Controlled Formation of Microheterogeneous Polymer Networks: Influence of Monomer Reactivity on Gel Structure

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ABSTRACT: The mechanistic pathway taken in constructing a three-dimensional polymer network was greatly influenced by the reactivity and the functionality of the monomers and cross-linkers used. An increase in the cross-linker reactivity and functionality were found to promote properties in the polymer network that are consistent with a microgel type polymer structure. The microstructure of polyacrylamide (PAAm) gels synthesized from acrylamide (AAm) with a variety of cross-linkers was investigated using temperature profiles and polymerization rates, electrophoresis, scanning electron microscopy, and clarity tests. It was discovered that the heterogeneity of these polymers is largely due to the increased reactivity of the cross-linkers and ultimately responsible for improved properties of PAAm gels such as optical clarity and enhancement of electrophoretic separation.

Introduction

The classical gelation theory published by Flory[1] and Stockmeyer[2] was based initially on step growth polymerization and of an idealistic homogeneous polymer network. Since then, it has been widely accepted that a kinetically controlled process dictated by the functionality, reactivity, and concentration of the monomeric components generally govern the formation of a three-dimensional polymer network.[3] Most common cross-linked polymers are formed by a chain polymerization and composed of a monomer that has at least one reactive double bond (bifunctional) and a cross-linker with at least two reactive double bonds (tetrafunctional).[4] Traditionally, cross-linking monomers with similar reactivity to the chain building monomer were selected to statistically form a polymeric network with a relatively even cross-linking density and high degree of homogeneity. The formation of an irregular structured polymer network is widely accepted as one explanation for discrepancies found between the predicted and observed behavior of polymers.[5−7] Such structural heterogeneity has often been regarded as undesirable since it was found to effect the physical properties of the polymers such as permeability, elasticity, and optical clarity.[8−10] It has since been proposed that structural heterogeneity predominantly stem from intramolecular cyclization or from differences in the reactivity of the monomers and pendant double bonds.[11−13]

In this paper PAAm gels were used to encompass previous explanations and theories detailing the microstructure of a three-dimensional polymeric network. It was found that certain heterogeneity might be responsible for improved properties of polymer networks including enhanced optical clarity and improved electrophoretic separation of charged macromolecules. The importance and influence of monomer reactivity and structure to the deliberate development of a heterogeneous matrix and the consequential properties of the polymers are reported.

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Experimental Section

Materials. Sodium hydroxide (NaOH) and calcium chloride ($CaCl_2$) were purchased from BDH Laboratories (Boronia, VIC, Australia). Trioxane, phosphorus pentoxide (P_2O_5), acrylonitrile which was dried with $CaCl_2$ and distilled prior to use, methacrylonitrile, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate (APS) (electrophoresis grade, >99.5%), and sodium dodecyl sulfate (SDS) were purchased from Aldrich Fine Chemicals (Castle Hill, NSW, Australia). Hydrochloric acid (HCl), glycine, carbon tetrachloride (which was distilled over P_2O_5 prior to use), sulfuric acid, methanol, ethanol, and acetic acid were purchased from AJAX Finechem (Auburn, NSW, Australia). Acrylamide (AAm) and *N*,*N*-methylenebis(acrylamide) (Bis) (electrophoresis grade, >98%), and tris(hydroxymethyl)aminomethane (Tris) (Ultrapure) were obtained from ICN Biochemicals Inc. (Aurora, OH). *N*,*N*′-Methylenebis(methacrylamide) (mBis) (electrophoresis grade) was purchased from PolyScience Inc. (Warrington, PA). A SDS broad range protein molecular weight marker was purchased from Bio-Rad Laboratories (Hercules, CA) and prepared according to the supplier's specifications. Coomassie brilliant blue G250 stain obtained from Gradipore Ltd. (Sydney, Australia). All reagents, unless specified, were of analytical grade and were used without further purification while distilled water was used at all times.

Preparation of the Cross-Linkers. Bis and mBis were of electrophoresis grade and used without further purification. 1,3,5-Triacrylylperhydro-*s*-triazine (**1**)[14] and 1,3,5-trimethacrylylperhydro-*s*-triazine (**2**)[15] were prepared as previously reported, recrystallized from ethanol, and employed after purity was confirmed.

