

Synthesis and Characterization of Poly(ethylene glycol) Dimethacrylate Hydrogels

Sheng Lin-Gibson,*¹ Sidi Bencherif,¹ Joseph M. Antonucci,¹
Ronald L. Jones,¹ Ferenc Horkay²

¹Polymers Division, National Institute of Science and Technology
Gaithersburg, MD 20899-8543, USA

E-mail: slgibson@nist.gov

²Section on Tissue Biophysics and Biomimetics, Laboratory of Integrative and
Medical Biophysics, NICHD, National Institutes of Health, Bethesda, MD
20892, USA

Summary: Facile synthesis and detailed characterization of photopolymerizable and biocompatible poly(ethylene glycol) dimethacrylates (PEGDM) and their hydrogels are described. Combined analyses of ¹H NMR and MALDI-TOF MS confirmed the formation of prepolymers of high purity and narrow mass distribution (PD < 1.02). A systematic investigation into the structure and mechanical properties of PEGDM hydrogels was performed to characterize the relationships between the network structure and gel properties. Small-angle neutron scattering was used to characterize the structural features of hydrogels with respect to their semidilute solution precursors. A well-defined structural length scale (correlation length) manifested as a maximum in the scattering intensity was observed for hydrogels derived from high molecular mass PEGDMs and/or high oligomer mass fractions. Hydrogels derived from lower molecular mass PEGDMs and/or low oligomer mass fractions exhibited multiple correlation lengths suggesting the formation of inhomogeneous gel structures. The shear moduli, determined from uniaxial compression measurement, showed that the gel structures correlate well with the gel mechanical properties.

Keywords: gel structure; hydrogel; MALDI-TOF MS; mechanical properties; neutron scattering; poly(ethylene glycol); SANS

Introduction

Hydrogels produced by photopolymerization 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 as coatings for biosensors.^[1] The photopolymerization process allows the hydrogel to be generated *in vitro* or *in vivo* from a low viscosity solution of monomer, oligomer, or low molecular

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mass polymer (macromer) by a free radical pathway in a minimally invasive manner. The chemical crosslinking results in hydrogels that contain large water contents yet possess mechanical properties similar to those of soft tissues. Another advantage of hydrogels is their high permeability for oxygen, nutrients, and other water-soluble metabolites, making them particularly attractive as scaffolds in tissue engineering applications.

Several groups have used photopolymerizable poly(ethylene glycol) (PEG) based hydrogels for preparing tissue engineering scaffolds^[2-5] with the macromer molecular mass^[2] and concentration^[6] as parameters for adjusting the mechanical properties. We are interested in PEG dimethacrylates (PEGDM) and similar PEGDM derivatives as model systems because PEG alone is bio-inert,^[7] but can be modified easily to become functional. Crosslinking by dimethacrylates have been shown to be biocompatible with unreacted dimethacrylates having relatively low cytotoxicity.^[8] In addition, PEGDMs and their copolymers and derivatives have been successfully used by several groups both *in vitro* and *in vivo* as scaffold materials.^[9]

There is a general consensus that both the material properties and external stimulation strongly affect the cell response. The importance of PEGDM hydrogel crosslink density (controlled by PEGDM mass fraction in solution) on mechanical properties and on the chondrocytes' ability to produce cartilaginous tissues has been demonstrated.^[4] Despite the large number of studies currently available, there is still a lack of a clear understanding of the correlation between material properties and cell response. Furthermore, after years of research, the physical properties of hydrogels are still difficult to predict by theories due to non-idealities of the gel formation. These non-idealities include conversion dependent reactivity, cyclization and multiple crosslinking, and defects and nonhomogeneous crosslinking (also known as spatial gel inhomogeneity). Well-defined model materials are necessary for the preparation of hydrogels with high reproducibility and easily adjustable properties.

