Polymer Bulletin 61, 147–156 (2008)
DOI 10.1007/s00289-008-0940-8

Polymer Bulletin

Chelating polymer-based membranes. Preparation and use for metal ion scavenging and sorption of murine immunoglobulin G by immobilized Ni(II) ions

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Received: 11 June 2007 / Revised version: 8 January 2008 / Accepted: 22 April 2008
Published online: 6 May 2008 –

Summary

Copolymers of 2-(2-hydroxyethoxy)ethyl methacrylate and ethylene dimethacrylate in the form of homogeneous membrane sheets were modified by chelating groups of iminodiacetic acid (IDA) in a two step reaction. The obtained sorbents showed high chelating capacity for Ni(II), Cu(II) and Fe(III) ions (up to 1.3 mmol/g). The potential use of immobilized Ni(II)-IDA complexes for sorption of murine immunoglobulin G by the immobilized metal affinity (IMA) method was tested. The sorption behaviour was characterized by a Langmuir-Freundlich adsorption isotherm. It was shown that the chelating membranes could be used for the sorption of low amounts of protein.

Introduction

Metal-chelating adsorbants are widely used in both large and laboratory scale applications, such as selective complexation and separation of toxic and carcinogenic metal ions [1,2], preconcentration of metal ions [3], removal of trace metal ions in the manufacture of optical and electronic polymers [4] or ligand exchange chromatography [5]. The immobilized metal affinity (IMA) method introduced by Porath [6] is a special part of ligand exchange, in which immobilized „hard“ {Fe(III)} or „borderline“ {Ni(II), Co(II), Zn(II), Cu(II)} metal ions are utilized for reversible adsorption of proteins through formation of coordination bonding with amino acid residues on the protein surface.

The solid supports of chelating sorbents are based on the various matrices of natural (mainly polysaccharides) or synthetic materials. For treatment with protein solutions, highly hydrophilic materials are usually required to prevent protein denaturation. Of hydrophilic synthetic supports, copolymers or terpolymers of 2-hydroxyethyl methacrylate in the form of particles [7-10] or membranes [11,12] became more popular in recent years.

In this work, chelating membranes based on the 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA) monomer were prepared, which is even more hydrophilic in comparison with 2-hydroxyethyl methacrylate. After subsequent attachment of the

chelating groups of iminodiacetic acid (IDA) to the membrane sheets, the ability of resulting sorbents to form complexes with metal ions and their utility in the IMA method for sorption of model protein was tested. Murine immunoglobulin G was used for this purpose owing to its ability to bind metal ions through histidine residues at C-terminal end [13].

Experimental

Materials

2-(2-Hydroxyethoxy)ethyl methacrylate (DEGMA) (b.p. 75°C/ 6.67 Pa) was synthesized according to ref. [14] and distilled prior to use. Pyridine was dried with solid KOH followed by fractional distillation (b. p. 115°C). Ethylene dimethacrylate (EDMA, 98%) (crosslinker), p-tosyl chloride (TsCl, 98%), catalyst 4-(*N,N*-dimethylamino)pyridine (DMAP) and benzoin ethyl ether (initiator) were purchased from Aldrich or Fluka. All the other chemicals were of analytical grade. In all experiments, high-quality water (Millipore Q, ρ >18.2 MΩ) was used. Murine serum immunoglobulin G was provided by Seva-Imuno Ltd. (Prague, Czech Republic).
The concentration of metal ions in solutions was determined in acetylene-air flame by atomic absorption spectrometry (AAS) using a Perkin Elmer 3110 spectrometer. Polymerizations were performed with mercury lamp Tesla RVK 125W.

