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Approaches to the controlled formation of network polymers. 1: Synthesis and evaluation of monomers with vinyl differentiation

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The synthesis of novel crosslinking agents *N*-acryloyl,*N*-methacryloyl methylenediamine (**1**) and *N*-acryloyl,*N′*-methacryloyl ethylenediamine (**2**) with non-identical vinyl functionalities is described. When incorporated into crosslinked networks, the resultant polymers exhibit a higher degree of porosity than the more conventional systems where the vinyl functionalities are identical, as evidenced by protein mobility studies using electrophoresis and water permeability experiments. This may be attributed to the formation of a bimodal pore size distribution arising from a crosslinking mechanism which is dictated by the relative reactivities of the differentiated vinyl units within the novel monomers. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

The ability to control each step of the polymerization process is of current interest in modern polymer synthesis and many advances have been made in step and chain growth polymerizations to afford linear and branched macromolecules of defined architecture. However, crosslinked networks have not enjoyed the same level of activity, partly due to the difficulties associated with controlling their mechanism of formation and also with the evaluation of the resultant materials.

Generally, network polymers formed via chain reactions involve the copolymerization of a monovinyl and divinyl monomer. Examples include styrene with 1,4-divinylbenzene, methyl methacrylate with 1,2-ethylene glycol dimethacrylate and acrylamide with *N*,*N′*-methylenebisacrylamide. In the examples given, the divinyl or crosslinking component of the copolymer mixture has a similar vinylic structure to that of the monovinyl monomer. Thus, under free radical conditions, the crosslinking agent would be incorporated at statistical intervals along the copolymer chain, with the second double bond undergoing reaction as dictated by electronic and steric considerations. Research into polymer networks has studied the way in which polymer microstructure may alter the final physical properties of the resultant materials[1–5]. However, attempts to modify the physical characteristics of the networks by controlling their manner of construction have been limited by the crosslinking agents currently available.

In a free radical system, compounds with polymerizable functionalities of different chemical structure possess individual relative reactivities. It was therefore envisaged that different network polymers may be produced, and a degree of control could be imparted over the crosslinking reaction and resulting polymerization exotherm if a crosslinking monomer with differentiated polymerizable structures was incorporated within the three-dimensional network. A notable example of monomers with this potential are the allyl esters, and include compounds such as allyl acrylate and allyl methacrylate. However, these molecules were not suitable for this investigation as the allyl functionality is known to participate in degradative chain transfer reactions, leading to incomplete polymerization under free radical conditions[6]. Thus, this work was limited to studying compounds containing two different vinyl derived structures, each of which would be expected to be incorporated in a manner reflected by the reactivity ratios of model systems[7]. With such crosslinking agents, a higher degree of control over the network formation may be obtained.

Herein, the synthesis and evaluation of two novel diamide crosslinking agents, *N*-acryloyl,*N′*-methacryloyl methylenediamine (**1**) and *N*-acryloyl,*N′*-methacryloyl ethylenediamine (**2**) which incorporate acryloyl and methacryloyl functionalities within the same molecule, is reported.

RESULTS AND DISCUSSION

The technique of electrophoresis was employed as the primary method of evaluating the networks afforded by these novel crosslinking agents. This is a sensitive tool for assessing the effect of variations in the type of crosslinking agent on the generated networks as evidenced by differences in the separation profile of a set of standard proteins of predetermined molecular weight.

A common polymer used as supporting matrices for electrophoresis is conventionally prepared through the copolymerization of two monomeric species; acrylamide and the multi-functional crosslinking agent *N*,*N′*-methylenebisacrylamide, commonly referred to as BIS. *N*-acryloyl,*N′*-methacryloyl methylenediamine (**1**) may be

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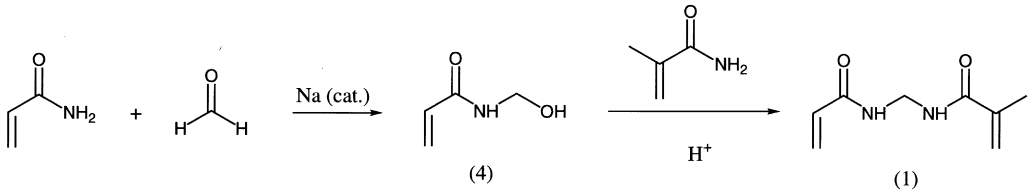

Figure 1 Synthesis of *N*-acryloyl, *N′*-methacryloyl methylenediamine (1)