In the current study, a series of controlled molecular mass (MM) PEGDMs of high purity and low polydispersity were prepared by straightforward synthetic approaches. The dimethacrylate products were characterized by proton nuclear magnetic resonance (¹H NMR) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). PEGDMs of different molecular masses were photo-crosslinked to form hydrogels. The structure of PEGDM semidilute solutions and corresponding hydrogels was investigated using small-angle neutron scattering (SANS). The effects of

molecular mass and oligomer mass fraction on the gel structures were determined. The structure of the hydrogels was correlated to the mechanical properties, specifically the shear modulus that was obtained using uniaxial compression testing.

Experimental

Materials

PEG (MM \approx 1000 g/mol (1k) to 8000 g/mol (8k)), methacrylic anhydride (MA), ethyl ether, and triethylamine (TEA) were purchased from Sigma-Aldrich* and used as received. Dichloromethane was 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 and characterization of PEGDM

PEGDM was prepared from the reaction of various PEGs and MA. An example of the synthesis of a 4k PEGDM is as follows. PEG (4.0 g, \approx 0.001 mol), a 2.2 equivalence of MA (0.34 g, 0.0022 mol) and TEA (0.20 mL) was reacted in \approx 12 mL dichloromethane over freshly activated molecular sieves (\approx 3 g) for 4 d at room temperature. The solution was filtered over alumina and precipitated into ethyl ether. The product was filtered, and then dried in a vacuum oven overnight at room temperature. We also explored the use of microwaves to prepare PEGDMs. The reaction required 5 min to reach completion under microwave irradiation as opposed to 4 d for solution reactions. In addition, microwave-assisted reactions did not require a solvent or catalyst, and the product could be precipitated simply by adding diethyl ether.

High-resolution, 270 MHz proton NMR spectra were taken on a 6.35 T JEOL GX270 spectrometer manufactured by JEOL, Ltd. (Akishima, Japan). Deuterated chloroform was used as a solvent and the polymer concentrations varied between 2.5 % and 3.0 % by mass fraction. All spectra were run at room temperature, 15 Hz sample spinning, 45° tip angle for the observation pulse, and a 10 s recycle delay, for 64 scans. The standard relative uncertainty for molecular mass calculated via ^1H NMR arises from the choice of baseline and is estimated to be 8 %.

* 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.

The MALDI matrix, dihydrobenzoic acid (DHB), and the PEGDM were dissolved in 1 mL THF. Sodium was used as the cationizing reagent in a 1:1 by volume ratio of THF solution (0.5 mg/mL solution in THF) and PEGDM/DHB solution. All MALDI samples were deposited on the target by electrospray. The MALDI-TOF MS was performed on a Bruker (Billerica, MA) REFLEX II in reflectron mode using delayed extraction and low-mass (i.e. matrix-ion) blanking as previously described.^[10] Estimated expanded uncertainty reported for MM moments arises from choice of baseline and laser power (5 %). The estimated standard uncertainty in overall signal intensity from repeatability studies is 15 %.

Preparation and characterization of hydrogels

Photopolymerized hydrogels were prepared according to a previously described procedure.^[4] PEGDM and aqueous I2959 solution (0.05 % by mass fraction) were mixed in distilled deionized water. Samples were cured with a long wavelength UV source (365 nm, 300 $\mu\text{W}/\text{cm}^2$) for 10 min to obtain hydrogels.

PEGDMs and the I2959 initiator were dissolved in D_2O . One-mm path length solution cells were used. SANS measurements of PEGDM samples were taken prior and after UV irradiation exposure (as specified previously to form hydrogels). SANS measurements were performed on the NG-7 30 m SANS instrument at the NIST Center for Neutron Research (NCNR). Sample-to-detector distances were 15 m and 2 m, and incident wavelengths (λ) of 8.44 Å were used to give q ranges ($q = 4\pi/\lambda \sin(\theta/2)$) of $0.0016 \text{ Å}^{-1} < q < 0.0164 \text{ Å}^{-1}$ and $0.0119 \text{ Å}^{-1} < q < 0.1992 \text{ Å}^{-1}$, respectively. Data were analyzed by established methods with the software provided by the NCNR.^[11]

The shear modulus was determined using uniaxial compression measurements performed using a TA.XT2I HR Texture Analyzer (Stable Micro Systems, UK). This apparatus measures the deformation ($\pm 0.001 \text{ mm}$) as a function of an applied force ($\pm 0.01 \text{ N}$). 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 $\sigma = G(\Lambda - \Lambda^{-2})$, where Λ is the macroscopic deformation ratio ($\Lambda = L/L_0$, L and L_0 is the length of the deformed and undeformed specimen, respectively). Measurements were carried out in triplicates at deformation ratios $0.7 < \Lambda < 1$. No volume changes or barrel distortions were detected.