Polymerization

A mixture of 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA), ethylene dimethacrylate (EDMA) (5.5, 3.0, 2.5, 2.0, 1.5, 1.0 or 0.5 wt %) (10 g of the monomer mixture) and benzoin ethyl ether (0.5 wt % relative to monomer mixture) was exposed to ultraviolet radiation in the mould for 20 min under nitrogen atmosphere.
The prepared polymer membranes were thoroughly washed with 50% ethanol (aq) and water.
Afterwards, swelling degrees of the membranes were determined according to [15] with slight modification. The wet samples were weighted after 48 h of treatment with the appropriate solvent (water or pyridine) and their weights were recorded every 30-60 s thereafter. The weight of the sample in swollen state (W_{sw}) was obtained by extrapolation to zero time of the plot of weight of wet sample vs. evaporation time. The degree of swelling was calculated as the ratio of the weight decrease after drying of the sample, to the weight of the dry sample.

Modification of poly(DEGMA-co-EDMA) membrane

I. Transformation of –OH groups of poly(DEGMA-co-EDMA) membrane to tosyloxy groups
a) The membrane flat sheet (5.0 g dry weight) was immersed successively into water/ pyridine mixtures (100/0, 80/20, 60/40, 40/60 and 20/80 vol %/ vol %) before treatment with neat pyridine. Tosylchloride (5.0 or 10.0 g, 0.9 or 1.8 equiv. relative to the calc. amount of OH groups - 5.7 mmol/g) and 4-(*N,N*-dimethylamino)pyridine (DMAP, 0.02 g) were dissolved in freshly distilled pyridine (100 ml). The solution

was cooled with ice water bath. Then, a membrane sheet was immersed and the reaction mixture was kept at 25°C for a period of 48 h under shaking. The modified membrane was washed with pyridine and then transferred into water in the same way as described above using pyridine/water mixtures with increasing proportions of water.
Found values 3.20-3.33 or 4.31-4.44 wt % S correspond to 1.02±0.02 or 1.36±0.02 mmol of tosyloxygroups per gram of dry membrane, respectively, depending on the TsCl content in the reaction mixture.

b) Tosylchloride (15.0 g, 13.7 equiv. relative to the calculated amount of membrane OH groups) was suspended in water (40 ml), DMAP (0.02 g) (catalyst) and a solution of methyl red in acetone (1 wt %, 0.02 ml) (pH monitor) were added. The membrane flat sheet (1.0 g dry weight) swollen in water was then immersed and the reaction mixture was kept in a water bath at 25°C under shaking. A solution of 5 M NaOH was dropped successively thereafter as long as the change in pH was observed. The membrane sheet was then thoroughly washed with water.
The found S content, 1.52-1.57 wt % of S corresponds to 0.48±0.01 mmol of tosyloxy groups per gram of dry membrane.

II. Reaction of tosyloxy groups with iminodiacetic acid (IDA)
The membrane modified with tosyloxy groups was immersed into a solution of iminodiacetic acid (IDA) (1.0 or 4.0 equiv. relative to OTs groups) in 1 M Na_2CO_3/1 M $NaHCO_3$ (50 ml). The reaction proceeded for 5 days in a water bath at 60°C under shaking. The resulting chelating membranes were then thoroughly washed successively with water, 0.5 M HCl and again with water.
The attached IDA groups were determined by nitrogen analysis.

Sorption of metal ions

Sheet of the chelating membrane, preswollen for 24 h in 0.1 M sodium acetate buffer, pH 4.5, was equilibrated with 0.1 M solutions of $NiCl_2$ or $CuSO_4$ in the same buffer for 12 h under shaking. The sheet was then repeatedly treated with pure acetate buffer. The liquid fractions were collected and their metal concentration was determined by AAS. The amount of metal ions chelated in membrane was calculated from the difference in metal contents of the initial and combined equilibrium solutions. $FeCl_3$ sorption was performed in 0.1 M acetic acid (pH 2.7) in the same way. The membrane loaded with metal ions was then treated with a buffer of pH 8 (0.05 M potassium phosphate in 0.5 M NaCl). After reaching equilibrium (ca. 8 h), the difference in the concentrations of the bonded metal was determined by AAS.

