Colloid Polym Sci 276:206–212 (1998)
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ORIGINAL CONTRIBUTION

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Fluorescence study of the interaction between metal ions and methyl methacrylate–methacrylic acid copolymers in aqueous solutions: thallium(I), calcium(II), and terbium(III)

Received: 10 June 1997
Accepted: 24 October 1997

Abstract The binding of the cations thallium(I), calcium(II) and terbium(III) to methyl methacrylate–methacrylic acid copolymers with different fractions of acid groups (x) has been studied in aqueous solution at various pH values using the fluorescence of covalently bonded 9-vinyl anthracene as a probe. In all cases, the extent of binding increases as a function of the charge of the polymer with either increasing fraction of carboxylic acids or of pH. However, differences are observed in the behavior of the three cations. With Tl(I), quenching of the anthracene group fluorescence is observed, indicating that the thallium(I) approaches the probe and suggesting that the alkylanthracene is probably in a relatively polar region. Binding constants have been determined from anthracene quenching data and from studies with the fluorescent-probe sodium pyrenetetrasulfonate. Good agreement is obtained between the two methods, and values for the binding constants increase from 250 to 950 M^{-1} as x increases from 0.39 to 1. It is suggested that the cation is held in the polyelectrolyte domain, partly by Debye–Hückel effects and partly by more specific interactions. Stronger binding is found with calcium(II) and terbium(III), and in this case increases in fluorescence intensity are observed on complexation due to the anthracene group being in a more hydrophobic region, probably as a result of conformational changes in the polymer chain. In the former case the stoichiometry of the interaction was determined from the fluorescence data to involve two carboxylate groups bound per calcium. Association constants were found using murexide as an indicator of free calcium to vary from 8400 to 37 000 M^{-1} as x increases from 0.39 to 1. It is suggested that in this case specific calcium(II)–carboxylate interactions contribute to the binding. With terbium(III), a greater increase in the probe fluorescence intensity was observed than with calcium, and it is suggested that the interaction with the polymer is even stronger, leading to a more pronounced conformational change in the polymer. It is proposed that the terbium(III) interacts with six carboxylic groups on the polymer chain, with three being coordinated and three attracted by electrostatic interactions.

Key words Polyelectrolytes – metal/polyelectrolyte interactions – polyelectrolyte conformations

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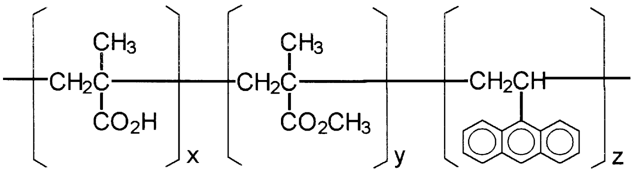
Introduction

Interactions between metallic cations and anionic polyelectrolytes are of theoretical and experimental interest both because of their importance in various technological applications and their relevance to the behavior of certain biological polymers in aqueous solutions. The structure and functions in vivo of many water-soluble biopolymers depend upon the reversible formation of complexes with such co-solutes. For example, the conformational stability and behavior of polynucleotides is dependent upon the formation of complexes with metal ions via electrostatic interactions [1]. From the technological viewpoint, much research has been directed towards the use of polyelectrolyte–cation complexes in areas as diverse as semiconductors, thermal stabilizers, redox reactions, pollutant treatment and metal-ion recovery [2]. Linear polyelectrolytes with high charge density along their chains have been used as supports for catalysts for various chemical and photochemical reactions [3, 4]. In photo-induced electron and energy transfer processes, these have been used to promote the forward reaction and to retard back transfer [5, 6]. Polyelectrolytes may form micelle-like structures, which can be used to increase local concentrations of reagents [7, 8], and hence to increase reaction rates.

Two distinct types of association between metallic cations and anionic polyelectrolytes have been suggested. In some cases, as with the alkali metal cations, the interaction is thought to be purely electrostatic within the ionic atmosphere at the somewhat diffuse polyelectrolyte–water interface. The binding is essentially non-directional, and the metal-ions retain some mobility. The second type of interaction involves binding with specific anionic sites in the polyelectrolytes, leading to the formation of intramolecular complexes [9]. These may affect the conformational structure of the polymers. In some cases, polyelectrolyte–cation complexes become more compact, and may be highly active as catalysts [10].

Various techniques have been used to study association in these systems, including dialysis [11], viscosimetry [12], UV spectroscopy [13], fluorescence [12, 14], and NMR spectroscopy [15]. Interest has been focused both on binding constants [16], the mode of binding of the metal ion and on the effect of this interaction on the polymer conformation.