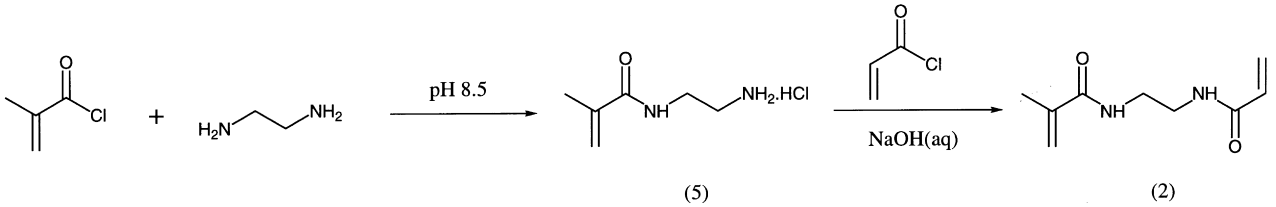

Figure 2 Synthesis of *N*-acryloyl, *N′*-methacryloyl methylenediamine (2)

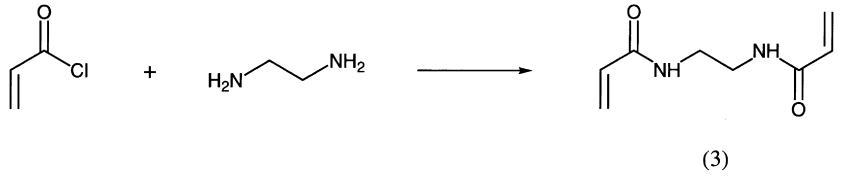

Figure 3 Synthesis of *N,N*-bisacryloyl, ethylenediamine (3)

considered as a derivative of BIS in that the acryloyl and methacryloyl units present within this compound are separated by a methylene bridge. In *N*-acryloyl,*N′*-methacryloyl ethylenediamine (**2**) the different vinyl functions are separated by an ethylene unit. Of these compounds, the monomer **2** has not previously been reported in the literature. Compound **1** has briefly been mentioned in the patent literature[8,9], however no structural characterization data exists and this compound has not been used in polymerization studies. Both molecules are investigated in this report as new crosslinking agents for polymer network formation. The monomer *N,N′*-bisacryloyl ethylenediamine (**3**) contains the same vinyl units as BIS, but with a longer, more flexible ethylene linkage separating the acryloyl functionalities. While this compound is known as a component in the preparation of beaded polymeric supports for solid phase peptide synthesis[10], it has not been reported as a crosslinking agent for polyacrylamide-based networks and for this work it serves as a useful example of another simple structural alteration for comparative studies.

Synthesis of diamide monomers

As the monomer **1** contains non-identical vinyl functionalities within its structure, a two-step synthetic procedure was required. The first step involved the reaction of acrylamide with paraformaldehyde at 50°C in the presence of a catalytic amount of sodium.[11] This afforded an *N*-methylolacrylamide intermediate (**4**), which was then treated with methacrylamide in an acid catalyzed reaction to give **1** in a yield of 51% (*Figure 1*). Herein, the first structural details for this compound are reported.

In the preparation of monomer **2**, it was anticipated that access to this molecule would be obtained through the use of a suitable *N*-monoacrylated intermediate. Attempts to synthesize *N*-acryloyl ethylenediamine by the reaction of acryloyl chloride with varying amounts of ethylenediamine afforded only poor yields of the required *N*-monoacrylated material. The low yield of the desired intermediate has been attributed to the preferential formation of the bis-acrylated compound via the process of transamination in which the free amino unit of *N*-acryloyl ethylenediamine reacts with further units of intermediate to give the bis-acrylated compound and re-generated ethylenediamine.

Harris and Wilson[12] described the isolation of *N*-acryloyl ethylenediamine monohydrochloride from a reaction which was conducted at a controlled pH. Presumably, this procedure involved the initial formation of a monohydrochloride salt of ethylenediamine which then reacted with acryloyl chloride to give the target compound. Using this protocol, the formation of *N*-methacryloyl ethylenediamine monohydrochloride (**5**) was successfully achieved using a biphasic reaction mixture (water/chloroform) at pH 8.5, with the required intermediate (**5**) being isolated from the aqueous portion. Treatment of **5** with 2 M sodium hydroxide and further reaction with acryloyl chloride then gave **2** in a yield of 37% (*Figure 2*).

Compound **3** was prepared according to literature procedure[13] by the reaction of ethylenediamine with acryloyl chloride and was obtained in a yield of 48% (*Figure 3*).

