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Approaches to the controlled formation of network polymers: 2. Studies of hybrid crosslinking agents

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Abstract

Polyacrylamide networks incorporating hybrid crosslinking agents in which amide and ester functional moieties form part of the vinylic structure have a higher degree of porosity than the more conventional systems as evidenced by electrophoresis and overswelling studies. The increase of porosity may be attributed to two mechanisms of network formation; star or non-star type, which are determined by the reactivity of the vinylic groups present within the monomer structure. The experimental results are in good agreement with the theoretical predictions.

Keywords: Crosslinking agents; Polyacrylamide; Porosity

1. Introduction

The study of polymer networks has been of interest as the foundation work of Flory and Stockmayer in the characterization of three-dimensional polymeric systems [1,2]. Network polymers can be generated through the copolymerization of a monovinyl and divinyl monomer and examples of such systems are commonly found throughout the polymer literature. In most cases, the divinyl or crosslinking component of the copolymer mixture has a similar vinylic structure to that of the monovinyl monomer, and the network polymer is constructed in a largely statistical fashion. Research into the manner of construction of polymer networks has implicated the influence of heterogeneities on the final properties of the resultant material [3–5]. However, by controlling the manner in which the network is assembled, it may be possible to alter the physical characteristics of the polymer and thereby afford the ability to control phase separation, crosslinking reactions and the resultant polymerization exotherm.

In earlier work, the design and synthesis of some novel acryloyl derived diamide crosslinking monomers with substitution at one α-vinyl carbon was described [6]. These compounds introduced the concept of differential vinyl reactivity. Preliminary results obtained from materials containing these monomers suggested that modification of the vinylic chemical structure lead to a measurable enhancement in polymer properties, for example, porosity. However, in addition to control over the network by introducing differences in vinylic reactivity, the desirable feature of differential functionality may also be achieved by manipulation of the functional moieties linking the vinyl units to the central alkylidene portion of the crosslinking monomer.

In this investigation, the concept of differential reactivity is extended further by reporting on the incorporation of hybrid crosslinking agents to form novel polymers. Like the diamide monomers reported previously [6], these hybrid monomers possess differential reactivity with respect to the vinylic structures present within the molecule. However unlike the diamide systems, functional differentiation is also achieved through distinguishing between the heteroatoms present within the monomer, such that compounds incorporating amide and ester functional groups are formed. A synthetic procedure able to afford four hybrid crosslinking agents (1–4) on commercial scale was developed previously, and has been reported elsewhere [7].

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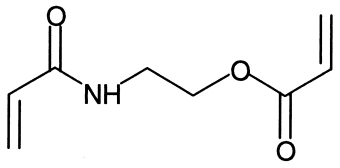

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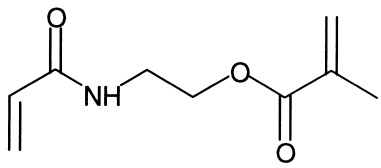

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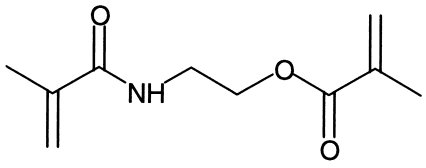

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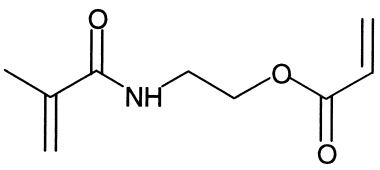

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2. Materials and method

2.1. Materials

Electrophoresis grade (>98%) acrylamide, sodium dodecyl sulfate (SDS) and 3-(dimethylamino)propionitrile (DMAPN) were obtained from Aldrich Chemical Company Inc. *N*, *N′*-methylenebisacrylamide (BIS) and ammonium persulfate of electrophoresis grade (>98%) were obtained from Sigma Chemical Company. Coomassie blue R-250 and SDS broad range protein molecular weight markers were purchased from Bio-Rad Laboratories and prepared according to the supplier's specifications. Tris(hydroxymethyl)aminomethane (Tris) and glycine were of analytical grade. All reagents were used without further purification.

