Effect of the molecular character and extent of crosslinking on the reactivity of attached amino groups in crosslinked polyacrylamide gels

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The dependence of the reactivity of the attached amino groups in crosslinked polyacrylamide gels on the extent of crosslinking and the molecular character of the crosslinks has been studied. Acrylamide has been copolymerized with N, N'-methylene-bis-(acrylamide) (NNMBA), tetraethyleneglycol diacrylate (TEG-DA) and divinyl benzene (DVB) in different proportions to afford crosslinked polyacrylamide gels of varying nature and extent of crosslinking. These gels have been functionalised with amino groups by treating with excess ethylenediamine. The extent of amino functionalisation is found to be dependent on the nature and degree of crosslinking. The reactivity of amino groups in these polyacrylamide gels has been investigated by following the course of aminolysis of p-nitrophenyl acetate with these resins. These studies reveal a significant influence of the nature and extent of crosslinking on the aminolysis reaction. The polarity of the crosslinking agent and its molar percentage affect the extent of swelling and solvation which are decisive in the facilitation of the aminolysis of the p-nitrophenyl active ester.

The reactivity of a group attached to a polymer backbone is governed by the characteristic structural features of the polymer support like its polarity, nature and extent of crosslinking and the solvation of the support and the bound species. Polyacrylamide-type supports have been used for the solid-phase synthesis of peptides and for the preparation of a number of polymeric reagents. These supports were found to have entirely different characteristics in terms of solvation and reactivity compared to the commonly used polystyrene supports. Investigations on the effect of the nature and extent of crosslinking on the reactivity of amino groups attached to polyacrylamides (PA) crosslinked with different crosslinking agents in varying amounts are described in this paper. Aminolysis of the chromogenic substrate, p-nitrophenyl acetate, was taken up for study as the model reaction for the analysis of these factors, since the reaction can be followed easily by measuring the optical density of the liberated p-nitrophenol which is a measure of the extent of reaction. A comparison of the reactivity of amino groups attached to different resins has been made.

Materials and Methods

Acrylamide and N,N'-methylene-bis-(acrylamide) (SRL, Bombay) were recrystallised and used. Di-vinyl benzene and tetraethyleneglycol diacrylate (Aldrich, W. Germany) were used as such. All solvents used were reagent grade and were purified before use by distillation.

Preparation of crosslinked polyacrylamides: Polyacrylamides crosslinked with N,N'-methylene-bis-(acrylamide) (2a-2f)

Ammonium persulphate (100 mg) was dissolved in water (70 ml) at 70°C. The monomer mixture was added to this solution with stirring until the whole substance was dissolved. Heating and stirring were continued till the polymer was precipitated. Water (50 ml) was added and contents heated at 80°C for 20 min. The lumps of polymer were powdered, filtered, washed with water (20 ml x 3 times), methanol (30 ml x 3 times) and dried in an oven at 80°C.

Extents of crosslinking were adjusted by varying the relative compositions of acrylamide and N, N'-methylene-bis-(acrylamide). Resins with 4, 8, 10, 12, 16 and 20 mole per cent crosslinking were prepared.

Polyacrylamides crosslinked with tetraethyleneglycol diacrylate (3a-3d)

Tetraethyleneglycol diacrylate was washed with sodium hydroxide solution (1%, 10 ml x 2 times) and with water (20 ml x 3 times) to remove the inhibitor. The mixture of monomers was dissolved in methanol,
ammonium persulphate (100 mg) was added to it and the contents were heated on a water bath. The mixture was stirred until the polymer precipitated; heating was continued for 30 min more and the polymer filtered, washed with water (20 ml × 3 times) and methanol (30 ml × 3 times) and dried at 70°C.

