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Graft polymerization of acrylic acid onto macroporous polyacrylamide gel (cryogel) initiated by potassium diperiodatocuprate

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Abstract

Potassium diperiodatocuprate-initiated graft polymerization was found to be an efficient and convenient method for grafting of acrylic acid (AAc) onto superporous polyacrylamide gels, so called cryogels (pAAm cryogels). It was possible to achieve grafting degrees as high as 70% with about 25% yield of grafted polymer with respect to the initial amount of monomer. The superporous structure of the cryogels promoted grafting by providing an ample surface of the gel for grafting, ensuring good mass transfer inside the gel sample and allowing to wash easily both homopolymer of AAc and insoluble by-products formed during the polymerization reaction. The grafted cryogels could be dried at 60 °C and re-swollen with retaining their properties. The adsorption of water vapours by dried pAAm cryogels was marginally dependent on the degree of grafting. The swelling of AAc-grafted pAAm cryogel increased pronouncedly with increasing pH. The adsorption of low-molecular weight ligand, Cu(II), by AAc-grafted pAAm cryogels increased linearly with the degree of grafting, while binding of high-molecular weight ligand, lysozyme, increased linearly till the degree of grafting of about 40% followed by a sharp, nearly three-fold increase in lysozyme binding when the degree of grafting increased from 60 to 70%. The results indicate that a 'tentacle'-type binding of lysozyme to grafted polyAAc takes place after a certain degree of grafting has been reached.

Keywords: Supermacroporous polyacrylamide gel; Graft polymerization; Protein binding

1. Introduction

Hydrogels are formed by physically or chemically cross-linked three-dimensional polymer network capable of holding a large amount of water while at the same time maintaining their shape [1]. A low interface tension and hydrophilic properties make hydrogels highly biocompatible allowing their numerous applications in biotechnology and biomedicine including their use as chromatographic materials, carriers for immobilisation of molecules and cells, matrices for electrophoresis and immunodiffusion, scaffolds for cultivation of microbial and mammalian cells, implants and drug delivery systems [2]. The increasing demands in hydrogel for different applications require access to new types of hydrogels with improved properties. Grafting polymer chains onto the backbone of polymer materials has been pointed out as a convenient method for improving properties of polymer materials [3,4].

The hydrogels with terminally bounded polymer chains (grafted hydrogels) could be prepared by several methods. Grafted hydrogels were formed when the polymerization mixture contained macromonomer [5–9] or as the result of cross-linking of preformed soluble graft copolymer [10–12]. New thermo- and pH-sensitive hydrogels were obtained by this way. However, this approach demands the preparation of macromonomers or graft copolymers, which is time consuming and sometimes rather complicated. Moreover, it is difficult to control the localization and density of grafted polymer chains in such grafted hydrogels.

Alternatively, grafting polymers to the gel surface could be achieved via chemical bonding between reactive groups on the gel surface and reactive terminal groups of the preformed polymer (so called *grafting to*) [13–17]. The obvious advantage here is that one can beforehand determine the properties (molecular mass, MW distribution)

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of the to-be-grafted polymer. The problem is that the hydrogel should have reactive groups suitable for grafting and the grafted chain should carry the proper functionality at the end. It is very difficult to achieve high grafting densities using the *grafting to* methods because of steric crowding of reactive sites at the gel surface by already bound polymer molecules. Moreover, the efficiency of *grafting to* methods is pretty low resulting in pronounced losses of the terminally modified polymer.

Surface-initiated polymerization using initiator bound to surface (also called *grafting from*) is a powerful alternative to control the density and thickness of polymer brushes. It requires the formation of active sites on the backbone of hydrogel-forming polymer, the desired polymerization is initiated from these active sites. During the polymerization reaction, the polymer chains 'grow' from the surface. The graft-type hydrogel with long chains and high density of polymer grafted can be prepared [18]. Some un-grafted polymer is also formed in solution during the reaction decreasing the grafting efficiency. Using Ce(IV) as initiator is a widely used approach for graft polymerization of various vinyl monomers onto hydrogels containing hydroxyl or epoxy groups [19–23]. The density of hydroxyl groups on the support surface and the amount of catalyst used determine the density of the grafting. Hydrogels with high graft density were prepared by using this method [21].

With *grafting from* approach, one could expect grafting to occur mainly at the interface of the hydrogel and the liquid phase, as the diffusion of the monomers inside the gel phase is restricted especially for the gels with high polymer density. Thus, an attractive system for a surface grafting would be a hydrogel with large interconnected pores ensuring high surface area available for grafting and efficient mass transport. With high density of the gel phase, grafting takes place mainly at the gel–liquid interface.

