Polymer Bulletin 32, 705–712 (1994)

Polymer Bulletin

Metal binding properties of poly[*N,N,N′,N′*-tetrakis(2-hydroxypropyl)ethylenediamine methacrylate]

J. A. Fulton and D. J. Smith*

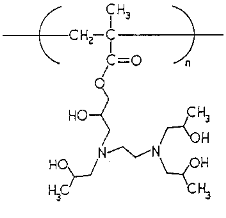
Department of Chemistry, University of Akron, Akron, OH 44325-3601, USA

Summary

When incorporated into a polymer hydrogel, the metal chelator, N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine (THPED), retains its metal binding properties. The resultant poly(THPED methacrylate) homopolymer is a polybase with displacement binding constants for copper(II) and zinc(II) of -3.11 and -6.55, respectively, as compared to values for THPED of -3.83 and -7.44. The chelating capacity of the polymer for various divalent metal ions at pH 5.5 follows the order Cu>Cd>Co>Zn>Mn, while calcium and magnesium do not bind. Metal ion release curves indicate that after 30 hours, Cd(II), Co(II), Zn(II), and Mn(II) are released at a slow, steady rate, while Cu(II) is not released under experimental conditions.

Introduction

N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine (THPED) is a tetra-alkanol substituted diamine that binds divalent metal ions in a 1:1 metal to ligand ratio (1,2). Covalent attachment of THPED to a polymethacrylate backbone via polymerization of THPED methacrylate monomer, creates a metal chelating homopolymer. Poly(THPED methacrylate)



is a polybasic hydrogel with evenly spaced pendant ligands which bind copper ions in a 1:1 ratio (3). Since the polymer also possesses immunostimulatory properties (4), it has been proposed for use as a wound dressing which could enhance the healing process and provide a source of therapeutic metal ions (5), such as zinc or copper. This paper investigates the metal binding properties of poly(THPED methacrylate) hydrogels. Potentiometric titration and equilibrium dialysis were used to determine binding constants, chelating capacities, and metal ion release characteristics.

---

*Corresponding author

Experimental

Spectrapor dialysis tubing (2,000 MWCO) was obtained from Spectrum Medical Industries (Los Angeles, CA). Tetraethyleneglycol dimethacrylate was purchased from Polysciences (Warring, PA). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), Aldrich Chemicals (Milwaukee, WI), and Fisher Chemicals (Cleveland, OH).

Polymer synthesis

Poly(THPED methacrylate) hydrogels were prepared by solution free radical polymerization of THPED methacrylate monomer using the method of Smith and Patel (3). Water soluble polymers were polymerized in dioxane with AIBN initiator, then extensively dialyzed against distilled water and freeze-dried.

Potentiometric titrations

Dissociation and binding constants were determined by pH potentiometric titrations. Vacuum oven-dried samples were weighed to the nearest 0.01 mg, and stock solutions containing approximately 0.005 M in polymer monomeric units were prepared with carbon dioxide-free distilled water. The concentration of each metal ion solution (0.004 M) was determined by atomic absorption spectroscopy (Perkin-Elmer 5000 AAS). Titrations were carried out in a Metrohm water-jacketed titration vessel (Brinkman Instruments Co., Westbury, NY) maintained at 25 ± 0.1°C. To one mL of sample stock solution, with and without an equal volume of metal ion solution, excess nitric acid was added to diprotonate the diamines. A constant ionic strength was maintained with potassium nitrate (0.5 M). After adjusting the volume to 6 mL, the samples were back-titrated with 0.1 M potassium hydroxide (KHP ref. std.) under nitrogen with constant stirring. Typically, 2 to 5 μL increments of titrant were added with an electronic pipetter (Rainin edp) fitted with a 250 μL tip. The $p[H^+]$ measurements were obtained with a 3-place digital pH meter (Corning model 130) and a calomel microprobe combination electrode (Fisher Scientific) calibrated to read hydrogen ion concentrations.