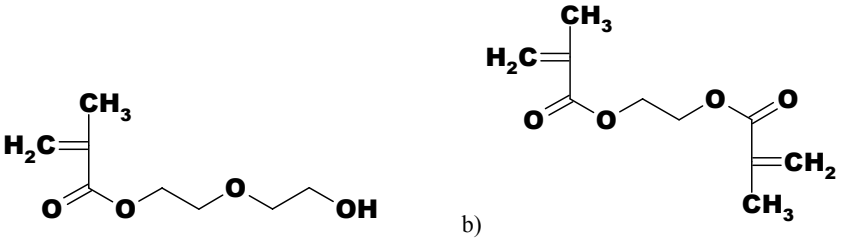
Protein adsorption studies

Adsorption of the murine serum immunoglobulin G was determined in batch experiments. The protein solutions were prepared in 0.05 M phosphate buffer (pH 8, 0.5 M NaCl). To a series of flasks containing an equal weight of the Ni(II)-IDA membrane flat sheet previously equilibrated in the same buffer, pH 8, equal volumes of buffered protein solutions of different concentrations were added. The flasks were incubated for 12 h in a water bath at 20°C. The protein concentration in the

supernatant was determined spectrophotometrically [16] and the amount of bound protein was calculated by mass balance.

Results and discussion

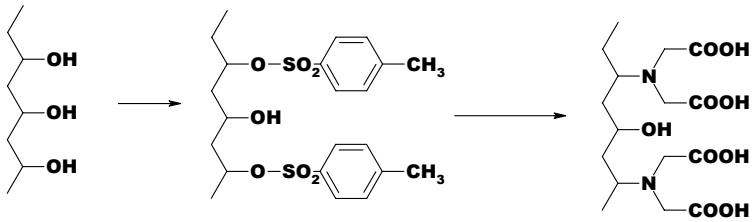
Homogeneous flat sheets of poly(2-(2-hydroxyethoxy)ethyl methacrylate-*co*-ethylene dimethacrylate) {poly(DEGMA-*co*-EDMA)} membranes of 73 μm thickness were prepared by radical polymerization initiated photochemically (with benzoin ethyl ether). The chemical structures of monomers are shown in Scheme 1. As the copolymerization parameters of the monomer pair are close to unity [17], the ideal copolymerization can be assumed and the degree of crosslinking is therefore determined by the crosslinker content in the monomer mixture. The degree of swelling of membranes in water and pyridine (Table 1) was evaluated. As expected, decreasing degree of crosslinking caused increase in the degree of swelling. The obtained results are in agreement with the properties of DEGMA-containing hydrogels [18,19]. The degree of swelling in pyridine of sheets with crosslinking greater than 1% was difficult to determine accurately owing to their poor resistance to the swelling pressures.



Scheme 1. Chemical structures of monomers 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA) (a) and ethylene dimethacrylate (EDMA) (b) used in the synthesis of poly(DEGMA-*co*-EDMA) membranes.

Flat sheets with a low crosslinking degree (0.5 %) were chosen for subsequent modification due to their most appropriate swelling as well as mechanical properties out of all prepared membranes. Modification included tosylation of membrane hydroxy groups followed by the replacement of the formed tosyloxy groups with chelating groups of iminodiacetic acid in order to obtain chelating sorbents (Scheme 2). Tosylation was first attempted in pyridine. Because the degree of swelling in pyridine is higher compared to that in water (Table 1), stepwise replacement of water with pyridine was required to prevent mechanical failure of the membrane. The conditioning included successive transfer of the membrane flat sheet from water to pyridine/water mixtures with increasing pyridine contents, and eventually with neat pyridine. Analogous conditioning of the membrane before the next modification step (in aqueous buffer) was performed using water/pyridine mixtures containing increasing proportions of water.

The degree of modification of membrane hydroxy groups to tosyloxy groups was dependent on the concentration of tosylchloride in pyridine solution and reached nearly 24 % (1.36 mmol/g of OTs group) in excess of tosylchloride. Lower amount of tosylchloride in the reaction mixture (0.9 equiv.) resulted in lower concentration of formed tosyloxy groups (1.02 mmol/g) (Table 2).