Results and Discussions

Synthesis and characterization of PEGDM

PEGDMs of high purity and low polydispersity are prepared as model materials for the formation of photo-crosslinkable hydrogels. Proton NMR and MALDI-TOF MS together provide comprehensive information regarding the degree of methacrylate conversion and product purity. The ^1H NMR spectrum (not shown) for PEGDM shows the expected peaks, and the lack of additional peaks suggests that unreacted methacrylate anhydride, methacrylic acid by-product, and triethylamine all have been quantitatively removed.

MALDI-TOF MS is a powerful technique from which the molecular mass, molecular mass distribution, and endgroup functionalities can be determined. The MALDI-TOF MS spectra of PEGDMs prepared from different molecular mass PEGs are shown in Figure 1. Intrinsic to MALDI analysis, the relative signal intensities decrease and the breadth of the peak appears to increase as the molecular mass is increased. Each molecular mass can be clearly distinguished with all oligomers displaying the expected molecular mass distribution.

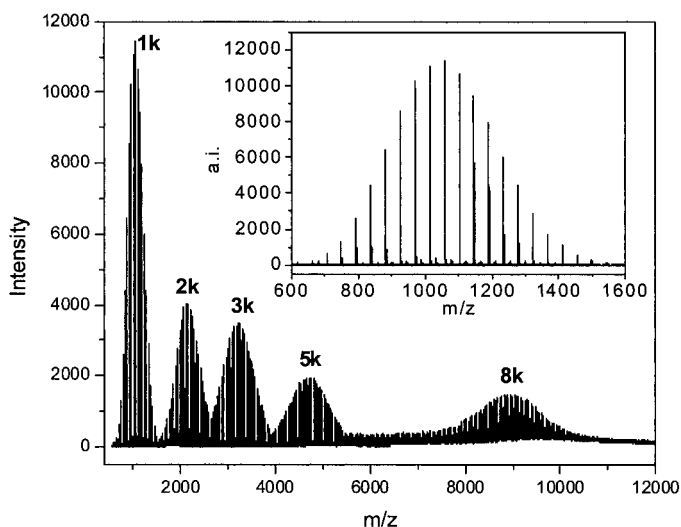


Figure 1. MALDI-TOF MS of a series of PEGDMs, insert shows the expanded spectrum for the 1k PEGDM.

Since MALDI detects all species within a discrete molecular mass range, it can be used to determine the amount of PEGDM versus the amount other impurities, such as PEGs with only one hydroxyl reacted (PEG mono-methacrylate) and unreacted PEG in a mixture. Insert of Figure 1 shows the expanded spectrum for the 1k PEGDM, which displays a main series of peaks due to Na^+ cationized PEGDM and two minor series of peaks due to H^+ and K^+ cationized PEGDM. No impurities are observed.

The molecular mass results of all PEGDMs are listed in Table 1. For all PEGDMs, the number average molecular masses (M_n) obtained by ^1H NMR match closely to those calculated by MALDI. Complementary techniques demonstrate the high reaction conversion and low impurity in these dimethacrylate.

Table 1. Molecular mass results (g/mol) of PEGDM obtained using ^1H NMR and MALDI-TOF MS.

| PEG | M_n (NMR) | M_n (MALDI) | M_w (MALDI) | PDI |
|-----|-------------|---------------|---------------|------|
| 1 k | 1050 | 1064 | 1085 | 1.02 |
| 2 k | 2220 | 2150 | 2178 | 1.01 |
| 4 k | 4490 | 4165 | 4199 | 1.01 |
| 8 k | 8330 | 8680 | 8776 | 1.01 |

Preparation and characterization of hydrogels

PEGDMs of high purity, high conversion (> 99 % conversion), and narrow polydispersity are appropriate model compounds for studying the structure-property relationships of hydrogels. SANS measurements were performed on semidilute PEGDM solutions and corresponding hydrogels made from different oligomer molecular masses and solution concentrations. The general approach for probing the mesoscale structure is to compare the excess scattering intensity from gel with respect to its semidilute solution precursor. It should be noted that all hydrogels under the current study have reached maximum methacrylate conversion, i.e., no additional crosslinking occurs upon further UV irradiation. The time required to achieve complete reaction has also been independently confirmed using in-situ rheology and mechanical testing.