An example of such material is macroporous polyacrylamide cryogels (pAAm cryogels) [24]. The pAAm cryogels (from the Greek κριοσ (kryos) meaning frost or ice) are produced by radical polymerization of acrylamide (AAm) and cross-linker *N*,*N*′-methylene-bis-acrylamide (MBAA) when the polymerization system is partially frozen after the onset of polymerization reaction. The ice crystals formed after partial freezing perform as porogen, while the dissolved monomers and initiator are concentrated in a small fraction of a non-frozen fluid in which polymerization proceeds. Despite the system looks apparently as a solid ice block, the polymerization proceeds in a very concentrated (non-frozen) monomer solution still left unfrozen and a dense polymer gel is formed. After melting, a continuous system of pores is formed in place of ice crystals, the pores are separated by walls of dense polymer gel. Macroscopically the gel has a sponge-like morphology.

The paper describes graft copolymerization of acrylic acid (AAc) on pAAm cryogel initiated by a free-radical technique using potassium diperiodatocuprate, $K_5[Cu(HIO_6)_2]$.

2. Materials and methods

Acrylic acid (AAc, 99% purity), acrylamide (AAm, more than 99.9% purity, electrophoresis reagent), *N*,*N*′-methylene-bis-acrylamide (MBAA), *N*,*N*,*N*′,*N*′-tetra-methyl-ethylenediamine (TEMED) and ammonium persulfate (APS) were from Aldrich (Aldrich, Steinheim, FRG). Lysozyme (from chicken egg white), copper sulfate and EDTA-tetrasodium salt were from Sigma (St Louis, USA). The buffer salts were of the best quality available.

2.1. Cryogenic co-polymerization of AAm with MBAA

The supermacroporous cryogels were prepared in glass tubes (total weight of monomers AAm + MBAA = 6%, AAm/MBAA = 8/1, the amount of TEMED as well as APS = 1.2% of total AAm + MBAA weight). The solution in the tubes was frozen at −12 °C and kept at this temperature for 20 h. After thawing and washing with water (200 ml) the gel matrix was dried at 60 °C and stored in dry state.

2.2. Preparation of potassium diperiodatocuprate (Cu(III)) solution

The Cu(III) solution was prepared according to Ref. [25] as follows: $CuSO_4 \cdot 5H_2O$ (3.54 g), KIO_4 (6.82 g), $K_2S_2O_8$ (2.20 g) and KOH (9.00 g) were added to 200 ml of deionised water. The mixture was boiled for 40 min. After cooling to room temperature, the mixture was filtered and the filtrate was diluted to 250 ml with deionised water. The final concentration of Cu(III) was 0.0562 M.

2.3. Graft polymerization of AAc onto pAAm cryogel monolith

Appropriate amounts of AAc and NaOH were mixed and the reaction solution was flashed with nitrogen for 10 min before Cu(III) solution was added. The total volume was adjusted to 10 ml with deionised water. Dry pAAm cryogel (0.15 ± 0.03 g) was soaked in the reaction solution. Polymerization was carried out for 2 hours at a defined temperature. The graft copolymerization was performed using different concentrations of NaOH, AAc and initiator and temperature. After the reaction was finished, then cryogels were washed with 0.1 M HCl followed by washing with an excess of hot deionised water.

The grafting is presented as grafting degree (*G*), density of AAc grafting (*D*) and grafting yield (*E*) of the grafting polymerization which were defined and calculated as follows:

$$G(\%) = \left[\frac{(W_1 - W_0)}{W_0}\right] \times 100\%$$

$$D_1(\text{mmol/g}) = \left[\frac{(W_1 - W_0)}{W_1}\right](1000/M_{\text{AAc}})$$

$$E(\%) = \left[\frac{(W_1 - W_0)}{W_2}\right] \times 100\%$$

where W_0 and W_1 are the weights (g) of original and grafted samples, respectively, and W_2 is a weight (g) of AAc added; M_{AAc} is the molecular weight of AAc, 72 Da. Alternatively, density of AAc grafting, D_2 was calculated from titration of grafted carboxy groups of AAc with NaOH (see below).

2.4. Characterization of grafted pAAm cryogel monoliths

The cryogel samples for scanning electron microscopy (SEM) was fixed in 2.5% glutaraldehyde in 0.12 M sodium phosphate buffer, pH 7.2 overnight, post-fixed in 1% osmium tetroxide for 1 h. Then the samples were dehydrated in ethanol (0–50–75–99.5%) and critical point dried. The dried sample was coated with gold/palladium (40/60) and examined using a JEOL JSM-5600LV scanning electron microscope.

The IR potassium bromide pellets with dried plain cryogel and grafted cryogel were recorded using an FTIR-8300 spectrophotometer (Shimadzu) in potassium bromide pellets.

