intensity deviates from those of the original semidilute solutions (not shown) at low and intermediate q, while the scattering intensity at very high q ($q >> \xi_{sol}$) remains relatively unchanged. At high q the scattering response is governed by the geometry of the polymer chains. A maximum in the scattering intensity at q* (calculated by peak fitting using a Kratky analysis) is observed for the 8k-PEGDM. Two predominate correlation lengths, one in the intermediate q (which will be referred to as ξ_{gel}) range and a much larger correlation length in the low q, are observed for hydrogels formed with lower molecular mass PEGDMs. It is interesting to note that the scattering intensities in the intermediate q range for the lower molecular mass PEGDMs do not appear as an intensity maximum but rather as a shoulder and become less defined as the molecular mass is decreased. A clear trend of increasing q*, corresponding to a decrease in ξ_{gel}, is observed as the molecular mass decreased.

A distinct maximum in scattering intensity similar to those observed for the 8k hydrogel has been observed for systems having a soft order, such as those prepared from multifunctional oligomers.[6] It has also been observed that the maximum in the scattering spectrum becomes more pronounced as the oligomers functionality increases. The present results suggest that crosslinking of PEGDM favors the formation of clusters. Although the PEGDM only has a functionality of 4 (2 on each oligomer chain end), the way in which the crosslinking process occurs allows the crosslinking sites to be more aggregated relative to the flexible PEG moieties, thereby creating clusters of methacrylate rich regions reminiscent of the gel structures formed by star-shaped oligomers. Although not shown, the scattering intensity for the 8k-PEGDM hydrogel also approaches that of the solution scattering at very low q suggesting that the gelation does not significantly alter concentration fluctuations from the uncrosslinked solution for this sample.

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* *Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation by the National Institute of Standards and Technology nor does it imply that the material or equipment identified is necessarily the best available for this purpose*

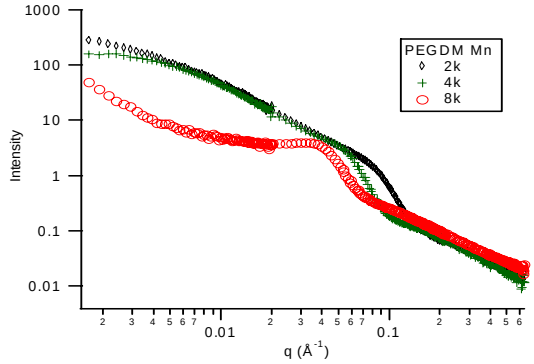

Figure 2. Scattering intensity as a function of the scattering vector q for 10 % hydrogels prepared from different molecular mass PEGDMs

The affect of PEGDM mass fraction on the gel structure for the 4k-PEGDM is illustrated in **Figure 3**. As one would expect, the PEGDM mass fraction is related to the degree of swelling, often examined in literature for gel scattering studies. However, the oligomers mass fraction and the degree of swelling are not directly proportional in the current study since the degree of conversion is not the same for hydrogels prepared from different mass fraction oligomers. Similar to the gel studies prepared by star-shaped oligomers where a minimum functionality is required before the scattering transitions from a shoulder to a maximum, the lower mass fraction hydrogels exhibit a shoulder but the higher mass fraction hydrogels form a maximum.

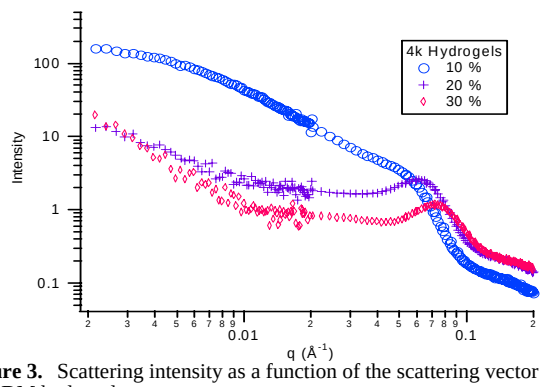

Figure 3. Scattering intensity as a function of the scattering vector q for 4k-PEGDM hydrogels

At higher oligomer concentrations, the scattering intensity exhibits a clear maximum indicating that the gel forms soft ordered methacrylate rich domains. For the lower oligomer mass fractions, the scattering intensity appears to have two (or possibly more) correlation lengths and these results are consistent with a network structure that exhibits inhomogeneities in the length scale range probed by SANS. The presence of a larger correlation length can also be considered as the result of the formation of more open structures, containing smaller, denser methacrylate rich regions. The methacrylate rich domains are more diffuse and hence appear as a broad shoulder in the SANS results. It is interesting to note that the gel structure clearly depends on both the molecular mass and the oligomer mass fraction. For the 2k-PEGDM, the 10 % and 20 % hydrogels show 2 pronounced correlation lengths; only the 10 % 4k-PEGDM hydrogel show 2 correlation lengths, and all 8k-PEGDM hydrogels had 1 correlation length.