The scattering intensity $I(q)$ vs. q for the 2k PEGDM solutions and gels are illustrated in Figure 2. For the solution scattering, a correlation length (ξ_{sol} , the solution blob size) is observed in the high q range and it decreases with increased solution concentration. A

scattering intensity upturn with a power law dependence is observed in the low q range for all semidilute solutions. Similar excess scattering intensities at low q , described as the Picot and Benoit effect,^[12] have been attributed to transient large-scale concentration fluctuations and are considered to be a “stray” effect.

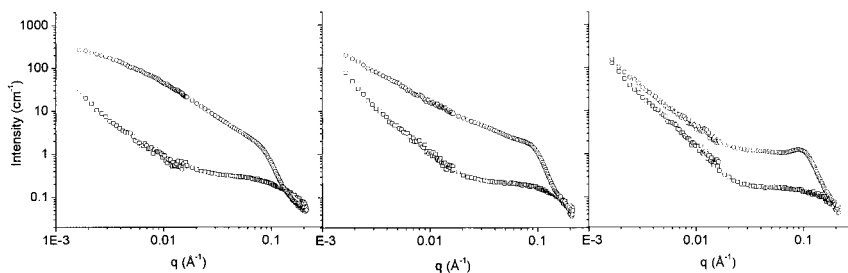


Figure 2. Scattering intensity as a function of the scattering vector for 2k-PEGDM solutions (\square) and hydrogels (\circ): 10 % (left), 20 % (middle), and 30 % (right).

Upon photo-crosslinking, the scattering intensities deviate significantly from those of the original semidilute solutions at low and intermediate q ranges, while the scattering intensity at the high q range ($q \gg \xi_{\text{sol}}$) remains practically unchanged. The scattering intensity overlap at high q is expected since it reflects the local properties of excluded volume chains or the polymer chain geometry. In the low and intermediate q ranges, there is a strong dependence on the scattering intensity with respect to q that cannot be described by an Ornstein-Zernicke function. A maximum in the scattering intensity at q^* is observed in the intermediate q range for the 30 % PEGDM hydrogel corresponding to a predominant characteristic length (ξ_{gel}). The presence of a peak in the scattering profile indicates the formation of well-defined clusters or domains. Multiple correlation lengths, one in the intermediate q range and at least one much larger correlation length in the low q , are observed for hydrogels formed with lower amounts of PEGDM. In addition, the scattering intensities in the intermediate q range for hydrogels containing lower PEGDM mass fractions do not exhibit a maximum but rather a shoulder that becomes less defined as the mass fraction is reduced. The presence of different structures with different correlation lengths indicates the structure richness of these hydrogels. A Kratky analysis was used to identify the q corresponding to the correlation length ξ_{gel} . As the PEGDM concentration is increased, q^* is increased and the correlation length is decreased.

The effect of molecular mass on ξ_{gel} is illustrated in Figure 3, which shows the scattering intensity versus q for 10 % hydrogels prepared from 1k, 2k, 4k, and 8k PEGDMs. A clear trend of increasing q^* , corresponding to a decrease in ξ_{gel} , is observed as the molecular mass is decreased. In addition, the correlation length becomes more distinct as the molecular mass is increased. The scattering for the 1k-PEGDM exhibits a broad shoulder, As the 1k-PEGDM is not fully soluble in water, the scattering intensity continues to increase even at the lowest q probed, suggesting the presence of structures on multiple length scales. Hydrogels prepared with 2k- and 4k-PEGDM also exhibit long-range fluctuations due to defects in the gel structure, but the scattering intensity appears to have reached a plateau at the lowest q probed.