**Polyacrylamides crosslinked with divinylbenzene** (4a-4d)

Divinylbenzene was washed with sodium hydroxide solution (1%, 10 ml × 2 times) and water (20 ml × 3 times) to remove the inhibitor. Acrylamide and divinylbenzene were dissolved in methanol (50 ml). Benzoyl peroxide (100 mg) was added to the solution and it was heated on a water bath with stirring. Heating and stirring were continued until the polymer got precipitated. The resin was collected by filtration, washed with water (20 ml × 3 times), methanol (30 ml × 3 times) and with benzene (30 ml × 3 times). The resin was dried in an oven at 80°C. Polymers with extent of crosslinking 5, 10, 15, and 20 mole percent were prepared by varying the monomer ratio.

**Trans-amidation of polyacrylamides to poly(N-2-aminoethylacrylamide): Preparation of resins (5, 6e-6f, 7a-7d, 8a-8d)**

Ethylenediamine (100 ml) was taken in a round-bottomed flask and crosslinked polyacrylamide (10 g) added with stirring. The addition was made gradually in order to avoid lumping, and the mixture was heated at 90°C for 9 h. The reaction mixture was poured into 500 ml of water containing crushed ice. The resin was filtered, washed with 0.1 M NaCl solution until the filtrate was free from ethylenediamine as indicated by the absence of colouration with the ninhydrin reagent. The gel was then washed with water and methanol, drained and dried in vacuo. A portion of the sample was heated with 2 ml of ninhydrin reagent. A deep blue colour was obtained indicating the presence of free amino group.

**Kinetics of hydrolysis of p-nitrophenyl acetate with the differently crosslinked amino resins: General procedure**

p-Nitrophenyl acetate (50 mg, 0.267 mmol) was dissolved in dioxane (5 ml) and the solution diluted to 100 ml with water. One ml of the solution was withdrawn, made up to 10 ml and was used as the blank solution. The amino resin (0.5 mmol) was added to this solution and the contents were shaken well. After 5 min, 1 ml of the solution was withdrawn, diluted to 10 ml and the optical density was measured spectrophotometrically at 400 nm using a Thoshniwal model VIS spectrophotometer. The experiment was repeated after the passage of 10, 20, 30, 40, 60 and 80 min. A standard curve was constructed by plotting the optical density against the concentration. From the curve, the amount of p-nitrophenol liberated at different time periods was determined. The rate constant for the reaction was calculated using the second order rate equation. The results are given in Table 1.

**Results and Discussion**

Acrylamide was copolymerized with varying mole percentages of different crosslinking agents to prepare the crosslinked polyacrylamides. The crosslinking agents selected were N, N'-methylene-bis-(acrylamide) (NNMBA), tetraethyleneglycol diacrylate (TEGDA) and divinylbenzene (DVB). The preparation of the differently crosslinked polyacrylamides is depicted in Scheme 1.

Polyacrylamides crosslinked with N,N'-methylene-bis-(acrylamide) (2a-2f) were prepared by free radical solution polymerization of the monomer mixture in water using ammonium persulphate as initiator. In the case of polyacrylamides crosslinked with tetraethyleneglycol diacrylate (3a-3d), polymerization reaction was carried out in methanol at 60°C in the presence of ammonium persulphate as initiator. The resulting polymers were characterized by IR spectroscopy and elemental analysis. The IR spectrum showed peaks at 1680 (C=O, amide) and 1740 (C=O, ester) cm⁻¹. The acrylamide-DVB copolymers (4a-4d) were prepared by the solution polymerization of the monomers in methanol/dioxane mixture at 70°C with benzoyl peroxide as the initiator. The extent of crosslinking of the polymers was adjusted by changing the mole percentage of the crosslinking agent. Linear polyacrylamide (1) was also prepared.

The crosslinked polyacrylamides were functionalized to poly (N-2-aminoethylacrylamides) by treatment with excess ethylenediamine. The amino resins were characterized by IR spectroscopy, nitrogen analysis and semiquantitative ninhydrin reaction. The resins gave a deep blue colour on heating with ninhydrin reagent. The preparation of poly (N-2-aminoethylacrylamides) is depicted in Scheme 2.

