Pergamon

0014-3057(94)E0032-Y

Eur. Polym. J. Vol. 30, No. 8, pp. 941–947, 1994

0014-3057/94 $7.00 + 0.00

IRON(III) CHELATING RESINS—IV. CROSSLINKED COPOLYMER BEADS OF 1-(β-ACRYLAMIDOETHYL)-3-HYDROXY-2-METHYL-4(1H)-PYRIDINONE (AHMP) WITH 2-HYDROXYETHYL METHACRYLATE (HEMA)

MIN-HUA FENG, LEEN VAN DER DOES* and ADRIAAN BANTJES
Department of Chemical Technology, Biomaterials Section, University of Twente, P.O. Box 217,
7500 AE Enschede, The Netherlands

(Received 20 April 1993; accepted 29 September 1993)

Abstract—Iron(III) chelating beads have been synthesized by copolymerization of 1-(β-acrylamidoethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone (AHMP) with 2-hydroxyethyl methacrylate (HEMA), and ethyleneglycol dimethacrylate (EGDMA) as the crosslinking agent. The synthesis of the AHMP–HEMA beads was performed by suspension polymerization of AHMP, HEMA and EGDMA in benzyl alchol—20% aqueous NaCl solution using 2,2′-azobisisobutyronitrile (AIBN) as the initiator and polyvinylalcohol (40–88) as a suspending agent.

The crosslinked copolymer beads were characterized by IR, and the AHMP content was determined by elemental analysis. The AHMP–HEMA beads were not too hydrophilic, and the copolymers absorbed at equilibrium only 40–50% water. It was found that the copolymer beads were very stable at 25°, but some degradation was observed at 121°.

The AHMP–HEMA copolymers were able to chelate iron(III) and the chelation was dependent on the conditions such as pH and temperature. However, the capacities towards iron(III) chelation were always found to be much lower than the calculated values. The influence of the polymeric matrix on the iron(III) chelating ability was studied with iron(III) chelating resins containing various polymeric matrices. It was found that the iron(III) chelating efficiencies of the resins were strongly affected by their hydrophilicities. The low chelating efficiency of the AHMP–HEMA beads (0–40%) is probably due to their poor swelling in water.

INTRODUCTION

Iron(III) chelating resins or gels for treatment of iron overload [1–5], removal of iron(III) from various systems [6–8] and for inhibition of bacterial growth [9] have attracted widespread interest. Several iron(III) chelating resins or gels have been synthesized in the past through the immobilization of natural chelators (e.g. desferrioxamine B) onto activated supports [3, 4, 7, 10] or by the reaction of activated polymers with specific ligands [8, 11, 12].

We studied in previous work several iron(III) chelating systems, prepared by immobilization of desferrioxamine (DFO), 1-(β-aminoethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone (HP) and L-mimosine onto Sepharose [7, 8]. These gels had a high affinity for iron(III) and were used for removing iron from milk, wine, whey and lactoferrin. However, the gels were not very stable mainly because of hydrolysis of their isourea bonds. For obtaining iron(III) chelating systems with a high stability, we therefore decided to study the polymerization of a monomer with a covalently bound chelating functional group. Because 3-hydroxy-2-methyl-4(1H)-pyridinone (HMP) derivatives are stronger chelators for iron(III) than desferrioxamine B [13–15], a monomer with an HMP group, 1-(β-acrylamidoethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone (AHMP) was selected as the functional monomer. This monomer was recently prepared in our laboratory and evaluated chemically and biologically [16]. 2-Hydroxyethyl methacrylate (HEMA) was used as a comonomer because poly(HEMA) and copolymers of HEMA have been widely used in the biomedical field for their non-toxicity, non-irritability and biocompatibility [17, 18]. Furthermore, for the production of insoluble copolymer networks, copolymerization of AHMP and HEMA was carried out with ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent.

Our interest of making copolymers was more concentrated on beads than on powders because it is more convenient to use and remove beads from treated systems. Preparation of beads by classical suspension polymerization is usually difficult with monomers such as AHMP and HEMA which are highly soluble in an aqueous medium. For this reason, attention has recently been devoted to the direct synthesis of poly(HEMA) particles by adding various salts to the water phase in order to diminish the water solutility of HEMA, and by using $MgCl_2$ $6H_2O$ or $Mg(OH)_2$ as suspending agent [19–21]. However, using $MgCl_2$ $6H_2O$ or $Mg(OH)_2$ has a

---

*To whom all correspondence should be addressed.