Preparation of the Stock Monomer Solutions. To ensure that the degree of cross-linking was the same for all cross-linking agents with equivalent potential functionality (equivalent number of polymerizable groups), all substitutions were calculated on a mole basis rather than a weight basis. When the potential functionality varied between the cross-linkers, the substitutions were calculated on an equivalent number of double bond basis with Bis. For an AAm and Bis system the accepted terminology of %T refers to the total concentration of the monomer AAm and the cross-linker Bis as a percentage (w/v) (eq 1). The term %C refers to the concentration of the cross-linker Bis (w/w) as a portion of %T (eq 2).

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$$\%T = \frac{\text{total mass of monomers (g)}}{\text{volume of solution (mL)}} \times 100 \quad (1)$$

$$\%C = \frac{\text{mass of the cross-linker (g)}}{\text{total mass of monomers (g)}} \times 100 \quad (2)$$

For cross-linkers with a potential functionality greater than four, calculations were made on an equivalent basis where the number of double bonds initially in the solution was the same. That is, for every 1 mol of Bis, $^2/_3$ mol of a hexafunctional cross-linker such as **1** is required.[16] This formulation will result in the real value of %T and %C of each PAAm gel cross-linked with a cross-linker other than Bis to vary from the Bis cross-linked AAm system. For example, a 10% T, 3% C gel would contain 9.7 g of AAm and 0.3 g of Bis per 100 mL. An equivalent 10% T, 3% C solution containing the hexafunctional cross-linker **1** would require 9.7 g of AAm and 0.32 g of **1**. This results in an actual concentration of 10.02% T, 3.19% C for the AAm and **1** system. For simplicity, the concentrations used to refer to Bis cross-linked gels and all the other cross-linked systems with similar concentrations are referred to as the equivalent Bis %T and %C concentration.

A 30% T, 3% C equivalent stock solution was made by dissolving AAm (29.10 g) with the cross-linker Bis (0.90 g), mBis (1.06 g), **1** (0.97 g), and **2** (1.13 g) in a 100 mL volumetric flask with distilled water. The solution was filtered through a Whatman No. 1 filter paper and stored at 4 °C prior to use. Similarly, a 40% T, 10% C equivalent stock solution was prepared using AAm (36 g) and the cross-linker Bis (4.00 g) or mBis (4.73 g) for a 100 mL stock solution and a 40% T, 0% C equivalent stock solution required AAm (40 g) for a 100 mL stock solution.

Preparation of Polyacrylamide Gels. An AAm and cross-linker solution (10 mL) was prepared by mixing the required amounts of the appropriate stock solution, distilled water, and 1.5 M Tris-HCl buffer (pH 8.8) (2.5 mL). Tris (27.23 g) was dissolved in water (80 mL) and adjusted to the pH of 8.8 with 6 N HCl to make a stock 1.5 M Tris-HCl buffer which was then brought to 150 mL with distilled water and stored at 4 °C prior to use. The gel solution was degassed by vacuum aspiration at room temperature for 40 min and then purged with nitrogen until the initiator system was added. The initiator system was composed of freshly made up 10% (w/v) APS (0.025 mL) and 10% (v/v) TEMED (0.025 mL), where the mole ratio of APS to TEMED was kept constant at 1:1. The gel solution (7 mL) was immediately cast between two glass plates (8 × 8 cm, 1 mm apart) that were purged with nitrogen and left to polymerize at room temperature for 3 h under a nitrogen environment prior to use. Measuring the extent of AAm and cross-linker conversion into PAAm ensured that a reproducible gel was formed, and the observations made were representative of the actual network. All gels have greater than 98% monomer and cross-linker conversion as determined using the HPLC method.

Temperature Profiles and Gel Times. The gel solution was prepared as described above (4 mL) and cast into small glass vials, which were purged with nitrogen and contained a thin temperature probe attached to a scanning thermocouple thermometer. The temperature of the reaction was recorded as soon as the gel solution was poured into the glass vials, initiated, and capped. The temperature readings were taken every 30 s for 2 h during the polymerization.

Clarity Tests. PAAm gels cross-linked with an equivalent Bis and mBis concentration at variable %T and %C gel concentrations were prepared similarly to the gels above and cast in Petri dishes purged with nitrogen. The optical clarity of each PAAm gel was noted and compared once the gels had polymerized.