Calculation of equilibrium constants

Apparent dissociation constant (pKa) values were determined for poly(THPED methacrylate) from the neutralization titrations with modified Henderson-Hasselbalch equations (6) as follows:

$$pH = pKa_1 - n_1 \log \frac{1 - \alpha}{\alpha} \tag{1}$$

$$pH = pKa_2 - n_2 \log \frac{2 - \alpha}{\alpha - 1} \tag{2}$$

Diprotonated, monoprotonated, and free ligand are represented by H_2L^{2+}, HL^+, and L, respectively. The degree of neutralization is represented by α, and n is a constant considered to be a measure of electrostatic interactions between neighboring groups on the polymer chain. Values for n_1 and pKa_1 were obtained from the slope and intercept of a plot of pH versus log $(1 - \alpha)/\alpha$. Values for n_2 and pKa_2 were determined from the slope and intercept of a plot of pH versus log $(2 - \alpha)/(\alpha - 1)$.

Binding constants were determined from complexation titrations with the modified Bjerrum method (7) for the displacement reaction

$$M^{2+} + H_2L^{2+} \rightleftharpoons ML^{2+} + 2H^+ \tag{3}$$

where M represents the metal ions. The displacement constant (B) was obtained from the half complexation point of a formation curve plotted as $\bar{n}$ versus log $[H^+]^2/[H_2L^{2+}]$, where the average ligand number, $\bar{n}$, is defined as

$$\bar{n} = \frac{C_L - [L] - [HL^+] - [H_2L^{2+}]}{C_M} \tag{4}$$

C_L is the total ligand concentration, and C_M is the total metal ion concentration. The free ligand concentration, [L], was determined with an expression for the dissociation constant in which the Ka value is a function of the degree of charging (z) on the polymer chain. For the case of a polybase (8) in a chelation reaction,

$$Ka_1 = \frac{[H^+][HL^+]}{[H_2L^{2+}]} \left(\frac{1}{z_1}\right)^{n_1 - 1} \tag{5}$$

and

$$Ka_2 = \frac{[H^+][L]}{[HL^+]} \left(\frac{1}{z_2}\right)^{n_2 - 1} \tag{6}$$

where z_1 is the ratio of dicharged to monocharged groups in the first dissociation reaction, and z_2 is the ratio of monocharged to uncharged groups in the second dissociation reaction. The overall dissociation constant is

$$Ka = \frac{[L][H^+]^2}{[H_2L^{2+}]} \left(\frac{[L]}{[HL^+]}\right)^{n_2 - 1} \left(\frac{[HL^+]}{C_L - [HL^+] - [L]}\right)^{n_1 - 1} \tag{7}$$

The concentration of the monoprotonated species can be calculated from the expression:

$$[HL^+] = \frac{[S][H^+]}{Ka_2} \tag{8}$$

The concentration of the diprotonated species can be calculated from the expression:

$$[H_2L^{2+}] = \frac{[HL^+][H^+]}{Ka_1} \quad (9)$$

The concentration of non-protonated species, [S], is based on the degree of neutralization and can be calculated from the expression:

$$[S] = \frac{(2 - \alpha)C_L - [H^+] + [OH^-]}{\frac{[H^+]}{Ka_2} + \frac{2[H^+]^2}{Ka_1\ Ka_2}} \quad (10)$$

An iterative process is then used to solve for [L] in equation 7.

Species distribution plots were generated with the programs SPE and SPEPLOT (9).

Equilibrium dialysis

Chelating capacities and metal ion release curves were obtained by using an equilibrium dialysis procedure. Ten mL aliquots of polymer solution (0.1 to 0.5 M monomer units) were dialyzed against one liter of metal acetate solutions (1.0 to 5.0 M, pH 5.5). After equilibrium was reached, the metal-loaded polymers were transferred to EDTA solutions to determine the maximum binding of metal ions. Following removal of all bound metal, the chelating capacities were calculated as mmoles metal bound per g polymer.