We report on the interaction between various metal cations and methacrylic acid–methyl methacrylate copolymers labelled with the fluorescent probe 9-alkylanthracene.



The photophysical behavior of these polymers in aqueous solutions was presented in a previous paper [17].

Experimental

Poly(methyl methacrylate-*co*-9-vinyl anthracene, poly(MMA-9VA)) was prepared by copolymerization of the monomers using azoisobutyronitrile as initiator. The product was purified by two cycles of dissolution in benzene and precipitation with hexane, filtered and vacuum dried. GPC and UV absorption spectral analysis gave values $M_w = 9.0 \times 10^4$, $M_n = 5.1 \times 10^4$ for the molar mass and showed that the polymer contained 0.12% (in moles) of bound 9-vinylanthracene. The terpolymers poly(methyl methacrylate-*co*-methacrylic acid-*co*-9-vinyl anthracene) were obtained by partial hydrolysis of the poly(MMA-9VA) copolymer with 20% NaOH in dimethylsulfoxide in the dark under a nitrogen atmosphere [17, 18]. The extent of hydrolysis was controlled by varying the reaction time and temperature, the terpolymer precipitated by neutralization with aqueous hydrochloric acid, washed with acidic methanol, precipitated with ethyl ether and vacuum dried. The amount of free acid in each polymer was determined by conductimetric titration of known masses of copolymers dissolved in methanol–water (20 : 80 v/v) with a freshly prepared standardized solution of NaOH. The degrees of hydrolysis of the copolymers, in terms of the fraction of methacrylic acid monomers (x), are given in Table 1 together with the charge-density parameter (ξ). Full details of the synthesis and characterization of these copolymers have been given in ref. [16].

All solutions were prepared in Millipore Milli-Q deionized water. The inorganic salts were of the purest grade commercially available and were used as received.

Copolymer solutions were prepared by dissolving known masses in a small volume of NaOH solution (0.01 M) and adjusting pH by addition of concentrated NaOH solution. Solutions were freshly prepared and stored in the dark. Stock solutions of quenchers were prepared, and small volumes added with a microsyringe to a known volume of the polymer solution directly in quartz cuvettes. Equilibrium was rapidly established on addition of the cations. Degrees of ionization of the polymers (α) were determined by titration, as described in ref. [17].

Table 1 Properties of the copolymers

Copolymer	COP1	COP2	COP3	COP4
x^a	0.39	0.67	0.87	1.00
ξ	1.12	1.86	2.42	2.78

[a] Fraction of methacrylic acid monomers.

Fluorescence spectra were recorded on Spex Flurolog 111, Aminco Bowman J8 and Edinburgh CD900 spectrofluorimeters. The polymers were excited at 375 nm and the emission measured at 418 nm. When pyrenetetrasulfonate was used as the probe, excitation and emission were performed at 338 and 387 nm, respectively. Absorption spectral measurements were made on Shimadzu UV-2100 and Hitachi U2000 spectrophotometers.

Results

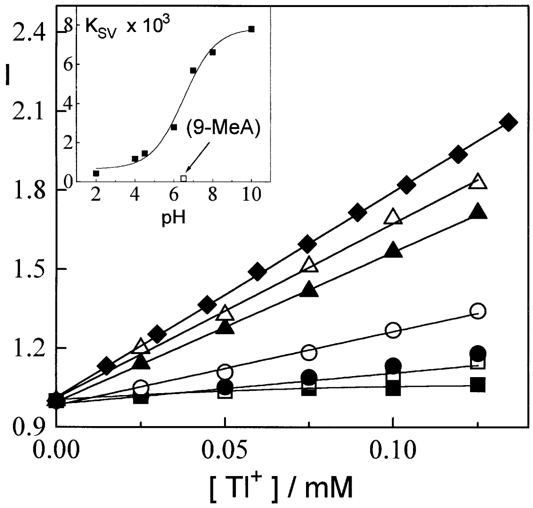
Association of Tl(I) ions

The association of thallium to the polyelectrolytes is expected to be dependent on the charge density on these polyelectrolytes [19]. Figure 1 shows Stern–Volmer plots for the fluorescence quenching of COP4 by Tl$^+$ ($\alpha \approx 1$) in aqueous solution. It can be noticed that the quenching efficiency increases with pH, that is with the increase of the electrostatic field around the poly-ions [20]. Due to the increase of the local concentration of the quenching ions near the probe, the quenching is much more efficient than the quenching of 9-methylanthracene in water (inset in Fig. 1).