2.2. Polyacrylamide gel preparation

Gel monomer solutions were initially prepared in a stock solution at a concentration of 30%T 3%C with respect to the acrylamide/BIS formulation, where %T refers to the concentration of total monomer (w/v) and %C refers to the concentration of crosslinking monomer (w/w) as a proportion of %T. Thus, 29.1 g acrylamide and 0.9 g BIS were dissolved in 100 ml of distilled water, filtered and kept at 4°C prior to use. A "30%T 3%C" stock solution for each of the new monomers was prepared by substituting the novel monomers for BIS on an equimolar basis. Gel solutions were diluted to the required concentration and prepared according to the method of Laemmli [8]. Subsequently, the gel solutions (30 ml) were degassed by vacuum aspiration at room temperature for 10 min, and at 10°C for 10 min, and then polymerized by the addition of 10% (w/v) ammonium persulfate (0.6 ml) and DMAPN (15 μl). The gels were cast as slabs between two glass sheets of 80 mm × 80 mm dimensions separated by 1 mm plastic spacers, at room temperature and under a nitrogen atmosphere. A 5%T 3%C BIS stacking gel used to aid in concentration of the protein sample prior to separation was also prepared in the same manner and polymerized on top of the network polymers under investigation. For each gel comprising the novel monomer, a reference BIS gel was prepared at the same time.

2.3. Electrophoresis

Electrophoresis was carried out at a constant voltage of 200 V using a Pharmacia constant power supply and a Gradipore Micrograd vertical electrophoresis unit. Gels containing the novel monomers and the equivalent BIS gel were electrophoresed alongside one another under identical conditions. Approximately 10 μl of broad range protein marker consisting of myosin (200 K), β-galactosidase (116.3 K), phosphorylase b (97.4 K), serum albumin (66.2 K), ovalbumin (45 K), carbonic anhydrase (31 K), trypsin inhibitor (21.5 K), lysozyme (14.4 K) and aprotinin (6.5 K), and containing bromophenol blue as the tracking dye was applied to four lanes in each gel, and electrophoresis performed under the SDS-PAGE discontinuous buffer conditions of Laemmli [8]. After electrophoresis, the gels were removed from the cassettes under running water and the protein bands visualized with Coomassie blue R-250. The degree of migration of the individual protein bands was determined as an R_f value, which is defined as the ratio of the distance moved by the protein to the distance moved by the tracking dye.

2.4. Overswelling

Overswelling refers to the excess mass of water

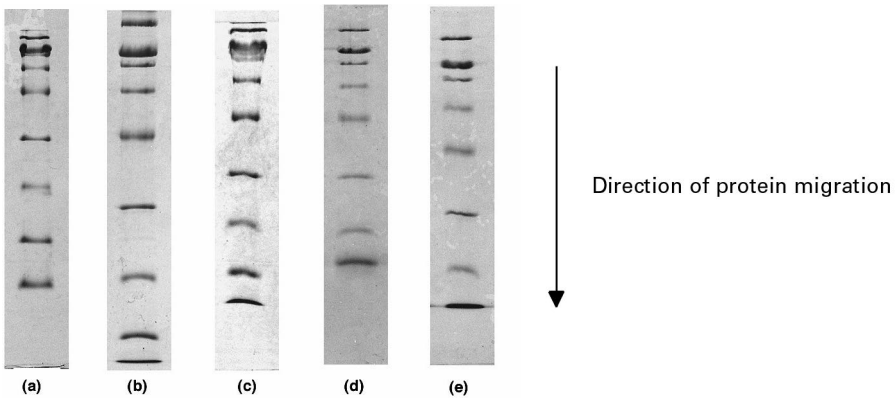

Fig. 1. Separation of the standard protein marker system in polyacrylamide gels containing the monomers: (a) BIS; (b) (1); (c) (2); (d) (3); (e) (4).

encapsulated by the polymer over its original wet weight. For these experiments, the gels containing the BIS monomer and the hybrid crosslinking agents were prepared according to the protocol outlined earlier and as such, contained the same molar content of monomer. Duplicate samples of approximately 35 mm × 40 mm were sectioned from the gels, blotted of excess surface moisture using Whatman 3MM paper and weighed immediately. The gel samples were then immersed in a bath of distilled water (150 ml) maintained at 15°C. At 10 min intervals over a period of 70 min, the samples were blotted of excess water and re-weighed.