Table 1. Copolymerization of AHMP and HEMA in the presence of various suspending agents

Suspending agent (SA)	Amount of SA(mg)	Weight recovery (%)	Nature of HEMA-AHMP copolymer formed
Cellulose-acetate-butyrate (17%)	100	99	Amorphous bulky solid
Carboxymethyl cellulose Na salt	50	99	Amorphous bulky solid
Polyvinyl alcohol (10–88)	50	99	Barlike particles
Polyvinyl alcohol (40–88)	100	99	Spherical beads, up to 0.2 mm
Polyvinyl alcohol (40–88)	50	98	Spherical beads, up to 0.3 mm

Copolymerizations were conducted with 54.0 mmol HEMA, 3.0 mmol AHMP and 3.0 mmol EGDMA in 12 ml benzyl alcohol and 40 ml 20% aqueous NaCl in the presence of 0.6 mmol AIBN for 4 h at 80°.

disadvantage because Mg^{2+} may contaminate the beads. Therefore, polymeric stabilizers were investigated as suspending agents in our polymerization system.

When polymers are intended for biomedical use, it is particularly informative to obtain knowledge on the properties of the beads at room temperature and at high temperature because high temperature (e.g. 121°) is usually used in sterilizing the materials using in biological or microbiological systems. Therefore, the properties such as swelling degree, stability and chelating ability of the copolymer beads were determined at both temperatures.

In this investigation, several AHMP–HEMA copolymer beads with various AHMP contents were synthesized and characterized by IR and elemental analysis. The swelling behaviour, stability and iron(III) chelating properties of the beads were determined. For exploring the effect of the polymeric matrix on the iron(III) chelating properties, another iron(III) chelating resin was also prepared by copolymerization of AHMP with acrylamide (AA). The water content of the swollen resin and its chelating capacity towards iron(III) were evaluated at the same conditions as used for the AHMP–HEMA beads. In addition, the water contents in the swollen resins, the ligand densities, iron(III) chelating capacities and efficiencies were compared with those of other iron(III) chelating polymeric systems.

EXPERIMENTAL PROCEDURES

Materials

1-(β-Acrylamidoethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone (AHMP) was prepared from 3-hydroxy-2-methyl-4-pyrone as described previously [16]. 2-Hydroxyethyl methacrylate (HEMA) and ehtylene glycol dimethacrylate (EGDMA) were obtained from Merck and used without purification. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized twice from methanol before use. Suspending agents: cellulose acetate-butyrate (butyryl content 17%, Serva), carboxymethyl cellulose sodium salt (Sigma), polyvinylalcohol (10–88, Aldrich) and polyvinylalcohol (40–88, Aldrich) were used as received. Polyvinylalcohol (10-88) and polyvinylalcohol (40–88), (88% hydrolysed polyvinylacetates), had molecular weights of 10,000 and 40,000, respectively. Acrylamide (AA, Aldrich), N,N′-ethylene-bis-acrylamide (EBAA, Aldrich), ammonium persulphate (Merck), sorbitan monostearate (Aldrich) and N,N,N′,N′-tetramethylethylenediamine (Merck) were used as received. All other chemical reagents were obtained from Aldrich or Merck.

Measurements

Infrared spectra were recorded on a Bio-Rad FTS-60 FT-IR spectrophotometer. Elemental analysis was performed by TNO, Zeist, The Netherlands. Visible absorption measurements were made with an Uvikon 930 spectrophotometer, and used for determination of iron(III) chelating capacities. The determination of the shape and size of the beads was determined with an Ortholux II Pol-BK microscope.

Crosslinked copolymers from AHMP and HEMA

Suspension copolymerizations were carried out as follows. AHMP (3.0 mmol, 666 mg), HEMA (54.0 mmol, 7.02 g), EGDMA (3.0 mmol, 594 mg) and the initiator, AIBN (0.6 mmol, 98 mg) in benzyl alcohol (12 ml) were suspended by rapid stirring in a previously prepared 20% aqueous NaCl solution (40 ml) containing a suspending agent (Table 1). The reaction flask was flushed with nitrogen for 15 min and heated at 80° for 4.0 h while stirring. The polymer was collected by filtration and washed consecutively with methanol, water and acetone. Then it was dried in vacuum at 80° for 48 h.