The capacities of the amino resins were determined by treating with excess HCl and back-titration. The capacities varied depending on the molecular character and the amount of the crosslinking agent in the copolymer. In the case of resins derived from PA-N-NMBA copolymers, the amino group capacities varied from 4.27 to 0.97 mmol/g from the linear polymer to the 20% crosslinked polymer. The amino resin derived from linear polyacrylamide showed maximum capacity. Among the PA-TEGDA resins, the amino
The reactivity of amino groups in crosslinked polyacrylamide gels was investigated. The effect of the extent of crosslinking on the reactivity of the amino group was analysed. Scheme 1 illustrates the synthesis of the crosslinked polyacrylamide gels.

The second order rate constants for the reaction were calculated. The method of log-linear least squares was used to fit the data to second order kinetics. In all the cases, the slope and hence the rate constant were calculated. The values approaching unity for the experimental rate constants agree with the second order kinetics. From the results obtained, the effect of the following structural features of the polymer support on the reactivity of the attached amino group was analysed.

The change in the rate of aminolysis with increase in the extent of crosslinking depends on the nature of the resins. In the case of PA-NNMBA resins, both acrylamide and N, N'-methylene-bis-(acrylamide) are hydrophilic due to the presence of polar amide groups and the solvation of the polymer is not very much dependent on the extent of crosslinking. The rate constant and the extent of aminolysis first decreased from linear to 4% crosslinked resin and then increased to maximum at 8% crosslinking. After that, there was a regular decrease in the rate as the degree of crosslinking increased, and the rate was found to be minimum for the 20% crosslinked resin.

With the amino polymers derived from acrylamide-TEGDA resins, the aminolysis occurred at a faster rate than that with PA-NNMBA polymers. With 5%
crosslinked resin (7a), about 60% aminolysis occurred after a period of 80 min with a rate constant of 2.604 l.mol\(^{-1}\)m\(^{-1}\). With 10% crosslinked resin (7b), the extent of aminolysis was 70% and the rate constant was 3.653 l.mol\(^{-1}\)m\(^{-1}\). The rate decreased gradually with increasing crosslinking after this.

In the case of amino polymers from PA-DVB resins, a considerable reduction in rate was observed with increasing extent of crosslinking. With 5% crosslinked resin (8a), the extent of aminolysis was only 29.2% and with the 20% crosslinked resin (8d), the percentage aminolysis was 5.8% after a period of 80 min.

In the solvolysis of p-nitrophenyl esters catalysed by crosslinked polyacrylamide resins containing 4-acrylamidopyridine residues, Kau et al.\(^{10}\), observed that the rate of the reaction increased with increase in the extent of crosslinking, passed through a maximum and then decreased. Thus, two opposing factors seem to affect the reactivity of the gel. The explanation suggested for this observation is that the catalytic pyridine groups are inaccessible to reagents in the continuous phase if the highly crosslinked resin is only slightly swollen and that the effective solvent medium is less favourable for the reaction if the swelling is too large. The increased rate with increase in the crosslink density is also attributable to the hydrophobic-hydrophilic balance of the reagent which makes it compatible with the substrate. A similar effect was observed in the oxidation of alcohols by N-bromopolyacrylamide where the rate was maximum for the reagent derived from the 10% crosslinked polymer\(^{11}\). The rate of hydrolysis for the linear resin 1 was faster than that for the NNMBA- and DVB-crosslinked resins and slower as compared to that for TEGDA-crosslinked resin.