The total number of carboxyl groups in the grafted pAAm cryogel was determined by conversion of the anionic form of the grafted cryogel to the protonated form by washing of samples with 0.1 M HCl. Excess acid was then removed by washing with deionized water. The sample of cryogel monolith was dried at 60 °C. The dried cryogel was cut on small pieces and 25 ml of 0.1 M NaOH containing 2 M NaCl was added. The H^+ ions were thus exchanged with Na^+ ions over 24 h to ensure the complete exchange. Then 10 ml supernatant was removed and titrated with 0.1 M HCl to pH 6.9–7.3 at slow stirring. The titration data were used to calculate the grafting density, D_2. The grafting density was determined as mmol of carboxyl groups per gram of dried cryogel.

The swollen cryogels (1 ml sample) were washed with 0.1 M NaOH and then with water until pH 7.0. Samples were dried in an oven at 60 °C till constant weight and the weight of the dried polymer was determined (m_1). The dried sample was placed into a water-saturated chamber with no direct contact of the sample with water. The increase in sample weight with time due to absorbed water vapor (m_2) was checked for 7 days. The amount of adsorbed water (q) was calculated as:

$$q(\text{g/g}) = \frac{(m_2 - m_1)}{m_1}$$

The swelling of AAc-grafted pAAm cryogel ($G = 70\%$) was measured by equilibrating the cryogel sample in buffer solutions with different pH values. The ionic strength of solutions was adjusted to 0.15 M by adding the required amount of sodium chloride salt. A monolith of grafted cryogel was put into a glass column (inner diameter 10 mm) equipped with upper and lower adapters and washed with 200 ml of buffer with a given pH value. Then cryogel was removed from the glass tube and immersed into buffer solution for 48 h. The weight of completely swollen cryogel was measured. Swelling capacity was determined as follows:

$$S(\text{g/g}) = \frac{(m_3 - m_1)}{m_1}$$

where m_3 is the weight (g) of swollen cryogel and m_1 is the weight (g) of dried cryogel, respectively.

Adsorption of Cu(II) ions by AAc-grafted pAAm cryogel was measured as follows. A monolith of grafted cryogel was put into a glass column (inner diameter 10 mm, 4 ml settled volume) equipped with upper and lower adapters and washed with an excess of 0.2 M $CuSO_4$. Then the column was washed with distilled water until the 730 nm readout was down to the base line. Elution with 0.1 M EDTA pH 7.3 was performed. Fractions of 5 ml were collected and optical density at 730 nm was measured. The quantity of metal ions adsorbed per unit of dry weight of the adsorbent (mmol/g) was calculated.

Adsorption of lysozyme by AAc-grafted pAAm cryogel was measured as follows. A monolith of grafted cryogel was put into a glass column (inner diameter 10 mm, 4 ml volume) equipped with upper and lower adapters and the column was connected to Biologic HR Chromatographic system (BioRad, Hercules, CA, USA). Lysozyme solution (1 mg/ml in running buffer, 20 mM Tris–HCl buffer, pH 7.0) was applied to the column followed by washing with running buffer until 280 nm readout was down to baseline. Elution was performed with 1.5 M NaCl solution in running buffer. Fractions of 5 ml were collected and optical density at 280 nm was measured. Lysozyme content was calculated using calibration curved (0.1–1 mg/ml) developed at 280 nm.

3. Results and discussion

The radical co-polymerization of AAm with MBAA in partially frozen reaction media, followed by defrosting and intensive washing results in the formation of sponge like hydrogel with highly porous structure (Fig. 1). The main part of the pAAm cryogel volume is composed of the interconnected large pores filled with water. Dry polymer constitutes only 3–4% of the total weight of completely swollen cryogel. Cryogels produced from polyacrylamide can be dried at 60 °C and re-swollen when submerged into water within less than a minute without deterioration of their supermacroporous structure [24]. Thus, during graft

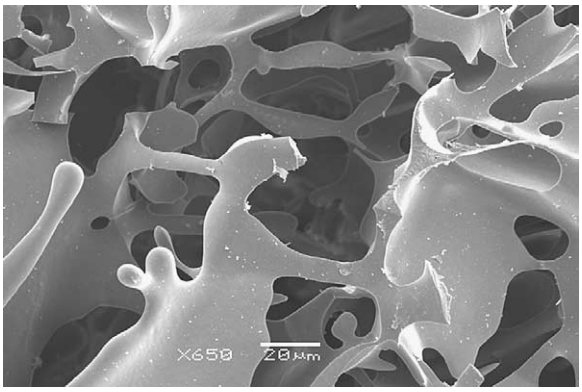

Fig. 1. SEM microphotograph of diametrical cross-sections of cryogel. The cryogel sample was fixed in 2.5% glutaraldehyde in 0.12 M sodium phosphate buffer, pH 7.2 overnight, post-fixed in 1% osmium tetroxide for 1 h. Then the sample was dehydrated in ethanol and critical point dried (see methods). The dried sample was coated with gold/palladium (40/60) and examined using a JEOL JSM-5600LV scanning electron microscope.