AHMP–HEMA crosslinked copolymer beads were prepared by this procedure with polyvinylalcohol (40–88) as the suspending agent and with various concentrations of monomer, comonomer and crosslinking agent (Table 2). The copolymer beads were isolated and treated as previously mentioned.

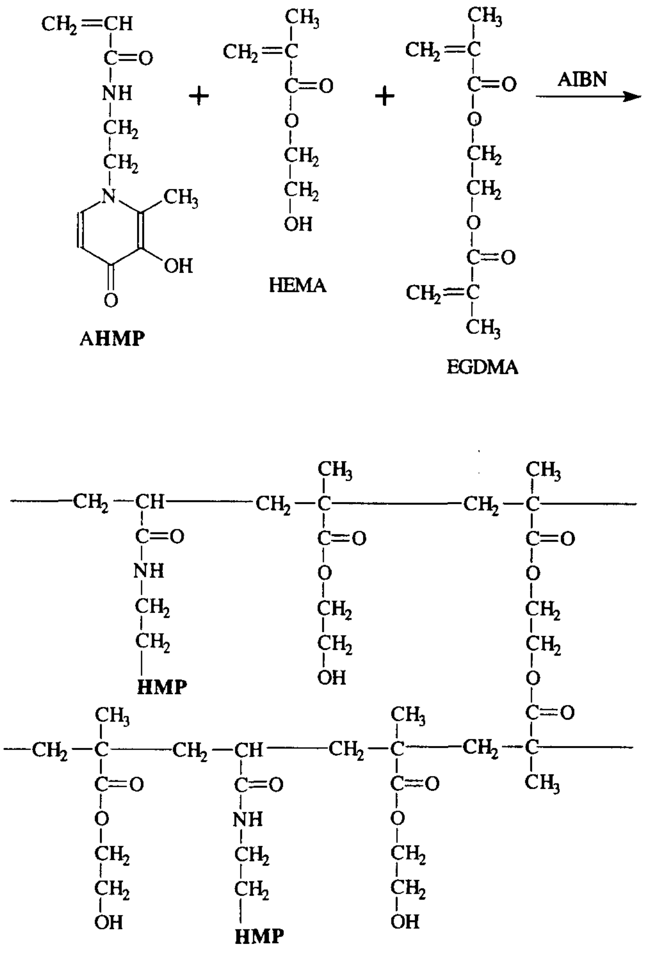
Preparation of a crosslinked AHMP-AA resin

To a 500 ml reactor were added AHMP (3.0 mmol, 666 mg), EBAA (3.0 mmol, 504 mg), AA (54.0 mmol, 3.8 g), water (40 ml), ammonium persulphate (0.6 mmol), hexane (100 ml) and carbon tetrachloride (60 ml). While stirring 100 mg sorbitan monostearate was placed into the reactor, and the mixture was flushed with nitrogen for 20 min. Then N,N,N′,N′-tetramethylethylenediamine (0.7 mmol) was

Table 2. Preparation of crosslinked AHMP–HEMA copolymer beads

Copolymer	Initial composition (mole %)			Weight recovery (%)	Diameter of dry beads (mm)
	AHMP	HEMA	EGDMA		
1	0	95	5	100	0.20–0.25
2	5	90	5	98	0.15–0.25
3	10	85	5	94	0.15–0.30
4	20	75	5	93	0.15–0.30
5	95	0	5	0	

Copolymerization conditions: total monomer amounts of 60 mmol were used; total weight of the monomers in benzyl alcohol = 0.70 g/ml; ratio of organic phase/water (v/v) = 0.5. The experiments were carried out at 80° for 4 h in the presence of AIBN (1 mol %), and polyvinyl alcohol (40–88) at 0.5% to the total monomer weight.



Scheme I. Synthesis of crosslinked copolymers from AHMP and HEMA.

added and the polymerization took place at 30° for 2 h. The resin was filtered and washed with isopropanol, methanol, water, ethanol, carbon tetrachloride, ethylacetate, and dried in vacuum at 80° for 48 h (4.10 g, 82% weight recovery).