Scanning Electron Microscopy (SEM). A piece of the PAAm gel (5 × 5 mm) made above was mounted vertically onto a SEM stub with nonconductive glue and cryogenically fractured in liquid nitrogen. The water from the gels fractured surface was sublimed at −95 °C for 90 min. The gel was then cooled to −198 °C and coated with platinum using argon gas and plasma for 2 min. Images of the fractured polymer were taken at various magnifications using a XL30 field emission scanning electron microscope (FESEM) and a Fission Polaron LT7400 Cryoprep platinum coater cryosystem.

Electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed and prepared under the discontinuous conditions of Laemmeli[17] using a mini vertical gel system and a micrograd II electrophoresis unit. A PAAm stacking gel with a concentration of 5% T, 3% C used to make the mold for the sample wells was prepared similarly to the gels above and poured into the empty section at the top of the glass cassettes already containing the resolving PAAm gels. SDS-PAGE was performed using a constant voltage of 150 V and 500 mA for 75 min and a Tris-glycine electrophoresis running buffer. The electrophoresis running buffer was prepared by dissolving Tris (9 g), SDS (3 g), and glycine (43.2 g) in 100 mL of distilled water and diluting 1:5 with distilled water before use. A 10 μL broad range protein molecular weight marker was syringed into the sample wells embedded in the stacking gel and separated using SDS-PAGE. The gels were stained after electrophoresis with a Coomassie brilliant blue G250 stain for 24 h and then destained with 10% acetic acid to observe the protein migration pattern.

Water Swelling Tests. The amount of water absorbed and the degree of swelling of each PAAm gel were measured. A piece of each gel (5 × 5 cm) was made, cut, dried in a 60 °C oven for 24 h, weighed, and then immersed in 100 mL of distilled water at 20 °C. Every 10 min for 2 h the gel was removed from the water, blotted with filter paper to remove any excess surface water, weighed, and returned to the water.

Results

Evaluation of Polyacrylamide Gels. An aqueous free radical polymerization between the monomer AAm and the cross-linker Bis is the most commonly used method to synthesize PAAm gels. Temperature profiles and polymerization rates, clarity tests, SEM, SDS-PAGE, and water swelling tests are well-established techniques that were chosen to characterize the properties of the resultant PAAm gels.

Bis contains two vinyl double bonds that are separated by a methylene unit and have a similar reactivity to AAm. The cross-linker mBis is similar to Bis in shape but has the more reactive methacrylamide type double bonds relative to AAm. The cross-linker mBis was initially chosen to investigate the importance of the cross-linker reactivity toward the polymeric structure. The model that emerged between the monomer AAm and the cross-linker Bis and mBis was further refined by using the cross-linkers **1** and **2** (Figure 1). Cross-linkers **1** and **2** are cyclic in shape, and within each molecule there are three equivalent reactive double bonds that are separated by a methylene unit. The nature of the vinyl double bonds of cross-linker **1** is of acrylamide. Cross-linker **2** has the more reactive methacrylamide type vinyl double bonds.

Temperature Profiles and Polymerization Rates. Temperature changes during the exothermic free radical polymerization between AAm and each cross-linker were monitored over time and plotted in Figure 2. The curve obtained contains a flat line (induction period) at the beginning of the reaction, which is sensitive to inhibitors such as oxygen that may delay the onset of the polymerization. This is followed by a sharp rise in temperature. The gradient of this rise is used to calculate the relative rate of the polymerization at room temperature and detect the temperature maximum.

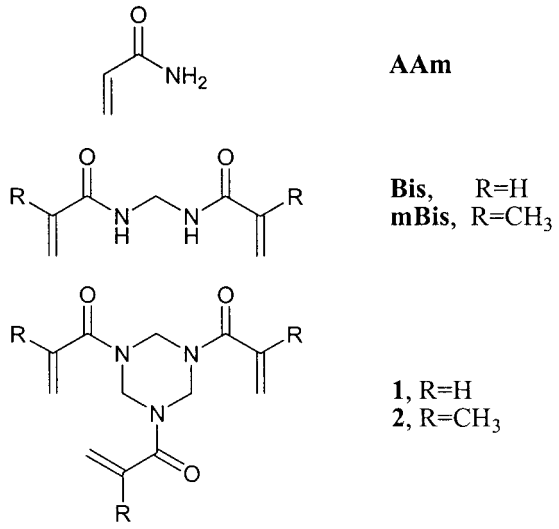

Figure 1. Molecular structure of the monomer AAm and the cross-linkers Bis, mBis, **1**, and **2** used to synthesize PAAm gels.