polymerization dry samples of pAAm cryogel were submerged into reaction solution reducing the time of contact of the polymer with highly alkaline reaction solution used. It was found that graft polymerization was mainly completed within 1 h (data not shown). In all our experiments reaction time was increased to 2 h to ensure the completion of the reaction. Potassium diperiodatocuprate (initiator of graft polymerization) was converted during reaction into insoluble products that precipitated inside the porous cryogel. Due to a highly porous structure of cryogel, the precipitate was easily removed by washing with 0.1 M HCl. A homopolymerization of AAc occurred also during graft polymerization. A homopolymer of polyacrylic acid formed was easily washed out of cryogel with an excess of hot water (around 45 °C).

The grafting was confirmed by comparing the FTIR spectrum of plain pAAm cryogel with that of the AAc-grafted pAAm cryogel (Fig. 2). The spectrum of AAc-grafted pAAm cryogel has a strong band at 1725 cm^{-1} corresponding to the carboxyl groups. This band is absent in the FTIR spectrum of plain pAAm cryogel. The carboxyl groups presented in the AAc-grafted pAAm cryogel could be related to the grafted AAc chains as well as carboxyl groups formed via partially hydrolysis of amide group during grafting under alkaline condition. There are the differences in both spectra at the wave numbers of 1100–1300 cm^{-1} with a band at 1245 cm^{-1} in the spectrum of AAc-grafted pAAm cryogel. This band could be related to the C–N stretching vibration and NH in-plane deformation of secondary amides [26]. The band at 788 cm^{-1} in the spectra of grafted pAAm cryogel could indicate NH out-of-plane deformation of –C(=O)NHR bond [26]. These data confirm initiation of the graft polymerization from the amide group of pAAm cryogel and formation of grafted polymer chain.

The presumable mechanism of the graft copolymerization of AAc onto pAAm cryogel is shown in Fig. 3. The amide group of the acrylamide cryogel backbone is activated by redox reaction with Cu(III) of potassium diperiodatocuprate ($K_5[Cu(HIO_6)_2]$) to form an amidyl radical, which is capable of initiating the polymerization of vinyl monomers [27].

The grafting procedure has been optimized with respect to the concentrations of NaOH, Cu(III) and AAc as well as reaction temperature.

Sodium hydroxide was used in grafting reaction for converting AAc to sodium salt and for adjusting pH of the reaction solution to highly basic conditions. This is essential because Cu(III) salts are stable only in highly alkaline medium [25]. Fig. 4 shows the effect of the NaOH concentration on graft polymerization parameters as grafting degree (G) and density of grafted AAc chains (D_1 and D_2). The grafting degree increased significantly with increasing NaOH/AAc ration up to 4.8 mol/mol. The increase in NaOH concentration could affect the equilibrium between different Cu(III) complexes formed with $H_3IO_6^{2-}$ and $H_2IO_6^{-3}$ anions hence affecting the efficiency of grafting reaction [28].

It should be noted that the density of grafted AAc chains, calculated from the weight of grafted AAc, D_1 was somewhat lower, than D_2 calculated from the titration of carboxyl groups. The reason could be that some of amide groups of polymer backbone are hydrolyzed under highly alkaline conditions forming 'additional' carboxy groups not accounted for AAc grafting. To reduce the hydrolysis of amide groups, the contact time of pAAm cryogel with alkaline reaction media was reduced to a minimum. However, at high grafting degrees the contribution of hydrolysis was less pronounced and the total number of carboxyl groups titrated was almost equal to the amount of AAc grafted.

The graft copolymerization of AAc onto pAAm cryogel was carried out at different temperatures ranging from 25 to 75 °C, respectively (Fig. 5). The grafting degree increased with increasing the reaction temperature from 25 to 45 °C. Further increase in the reaction temperature resulted in the decrease of the G and D_1 probably due to the increased rate of termination of grafted polymer chains. The difference between D_1 and D_2 was small at temperatures below 50 °C but increased progressively with temperature above 50 °C. As the temperatures above 50 °C were considered inappropriate for AAc grafting to pAAm cryogels due to the increased hydrolysis of amide groups, the optimum reaction temperature of 45 °C was selected for further experiments.