3. Results

Previously, divinyl crosslinking agents in which the vinyl units were structurally different from one another have been used to investigate the influence of monomer structure on polymer assembly [6]. A study of the reactivity ratios of model systems suggests that even greater differences in reactivity may be induced with ester and amide functional moieties, leading to large reactivity differentials that have the potential to have a marked influence on the polymer macro-structure [9]. Thus, two techniques were employed to evaluate the polymers arising from the incorporation of such crosslinking agents, for differences in physical properties. The major technique used was that of electrophoresis, which was performed under the SDS discontinuous buffer conditions of Laemmli [8], such that the predominate influence on protein separation was the molecular sieving capabilities of the polymer. The relative migration (R_f value) for a commercial sample of protein molecular weight markers were determined. These results were then correlated with studies of overswelling of the polymer networks as a means of detecting differences in polymer porosity.

In this investigation, the various crosslinking agents to be evaluated were solubilized in aqueous acrylamide prior to free radical initiation. The polyacrylamide networks were studied at a concentration of 15%T 3%C, and a standard protocol for the preparation of the polyacrylamide networks is described in the experimental section and elsewhere [6]. A molar equivalent of the novel crosslinking agents was used to replace the BIS monomer, with the new networks being compared with the BIS crosslinked polymer, which was maintained as a reference system throughout the investigation.

It has been shown in a preliminary work that the use of a thin film provides a suitable format for evaluation of the novel crosslinking agents in polyacrylamide networks, and is also likely to assist in the rapid dissipation of the heat generated from the polymerization exotherm. Thus, the various network polymers were formed as 1 mm films at room temperature and under a nitrogen atmosphere, using a redox initiating system composed of ammonium persulfate and 3-dimethylaminopropionitrile at a standard concentration of 0.2 mol%.

3.1. Monomer hydrophilicity and gel appearance

During this work, it was observed that each of the hybrid crosslinking monomers has sparing solubility in water, but were soluble in acrylamide solution. As polyacrylamide gels are formed within an aqueous environment, a major requirement of any novel monomer for these polymer systems is a high degree of hydrophilicity, which may be tested for by partitioning between *n*-octanol and water [10]. While the hydrophilicity of a monomer is an important consideration, it is also worthwhile to regard the carbon to heteroatom ratio within the compound such that the monomer remains soluble in acrylamide solution.

Polyacrylamide gels formed using the BIS crosslinking monomer were optically transparent and dry to the touch. At the equivalent concentration, gels incorporating the monomers (1) and (4) were also optically clear, but were slightly tacky to touch. Gels prepared with the monomers (2) and (3) were not transparent and were opaque in appearance, but in other respects were similar to the BIS gels and dry to the touch. The appearance of these gels and the possible reasons for the opaqueness is discussed further in this report.

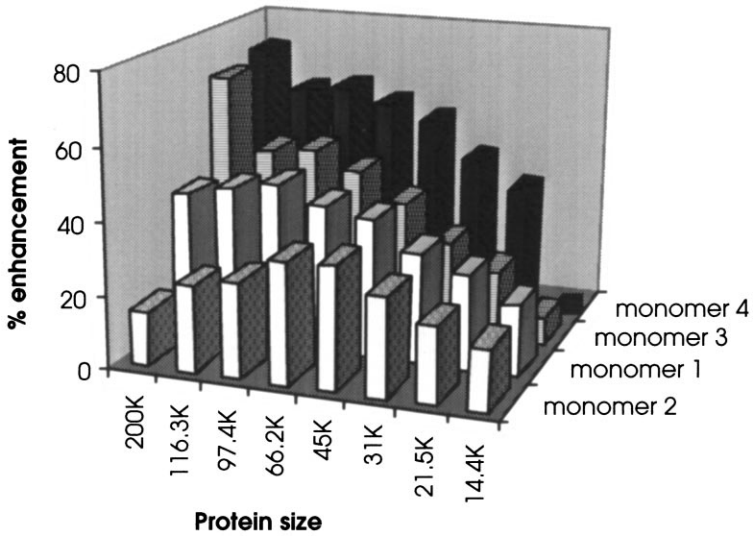

Fig. 2. Graphs of percentage differential in protein separation in gels prepared with the synthesized monomers as compared to the equivalent BIS matrix.