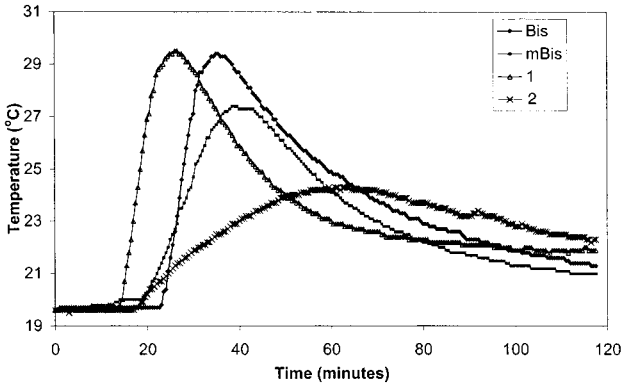

Figure 2. Temperature profile obtained during the aqueous free radical polymerization between the monomer AAm and the cross-linker Bis, mBis, **1**, or **2** (10% T, 3% C equivalent).

Table 1. Maximum Change in Temperature and the Rate of Polymerization during the Aqueous Free Radical Polymerization between AAm and the Cross-Linker Bis, MBis, 1, and 2

cross-linker	maximum temperature change (°C)	polymerization rate (°C/min)
Bis	11.2	0.56
mBis	9.0	0.28
1	10.6	0.50
2	3.7	0.08

The polymerization rate was slower, and the "Trommsdorff" effect[18] was depressed for PAAm gels cross-linked with the methacrylamide type cross-linkers mBis and **2** compared to equivalent gels cross-linked with the acrylamide type cross-linkers Bis and **1**, respectively (Table 1).

Clarity Tests. Comparison of the optical clarity between equivalent Bis and mBis cross-linked PAAm gels at different concentrations was made (Table 2). At concentrations below 5% C, both Bis and mBis gels were transparent regardless of the value of %T as was reported for Bis cross-linked PAAm gels.[19] At 10% T, 5% C Bis gels were cloudy and at 10% T, 7% C became completely opaque. mBis gels were only slightly cloudy at 10% T, 7% C and were not observed to be completely opaque until a concentration of 10% T, 20% C was reached. Similarly, PAAm gels cross-linked with **1** were slightly cloudy at 10% T, 3% C while equivalent PAAm gels cross-linked with **2** were clear.

Table 2. Optical Clarity of Equivalent PAAm Gels Cross-Linked with Bis and mBis at Variable %T, %C Concentration, Where the Appearance of the Gel Is Graded Clear for High Optical Clarity, Slightly Cloudy for Medium Optical Clarity, Cloudy for Poor Optical Clarity, and Opaque for Low Optical Clarity

concentration (%T %C)	bis clarity	mBis clarity
5, 5	clear	clear
5, 7	cloudy	clear
10, 3	clear	clear
10, 5	cloudy	clear
10, 7	opaque	slightly cloudy
10, 10	opaque	cloudy
10, 20	opaque	opaque
40, 3	clear	clear

SEM. SEM images were taken to visualize the apparent pore size distribution between each cross-linked PAAm network (Figure 3). From these images it appears that the methacrylamide type cross-linked gels mBis and **2** have an uneven pore size distribution and larger pores than their corresponding acrylamide type cross-linkers Bis and **1**, respectively.

SDS-PAGE. SDS-PAGE is a relatively sensitive and known technique that can give an indication of the relative PAAm gel pore properties such as the pore size distribution and pore sizes since it has a sieving effect on the protein mixtures enabling them to be separated by molecular weight.[20] The retardation factor (R_f), which is the distance migrated by each protein fraction over the distance traveled by the dye front, was calculated for each protein fraction separated on each cross-linked PAAm gel during electrophoresis. R_f values were compared, and an increased protein migration was observed for all the PAAm gels cross-linked with the more reactive methacrylamide type cross-linkers mBis and **2** compared to the gels cross-linked with the equivalent acrylamide type cross-linkers Bis and **1**, respectively (Figure 4). There is much big effect from Bis to mBis in comparison with **2** to **1**.

Water Swelling Tests. The swelling and permeability of PAAm gels have been studied and shown to be sensitive to the network microstructure. Water swelling tests were used to correlate the cross-linker properties to the porosity and heterogeneity of the network.[21,22] The water swelling properties of each polymeric network were presented by the ratios of the mass (g) of water absorbed by the gel over the weight of the dry gel vs time (Figure 5). Gels cross-linked with mBis and **2** have greater swelling in water compared to gels cross-linked with Bis and **1**, respectively.