Polyacrylamide gel preparation

In the preparation of polyacrylamide gels for the evaluation and comparison of the synthesized monomers, the crosslinking agent was solubilized in aqueous acrylamide prior to free radical initiation to produce a three-dimensional network polymer. For this investigation, polyacrylamide gels were studied at a concentration of 15%T, 3%C, 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 (as defined in Section 4). In studying the gels which contained

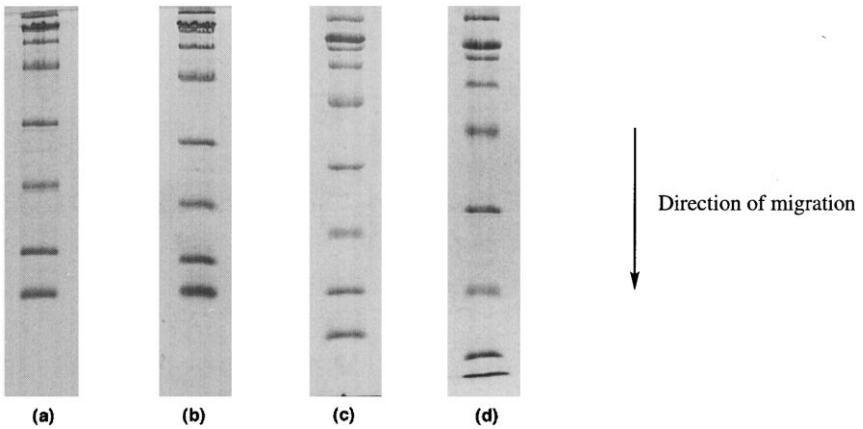

Figure 4 Separation of the standard protein marker system in polyacrylamide gels containing the monomers (a) BIS, (b) *N′*-acryloyl,*N′*-methacryloyl methylenediamine, (c) *N*,*N′*-bisacryloyl ethylenediamine and (d) *N*-acryloyl,*N′*-methacryloyl ethylenediamine

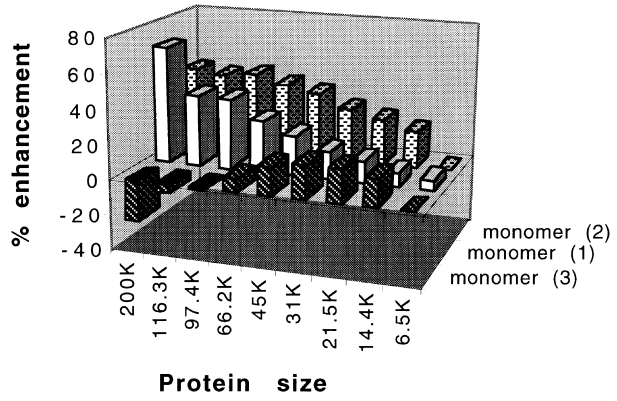

Figure 5 Graphs of percentage differential in protein separation in gels prepared with the synthesized monomers as compared with the equivalent BIS matrix

the synthesized crosslinking agents, the standard BIS monomer was replaced by the crosslinking agent of interest on a mole to mole basis to ensure that an equivalent crosslinking potential exists in the final monomer solution, allowing a more direct evaluation to be made between the structure of the monomer and the properties of the resultant network polymer. The networks containing the novel monomers were then able to be compared with those containing the BIS crosslinking agent, which was maintained as a reference system throughout the investigation.

The various network polymers were formed as 1 mm films at room temperature and under a nitrogen atmosphere. The use of a thin film ensures a suitable format is obtained for electrophoretic analysis consistent with the requirements of the Laemmli discontinuous buffer system[14]. Moreover, it also assists in dissipating the heat generated from the resulting polymerization exotherm. Based on previous work[15], a redox initiating system composed of ammonium persulfate and 3-dimethylaminopropionitrile was adopted, with the amount of total initiator relative to monomer maintained at a standard concentration of 0.2 mol.% throughout the investigation. After initiation, the prepared monomer solutions were carefully introduced into the glass gel forming moulds or 'cassettes' under a nitrogen atmosphere. The polymerization of the networks was allowed to proceed for 1 h, after which a stacking gel comprising the BIS crosslinking agent at a concentration of 5%T, 3%C was layered on top of the 15%T, 3%C resolving gel. For the protein sample being used, the low concentration stacking gel has no effective separative properties and only served to concentrate the proteins into a thin zone prior to separation in the higher concentration gel. After the stacking gel had been left to polymerize for a further 1 h, the cassettes were cleaned and the gels stored at 4°C for 18 h prior to electrophoretic evaluation.

Electrophoresis of the hydrophillic networks was performed under the sodium dodecyl sulfate (SDS) discontinuous buffer conditions of Laemmli[14], such that protein separation was primarily influenced by the molecular sieving capabilities of the polymer. After electrophoresis, the relative migrations (R_f value) for a set of proteins in a commercial sample of molecular weight markers were determined and compared. When correlated with overswelling studies, a powerful and sensitive means of detecting differences in the porosity of the systems under investigation was possible.