The grafting degree increased with increasing initiator concentrations leveling at about 0.015 M Cu(III) (Fig. 6). The increase may be attributed to increasing number of free radicals sites formed on the pAAm backbone. However, further increase in initiator concentration did not promote grafting. This may be due to a few factors. First, at higher concentrations, Cu(III) are able to participate in termination reactions thus decreasing the efficiency of grafting [28]. At the same time, a high number of macroradicals could

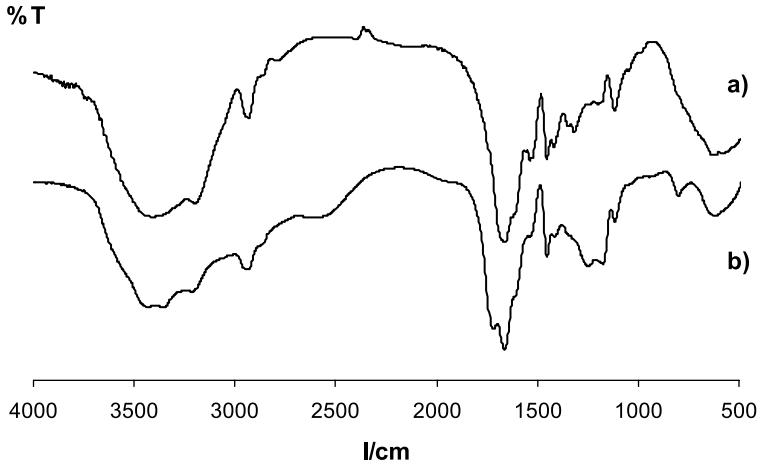

Fig. 2. FTIR spectra of plain (a) and AAc-grafted (b) pAAm cryogel.

accelerate the chain transfer reaction, leading mainly to homopolymer formation in solution. The increase in homopolymer formation decreased the amount of monomer available for graft polymerization. The formation of soluble homopolymer of AAc increased drastically the solution viscosity restricting also the mass transport of monomer and initiator to the gel surface. Moreover, as graft polymerization on the pAAm cryogel is induced by the redox system acrylamide-potassium diperiodatocuprate, the availability of amide groups of pAAm backbone defined the concentration of amidyl radicals formed. Thus, the degree of grafting increased with increasing Cu(III) concentration until the grafting started to be restricted by the concentration of amide groups available for the reaction with Cu(III).

Fig. 7 shows the effect of AAc concentration on grafting. The degree of grafting increased nearly linearly with increase in AAc concentration up to 0.6 M and then started to level off at concentrations 0.8–1.0 M. It should be noted that at grafting degrees above 30% the contribution of amide hydrolysis was negligible and both D_1 and D_2 were essentially equal.

The yield of graft polymerization (E), presenting the relative efficiency of graft polymerization versus homopolymerization, reached maximum at AAc concentration of about 0.7 M (Fig. 8). Nevertheless, the maximal yield of graft polymerization did not exceed 25% indicating that homopolymerization was the predominant reaction under all the conditions studied.

Thus, when using the redox system acrylamide-potassium diperiodatocuprate it was possible to achieve grafting of AAc on pAAm cryogel with grafting degrees up to 70%. The superporous structure of the cryogels used was beneficial for grafting as it provided an ample surface of the gel for grafting, good mass transfer inside the gel sample

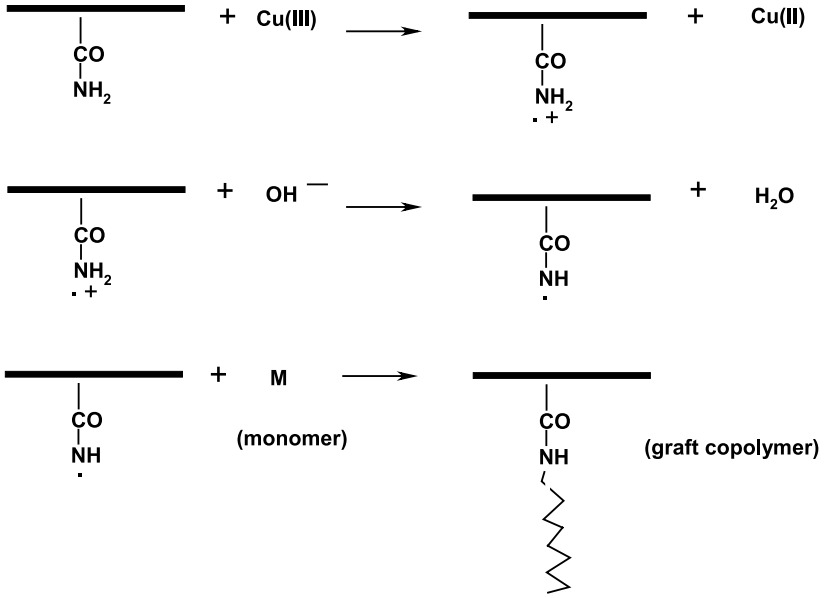

Fig. 3. The mechanism of initiation of graft polymerization onto pAAm cryogel using potassium diperiodatocuprate.