Discussion

The system of AAm and a cross-linker most conveniently exemplifies the concepts we wish to develop and is well suited to the study of network formation. Even though AAm and Bis have approximately equal reactivity, it has been argued that the slightly higher reactivity ratio of Bis contributes to network heterogeneity.[23] In response, others have argued that the difference between Bis and AAm was too small to account for the extent of the abnormalities observed and that the large-scale heterogeneity in PAAm gels was due to intramolecular cyclization.[24,25]

In this study the results suggest that one of the most dominant influences toward the structural formation and properties of a PAAm gel is the reactivity of the

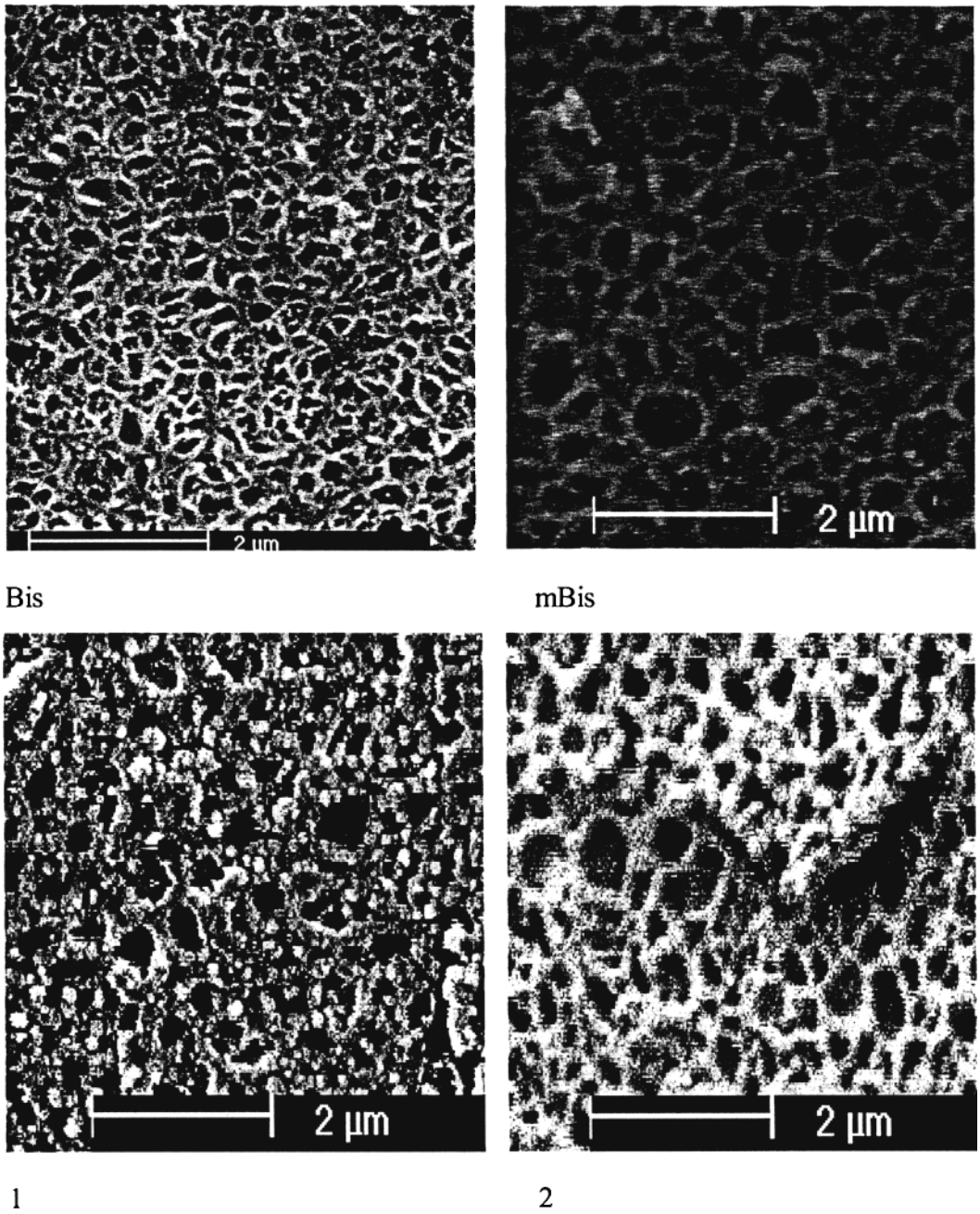

Figure 3. SEM images obtained from 10% T, 3% C PAAm gels cross-linked with Bis, mBis, **1**, or **2**.