The association, or binding of ions (I) to polymers (P) can be represented by an equilibrium

$$P + I^+ \rightleftharpoons P - I^+ \, . \tag{1}$$

Fig. 1 Stern–Volmer plots for the quenching of 9VA-PMA (COP4) by Tl$^+$ as a function of pH: (■) 2.0, (□) 4.0, (●) 4.5, (○) 6.0, (▲) 7.0, (△) 8.0, (◆) 10.0. **Inset**: plot of the Stern–Volmer constants versus pH. [COP4] = 0.5 g/l. The arrow indicates the position for 9-methylanthracene



From where a formal equilibrium constant can be defined as

$$K = \frac{[P - I^+]}{[P][I^+]} \tag{2}$$

where $[P - I^+]$, $[P]$ and $[I^+]$ represent the concentrations of ions bound to the polymer, of free binding sites on the polymer and of free positively charged ions, respectively.

Two different approaches were used to determine the constants of association of thallium(I) ions to the polyelectrolytes. In the first, the quenching of fluorescence of the probe bound to the polyelectrolyte by the ions was used. The relative fluorescence intensity I_0/I (in the absence and presence of quencher, respectively) will depend on the fraction of the quenching T1$^+$ ions localized in the polyelectrolyte domain and on the fraction in the neighborhood of the polymer due to Debye–Hückel-type interactions [21]. Considering that the binding sites on the polymer will be the carboxylate groups, and the metal ions present in the system are the quenchers for the probe, Eq. (1) can be written as

$$K = \frac{[Q_b]}{[COO^-][Q_f]} = \frac{f}{[Q_f]} \tag{3}$$

and

$$[Q_t] = [Q_b] + [Q_f] = f[COO^-] + f/K \, , \tag{4}$$

where f is the ratio between bound quencher molecules and ionized groups on the polyelectrolyte ($[Q_b]/[COO^-]$); $[Q_t]$ is the total concentration of thallium ions, and $[Q_b]$ and $[Q_f]$ the concentrations of bound and free thallium ions, respectively. $[COO^-]$ is the concentration of ionized carboxylate groups on the polyelectrolyte.

Using a modified version of the Encinas and Lissi method for determination of constants for the association of quenchers to microheterogeneous domains [16], the constants for the association of T1$^+$ to the polyelectrolytes were obtained. The Stern–Volmer plots for the quenching of COP2 by thallium ions in the presence of various polyelectrolyte concentrations are shown in Fig. 2, with the inset showing the plots according to Eq. (4), from where the K values can be extracted.

The second method uses sodium pyrenetetrasulfonate (NPTS) as a probe, which is excluded from the polyelectrolyte domain by electrostatic repulsion. This probe has been used successfully for the determination of association constants of tempol to SDS [22]. Its lifetime ($\sim$10 ns), fluorescence quantum yield and emission spectrum are not affected by the presence of the copolymer, indicating that there is no interaction between it and the polyelectrolytes. Although a small overlap exists between the spectra of NPTS and the anthracene moiety on the

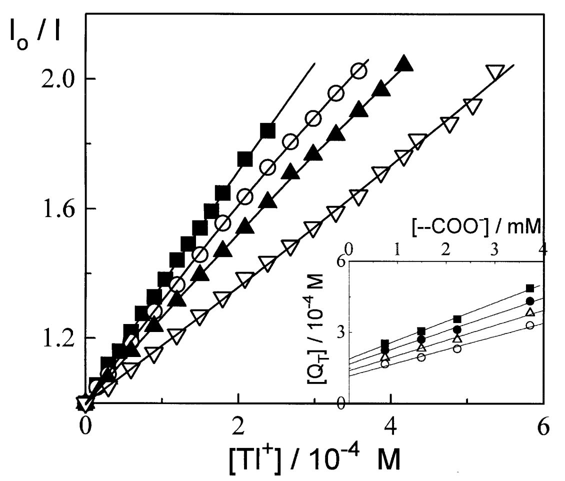


Fig. 2 Stern–Volmer plots for the quenching of COP2 by Tl^+ as a function of copolymer concentration at pH 10: (■) 0.1, (○) 0.2, (▲) 0.3, (▽) 0.5 g/l. **Inset**: plots of Eq. (2) for I_0/I (■) 1.9, (●) 1.8, (△) 1.7 and (○) 1.6

copolymers, the experimental conditions used for the determinations were such as to minimize these effects and make them negligible when compared with the NPTS fluorescence.