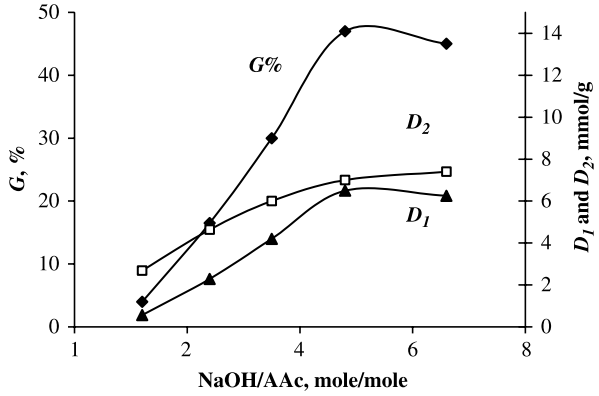

Fig. 4. The effect of NaOH on AAc grafting onto pAAm cryogel. G (%) is the degree of grafting, D_1 and D_2 (mmol/g) are the density of grafting calculated gravimetrically and by pH titration, respectively (see methods). Reaction conditions: Cu(III) concentration 0.021 M, AAc concentration 0.5 M, 45 °C.

and allowed to wash easily both homopolymer of AAc and insoluble by-products formed in the polymerization reaction.

Below are presented some properties of the AAc-grafted pAAm cryogels. One of the interesting properties of cryogels is that cryogels could be dried and re-swollen without loosing their properties [24]. AAc-grafting affected marginally water vapour adsorption by dried cryogels (Fig. 9). However, swelling of cryogel changed significantly with grafting and became pH-dependent (Fig. 10). Contrary to plain pAAm cryogel, swelling of grafted pAAm cryogel ($G=70\%$) was lower in acidic media while it increased two-fold in the pH-range 4.5–8.0, probably due to the ionization of carboxyl groups of grafted AAc. Both the repulsion of the negatively charged polymer chains and the presence of free counter-ions in the gel, which cause a high osmotic swelling pressure, could contribute to increased swelling.

We were trying to estimate the accessibility of carboxyl groups of grafted poly-AAc chains by measuring binding of both low-molecular-weight ligand (Cu(II) ions) and high-molecular-weight ligand (lysozyme, a protein with molecular weight of 14.4 kDa and positively charged under the conditions used) to COO^- groups.

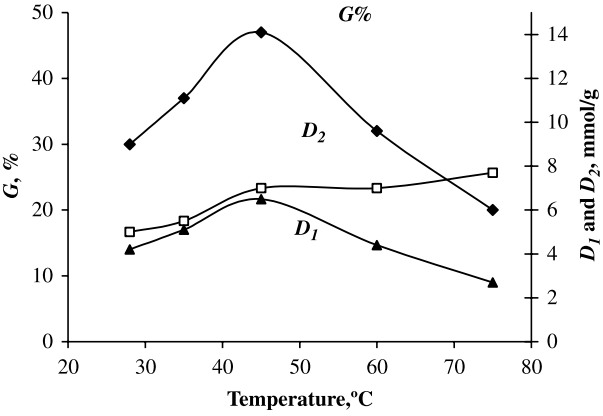

Fig. 5. The effect of temperature on AAc grafting onto pAAm cryogel. For explanation, see Fig. 4. Reaction conditions: Cu(III) concentration 0.021 M, AAc concentration 0.5 M, NaOH/AAc = 4.8 mol/mol.

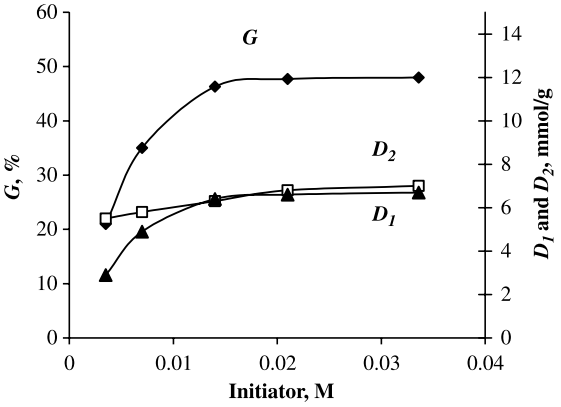

Fig. 6. The effect of Cu(III) concentration on AAc grafting onto pAAm cryogel. For explanation, see Fig. 4. Reaction conditions: AAc concentration 0.5 M, NaOH/AAc = 4.8 mol/mol, 45 °C.

The amount of titratable COOH groups of grafted AAc chains increased linearly from 4.0 to 9.0 mmol/g with increasing degree of grafting from 7 to 70%, respectively (Fig. 11, curve 1). At the same time, binding of Cu(II) ions increased linearly from 0.9 to 3.7 mmol/g (Fig. 11 curve 2). It is interesting to note that at low grafting degrees, one Cu(II) ion was bound to about four carboxyl groups, while at high grafting degrees, the number of carboxyl groups required for binding one Cu(II) ion was close to stoichiometric, i.e. 2. Probably, carboxyl groups of AAc grafts, located close to the dense cryogel surface are not available for Cu(II) binding. The longer the grafts, the higher the relative number of carboxyl groups accessible for Cu(II) binding, approaching the stoichiometric binding ratio.