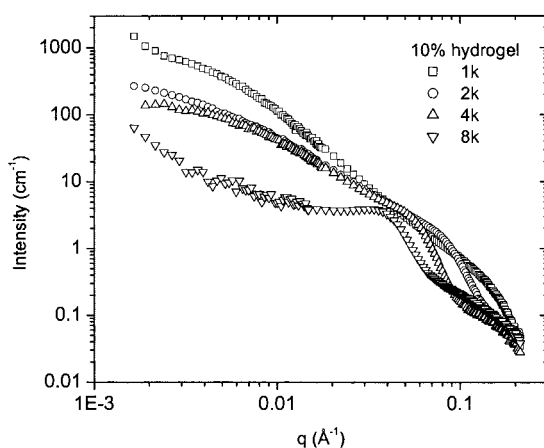


Figure 3. Scattering intensity as a function of q for 10 % hydrogels prepared from different molecular mass PEGDMs.

A distinct maximum in scattering intensity has been observed for hydrogels having a soft order, such as those prepared from multifunctional oligomers.^[13] It was also noted that the maximum in the scattering spectrum becomes more pronounced as the oligomer functionality is increased. Although the PEGDM only has a functionality of 4 (2 on each oligomer chain end), the way in which the crosslinking process occurred allowed the crosslinking sites to be more clustered relative to the flexible PEG moieties. Intermolecular reaction leads to branched microgels similar to those formed in bulk photopolymerization of dimethacrylates,^[14] thereby creating clusters of methacrylate rich

regions reminiscent of the gel structures formed by star-shaped oligomers. As the concentration decreases, the amount of oligomer present becomes insufficient to form gels with uniformly dispersed clusters, thereby creating large “defects” in the network. These inhomogeneities in the network structure are manifested as larger correlation lengths observed in the low q scattering. The larger correlation lengths 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 diffused and hence appear as a broad shoulder in the SANS results. These results imply 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 inhomogeneous networks.

The variation of correlation length with polymer volume fraction ϕ for 2k and 4k-PEGDM are shown in Figure 4. As expected, ξ_{gel} of PEGDM hydrogels increases as the molecular mass is increased. In addition, ξ_{gel} increases as the oligomers concentration is decreased. An exponent (α) between -0.31 and -0.41 is observed following the relationship $\xi \sim \phi^\alpha$. Interestingly, previous studies on crosslinked of star shaped oligomers also found an exponent near -0.4.^[13]

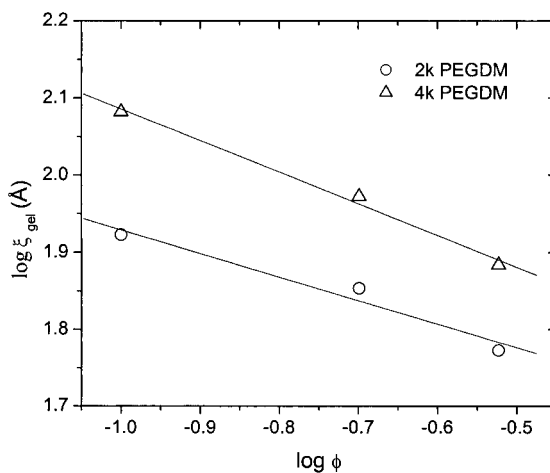


Figure 4. Correlation length as a function of polymer volume fraction. The error bar is smaller than the size of the symbol. Line drawn is the linear fit of the data.

The shear moduli of hydrogels were measured using a uniaxial compression test and calculated using equations derived from the strain-energy function. Figure 5 shows the nominal stress σ as a function of the deformation ratio Λ for three gels. Note that σ is negative due to the compressive forces used. Insert of Figure 5 plots the normal stress versus Λ according to the Mooney-Rivlin representation, $\sigma = 2(\Lambda - \Lambda^{-2})(C_1 + C_2/\Lambda)$, where C_1 and C_2 are constants. It is apparent that the value of C_2 is approximately zero. This result is consistent with many previous findings reported for swollen polymer networks. $2C_1$ can be identified with the shear modulus of the gels.