Water content of swollen copolymers

The water contents in the swollen AHMP–HEMA beads or AHMP–AA resin were determined as follows. Approximately 0.5 g of the dried beads or resin was weighed into a flask and swollen with 40 ml of distilled water for 24 h at 25°, or for 60 min at 121° in an autoclave and then cooled to room temperature. The swollen beads and/or resin were weighed and the water content (%) was calculated according to the formula:

$$\text{Water content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100.$$

Stability of AHMP–HEMA beads

As an indication for the stability of the copolymer beads, the release of AHMP groups from the beads in water was determined at 25 and 121°. About 0.2 g of the beads was placed into a flask with 20 ml of water. The flask was rotated at 25° or incubated in an autoclave at 121° for a determined time. The amount of released AHMP in the supernatant was measured by its maximal absorbance at 280 nm.

Iron(III) chelation

The chelating capacities of the AHMP–HEMA beads or AHMP–AA resin towards iron(III) were determined using the batch method. A sample of *c*. 100 mg was added to 25 ml of a 1.8 mM solution of iron(III) in a buffer. The mixutre was rotated at 25° or incubated at 121° in an autoclave. The amount of iron chelated on the beads or resin was calculated from the change in the iron(III) concentration. Iron(III) in

Table 3. Elemental analysis data and AHMP contents of the AHMP–HEMA beads

	AHMP in beads		AHMP in feed	
Copolymer	% N content[a]	μmol/g	% N content[b]	μmol/g
2	0.60	214	1.00	362
3	1.36	486	2.00	701
4	2.62	936	3.70	1318

[a] Found.
[b] Calculated from initial monomer composition.

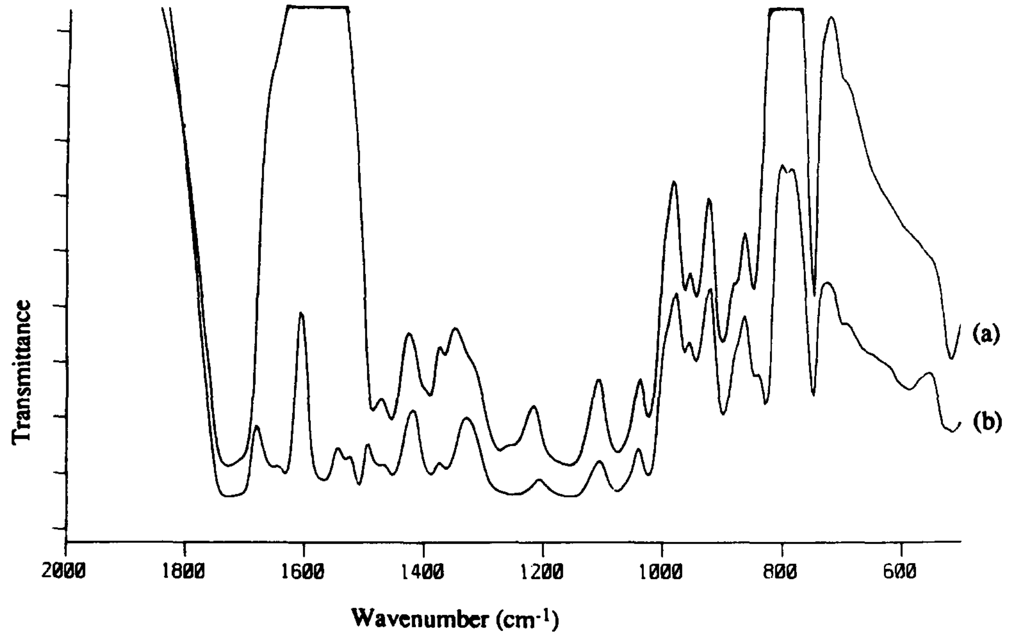


Fig. 1. IR spectra of (a) crosslinked poly(HEMA) and (b) crosslinked AHMP–HEMA copolymer.

aqueous solutions was quantitatively analysed by a spectrophotometric procedure with 1,10-phenanthroline [22].

RESULTS AND DISCUSSION

Scheme I shows the copolymerization of AHMP and HEMA, crosslinked with EGDMA, and the typical structure of the crosslinked AHMP–HEMA copolymers.