Polyacrylamide gel evaluation

The crosslinking agents investigated were all found to be soluble in buffered acrylamide solutions and copolymerized efficiently with acrylamide to afford polyacrylamide networks which were dry to the touch. The gels were also optically clear at the required concentration, although optical clarity was observed to diminish as the concentration of the crosslinking agent was increased, reflecting observations found with the supports containing BIS[16].

Polyacrylamide gels comprising the novel crosslinking agents were prepared and electrophoresed alongside equivalent gels prepared with the BIS monomer for each evaluation. After electrophoresis and visualization of the 15%T,3%C acrylamide supports, comparison of the protein mobilities indicated that in all cases, the proteins had a greater migration in the novel gels relative to those containing BIS (*Figure 4*).

As measured by percentage enhancement of protein mobility with respect to the standard BIS matrix (*Figure 5*), the polymer incorporating monomer **1** showed enhanced mobility in the large molecular size protein region (200–45 K), whereas the lower molecular weight markers ran only marginally faster than in the BIS equivalent. The reverse trend is essentially observed in the polyacrylamide network comprising monomer **3**, where the high molecular weight protein range exhibit migrations comparable with the BIS standard while the 31–14 K protein range show significant enhancement of mobility, with the smallest

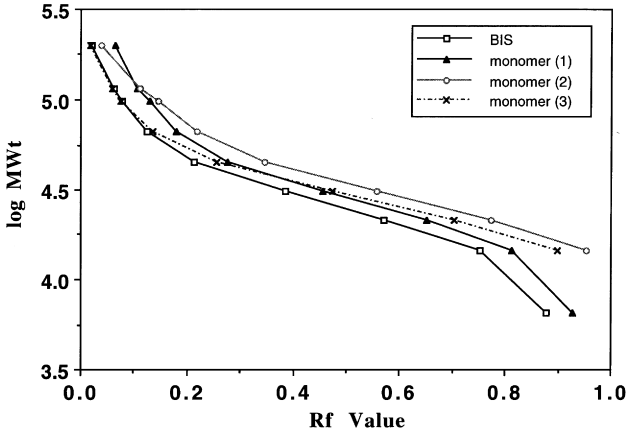

Figure 6 Ferguson plots of $\log_{10}$(molecular weight) *versus* protein mobility

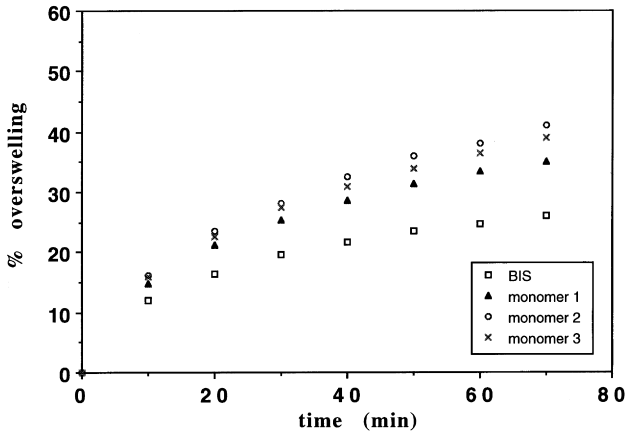

Figure 7 Graphs of percentage excess water uptake over time for gels incorporating the new monomers and for a standard BIS containing polymer