When metal-loaded polymers were dialyzed against sodium acetate solutions (equimolar to the metal acetate solutions), metal ion release could be measured. Dialysis bags containing only metal acetate solutions and no polymer were used as controls. Samples of the dialysates were taken for analysis at regular time intervals up to 200 hours. The metal ion concentrations of all solutions were determined by atomic absorption spectroscopy (Perkin-Elmer 5000 AAS).

Results and discussion

Dissociation constants

Dissociation constants were determined from the neutralization titration data represented by curve a in Figure 1. The protonated polymer behaved like a dibasic acid with two distinct endpoints. This indicates that the first amine hydrogen of each monomer unit was completely titrated before titration of the second began.

When pH was plotted against log $(1 - \alpha)/\alpha$, the linear range where the modified Henderson-Hasselbalch equation is valid, was from α equals 0.3 to 0.9. The plot of pH versus log $(2 - \alpha)/(\alpha - 1)$ gave a linear range from α equals 1.35 to 1.75. Table 1 lists the n and pKa values determined from the slopes and intercepts, respectively. Since interactions between ligands should not be present for the monomer analogue, THPED, slopes of about 1.0 were expected. In contrast, n values of 0.55 and 1.48 for the polymer indicate the presence of electrostatic interactions between neighboring groups in the matrix. Also, lower pKa values for poly(THPED methacrylate) indicate that it is a weaker base than THPED.

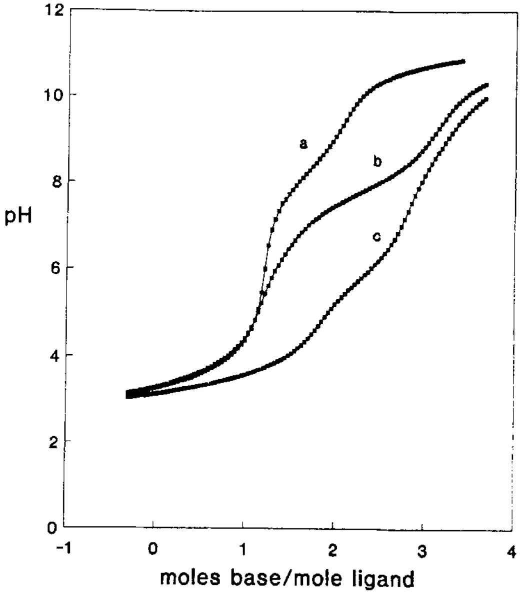


Figure 1. Potentiometric titration curves for poly(THPED methacrylate) (0.0054 M). Titrated with 0.1 M KOH at μ = 0.5 M (KNO_3) and 25°C: (a) without added metal; (b) in presence of Zn(II) (0.0038 M); (c) in presence of Cu(II) (0.0042 M).

Table 1. Dissociation and Binding Constants for THPED and Poly(THPED methacrylate)

Constants	Polymer	THPED
pKa_1	3.62 + 0.03	4.43 + 0.01
pKa_2	7.77 + 0.04	8.94 + 0.02
n_1	0.55 + 0.03	0.98 + 0.02
n_2	1.48 + 0.03	0.95 + 0.02
$B_{Cu(II)}$	-3.11 + 0.05	-3.83 + 0.03
$B_{Zn(II)}$	-6.55 + 0.05	-7.44 + 0.04

Binding constants

Copper and zinc were selected for binding constant determination because of their biological activity. As seen in Figure 1 (curves b and c), protons displaced during complex formation lower the pH values in relation to the affinity of the ligand for the metal ion. Thus, the presence of Cu(II) lowers the titration curve more than Zn(II). Copper(II) ions begin binding immediately and displace the two amine hydrogen atoms which are titrated together. After two equivalents of base have been added, the third and fourth protons from coordinated hydroxyl groups are titrated. In contrast, with Zn(II), the first proton is titrated before binding begins, and titration of the third proton begins before binding is complete.