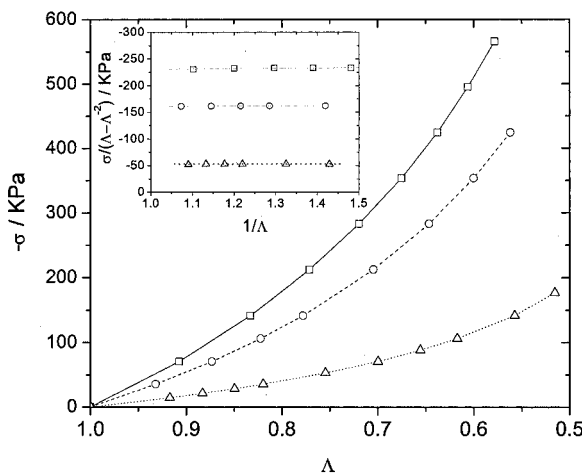


Figure 5. Nominal stress σ measured as a function of the deformation ratio Λ for 4k-PEGDM hydrogels, 10 % (Δ), 20 % (O), and 30 % (\square), insert shows the Mooney-Rivlin representation plotting the reduced stress vs $1/\Lambda$.

Figure 6 shows the shear modulus of PEGDM hydrogels prepared from different molecular mass oligomers (2k, 4k, and 8k) as a function of PEGDM mass fraction (varying from 10 % to 30 %). As expected, G increases as the oligomers mass fraction increases for PEGDMs of all molecular masses. The effect of molecular mass on G appears to depend on the oligomer mass fraction. For hydrogels made with a high PEGDM mass fraction (30 %), G decreased as the molecular mass increased. However, the variation between molecular mass and G is less pronounced for hydrogels made with lower PEGDM contents (i.e., 10 % and 20 %). Whereas hydrogels prepared from the 8k PEGDM have a lower G than those prepared from the 2k and 4k PEGDM, there are no

significant differences in the G of hydrogels prepared from the 2k and 4k PEGDM at lower PEGDM mass fractions.

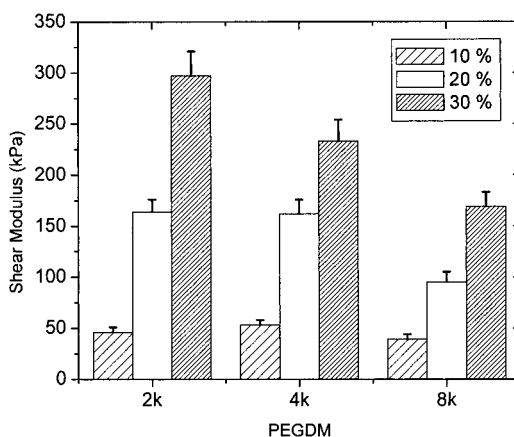


Figure 6. Shear modulus (G) for PEGDM hydrogels prepared with different molecular mass and at different PEGDM concentrations.

Conclusions

Facile synthesis and detailed characterization of PEGDMs and their hydrogels are presented. The combination of ^1H NMR and MALDI-TOF MS proves to be extremely effective in providing detailed characterization of reaction conversion, molecular mass, and molecular mass distribution.

PEGDMs (between 1k and 8k) are photopolymerized to form hydrogels. SANS is used to characterize the structure of hydrogels with respect to their semidilute solution precursors. A well-defined characteristic length (ξ_{gel}), appeared as a maximum in the scattering intensity, is observed for hydrogels formed from higher molecular mass PEGDM and/or high oligomer mass fractions. Hydrogels derived from lower molecular mass PEGDMs and/or lower oligomer mass fractions tend to form polydisperse structures and the ξ_{gel} appears as a shoulder in the scattering.

The network structure clearly affects the gel mechanical properties. The shear modulus (G) shows these hydrogels are mechanically robust, consistent with gels structures that contains reinforcing clusters. The current study demonstrates the subtle balance between

macromer molecular mass and mass fraction in designing hydrogels with desired structures and mechanical properties.

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