3.2. Protein migration

For the determination of relative protein migration and hence porosity, the polyacrylamide gels incorporating the hybrid crosslinking agents were cast and electrophoresed alongside an equivalent gel containing the BIS monomer. After electrophoresis and visualization of the proteins by staining, it was observed (Fig. 1) that all gels containing the different hybrid monomers show greatly enhanced protein migration and higher R_f values for equivalent proteins when compared with the standard BIS gels. This enhancement of protein migration is most evident in gels formed with the monomer (4), with monomer (1) and (3) enhanced in the lower and higher pore size regions, respectively.

When viewed as a percentage enhancement of protein mobility as compared with the standard polymer network containing the BIS monomer, it may be seen that in all the new systems under investigation, the trend is for a marked increase in protein migration for all the polymer networks containing the hybrid crosslinking agents. The gels with the monomers (1) and (2) appear to show an enhancement of movement for the mid-range proteins (97–45 K), while the proteins which have passed through the polymers containing the compounds (3) and (4) show an increased migration in the high molecular weight region (200 K). In contrast to the BIS containing gel, the lowest molecular weight protein (6.5 K) runs co-incident with the dye front in the polymers containing the monomers (1–3), reflecting an overall increase in the polymer pore size. In the network incorporating the hybrid crosslinking agent (4), the two lowest molecular weight proteins (14.4 and 6.5 K) run co-incident with the tracking dye, suggesting an even larger pore structure than the other hybrid systems (see Fig. 2).

When translated to a standard correlation curve (Ferguson plot [11]) of $\log_{10}$(molecular weight) vs. protein mobility, this enhancement in migration is manifested as a shift of the curve to the right. The gel comprising the monomer (4) exhibits the largest shift of the curve, while the other novel gels have approximately the same degree of overall shift. It is interesting to note that the point corresponding to the highest molecular weight fragment of 200 K is shifted further to the right in gels with the monomers (3) and (4), both of which possess a methacrylamide vinylic unit.

3.3. Overswelling

The polyacrylamide gels with the hybrid monomers also undergo a greater degree of overswelling than the BIS gels, which indicate a more porous structure. Gels incorporating the BIS monomer overswells by 26% over its original mass. The hybrid gels with the monomers (1), (3) and (4) were able to incorporate 59–60% extra water, while the gel with the compound (2) as the crosslinking agent was able to encase 39% excess water. These overswelling results are supportive of the previous electrophoresis studies (see Fig. 4).

4. Discussion

In these studies, a systematic approach to the design of a number of novel crosslinking agents is presented. Earlier work investigated the effect which elongation of the

Table 1
Comparison of literature reactivity ratios for model systems

M_1/M_2	r_1	r_2	Reference
Acrylamide/ Methacrylamide	0.74	1.1	[20]
Methyl acrylate	1.30	0.05	[9]
Methyl methacrylate	0.44	2.60	[21]