Scheme 2. Incorporation of chelating groups into poly(DEGMA-*co*-EDMA) membranes. The derivatization includes transformation of hydroxy groups into tosyloxy groups followed by their replacement with the iminodiacetic acid chelating groups.

Table 1. Swelling properties of poly(DEGMA-*co*-EDMA) membranes in dependence of cross-linking degree.

Degree of crosslinking (%)	Degree of swelling (%)	
	in water	in pyridine[a]
5.5	69	-
3	77	-
2.5	85	-
2	96	-
1.5	102	-
1	146	320
0.5	240	380

[a] Crashing of the membrane sheet with crosslinking greater than 1 % occurred under the swelling pressure.

In the subsequent reaction step, tosyloxy groups were replaced practically quantitatively in excess of iminodiacetate. When equivalent of iminodiacetate was used, the resulting concentrations of attached IDA groups were slightly lower. The results are given in Table 2.

In addition, the tosylation of polymer hydroxy groups was alternatively performed in aqueous medium in order to simplify the modification procedure, and to avoid any mechanical failure of the membrane flat sheet. This technique is in fact analogy of the Schotten-Baumann method, which is applied in the formation of esters from alcoholes and acid chlorides in aqueous medium with the use of alkali hydroxide to drive the equilibrium. However, in the case of tosylation of poly(DEGMA-*co*-EDMA) membrane sheets, this reaction manner was slow and the maximal resulting concentration of tosyloxy groups (0.48 mmol/g) was approximately three times lower than in analogous tosylation in pyridine (Table 2). The tosyloxy groups were then transformed into the groups of iminodiacetic acid in the same way as in the previous case, i.e. in aqueous buffer.

The ability of prepared chelating sorbents to form metal complexes was tested (Table 2). The obtained data confirm that sorbents show high capacities for coordination of studied metal ions Ni(II), Cu(II) and Fe(III), which are almost one order of magnitude higher in comparison with poly(DEGMA-*co*-EDMA) chelating

Table 2. Characteristics of modified poly(DEGMA-*co*-EDMA) membranes.

OH group (mmol/g)	OTs[a] group (mmol/g)	IDA[b] group (mmol/g)	Complexed ion (μmol/g)[c]			Retained complexes (%) on pH change to 8[c]		
			Ni(II) (pH 4.5)	Cu(II) (pH 4.5)	Fe(III) (pH 2.7)	Ni(II)	Cu(II)	Fe(III)
IDA introduced in pyridine								
5.72	1.02	0.79	785	652	150	95.7	99.2	100.0
		0.97	951	944	259	98.9	96.1	99.8
	1.36	1.09	1092	1069	793	96.3	99.1	97.1
		1.35	1258	1196	805	98.8	96.8	95.6
IDA introduced in aqueous NaOH								
5.72	0.48	0.38	369	318	-	97.7	99.1	-
		0.44	421	398	-	98.1	96.2	-

[a] ± 2 % by sulphur analysis
[b] ± 5 % by nitrogen analysis
[c] determined by AAS

sorbents in the form of homogeneous beads 15-60 μm in diameter, with the degree of crosslinking 10 %, prepared in our previous work [20]. An explanation of the phenomenon is a better swelling of the membrane polymer network as a consequence of the low crosslinking degree allowing attachment of a sufficient amount of chelating groups, which are subsequently engaged in coordination with metal ions. In addition, both poly(DEGMA-*co*-EDMA) chelating sorbents differ in the polymer support shape (membrane, bead) and in the type of attached chelating groups.

The stability of complexes to the pH change is high for all the sorbents. Sorption of metal ions was performed in an acid buffer (pH 4.5), in the case of Fe(III), pH was lowered to 2.7 in order to prevent hydrolysis of $FeCl_3$. The change in pH to 8 caused a negligible loss of the immobilized metal ions; at least 96 % of them remained in the immobilized complex (Table 2).

The obtained values of sorption capacities for metal ions of chelating poly(DEGMA-*co*-EDMA) membranes are comparable with other adsorbents using ion-exchange mechanism for metal ion removal from aqueous solutions [9,21], which presumes their potential use as scavengers of toxic metal ions from polluted aqueous solutions.