For investigating the influence of the suspending agent on the formation of AHMP–HEMA copolymer beads, various polymeric stabilizers were used at the same conditions, and the results are given in Table 1. It can be seen that spherical beads were obtained when using polyvinylalcohol (40–88) as suspending agent. However, it is not clear why only with polyvinylalcohol (40–88) beads were formed.

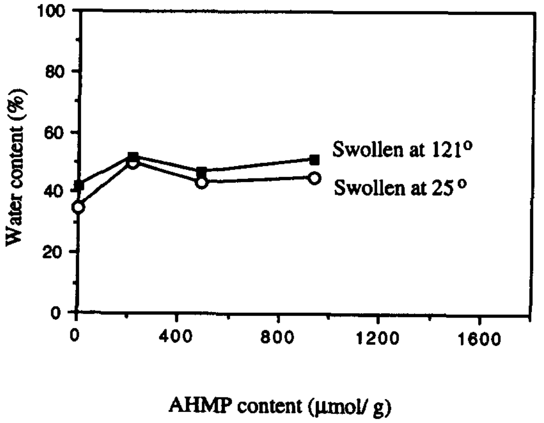


Fig. 2. Effect of AHMP content on the water content of swollen AHMP–HEMA copolymer beads.

In further experiments polyvinylalcohol (40–88) was used as the suspending agent and Table 2 gives additional results about the synthesis of AHMP–HEMA copolymer beads. It is obvious that HEMA was completely converted into the crosslinked homopolymer, whereas under identical conditions no polymerization was observed for AHMP. Copolymerization of AHMP with HEMA and crosslinking by EGDMA occurred, resulting in the formation of water-insoluble beads. The weight recovery (%) of the copolymers decreased with higher AHMP concentration in the feed.

The presence of AHMP in the copolymer beads was indicated by their N content, and the results are given in Table 3. The content of AHMP ligands in the beads increased with increasing AHMP monomer fraction in the feed, but the AHMP content in the copolymers was always lower than in the feed, which also indicates that part of the AHMP had not reacted. The relatively low conversion of AHMP and no homopolymerization may result from an inhibiting effect of the phenol group on the radical polymerization, whereas the bulky group will make the polymerization more difficult.

Figure 1(a) and (b) show the IR spectra of poly(HEMA) and of AHMP–HEMA copolymer, respectively. Peaks near 1730, 1280 and 1160 cm^{-1} are assigned to the C=O and C—O—C absorption of the ester. Besides these peaks, several bands appear only in AHMP–HEMA copolymer, which suggests the presence of AHMP in the copolymer. In our opinion it is allowed to conclude that the bands at 1660 and 1630 cm^{-1} are from C=O stretching vibrations of the amide and the ring. The absorption at 1570 cm^{-1} due to the bending vibration of N–H and C–N, and the band around 830 cm^{-1} results from C–H in plane bending of the two hydrogens at the pyridinone ring.

Table 4. Stability of AHMP–HEMA copolymer beads in water

Copolymer code	AHMP groups released at various conditions (%)				
	25°, 24 h	25°, 48 h	25°, 120 h	121°, 15 min	121°, 60 min
2	0.1	0.3	0.5	1.9	3.6
3	0.1	0.3	0.6	2.3	4.4
4	0.3	0.4	0.8	5.0	7.8

The water content (%) of the swollen copolymers was measured under two swelling conditions, and the results are shown in Fig. 2. Without AHMP the water content of swollen poly(HEMA) was 35–45%. The introduction of AHMP increased the water content in the copolymer, however, the water content was not an additive function of the AHMP content, which indicates that specific interactions were present. This observation is consistent with the finding of Dusek *et al.* with acrylamide-HEMA gels [23]. The water content of the AHMP–HEMA copolymers is higher than that of poly(HEMA), which may be explained by Dusek's suggestion that the introduction of more hydrophilic groups into the HEMA chain brings about destruction of the hydrophobic associates which are the main cause of the low water content of poly(HEMA). Because the AHMP group is not so hydrophilic, the increase of the water content by the introduction of AHMP was limited and only about 40–50% water was contained in the swollen copolymers. Although the water contents of beads swollen at 121° were higher than those of beads swollen at 25°, the differences were small.