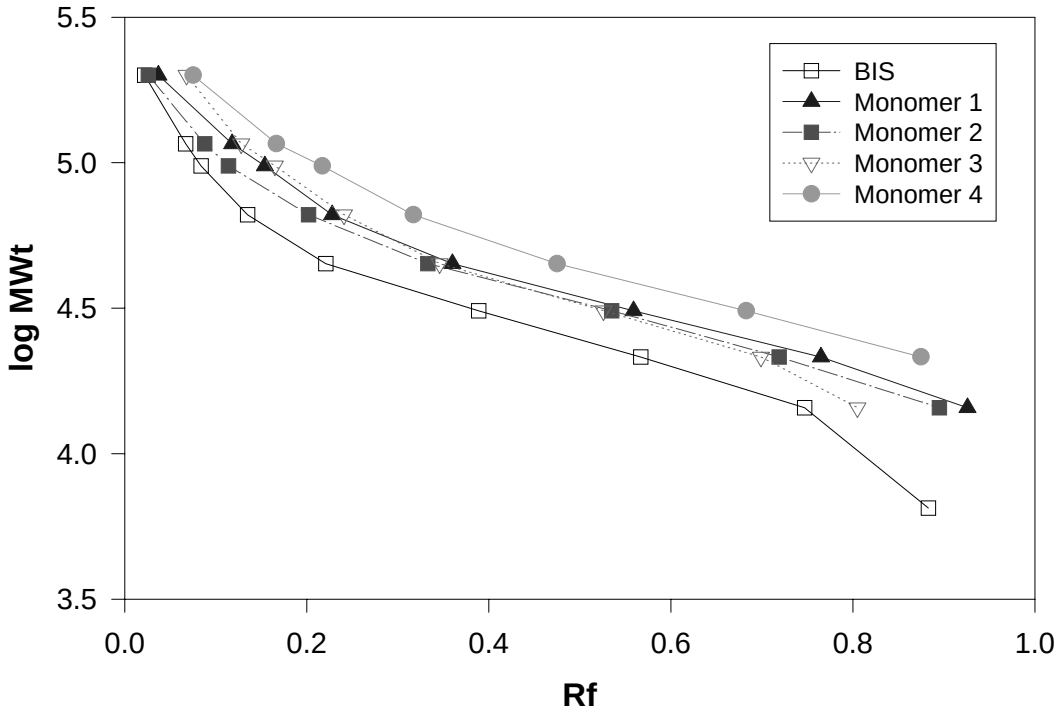

Fig. 3. Ferguson plots of $\log_{10}$(molecular weight) vs protein mobility.

alkylidene spacer linking the two vinylic units, and modification of vinylic structure may have on the manner of network construction [6]. Further changes in monomer structure by the inclusion of different heteroatoms in the hybrid crosslinking agents (1–4) to give a greater vinylic reactivity differential was shown to exert a significant influence on polymer architecture.

Literature reports have generally stated the conversion of monomer in the formation of crosslinked polyacrylamide is greater than 90% [12]. In a typical experiment, we confirmed these findings by the analysis of residual monomer extracted from a 15%T 3%C polyacrylamide gel crosslinked with the BIS monomer using. The conversion of the monomers was found more than 99.6% according to a ^{1}H NMR measurement of the residual monomers. Therefore, we assumed that the conversion of the gels prepared with different crosslinkers under the same condition is equivalent on the same molar bases.

To explain the observations made during this study, it is worthwhile to consider the expected behaviour of each novel hybrid crosslinking monomer under free radical conditions, based on the reactivity ratios of model systems. Initially, we attempted to measure reactivity ratios in an actual system using ^{1}H NMR, according to the work of Nieto [13]. Although the technique was advantageous in that it enabled the distinguishing of all the double bonds present in the system, the high concentration of crosslinking agent required for visualization of the various vinyl units led to an insoluble gel. The use of hydroquinone, to limit the chain lengths in order to obtain a high concentration of crosslinked polymer in the solution, did not prove satisfactory and introduced more variables into the system. Consequently, we decided to use literature reactivity ratios to calculate the polymer network formed when the various crosslinking agents are incorporated into the network system. These ratios are derived from monomers which are structurally equivalent to the components in the crosslinking agents of interest, such as alkyl substituted model systems, and Table 1 gives their typical values.

The calculations of the network were performed using the reaction probability models [14] for a three-component copolymerization in combination with the conversion–composition equation [15,16] with a non-numerical procedure. From the calculation, the relations between the total conversion of the monomers to the gel polymer and the individual conversions of the monomers are achieved, hence the gel network structure can be predicted. The details of the calculation method will be published elsewhere.

As the crosslinking agent would only comprise 1.4 mol.% of the total monomer present in solution, the likelihood of encountering another molecule of crosslinker is small compared with a possible reaction with acrylamide. Therefore, assuming that each vinylic unit of the crosslinking monomer reacts independently of the other, and no secondary reactions such as cyclization occur, consideration need only be given to the relative rates of reaction of each vinylic moiety with the comonomer, acrylamide.