In general, practically all of the chelating groups are engaged in complexing Ni(II) or Cu(II) ions. On the other hand, somehow lower capacities for Fe(III) complexation were observed. The possible explanation of the phenomenon could afford the hard-soft acid-base theory of Pearson [22], which classifies metals to soft, borderline and hard on the basis of their polarizability. The nitrogen atom in IDA ligand has higher affinity to borderline Ni(II) and Cu(II) ions than to hard Fe(III) ion. However, also carboxylates are responsible for binding to the metal. These could be protonated to some extent at lower pH under which the Fe(III) experiment was conducted and lose therefore their binding ability. As a consequence, lower capacities for iron could be indicated.

High concentrations of immobilized Ni(II) ions suggest their possible use in ligand exchange of the water molecules in their coordination sphere for other ligand. Due to

high affinity of Ni(II) ions to immunoglobulins [13], the chelating Ni(II)-IDA-DEGMA-*co*-EDMA membranes were tested for sorption of murine immunoglobulin G by the IMA („immobilized metal affinity“) method. Sorption studies were performed in batch experiments.

Interaction of protein with immobilized metal ion can be explained on the basis of various isotherm models expressing equilibria between the sorbate and the solution. Murine immunoglobulin G sorption on the Ni(II)-IDA complexes of the poly(DEGMA-*co*-EDMA) membrane (Fig.1) can be characterized by the Langmuir-Freundlich model (eq. 1), which has already been used for representation of sorption of monoclonal IgG_1 (murine anti-TNP) on Zn(II)-IDA poly(ethene-vinyl alcohol) membrane [23] or sorption of lysozyme, conalbumin, wheat germ agglutinin and bovine serum albumin on Ni(II)-IDA and Cu(II)-IDA agarose [24].

The Langmuir-Freundlich equation is expressed as

$$q^* = \frac{q_{m(LF)} * (C^*)^n}{K_d^* + (C^*)^n}, \qquad (1)$$

where q^* is the amount of the adsorbate, C^* is the equilibrium concentration of the solute, K_d^* is apparent dissociation constant, $q_{m(LF)}$ is the maximum binding capacity of the sorbent and n is a dimensionless exponent related to energy of adsorption. This model describes cooperative behaviour of the binding sites on the solid surface in the course of their interaction with sorbate. This means that the occupied binding site influences adjacent binding sites positively ($n > 1$) or negatively ($0 < n < 1$) to interact with the sorbate. In the case of independent and non-interacting sites, the value of n is equal to 1. The apparent dissociation constant K_d^* includes a contribution of the binding of one molecule of the sorbate as well as binding of its associated forms (in the case of proteins, their dimers, trimers and oligomers).

The Langmuir-Freundlich isotherm model that contains three parameters reduces to two-parameter models under particular conditions. For low concentrations, to the Freundlich model ($q = FC^n$, in which F is the Freundlich constant) and for homogeneous surfaces to Langmuir model $q = q_m C/(K_d+C)$, in which q_m is the maximal binding capacity and K_d is dissociation constant. However, these two-parameter models do not sufficiently describe the sorption behaviour of immunoglobulin G on flat sheets of the Ni(II)-IDA-DEGMA-*co*-EDMA membranes (Fig.1).

With the help of the Langmuir-Freundlich model, the influence of the concentration of Ni(II)-IDA complexes immobilized on poly(DEGMA-*co*-EDMA) membranes on the sorption of immunoglobulin G was studied. From isotherm curves, which were obtained by fitting the experimental points with Langmuir-Freundlich equation, the values of thermodynamic parameters $q_{m(LF)}$, n and K_d^* were determined (Table 3). For all the studied Ni(II)-IDA concentrations, the existence of positive cooperation of binding sites ($n > 1$) was observed, which is probably a consequence of protein-multiple ligand interaction. However, the influence of the cooperation of the binding sites seems to diminish with a decrease in Ni(II)-IDA concentration, which manifests itself in decreasing values of parameter n. The values of K_d^* of the order of magnitude 10^{-6} mol.l^{-1} correspond to the affinity of the pseudobiospecific sorbents. Nevertheless, the values of maximum binding capacities $q_{m(LF)}$ of the membrane sorbents for murine immunoglobulin are generally low in comparison with other sorbents [23]. The most appropriate explanation for this phenomenon is the poor accessibility of some