Assuming that the decrease of the quenching of NPTS by Tl^+ in the presence of the copolymers is due to their association to the anionic sites of the polyelectrolyte, an equilibrium will be established

$$Q_f + P \rightleftharpoons Q_b \ , \tag{5}$$

$$K = \frac{[Q_b]}{(P_0 - [Q_b])[Q_f]} \ , \tag{6}$$

where the meaning of $[Q_b]$ and $[Q_f]$ is the same as before and P_0 corresponds to the concentration of ionized carboxylic groups. For a given thallium concentration, where $[Q_t] = [Q_b] + [Q_f]$, it can be assumed that the quencher concentration corresponding to I_0/I, will be due to $[Q_f]$. Thus, Eq. (6) can be rearranged to give Eq. (7), and the association constants can be extracted from plots of $[Q_b]/[Q_f]$ as a function of $[Q_b]$, as shown in Figs. 3(a) and (b) for COP3.

$$[Q_b]/[Q_f] = P_0K - K[Q_b] \ . \tag{7}$$

The association constants determined by both methods, as well as the linear charge densities for each copolymer are shown in Table 2. The charge densities were calculated assuming that the polyions can be considered as rigid cylinders (at least locally), in which the charges are randomly distributed [23].

The values obtained by the two methods are in reasonable agreement. It can be noticed that the constants decrease for polyelectrolytes with the smaller linear charge densities.

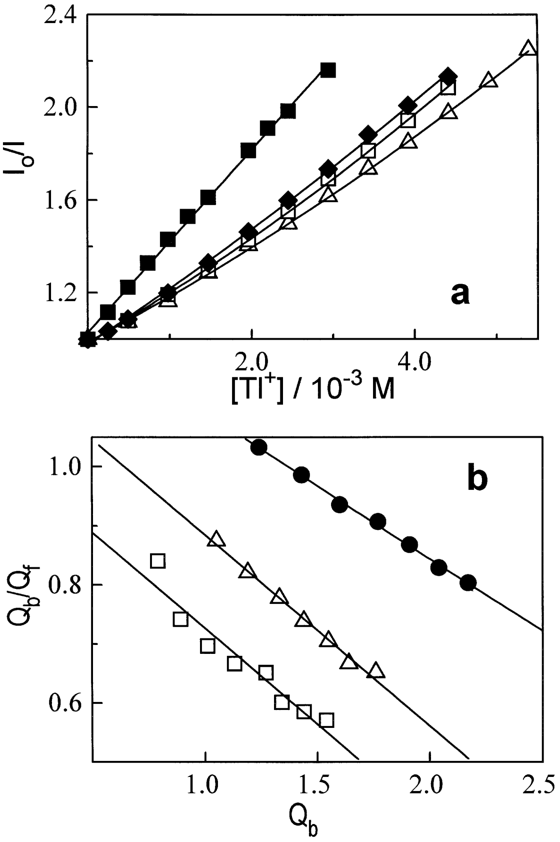


Fig. 3 (a) Stern–Volmer plots for the quenching of pyrenetetrasulfonate by Tl^+ at pH 10 in water (■) and in the presence of COP2. Copolymer concentration: (◆) 0.2, (□) 0.3, (△) 0.4 g/l. (b) Data plotted according to Eq. (5). Copolymer concentration: (●) 0.2, (□) 0.3, (△) 0.4 g/l

Table 2 Association constants (M^{-1}) of poly(methacrylic acid) and its copolymers to Tl^+ and Ca^{2+} ions at high pH and 25 °C

	COP1	COP2	COP3	COP4
Tl^+ (a)	250 (±50)	350 (±50)		950 (±100)
(b)		440 (±25)	800 (±10)	800 (±50)
Ca^{2+}	8400	29300	37 700	37 000

(a) Determined using pyrenetetrasulfonate.
(b) Determined using the fluorescence quenching of bound anthracene.