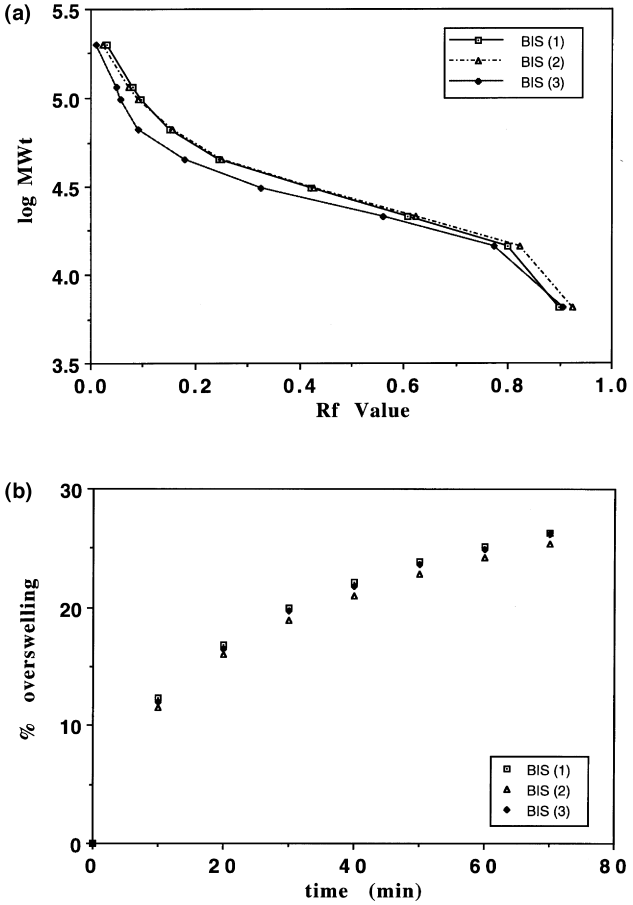

Figure 8 Comparison of BIS gels prepared on different days for (a) separation profile for a standard set of proteins and (b) overswelling results

protein (6.5 K) in the sample co-incident with the dye front. The network containing **2** displays increased mobility over the full range of molecular dimensions investigated, with the smallest protein (6.5 K) also running with the dye front, similar to what is observed in the gel prepared with monomer **3**. As with the other crosslinking agents evaluated, the polymer containing **2** does show preference for a specific molecular size, with proteins in the order of 100 K showing a substantial mobility differential with respect to the BIS crosslinked support.

Ferguson plots of the resultant protein mobilities clearly display this enhancement in migration via the translation to the right of the data points in all new crosslinking systems investigated (*Figure 6*). Furthermore, the classical line shape between $\log_{10}$(protein molecular weight) and mobility (R_f) holds in these novel gel systems, with the curve corresponding to the gel with **2** approaching linearity over the full molecular range investigated. The 6.5 K protein does not appear on the Ferguson plot for the gels containing monomers **2** and **3** due to its proximity to the tracking dye.

The increased overall porosity of these systems also manifests itself in the overswelling experiment. All gels incorporating the new crosslinking agents were found to uptake the solvent (water) more rapidly and encapsulate a greater amount of water than the equivalent BIS gel. As can be seen in *Figure 7*, polyacrylamide gels with the BIS monomer overswells by 26%, while the gels incorporating the crosslinking agents **1**, **2** and **3** overswell by 35, 39 and 41% respectively. Water uptake occurred rapidly in the initial stages with a plateau reached in a similar time to that observed with the control gel prepared with BIS. These observations for polymer overswelling are consistent with the increased porosity found in the electrophoretic evaluation of the novel crosslinking agents, where the order of increase on migration is reflected in the order of increased overswelling ability.

Comparison of the BIS crosslinked material prepared on different days show that reproducibility of the acrylamide supports prepared at room temperature was good, in accordance with the findings of Weiss et al.[17]. The reproducibility was evidenced by comparison of the protein mobilities as well as the overswelling profiles of the gels containing the BIS monomer prepared under the standard conditions (*Figure 8a* and *b*). This suggests that the polymer macrostructure was consistent under the polymerization conditions employed.

The novel crosslinking agents evaluated here may be envisaged as stepwise refinements of BIS. Elongation of the methylene unit in the BIS monomer to the ethylene tether contained in **3** increases the distance between crosslinking functionalities in molecular terms. This elongation effect has been implicated in other acrylamide networks where it was found to make significant changes to the resultant network[18]. Replacement of an acrylamide reactive functionality by a methacrylamide unit as in **1** alters the relative reactivity of the polymerizable moieties and affects the manner in which the network is assembled. Both of these

features are incorporated into **2**, which comprises an elongated tether and differentiated vinyl functionalities.

In all cases the crosslinking agents investigated afforded acrylamide networks which, from their electrophoretic and overswelling performance, are different to that of the BIS crosslinked material. As seen from *Figure 6*, proteins migrating through a polymer comprising of monomer **1** displayed an appreciable enhancement in mobility in the high molecular weight region of the protein sample, while the converse trend was observed for the polymer prepared with monomer **3**. When these two features were coupled together in **2**, the afforded material exhibits an additive effect of the two structural refinements to show the greatest overall increase in porosity of the crosslinking agents evaluated.

Weiss et al.[17] proposed a two-phase model for the formation of polyacrylamide networks, in which highly crosslinked, small pore regions resembling microgel-like structures were generated during the initial stages of the polymerization, consuming the majority of the crosslinking monomer present within the monomer solution. Subsequent interchain crosslinking then produced an overall large pore structure for the gel due to depletion of the crosslinking agent within the remaining monomer mixture.