In a mode different from of Cu(II) binding, the binding of the high-molecular-weight ligand, lysozyme, increased avalanche-like with increasing degree of grafting. A sharp, nearly three-fold increase in binding occurred when grafting

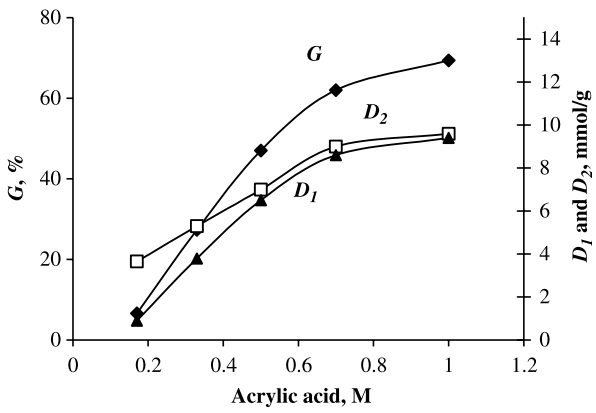

Fig. 7. The effect of AAc concentration on AAc grafting onto pAAm cryogel. For explanation, see Fig. 4. Reaction conditions: Cu(III) concentration 0.021 M, NaOH/AAc = 4.8 mol/mol, 45 °C.

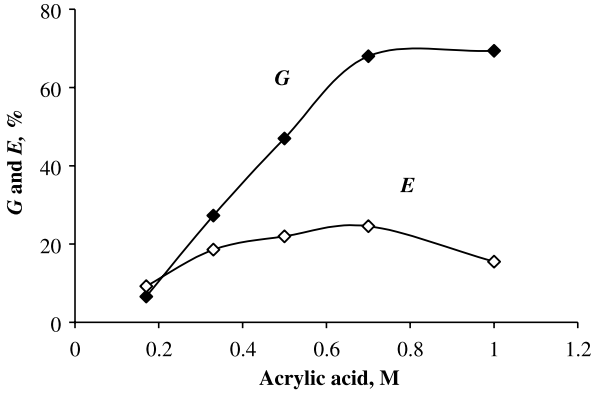

Fig. 8. The effect of AAc concentration on grafting yield (E). Reaction conditions: Cu(III) concentration 0.021 M, NaOH/AAc = 4.8 mol/mol, 45 °C.

degree increased from 60 to 70% (Fig. 11, curve 3). The rationale behind this finding could be as follows. At low degrees of grafting (below 40%), the AAc-grafted pAAm cryogel behaved as an adsorbent with ion-exchanging groups located at the interface of gel phase and liquid phase. Certainly, the capacity for lysozyme binding is increased as the number of ion-exchange groups increases with increased density of grafting. The capacity for protein binding remained in the range of capacities typical for modification of cryogel with monomeric ion-exchange groups [24,29]. However, after some critical degree of grafting (about 60%), the grafted poly-AAc chains started to protrude in solution performing as 'tentacles' capable of multipoint interactions with positively charged lysozyme molecules. The flexibility of these tentacles allowed them to change their conformation in order to adapt for the most efficient lysozyme binding and thus to increase the 'efficiency' of utilization of negative charges (Fig. 12).

Similar results on non-linear increase in protein binding were obtained for the anion-exchange resin having grafted polyglycidylmethacrylate chains modified with

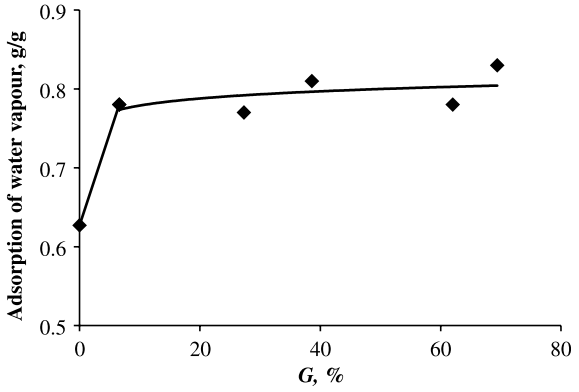

Fig. 9. Water vapor adsorption by dried AAc-gafted pAAm cryogels with different degrees of grafting. Dried samples were placed into a water-saturated chamber with no direct contact of the samples with water. The increasing of sample weight with time due to absorbed water vapor was checked for 7 days.