Association of Ca(II) and Tb(III)

The addition of calcium ions to the copolymers results in a noticeable increase of the fluorescence quantum yield of the anthracene moiety bound to the polymer chain. This increase is shown in Fig. 4 for COP2 at low and high degrees of ionization. The increase in the fluorescence intensity must be due to a change in the microenvironment sensed by the anthracene group. Most probably this is a result of conformational changes in the macromolecules

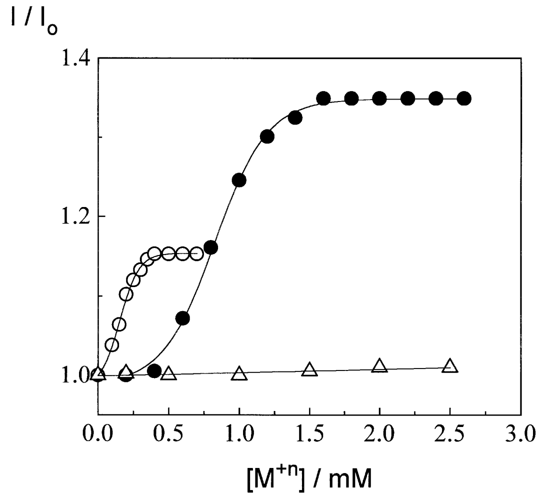


Fig. 4 Fluorescence intensity of COP2 (0.5 g/l) as a function of the concentration of (△) NaCl and Ca^{2+} at different ionization degrees: (○) $\alpha = 0.41$ (pH 6.7), (●) $\alpha = 0.8$ (pH 8.0)

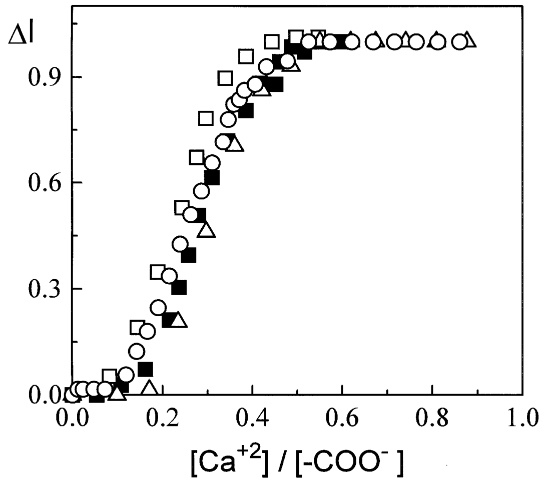


Fig. 5 Fluorescence titration of the anthracene containing copolymers with Ca^{2+}: (○) COP1, (△) COP2, (□) COP3, (■) COP4. Copolymer concentrations 0.5 g/l

induced by the calcium ions. For higher degrees of ionization (high pH), the conformations of the chain are more extended, so that the anthracene is in a more aqueous environment [24]. In these cases the addition of the divalent calcium ions will induce an entangling of the chains, by reducing the electrostatic repulsive interactions between different segments. This produces more hydrophobic microenvironments for the anthracene, resulting in a higher emission intensity. On the other hand, at low pH, when there are not many effective charges along the chain, the probe is already placed in a relatively hydrophobic region before the addition of calcium, so that the effect of the ions will be much lower than at higher pH, and a steady-state plateau will also be reached at lower Ca^{2+} concentrations. The importance of changes in ionic strength on the fluorescence behavior was checked by the addition of NaCl. In this case, there is practically no change in the emission intensity, proving that the effect of calcium is to induce an entangling, or cross linking of the chains due to electrostatic interactions between Ca^{2+} and the anionic sites of the polyelectrolytes.

The stoichiometry of the interaction between Ca^{2+} ions and the polyelectrolytes can be obtained from the behavior of the emission intensity of the probe bound to the polymer backbone, at high degrees of ionization. Plots of the emission intensities as a function of the $[Ca^{2+}]/[COO^-]$ ratio are shown in Fig. 5 for all the copolymers. It can be seen, that, when normalized, the plots for all the systems are more or less coincident, and that, furthermore, the plateau is reached in all cases when the ratio $[Ca^{2+}]/[COO^-]$ approaches ~0.5.

Qualitatively, the addition of Tb(III) ions has the same effect as Ca(II), although the increase of the emission intensity is larger for the former cation. This behavior was observed for all the copolymers and is shown in Fig. 6 for COP2, where it can be seen that in the plateau region at high pH the addition of Tb(III) results in an increase in fluorescene intensity about three times that for Ca(II). This implies that, somehow, the nature of association between the cations and the polyelectrolytes is different in the two cases, possibly as a result of different conformational changes being induced in the polymer by the cation complexation.