Thus, the ability of the high molecular weight proteins to penetrate further into the gel when the crosslinking component is either monomer **1** or **2** is indicative of an increase in the average pore size within the three-dimensional networks generated. It is therefore proposed that the reactivity differential between the acryloyl and methacryloyl groups present in these compounds allow the more reactive methacryloyl portion of the crosslinking monomer to be the first unit incorporated into the growing copolymer chain. This produces highly crosslinked regions or 'clusters' prior to crosslinking, ultimately resulting in a bimodal pore size distribution within the three-dimensional network. Although the final effect is more pronounced as a result of the greater reactivity differential between the two vinylic units within the crosslinking agents, it is in general agreement with the proposal put forth by Weiss et al.[17], in which the network is generated by stepwise construction and comprised of small pore regions within the cluster and relatively large pores as a result of intermolecular bridges.

Additionally, there appears to be an increase in the overall pore volume exposed to the protein sample by polyacrylamide gels comprising monomers **2** and **3**, as measured by the significant enhancement of mobilities for the medium to small molecular weight proteins (*Figure 5*) and the overswelling experiments in which water was able to be encapsulated to a greater extent within the larger pore volume within these matrices (*Figure 7*). Moreover, the greatest mobility enhancement displayed by the medium sized proteins further supports a bimodal pore size distribution comprising small and large pores co-existing within the network structure. Within this type of configuration, proteins of molecular dimensions greater than the limiting pore size would be separated on the basis of molecular sieving as they move through the large pore structures within the gel, whereas small macromolecular systems would be exposed to the total pore volume and be impeded in their progression through the network. The greatest enhancement in mobility would therefore be seen for molecular sizes of less than the limiting pore size and greater than the minimum pores within the clusters. Consequently, these macromolecules would be channelled through the network with a minimum of gel volume. If the porosity of the systems was solely attributed to an overall increase in pore size within the network, a significant and proportional mobility enhancement would have been expected for all proteins whose size was less than the limiting pore size of the network.

MATERIALS AND METHODS

Instrumentation

Nuclear magnetic resonance (n.m.r.) spectra were obtained in deuterochloroform (99.9%) (Cambridge Isotope Laboratories), using a Varian Unity 300 or Varian Unity Plus 400 spectrometer. High and low resolution mass spectra were recorded either on a V.G. Micromass 7070F instrument or a JEOL JMS-AX505H Mass spectrometer using positive ion electron ionization (EI) techniques. Infrared (i.r.) spectra were recorded on a Bio-Rad FTS-60A FTIR spectrophotometer as potassium bromide (KBr) discs. Microanalyses were performed by Chemical and Micro Analysis Services Pty. Ltd. (CMAS) (Melbourne, Australia) or NAL Australian Microanalytical Services (Melbourne, Australia).

Reagents

Acryloyl and methacryloyl chloride (Aldrich Chemical Company) were filtered through basic alumina and distilled prior to use. Ethylenediamine (Fluka) was dried over calcium oxide and potassium hydroxide, and distilled before use following the method described by Perrin and Armarego[19]. Electrophoresis grade ($>$ 98%) acrylamide, sodium dodecyl sulfate (SDS) and 3-(dimethylamino)propionitrile (DMAPN) were obtained from Aldrich Chemical Company Inc. Methacrylamide, *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), glycine and all organic solvents were of analytical grade. 1,2-dichloroethane and acetonitrile were distilled over calcium hydride and stored over 4 Å molecular sieves.

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. Gels solutions were diluted to the required concentration and prepared according to the method of Laemmli[14]. Subsequently, the 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 cassettes of 80 mm $\times$ 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 was also prepared in the same manner and

polymerized on top the network polymers under investigation. For each gel comprising the novel monomer, a reference BIS gel was prepared at the same time.

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 β (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 each gel, and electrophoresis performed under the SDS-PAGE discontinuous buffer conditions of Laemmli[14]. 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 R_f values, which is defined as the ratio of the distance moved by the protein to the distance moved by the tracking dye.

Overswelling

For overswelling studies, duplicate samples of approximately 35 × 40 mm were sectioned from freshly prepared gels, blotted of excess surface moisture using Whatman 3MM paper and weighed immediately. 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 gels were blotted of excess water and reweighed.