**Effect of the nature of the crosslinking agent**

The investigation of the aminolysis reaction with different resins revealed that the nature of the crosslinking agent in the copolymer had a definite influence on the reactivity of the bound amino group. The amino polymers 7a-7d derived from PA-TEGDA copolymers showed significant increase in the reactivity of the amino group. In the case of the amino polymers derived from PA-NNMBA copolymers (6a-6f), the reactivity of the crosslinked resins was less than that of the linear polymer (5). But in the former case the crosslinked polymers were more reactive than the linear ones and the reactivity increased with increase in the crosslink ratio. For the 10% crosslinked amino resin from PA-NNMBA copolymer, the aminolysis was only 35.9% after 80 min, but the extent of reaction after the same period for the PA-TEGDA polymer

### Table 1—Rate constants and % aminolysis of p-nitrophenyl acetate with crosslinked poly (N-2-aminoethylacrylamide) resins

<table>
<thead>
<tr>
<th>Resin</th>
<th>Amino group capacity (mmol/g)</th>
<th>Extent of crosslinking (%</th>
<th>Rate constant (l.mol(^{-1})m(^{-1}))</th>
<th>Correlation coefficient</th>
<th>% aminolysis*</th>
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<tbody>
<tr>
<td>5</td>
<td>4.27</td>
<td>0</td>
<td>1.4428</td>
<td>0.953</td>
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<td>6a</td>
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<tr>
<td>6b</td>
<td>2.59</td>
<td>8</td>
<td>1.2914</td>
<td>0.985</td>
<td>39.9</td>
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<tr>
<td>6c</td>
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<tr>
<td>6d</td>
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</tr>
<tr>
<td>6e</td>
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<td>16</td>
<td>0.7214</td>
<td>0.933</td>
<td>22.2</td>
</tr>
<tr>
<td>6f</td>
<td>0.97</td>
<td>20</td>
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<td>0.932</td>
<td>16.9</td>
</tr>
<tr>
<td>7a</td>
<td>2.68</td>
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<td>0.994</td>
<td>60.7</td>
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<tr>
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<td>3.1880</td>
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<tr>
<td>7d</td>
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<td>20</td>
<td>2.2770</td>
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</tr>
<tr>
<td>8a</td>
<td>2.07</td>
<td>5</td>
<td>0.8669</td>
<td>0.970</td>
<td>29.2</td>
</tr>
<tr>
<td>8b</td>
<td>1.45</td>
<td>10</td>
<td>0.6417</td>
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<tr>
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<td>20</td>
<td>0.1809</td>
<td>0.942</td>
<td>8.8</td>
</tr>
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</table>

* After 80 minutes.
was 70.2%. In the case of PA-DVB resins (8a-8d), the reactivity was found to be less than that in the previous cases, and the rate of reaction decreased gradually as the degree of crosslinking increased. The reactivities of the crosslinked resins were less than that for the linear polymer.

The PA-TEGDA resins are more flexible than the PA-NNMBA resins due to the long tetraethyleneglycol diacrylate crosslinks. The enhanced reactivity of the PA-TEGDA resins can be attributed to this increased flexibility and also to the compatibility of the substrate and solvent. In the case of PA-DVB resin, crosslinking makes the resin more rigid and hydrophobic and the compatibility of the crosslinked resin with water becomes reduced. The swelling of the PA-DVB resin in water was found to decrease as the crosslink ratio was increased, and for the 20% resin actually no swelling was observed. This increased rigidity and decreased swelling can be the reasons for the low reactivity of these resins. A comparison of the rates of hydrolysis of p-nitrophenyl acetate with different resins is depicted in Fig. 1.

The foregoing results on the correlation between the reactivity of the bound amino functional groups and the structural parameters of the macromolecular matrix like the molecular character and the amount of the crosslinking agent in the crosslinked polyacrylamide gels are helpful in designing tailor-made polymers for use as supports for peptide synthesis, polymeric reagents and as matrices for enzyme immobilization. The reactivity of the bound functional groups, the polarity of the macromolecular matrix, degree of solvation and swelling, hydrophilic-hydrophobic balance and the mechanical integrity of the polymer matrix are mutually interdependent factors. Quantification of these closely interrelated parameters is necessary for a judicious selection of the structural variables of a polymer designed for a specific use as support for reagent functions, peptide synthesis or enzyme immobilization.

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