The stability of the AHMP–HEMA beads in water was measured at various conditions, which referred to the degree of AHMP groups released from the beads. As shown in Table 4, the AHMP–HEMA beads are very stable in water at 25°, and for instance, only 0.1–0.3% AHMP groups released from the beads at 25° within 24 h. Previously, we reported about iron(III) chelating systems, obtained by immobilization of iron(III) chelators to Sepharose [7, 8]. The gels released at the same conditions *c.* 1.0% of the functional groups, probably becasue of hydrolysis of the isourea bonds. Obviously, the AHMP–HEMA copolymer beads are more stable because the AHMP groups are linked by amide bonds to the polymer matrix. The stability of the AHMP–HEMA beads in water was also tested at high temperature (121°). The release of AHMP groups was much higher at 121 than 25°, possibly resulting from degradation of the beads. Furthermore, the amount of AHMP groups released increased with the incubation time, whereas the beads with higher AHMP content were always less stable.

The iron(III) chelating capacities were determined for one type of bead at various conditions. Dry beads were used although non-reversible effects on solvent uptake in the copolymer beads might occur. In Table 5 the calculated and experimental iron(III) chelating capacities for beads with an AHMP content of 214 μmol/g are shown. The calculated values were based on the N content of the sample and assuming the formation of a 3:1 complex at pH 6.8 and 3.9, and a 2:1 complex at pH 2.3 between AHMP groups and iron(III) [14, 16]. It was observed that the capacity of the beads towards iron(III) was dependent on pH, temperature and time. At physiological pH, the beads did not chelate iron(III) at 25° but chelation occurred at a higher temperature. When the pH was decreased, the capacity increased both at 25 and 121°. The temperature of incubation had an obvious influence on the capacity, and the capacity was much higher at higher temperatures. In addition, the capacity increased with time, which suggests that the chelation equilibrium was not reached after 15 min incubation. From the data in Table 5 it can be seen that the experimental capacities were always lower than the calculated values.

In Fig. 3 the calculated and experimental capacities determined at the same conditions for beads with various AHMP content are compared. Also in these experiments the observed values are much lower than the calculated ones, and the difference even increases with increasing AHMP content.

Because the type of polymer matrix might have an effect on the iron(III) chelating ability, data are given in Table 6 about the ligand densities, water contents and iron(III) chelating capacities as well as efficiencies for AHMP–HEMA beads and other iron(III) chelating resins with various polymeric matrices. It can be seen that the AHMP–HEMA beads possess a high ligand density, but the iron(III) chelating efficiency is zero under this condition. The difference in the ability and efficiency between the AHMP–HEMA beads and other iron(III) chelating resins is probably due to

Table 5. Iron(III) chelating capacity of AHMP–HEMA copolymer beads at various conditions[a]

	Chelating conditions				Capacity (μmol/g)[b]	
Iron(III) ions	pH	Buffer	Temp. (°)	Time	Calc.[c]	Found
Iron(III) citrate	6.8	PBS (pH 7.4)	25	24 h	71	0
Iron(III) citrate	6.8	PBS (pH 7.4)	121	15 min	71	8.4
$(NH_4)Fe(SO_4)_2$	3.9	Acetate (0.2 M)	25	24 h	71	9.7
$FeCl_3$	3.9	Acetate (0.2 M)	25	24 h	71	10.5
$FeCl_3$	2.3	Citrate (0.1 M)	25	24 h	107	16.1
$FeCl_3$	2.3	Citrate (0.1 M)	121	15 min	107	31.1
$FeCl_3$	2.3	Citrate (0.1 M)	121	60 min	107	40.4

[a] Copolymer **2** (Table 2).
[b] Dry beads.
[c] Based on data from Table 3.