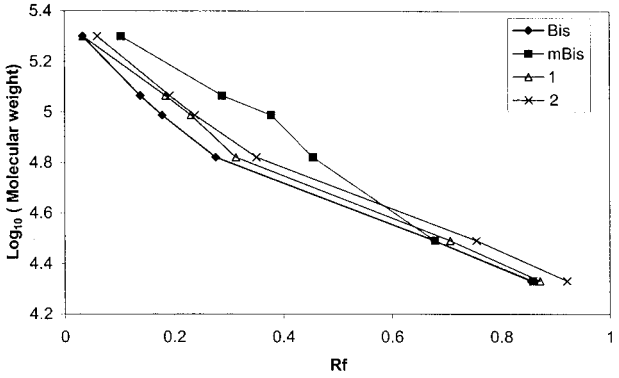

Figure 4. Plot of the protein R_f migration pattern obtained on each cross-linked PAAm gel (10% T, 3% C equivalent) against the log molecular weight.

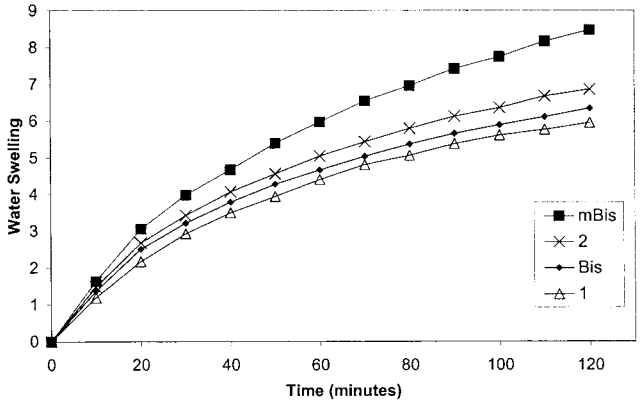

Figure 5. Water uptake over time experienced by each cross-linked PAAm gel (10% T, 3% C equivalent).

monomers, provided that the reactivity of AAm and the cross-linker are considerably different. Two sets of cross-linker pairs, Bis with mBis and **1** with **2**, were chosen to react with AAm. The reactivity ratio of the meth-

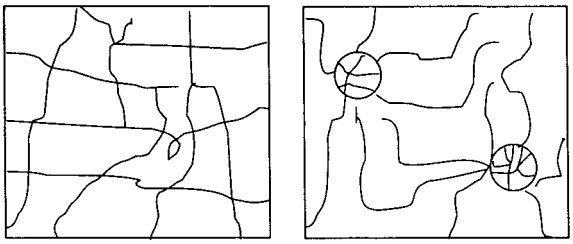

Figure 6. Schematic representation of the polymer network structures formed with (a) statistically distributed cross-linkers and (b) cross-linkers that form highly cross-linked loci.

acrylamide type double bonds over the acrylamide type double bonds has been reported to be approximately 1.35.[26]

Bis and mBis Cross-Linked PAAm Gels. The "Trommsdorff" or gel effect is often observed as a sudden increase in temperature at the point the polymer chain mobility becomes restricted because of the increased number of cross-linking points and subsequent increased viscosity. At this point the inability of the chain radicals to terminate by self-reaction allows chain growth to continue until the monomers are consumed since termination is diffusion-controlled.[27] A decreased temperature maximum and "Trommsdorff" effect were observed for mBis compared to Bis. This observation was unexpected since mBis is more reactive and should thus enter the network before Bis. An explanation of what may happen is as follows.

As AAm and Bis have a similar reactivity, the PAAm gels polymerized with Bis form a macroscopic network with a relatively statistical distributed cross-linking network as shown schematically in Figure 6a. On the other hand, mBis is more reactive than AAm and should potentially be incorporated into the polymer chain much earlier than Bis during the early stages of the polymerization. This will result in the polymer network containing areas with highly concentrated mBis units. This early incorporation of mBis into the polymer network creates a number of highly cross-linked loci which are small due to the low concentration of the cross-linker used compared to AAm but are highly concentrated in possible cross-linking points. Once mBis is consumed into the polymer network, the remaining AAm in the solution will continue to react and build relatively linear polymer chains branching away from these cross-linked loci, linking them together and forming the resultant three-dimensional polymer network. The second type of network formed is shown schematically in Figure 6b. During the growth of the AAm polymer chains there is considerable flexibility and mobility within the reaction mixture to allow termination of the radicals present on the growing chains. Therefore, we observe a smaller "Trommsdorff" effect with mBis gels compared to Bis gels.