The displacement binding constants (B) are listed in Table 1 and indicate that poly(THPED methacrylate) binds metal ions more strongly than its monomer analogue, THPED. Also, the binding constant for Cu(II) is more than three orders of magnitude greater than the Zn(II) constant for both THPED and poly(THPED methacrylate).

Species distribution plots provide an alternate means of examining titration data. The plot for Cu(II) (Figure 2A) shows that complex formation begins at a pH of about 2.5 and reaches a maximum by pH 4.5 with the monoprotonated species remaining minor. In contrast, the Zn(II) curves (Figure 2B) show that the monoprotonated species becomes dominant and then complex formation begins about pH 4. Maximum complexation is not reached until about pH 7. This could be an important consideration when looking at a physiological system. For example, a slightly acidic environment would greatly enhance the release of zinc ions from the polymer but have little effect on copper ions.

Chelating capacities

The chelating capacity is an indication of the maximum amount of metal ion that can be loaded onto the polymer at any given pH. Since the microenvironment of a wound is acidic (10), chelating capacities of poly(THPED methacrylate) for various metal ions were determined at pH 5.5. The results are given in Table 2. The polymer binds the divalent metal ions in a decreasing order as follows: Cu > Cd > Co > Zn > Mn, while calcium and magnesium binding was not detected.

Table 2. Chelating Capacities for Poly(THPED methacrylate)

	Chelating Capacity (mmol metal/g polymer)
Cu(II)	2.4 + 0.1
Cd(II)	1.6 + 0.1
Co(II)	1.2 + 0.1
Zn(II)	0.9 + 0.1
Mn(II)	0.2 + 0.1
Mg(II)	No detectable binding
Ca(II)	No detectable binding

Metal ion release

Release curves in Figure 3 show the cumulative amount of metal ion released per gram of polymer as a function of time. The entire release process can be divided into three overlapping phases. First, the metal ions in the solution surrounding the polymer diffuse out of the dialysis bag.

Analysis of a blank solution containing only metal ions indicated that diffusion was complete within five hours. Secondly, diffusion of unbound metal ions from the polymer matrix requires about 30 hours. This can be seen from the plot of copper since the high affinity of the polymer for copper prevents the release of bound ions. Lastly, the bound metal ions are released and diffuse out of the matrix at a relatively steady rate. These results suggest that the polymer could be used for slow release of metal ions.