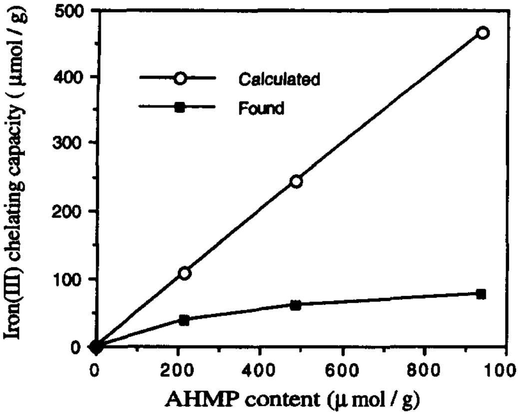


Fig. 3. Iron(III) chelating capacity vs AHMP content for AHMP–HEMA copolymer beads (pH 2.3). The experiments were performed at 121° for 60 min with copolymers **1**, **2**, **3** and **4**.

differences in the type of the polymeric matrices. Concerning the physical properties of the iron(III) chelating resins presented in Table 6, large difference in their water contents was found. The water content of the AHMP–AA resin was considerably higher than that of the AHMP–HEMA beads. Although the ligand density of the AHMP–AA resin probably differs from the one indicated in Table 6, it seems reasonable to assume that the very high efficiency is due to the high water content, as compared to the value for the AHMP–HEMA beads. In addition, the DFO–Sepharose and HP–Sepharsoe gels have a high water content, resulting in a high iron(III) chelating efficiency. From these results, it may be concluded that the low iron(III) chelating efficiency of the AHMP–HEMA copolymer beads is due to the poor swelling of the beads. The low hydrophilicity of the copolymer beads may influence the diffusion rate of iron(III) into the beads and induce steric hindrance for the ligands, which is in agreement with our observation of the effect of temperature and time on the iron(III) chelating capacity. Furthermore, generally substituted acrylamides and methacrylates do not copolymerize well, which could provide an inhomogeneous chain composition with AHMP being incorporated later in the polymerization. This may have some effect on site distribution and hence on the accessibility of iron(III).

The effect of the type of polymeric matrix on the chelating ability and efficiency of chelating resins has also been observed by other workers. Crosslinked polymers containing ligands based on derivatives of N,N′-bis(2-pyridylmethyl)-2,2′-diaminobiphenyl and N-(2-pyridylmethyl)-2,2′-diaminobiphenyl were studied by Melby [24]. Such N-alkylated polymers showed very low chelation capacity for Cu(II) (2–10% of the calculated) and little or no ability to discriminate between Cu(II) and Fe(III), probably because the N-benzyl groups, which are part of the strained and rigid polymer backbone, are also part of the coordination sites and are expected to distort them very badly. Janus *et al.* [25] found that both linear and crosslinked copolymers of vinylamine (VA) with vinylpyrrolidone or methyl methacrylate had a very low chelating efficiency, which was probably due to the fact that some VA groups are not accessible to the metal ion or by steric constraints preventing complete coordination of the metal ion in the crosslinked copolymer. By comparing the metal sorption behaviour of macroreticular poly(4-vinylpyridine), 4 VP resins crosslinked with hydrophilic and hydrophobic crosslinking agents, Atsushi *et al.* [26] concluded that the difference in crosslinkers strongly affected the sorption behaviour, such as the pH profile and the capacity for sorption of metal ions, and they suggested that the hydrophilicity of the resin matrix, other than the chelating groups, should be taken into account in the design of chelating resins.

For improving the iron(III) chelating ability and efficiency of AHMP containing copolymers, the use of a highly hydrophilic comonomer might result in a polymeric matrix with more suitable properties. For this aim, copolymerization of AHMP and N,N-dimethylacrylamide has been investigated in our laboratory. In the next part of this series the design, synthesis, iron(III) chelating properties and applications of the resins will be demonstrated.

CONCLUSIONS

Crosslinked AHMP–HEMA copolymer beads were synthesized by suspension polymerization of AHMP, HEMA and EGDMA.

The AHMP–HEMA beads possess a high ligand density (214–936 μmol/g dry beads), and are more stable in water at room temperature than HP–Sepharose gels. The water content of the swollen beads was relatively low (40–50%) because of the limited hydrophilicity of AHMP as well as of HEMA.

Table 6. Ligand densities, water contents, iron(III) chelating capacities and efficiencies of iron(III) chelating resins with various polymeric matrices

Iron chelating resin	Ligand density (μmol/ml)	Water content (%)[a]	Iron chelating capacity (μmol/ml)[b]	Chelating efficiency (%)
AHMP–HEMA beads[c]	68	50	0	0
AHMP–AA resin	39[d]	87	12	92
DFO–Sepharose gel (G2)[e]	23	95	18	78
HP–Sepharose gel (G3)[f]	25	95	20	80

[a] Swollen at 25°.
[b] Determined at pH 6.8 after contacting 24 h at 25°.
[c] Copolymer **2** (Table 2).
[d] Value calculated from initial composition.
[e] Reference [7].
[f] Reference [8].