The description given above for a mBis cross-linked AAm polymer appears to have some similarities to that of a microgel type system. Microgels are "intramolecularly" cross-linked macromolecules in solution with extended linear polymeric arms that are usually swollen and transparent.[28] The greater mobility of the AAm chains and more self-termination observed for the mBis and AAm system appear to have similar characteristics to the novel cross-linked microgels previously synthesized in our lab.[29,30] The type of microgels synthesized in our laboratory is star-shaped macromolecules with small highly cross-linked loci or cores that have numerous long chains branching from them. This type of polymeric structure is completely solvated and remains transparent even at high monomer concentrations. If mBis cross-linked PAAm gels are solvated in water similar to microgels, they too should remain transparent at fairly high concentrations. As outlined in Table 2, mBis cross-linked PAAm gels were found to have better optical clarity compared to equivalent Bis cross-linked PAAm gels at higher concentrations while maintaining similar mechanical strength. The experimental results support the described hypothesis of mBis forming a microgel type network. The difference in the hydrophilic and hydrophobic balance between Bis and mBis (two more methyl groups) does not appear to be a determining factor in this scenario. However, at very high concentrations the insolubility of the monomers may influence the clarity of the gel and may make the investigation unreliable. Therefore, gel concentrations greater than 40% T, 3% C and 10% T, 20% C were avoided.

The SEM images (Figure 3) taken show that mBis cross-linked gels have bigger pores and a greater pore size distribution than Bis gels, which is in agreement with the star like network structure described. SDS-PAGE analysis was used to further investigate the pore size and electrophoretic properties of the matrix. mBis cross-linked PAAm gels were found to have enhanced protein migration during electrophoresis compared to Bis cross-linked PAAm gels, which suggests that mBis cross-linked gels have larger pores than Bis as was brought to our attention with SEM and the model proposed. Water swelling tests (Figure 5) also show that mBis cross-linked gels have greater water uptake capability and as expected a greater porosity than Bis.

PAAm Gels Cross-Linked with 1 and 2 Cross-Linkers. The cross-linkers **1** and **2**, which have a potential functionality greater than Bis and mBis, were investigated. In a separate publication we have reported that the potential functionality of the cross-linker is a significant factor toward controlling the type of network formed in conditions similar to those we have used.[31] An increase in the potential functionality of the cross-linker was found to promote a similar microgel type network.

The "Trommsdorff" effect during gel formation with the methacrylamide type cross-linker **2** was greatly depressed compared to the system containing the acrylamide type cross-linker **1** (Table 1). The SEM images (Figure 3) indicate that **2** cross-linked gels have a slightly more heterogeneous network with larger pores than **1**-cross-linked gels. SDS-PAGE and water swelling tests for PAAm gels cross-linked with **2** also suggest that these gels have bigger pores than gels cross-linked with **1** as they gave a greater protein separation and water swelling.

The experimental trends between the gel cross-linked with **1** and **2** were similar to those observed between Bis and mBis gels. However, the extent of the differences between the gels cross-linked with **1** and **2** were comparatively smaller than the Bis and mBis gels. We have previously reported that an increased potential functionality of a cross-linker promotes networks with bigger pores.[32] This is also observed by SEM experiments (Figure 3, comparing Bis to **1**). The phase separation experienced by **1** cross-linked gels[31] and the

added functionality effect on the heterogeneity of the network[32] may contribute to the reduced reactivity effect observed between the two gel networks with **1** and **2**.

Conclusions

The properties of polymer networks appear to stem from the difference in the double-bond reactivity between the monomer and the cross-linker. When AAm is reacted with the more reactive methacrylamide type cross-linkers mBis or **2** in formation of PAAm gels, an alternative polymerization pathway was followed, leading to a "microgel" type network structure. Such gel networks have bigger pores and a more uneven pore size distribution, which give the formed polymeric network greater heterogeneity. The formed gels had an enhancement of optical clarity and protein separation while maintaining mechanical strength.

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