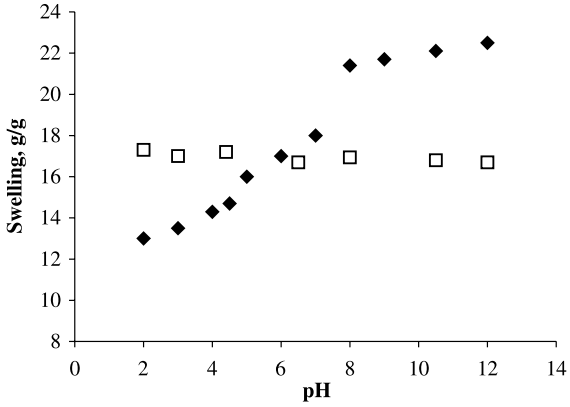

Fig. 10. Swelling of plain dried pAAm cryogel (open squares) and dried AAc-grafted pAAm cryogel with $G = 70\%$ (closed rhombus) in aqueous media with different pH. The ionic strength was justified to 0.15 M in each solution by adding the required amount of sodium chloride.

diethylamine [20]. A sudden increase in protein binding capacity occurred at a 'critical accessible ion ligand density' corresponding to a 'critical degree of grafting'. The same approach for promoting protein binding by using grafted charged polymers is used in so-called tentacle chromatography [30–33].

4. Conclusion

The potassium diperiodatocuprate was found to be an effective and cheap method for graft polymerisation of AAc onto pAAm cryogels. It was possible to achieve grafting degrees as high as 70% with about 25% yield of grafted polymer with respect to the initial amount of monomer. The

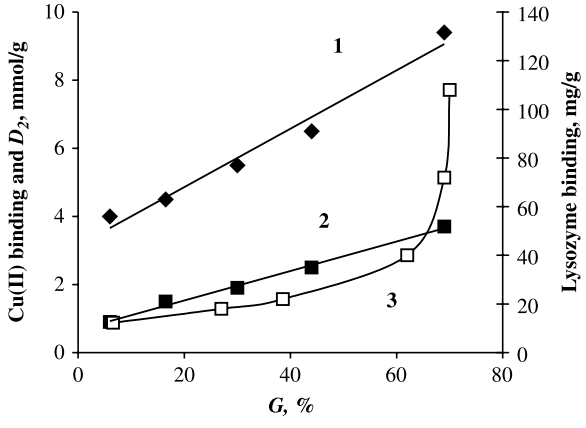

Fig. 11. Binding of Cu(II) (curve 2) and lysozyme (curve 3) by AAc-grafted pAAm cryogel with different degrees of grafting. For comparison, the amount of titrated COOH groups on AAc-grafted pAAm cryogel is presented (curve 1). Cu(II) binding was measured by saturating AAc-grafted pAAm cryogel with a solution of 0.2 M $CuSO_4$, washing unbound Cu(II) ions with water and elution of bound Cu(II) ions with 0.1 M EDTA pH 7.3. Lysozyme binding was measured by saturating AAc-grafted pAAm cryogel with lysozyme (1 mg/ml in 20 mM Tris–HCl buffer, pH 7.0), washing unbound lysozyme and elution with 1.5 M NaCl in 20 mM Tris–HCl buffer, pH 7.0.

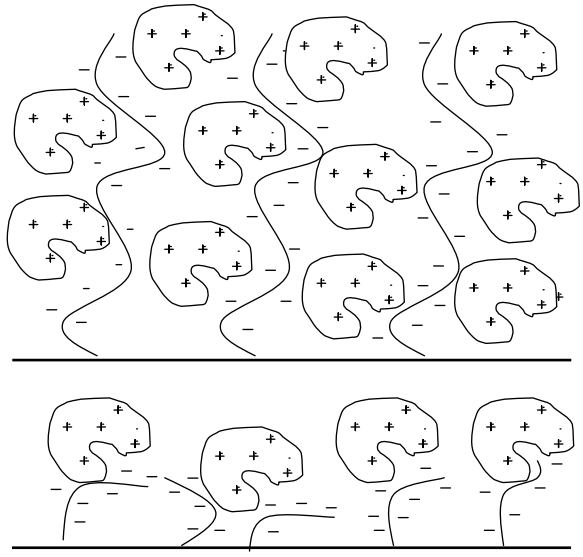

Fig. 12. Schematic presentation of regular (bottom) and tentacle-type (up) lysozyme binding to AAc-grafted pAAm cryogel.

superporous structure of the cryogels promoted grafting providing an ample surface of the gel, good mass transfer inside the gel sample and allowed to wash easily both homopolymer of AAc and insoluble by-products formed in the polymerization reaction.

The most interesting property of AAc-grafted pAAm cryogels was a drastic nearly three-fold increase in lysozyme binding when grafting degree increased from 60 to 70%. This behaviour was explained as tentacle-like binding of protein to the polymer.

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