The reported literature reactivity ratios of acrylamide and

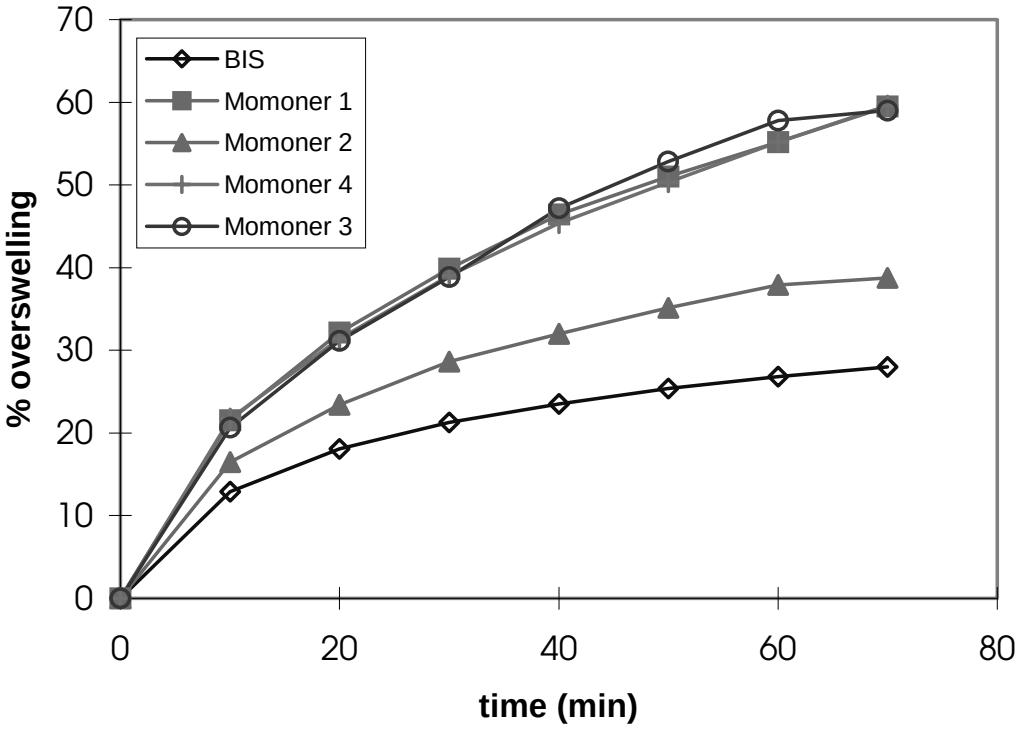

Fig. 4. Graphs of percentage excess water uptake over time for gels incorporating the new monomers and for a standard BIS containing polymer.

methyl acrylate imply that the ester derived monomer is much less reactive than that of acrylamide (Table 1). Thus, in the crosslinking monomer (1) which has one acrylamido and one acrylate group, it is expected that the acrylamido unit would be incorporated in a statistical manner into the growing polymer chain at a similar rate to acrylamide, whereas the much less reactive acrylate unit would remain as a pendant functionality until a concentration is reached at which crosslinking would occur. The calculated network structure crosslinked with monomer (1) shows that, when the acrylamido units of monomer (1) are completely incorporated in the polymer, the total conversion of the monomers is about 78% and the conversion of acrylate units of monomer (1) is about 40%. The similar reactivity of the acrylamido unit with acrylamide ensures a more statistical distribution of the crosslinking agent while the delayed crosslinking also allows the polymerization exotherm to be dissipated more readily.

When reactivity ratios are extrapolated to the hybrid crosslinking monomer (2), which has one acrylamido and one methacrylate group, the methacrylate unit is considered to be much more reactive than the acrylamido unit as the methyl substituent at the α-position is more readily able to stabilize the resultant free radical. In this monomer, the methacrylate unit enters at a much faster rate into the copolymer chain such that initially, there is a higher degree of branching. The pendant acrylamido units would then crosslink and be incorporated at a rate similar to acrylamide. Effectively, this would result in the formation of a network polymer from an initial region of high crosslink density. As the polymerization proceeds, the resulting polymer undergoes phase separation as the methacrylate-rich portions are less likely to be soluble in an aqueous environment. The calculation shows that there is a fast incorporation of methacrylate units into the polymer and it is completed at a very early stage of total monomer conversion (36%). At the same time, only 20% of the acrylamido units of (2) have reacted. Any further reaction of the monomers will mainly involve the double bonds of the unreacted acrylamido units pendant from the polymer formed, and these reactions will form long chains to give a star-type polymer network. The existence of highly crosslinked structures within the network polymer is most likely to account for its opaque appearance as a result of the increased incidence of scattered light.