EXPERIMENTAL

N-*methylolacrylamide (4)*

Sodium (5 mmol, 0.12 g) was finely cut and dispersed in 1,2-dichloroethane (60 ml) and heated at 50°C with stirring for 20 h. To this was then added acrylamide (50 mmol, 3.5 g) and crushed paraformaldehyde (50 mmol, 1.5 g), and the solution heated at 50°C for 30 min. The hot solution was then filtered and the filtrate cooled to − 4°C to afford *N*-methylolacrylamide (**6**) as colourless needles (4.3 g, 84%), m.p. 66–68°C (literature m.p.[11], 74–75°C). ν_{max} (KBr)/cm^{-1}: 3263, 3070, 2988, 2945, 2889, 1672, 1629 and 1554. δ_H (300 MHz): 6.96 (1H, br s), NH; 6.35 (1H, dd, J = 16.5, 1.2 Hz), CH=CH$_a$H$_b$; 6.15 (1H, dd, J = 16.7, 10.3 Hz), CH=CH$_a$H$_b$; 5.74 (1H, dd J = 10.3, 1.2 Hz), CH=CH$_a$H$_b$; 4.83 (2H, d, J = 6.9 Hz), CH$_2$. δ_C (75.4 MHz): 166.7, C=O; 130.4, CH=CH$_2$; 128.0, CH=CH$_2$; 67.1, CH$_2$. *m*/*z* 101 (M$^+$., 1.6%, 70 eV), 100 (5), 85 (28), 84 (53), 54 (100).

N-*acryloyl*,N′-*methacryloyl methylenediamine (1)*

A mixture of *N*-methylolacrylamide (**4**) (80 mmol, 8.1 g) and methacrylamide (80 mmol, 6.8 g) in 1,2-dichloroethane (60 ml) and conc. HCl (0.5 ml) was heated at reflux for 30 min. The hot solution was filtered and the filtrate was cooled to room temperature. The crude product was collected and recrystallized from ethanol to give *N*-acryloyl,*N*′-methacryloyl methylenediamine (**1**) as colourless needles (6.92 g, 51%), m.p. 163–165°C decomp. (lit. m.p.[9], 160–162°C decomp.). (Found: C, 57.0; H, 7.2; N, 16.3. Calc. for $C_8H_{12}N_2O_2$: C, 57.12; H, 7.21; N, 16.65%). ν_{max} (KBr)/cm^{-1}: 3285, 3061, 2961, 2927, 1654, 1611 and 1536. δ_H (400 MHz): 7.65 (2H, br s), 2 × NH; 6.30 (1H, dd, J = 17.1, 2.1 Hz), CH=CH$_a$H$_b$; 6.18 (1H, dd, J = 17.1, 9.7 Hz), CH=CH$_a$H$_b$; 5.75 (1H, br m), (H$_3$C)C=CH$_c$H$_d$; 5.65 (1H, dd, J = 9.8, 2.0 Hz), CH=CH$_a$H$_b$; 5.36 (1H, br m), (H$_3$C)C=CH$_c$H$_d$; 4.70 (2H, t, J = 6.0 Hz), CH$_2$; 1.95 (3H, s) CH$_3$. δ_C (75.4 MHz): 169.3, O=C(H$_3$C)C=CH$_2$; 166.4, O=CCH=CH$_2$; 139.0, (H$_3$C)C=CH$_2$; 130.5, CH=CH$_2$; 127.2, CH=CH$_2$; 120.9, (H$_3$C)CH=CH$_2$; 44.9, CH$_2$; 18.4, CH$_3$. *m*/*z* 168 (M$^+$., 2.2%, 10 eV), 113 (41), 99 (66), 69 (100), 54 (91). Found: M$^+$., 168.0900. $C_8H_{12}N_2O_2$ requires 168.0898.