The iron(III) chelating capacity and efficiency of the AHMP–HEMA copolymer beads are dependent on the chelation conditions. The hydrophilicity of the polymeric matrix plays a very important role in the iron(III) chelation. The low swelling degree of the AHMP–HEMA beads resulted therefore in a low chelating efficiency twoards iron(III) (0–40%).

REFERENCES

1. J. R. Mahoney Jr, P. E. Hallaway, B. E. Hedlund and J. W. Eaton. *J. clin. Invest.* **84,** 1362 (1989).
2. A. Winston, D. V. P. R. Varaprasad, J. J. Metterville and H. Rosenkrantz. *J. pharmacol. exp. Ther.* **232,** 644 (1985).
3. P. E. Hallaway, J. W. Eaton, S. S. Panter and B. E. Hedlund. *Proc. natn. Acad. Sci. U.S.A.* **86,** 10,108 (1989).
4. D. Horowitz, S. Margel and T. Shimoni. *Biomaterials* **6,** 9 (1985).
5. A. Winston, D. V. P. R. Varaprasad, J. J. Metterville and H. Rosenkrantz. *Polym. Sci. Technol. (Plenum),* **32,** *(Polym. mater. Med.),* 191 (1985).
6. G. J. Loubser. S. African ZA 81, 03,988 (1981).
7. M. H. Feng, L. van der Does and A. Bantjes. *J. biomater. sci. Polym. Edn.* **4,** 99 (1992).
8. M. H. Feng, L. van der Does and A. Bantjes. *J. biomater. sci. Polym. Edn.* **4,** 145 (1992).
9. I. W. DeVoe and B. E. Holbein. U. S. Pat. 4,530,963 (1985).
10. R. S. Ramirez and J. D. Andrade. *J. macromol. sci. Chem.* **A10,** 309 (1976).
11. A. Winston and G. R. McLaughlin. *J. Polym. sci. Polym. Chem. Edn.* **14,** 2155 (1976).
12. A. Winston and D. Kirchner. *Macromoleucles* **11,** 597 (1978).
13. G. J. Kontonghiorghes. *Biochim. biophys. Acta* **882,** 267 (1986).
14. G. J. Kontoghiorghes, L. Sheppard and S. Chambers. *Arzneim.-Forsch./Drug Res.,* **37**(II), Nr. 10, 1099 (1987).
15. R. C. Hider and A. D. Hall. *Perspect. bioinorg. Chem.* **1,** 209 (1991).
16. M. H. Feng, L. van der Does and A. Bantjes. *J. med. Chem.* **36,** 2822 (1993).
17. A. S. Hoffman and C. Harris. *Am. chem. Soc., Div. Polym. chem., Polym. Prepr.* **13,** 740 (1972).
18. S. D. Bruck. *J. biomed. mater. Res.* **6,** 173 (1972).
19. O. Okay and C. Gurun. *J. appl. Polym. Sci.* **46,** 401 (1992).
20. A. Jayakrishnan, M. C. Sunny and B. Chithambara Thanoo. *Polymer* **31,** 1339 (1990).
21. A. B. Scranton, A. G. Mikos, L. C. Scranton and N. A. Peppas. *J. appl. Polym. Sci.* **40,** 997 (1990).
22. H. H. Willard, L. L. Merritt and J. A. Dean Jr. *Instrumental Methods of Analysis,* 5th Edn, p. 113. New York (1974).
23. K. Dusek and J. Janacek. *J. appl. Polym. Sci.* **19,** 3061 (1975).
24. L. R. Melby. *J. Am. chem. Soc.* **74,** 4044 (1975).
25. L. Janus, J. Morcellet, M. Delporte and M. Morcellet. *Eur. Polym. J.* **28,** 1185 (1992).
26. S. Atsushi, O. Naotake, H. Kumiko and N. Koichi. *Analyt. Sci.* **4,** 399 (1988).