The crosslinking agent (3) has one methacrylamido and one methacrylate group, which are both more reactive than acrylamide. This implies that the crosslinkers will be incorporated quickly into the copolymer chain in a manner dictated by their relative reactivities and the crosslinking reaction will also proceed relatively quickly, producing regions of high crosslink density with large spaces or pores in between each region. As the methacrylate units of (3) are far more reactive, a star-type network is also predicted. Correspondingly, the polymer formed with the monomer (3) appears opaque due to phase separation as a result of the presence of methacryloyl groups.

The hybrid agent (4) contains one methacrylamido and one acrylate group. The methacrylamido unit, being much

more reactive than acrylamide, will be incorporated quickly into the copolymer chain. The acrylate unit, being less reactive than acrylamide will remain as a pendant moiety. Similar to the monomer (1), the crosslinking reaction will be significantly delayed to give a more open and porous structure. The calculation also predicted a non-star type polymer network (methacrylamido units completely incorporated into the polymer at 71% of the total conversion of monomers). The possibility of regions of high crosslink density forming is also possible within this network, however the optical transparency of this polymer suggests that visible phase separation does not occur and that the opacity observed for the gels containing the monomers (2) and (3) is due to the presence of a methacrylate functionality.

As evidence to support the theory of delayed crosslinking associated with the acrylate functional moiety, the polyacrylamide gels containing the compounds (1) and (4) were slightly tacky to the touch, suggesting different types of crosslinking. This is in contrast to the gels synthesized with the monomers (2) and (3) which were dry to the touch.

Additionally, the presence of methacrylamido unit as a part of the hybrid monomer structure appears to enable a larger average pore size to be obtained. Inspection of the standard correlation curves produced from protein migrations in the various novel matrices (Fig. 3) showed the greatest enhancement in mobility for the largest protein fragment in the networks incorporating either monomer (3) or (4).

The gels containing the crosslinking agents (2) and (3) were more opaque than the gels with the monomers (1) and (4) and also the standard BIS gel which were optically transparent at the concentration of 15%T 3%C. The opaque appearance is due to a higher amount of scattered light, suggesting the existence of more highly crosslinked regions in the gel network and a greater degree of inhomogeneity and incompatibility with the surrounding environment with phase separation. As suggested from the study of reactivity ratios for model systems, phase separation arising from the greater relative reactivity of the methacryloyl derived group is responsible. At a higher crosslink concentration (15%T 6%C) the gels with the monomers (2) and (3) become very opaque, being almost white in appearance. Corresponding to this, the values measured for protein migration were much greater than anticipated from previous work, implying an overall increase in pore size. However, the protein bands are not well resolved or well defined, but appear fuzzy and diffuse as if the proteins needed to move around thickened polymer chains. This was not observed for protein band appearance or migration for the gels incorporating the monomers (1) and (4) at the higher 15%T 6%C concentration.

Increased fibre thickness produces an overall decrease in the number of fibres per unit of gel volume, which may also be coupled with an increase in network pore size. This effect has been observed by other authors when a hydrophilic polymer such as poly(ethylene glycol) has been incorporated into the monomer solution [17] or when a high level of crosslinking is formed within the polymer network of a given %T concentration [18].

The crosslinking agent ethylene glycol diacrylate has been used to advantage in polyacrylamide gels where it is desirable to destroy the network for recovery of separated material [19]. The presence of a readily hydrolyzable ester moiety within these new hybrid crosslinking agents also presents the attractive option of being able to dissolve the network. As the acrylate group present within the crosslinking agents (1) and (4) is less stable under the alkaline conditions required for hydrolysis than the more substituted methacrylate group present in the monomers (2) and (3), it is expected that gels comprising (1) and (4) would be more readily destroyed. This was observed experimentally after gels containing (1) and (4) stored in Tris buffer (pH 8.8) for extended periods of time produced a liquefied mass.

5. Conclusion

In this work we continue with the systematic design and development of a number of novel crosslinking agents. Unlike the commonly used monomers for network formation, the hybrid crosslinking agents exhibited the potential to control the mechanism of network formation to give networks that are different to the conventional BIS crosslinked systems. This was manifest as an overall increase in polymer porosity, which may be attributed to two modes of network formation; delayed crosslinking and phase separation. Both phenomena may be related to the differential reactivity of the two vinylic units present within these molecules. It is envisaged that future developments in the studies of controlled network formation would eventually result in an array of crosslinking agents able to impart specific properties to network polymers for specialty applications.

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