N-*acryloyl*,N′-*methacryloyl ethylenediamine (2)*

A solution of ethylenediamine (15 mmol, 10.1 ml) in water (150 ml) at 0°C was adjusted to pH 8.5 with 3 N HCl. To this, was added a solution of methacryloyl chloride (16.5 mmol, 16.1 ml) in chloroform (100 ml), dropwise, over 2 h. After the addition was complete, the reaction mixture was stirred at 0°C for a further 2 h. The organic and aqueous layers were then separated, and the aqueous layer extracted with chloroform (3 × 50 ml). The aqueous layer was then collected and concentrated under reduced pressure to give a white solid. The residue was washed repeatedly with room temperature methanol. Solid residues were removed and the filtrate concentrated to give the intermediate *N*-methacryloyl-ethylenediamine monohydrochloride (**5**) as a clear, yellow oil (14.32 g, 58%). The monohydrochloride intermediate (**5**) (16 mmol, 20.0 g) was then stirred in 2 M NaOH (60 ml) for 10 min. Water (100 ml) was then added and the solution cooled to 0°C. To this mixture was added dropwise, a solution of acryloyl chloride (17.6 mmol, 14.3 ml) in chloroform (300 ml) over 3 h. After the addition was complete, the reaction mixture was then stirred at 0°C for a further 2 h. The organic and aqueous layers were separated, and the aqueous portion further extracted with chloroform (3 × 100 ml). The organic extracts were combined and concentrated to give the crude product as a white solid. Recrystallization from acetonitrile then gave *N*-acryloyl,*N*′-methacryloyl ethylenediamine (**2**) as colourless needles (10.70 g, 37%), m.p. 140–142°C. (Found: C, 59.5; H, 7.7; N, 15.2. $C_9H_{14}N_2O_2$ requires: C, 59.3; H, 7.8; N, 15.2%). ν_{max} (KBr)/cm^{-1}: 3298, 3087, 2943, 2864, 1656, 1614 and 1544. δ_H (400 MHz): 6.84 (1H, br s), NH; 6.74 (1H, br m), NH; 6.27 (1H, dd, J = 16.9, 1.5 Hz), CH=CH$_a$H$_b$; 6.13 (1H, dd, J = 17.0, 10.2 Hz), CH=CH$_a$H$_b$; 5.74 (1H, br m), (H$_3$C)C=CH$_c$H$_d$; 5.65 (1H, dd, J = 10.3, 1.6 Hz), CH=CH$_a$H$_b$; 5.34 (1H, br m), (H$_3$C)C=CH$_c$H$_d$; 3.49 (4H, m), 2 × CH$_2$; 1.95 (3H, s), CH$_3$. δ_C (75.4 MHz): 169.4, O=C(H$_3$C)C=CH$_2$; 167.0, O=CCH=CH$_2$; 139.2, (H$_3$C)C=CH$_2$; 130.7, CH=CH$_2$; 126.2, CH=CH$_2$; 120.1, (H$_3$C)C=CH$_2$; 40.4, CH$_2$CH$_2$; 39.5, CH$_2$CH$_2$; 18.4, CH$_3$. *m*/*z* 182 (M$^+$., 0.3%, 10 eV), 111 (41), 99 (25), 98 (42), 97 (24), 84 (36), 69 (100), 53 (53). Found: M$^+$., 182.1059. $C_9H_{14}N_2O_2$ requires 182.1055.

N,N′-*bisacryloyl ethylenediamine (3)*

A stirred solution of acryloyl chloride (37.5 mmol, 3.1 ml) in acetonitrile (50 ml) was added dropwise to a solution of ethylenediamine (37.4 mmol, 2.5 ml) in acetonitrile (50 ml), maintaining the temperature between 0 and 4°C. The reaction was subsequently warmed to room temperature and stirred for a further 3 h. The resulting precipitate was removed and washed with hot acetonitrile (50 ml). The filtrates were combined and concentrated to give a white solid which was recrystallized from acetonitrile

to afford *N*,*N*′-bisacryloyl ethylenediamine (**3**) as colourless plates (1.51 g, 48%), m.p. 142–143°C (lit. m.p.[10], 144–145°C) (Found: C, 57.1; H, 7.2; N, 16.4. Calc. for $C_8H_{12}N_2O_2$: C, 57.1; H, 7.2; N, 16.4%). ν_{max} (KBr)/cm^{-1}: 3254, 3082, 12992, 2960, 2941, 2883, 1657, 1624 and 1565. δ_H (400 MHz): 6.78 (2H, br s), 2 × NH; 6.26 (2H, dd, J = 17.0, 1.6 Hz), 2 × CH=CH$_a$H$_b$; 6.13 (2H, dd, J = 17.1, 10.3 Hz), 2 × CH=CH$_a$H$_b$; 5.65 (2H, dd, J = 10.3, 1.5 Hz), 2 × CH=CH$_a$H$_b$; 3.50 (4H, m), 2 × CH_2. δ_C (100 MHz): 166.9, C=O; 130.6, CH=CH_2; 129.7, CH=CH_2; 40.0, CH_2. *m*/*z* 168 ($M^{+\cdot}$, 0.15%, 15 eV), 97 (42), 85 (26), 84 (46), 54 (100). Found: M^+. 168.0897. $C_8H_{12}N_2O_2$ requires 168.0898.

CONCLUSION

From investigations based on electrophoretic migrations and overswelling studies, it has been shown that simple structural changes to the crosslinking monomer afforded networks with a larger average pore size and pore volume than the traditional BIS crosslinked polymers, and hence addresses some of the limitations previously observed for BIS[13]. From these observations, we have instigated a rational design and evaluation of crosslinked polyacrylamide gels with the aim to obtain a greatly increased number of crosslinking agents which can be applied to different problems as is currently possible in affinity chromatography supports.

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