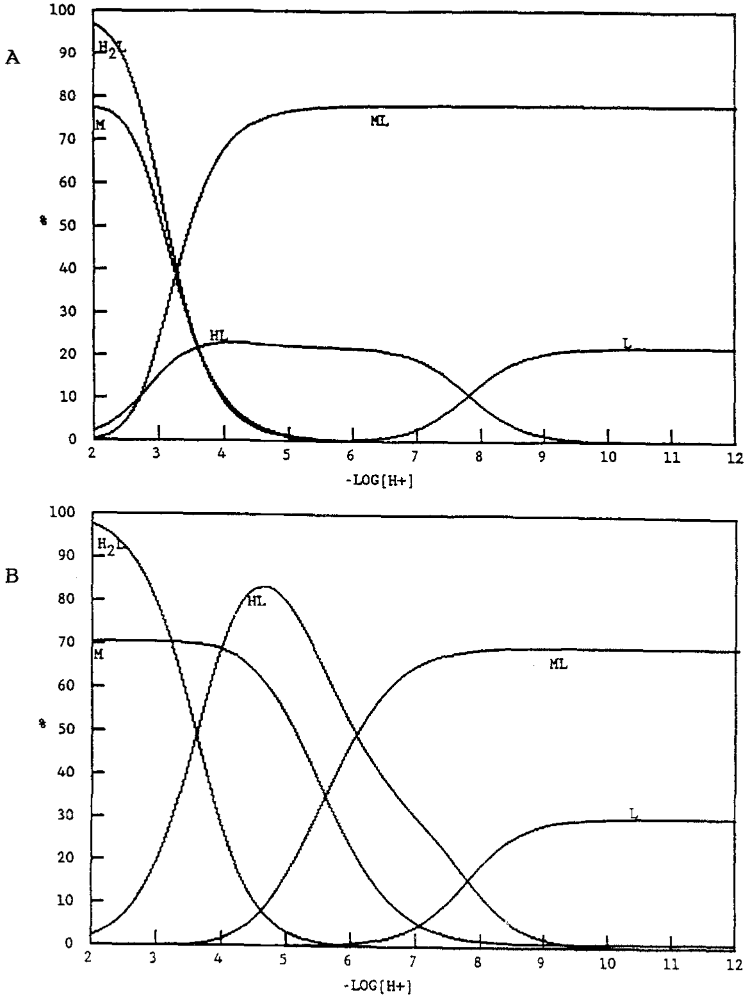
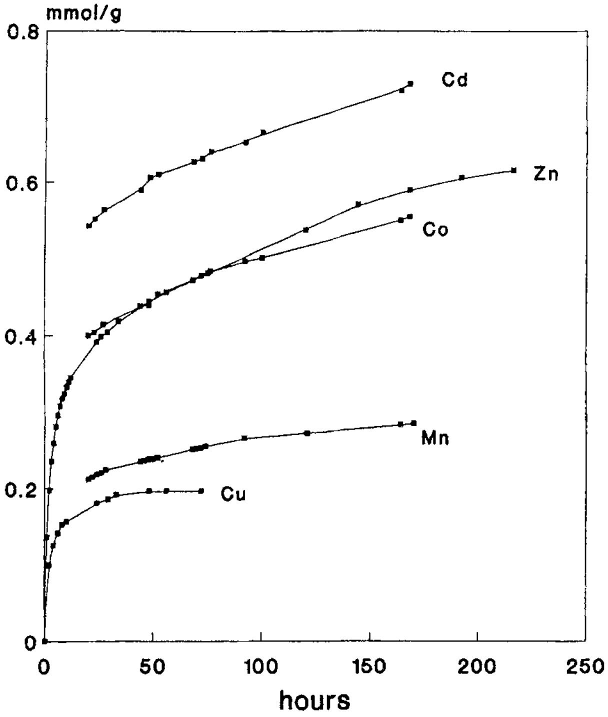


Figure 2. Species distribution diagrams for 0.0054 M poly(THPED methacrylate) and A) 0.0042 M Cu(II); B) 0.0038 M Zn(II) at μ = 0.5 M (KNO_3) and 25°C. Where M = copper(II) ions (A) or zinc(II) ions (B); H_2L = diprotonated ligand; HL = monoprotonated ligand; L = free ligand; ML = metal-ligand complex.

Figure 3. Metal ion release curves. The cumulative amount of divalent metal ion released per gram of polymer per hour.



Acknowledgment. This work was supported in part by a grant from the Edison Polymer Innovation Corporation.

References

1. Hall JL, Dean WE, Pacofsky EA (1960) *J Am Chem Soc 82:* 3303
2. Orama M, Saarinen H, Kaila L (1979) *Finn Chem Lett 6:* 182
3. Smith DJ, Patel S (1985) *J Polym Sci, Polym Lett Ed 23:* 633
4. Bhide MV, Smith DJ (1987) *Immunopharmacol Immunotoxicol 9:* 129
5. Smith DJ, Patel SR (1988) *U.S. Patent* 4781921
6. Katchalsky A, Spitnik P (1947) *J Polym Sci 2:* 432
7. Gregor HP, Luttinger LB, Loebl EM (1955) *J Phys Chem 59:* 34
8. Gold DH, Gregor HP (1960) *J Phys Chem 64:* 1461
9. Martell AE, Motekaitis RJ (1988) *Determination and Use of Stability Constants.* VCH Publishers New York
10. Hunt TK, Twomey T, Zederfeldt B, Dunphy JE (1967) *Am J Surg 114:* 302

Accepted March 24, 1994 K