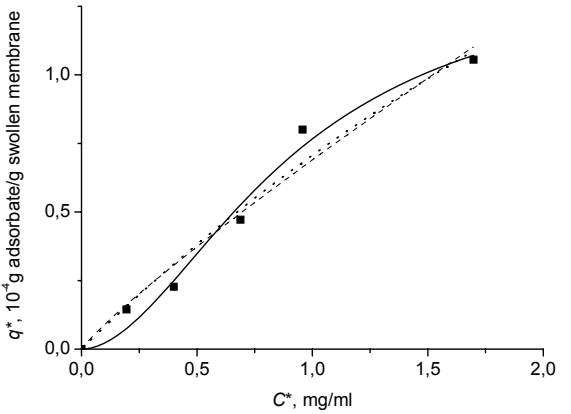


Figure 1. Sorption of murine immunoglobulin G onto Ni(II)-IDA-DEGMA-*co*-EDMA in 0.05 M phosphate buffer, pH 8, 0.5 M NaCl at 20°C. Concentration of Ni(II)-IDA complexes is 1.1 mmol/g. The lines correspond to fitting experimental values to the isotherm models: Langmuir-Freundlich (solid line), Langmuir (dotted line) and Freundlich (dashed line).

immobilized Ni(II) ions, which are buried inside the polymer network of homogeneous membrane. Therefore, the utility of chelating poly(DEGMA-*co*-EDMA) membranes in IMA method is limited to the sorption of analytical amounts of protein.

The retention of protein on the gel containing immobilized metal ions is due not only to coordinative interaction between the protein and metal ions, but also to noncovalent interactions between the protein and solid support. In the case of poly(DEGMA-*co*-EDMA) membranes, these interactions were suppressed by high hydrophility of the solid matrix and ionic strength of the used buffers; measurable sorption of immunoglobulin G on unmodified poly(DEGMA-*co*-EDMA) membranes was not observed. This implies a dominant contribution of coordination interactions in the course of sorption of immunoglobulin G on Ni(II)-IDA complexes of poly(DEGMA-*co*-EDMA) membranes.

Table 3. Sorption parameters determined from the Langmuir-Freundlich isotherm model for murine immunoglobulin G sorption onto Ni(II)-IDA-DEGMA-*co*-EDMA at 20°C.

Ni(II)-IDA (mmol/g)[a]	$q_{m(LF)}$ (μg/g)[b]	n	K_d^* (μmol.l^{-1})
1.35	139±33	1.90±0.22	2.97±0.16
1.09	125±28	1.85±0.40	5.75±0.36
0.44	122±36	1.68±0.40	7.58±0.55

[a]values from Table 2
[b]related to the weight of the swollen membrane

Conclusions

Sheets of crosslinked hydrophilic polymer membranes were prepared by radical copolymerization of 2-(2-hydroxyethoxy)ethyl methacrylate and ethylene dimeth-

acrylate. After modification with the chelating groups of iminodiacetic acid (IDA), they showed a relatively high capacities in the sorption of metal ions {Ni(II), Cu(II), Fe(III)}. Their possible use in metal ion removal from wastewaters can be envisaged. The Ni(II)-IDA complexes immobilized on poly(DEGMA-*co*-EDMA) membranes were utilized in the exchange of water molecules in the coordination sphere of Ni(II) ion for an other ligand. Murine serum immunoglobulin G has been chosen for the purpose. As it was shown, sheets of Ni(II)-IDA-DEGMA-*co*-EDMA membranes can be used for the sorption of low amounts of the immunoglobulin.

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