Table 1 lists the radius of gyration (R_g), theoretical end-to-end distance (r), and the correlation length ξ_{gel} for various molecular mass PEGDMs at varied mass fractions. The R_g was calculated from dilute solution scattering (not shown), and the ξ_{gel} was calculated using the q*. As expected, the R_g of PEGDM oligomers increased as the molecular mass increased. The ξ_{gel} followed the same trend, but was significantly higher than the R_g. In addition, ξ_{gel} increased as the oligomers mass fraction decreased suggesting that the network becomes more "open" as the mass fraction decreased. We would argue that the openness of the network would also be related to the larger correlation length such that the diffusive properties should be significantly higher for networks containing two correlation lengths, but this will be the subject of a future study.

Table 1. The radius of gyration (R_g), end-to-end distance, and correlation length ξ_{gel} for PEGDM solutions and gels

PEGDM	R_g(Å)	*r*(Å)	$\xi_{10\%}$(Å)	$\xi_{20\%}$ (Å)	$\xi_{30\%}$ (Å)
1k	20.4	84	52.4	41.9	37.0
2k	22.3	167	83.7	71.4	59.3
4k	25.4	335	120.8	93.8	76.6
8k	32.2	670	157.1	--	--

- The R_g was determined using Guinier analysis on the 1 % by mass fraction solutions.
- The end-to-end distance (r) was calculated based on the molecular mass of the PEGDM and the length of ethylene oxide repeat unit (3.69 Å).
- The correlation length (ξ) was calculated from q*.

The shear moduli of hydrogels were measured using a uniaxial compression test and calculated using equations derived from the strain-energy function. **Figure 4** the shear modulus of PEGDM hydrogels prepared from different molecular mass oligomers (2k, 4k and 8k) and as a function of PEGDM mass fraction (varying from 10 % to 30 %). As expected, the shear modulus monotonically increased as the oligomer mass fraction increased for PEGDMs of all molecular masses. The effect of molecular mass on shear modulus appears to depend on the oligomer content. For hydrogels high in the PEGDM mass fraction (30 %), the shear modulus decreased as the molecular mass increased, presumably due to a decrease in the crosslink density. However, the variation between molecular mass and shear modulus is less pronounced for hydrogels with lower PEGDM contents (i.e. 10 % and 20 %). Whereas hydrogels prepared from the 8k PEGDM do have a lower shear modulus than those prepared from the 2k and 4k PEGDM, there are no significant differences in the shear modulus of hydrogels prepared from the 2k and 4k PEGDM at lower PEGDM mass fraction.

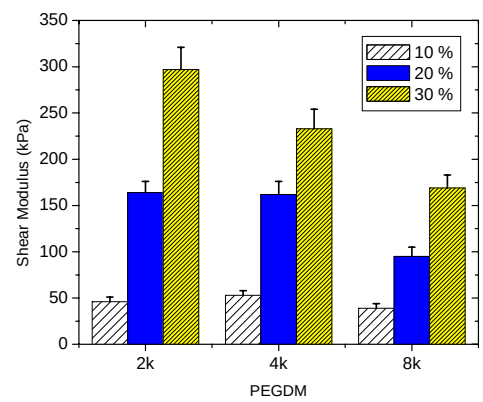

Figure 4. Shear modulus for hydrogels prepared with different PEGDM molecular masses and mass fractions.

Conclusions

The PEGDM hydrogel structure and mechanical properties were determined using SANS and uniaxial compression tests, respectively. Both the gel structure and shear modulus depend on the PEGDM molecular mass as well as the oligomer mass fraction. For PEGDM of all molecular masses, the shear modulus increased as ξ_{gel} decreased. These observations are consistent with the theory of rubber elasticity. The effect of molecular mass is less apparent for lower mass fraction hydrogels. For the 30 % hydrogels, the expected trend of increased shear modulus with decreased molecular mass is observed.

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