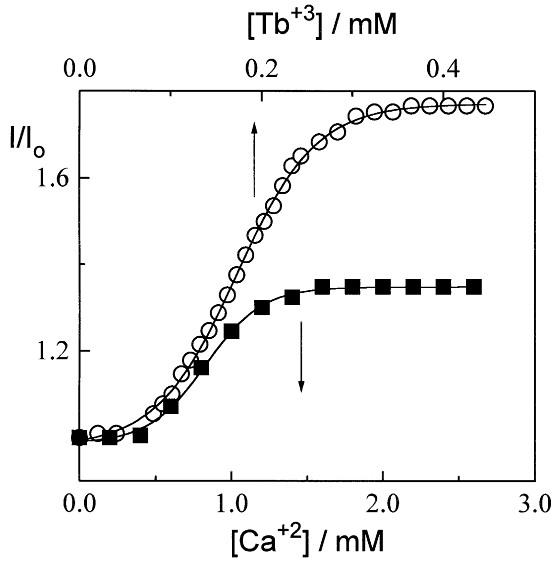


Fig. 6 Fluorescence intensity of COP2 (0.5 g/l) as a function of the concentration of (■) Ca^{2+} and (○) Tb^{3+}. $\alpha = 0.8$

The association constant for calcium ions has been determined assuming that there is fixed number of active sites

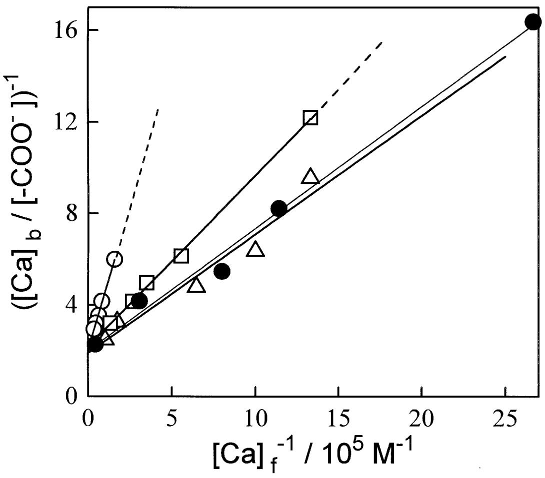


Fig. 7 Double-reciprocal (Klotz) plots for the binding of Ca^{2+} to (○) COP1, (△) COP2, (□) COP3, (●) COP4. Copolymers concentrations 0.5 g/l, pH 8.0 in Tris(hydroxymethyl) aminoethane buffer 2×10^{-3} M

on the polyelectrolytes, which is independent of the charge density on the chain. The constants were obtained from Klotz plots [25], which correspond to the relationship

$$\frac{[COO^-]}{[Ca^{2+}]_b} = \frac{1}{n} + \frac{1}{nK} \cdot \frac{1}{[Ca^{2+}]_f} , \tag{8}$$

where $[Ca^{2+}]_b$ and $[Ca^{2+}]_f$ correspond, respectively, to bound and free Ca^{2+} ions, and n is the number of sites on the chain per Ca(II) ion.

The concentrations of free and bound calcium were determined using murexide in Tris buffer (pH 8.0) as an indicator for free calcium. At this pH, the indicator has an association constant of 2200 [26]. The absorption maxima of free murexide and its Ca complex are at 522 and 489 nm, respectively. No interaction between the copolymers and the dye was detected, so that the determination of free calcium ions could be obtained in a straightforward fashion and used to construct plots according to Eq. (8) (shown in Fig. 7), from where the association constants shown in Table 2 were obtained. The behavior observed for these constants as a function of the charge density is similar to that found in the case of the thallium(I) ions.

Discussion

At low pH, when few carboxylic groups are ionized, the quenching process of the probe alkylanthracene fluorescene by Tl(I) is completely inhibited, proving that under these conditions the fluorophore is in a relatively hydrophobic region inaccessible to the cations. Thus, the association constants for Tl(I) to the copolymers will be directly related to the charge density along the chain. The increase of the degree of ionization of the carboxylic groups will expose the fluorophores to the external environment, as well as attracting the positive ions towards the polyelectrolyte ions, increasing the efficiency of the quenching process. Therefore, both the binding and the increase of quenching can be thought to be due, at least partially, to the condensation process of Tl(I) in the domain of the polyelectrolyte [27]. Taking into account the fact that the charged monomers are randomly distributed along the chain, the decrease in the charge-density parameter ξ shown in Table 1 can easily explain the decrease of the association constant by a factor of 3 when going from COP4 to COP1.

According to Manning's theory, the condensation of monovalent ions on the charged domain of a polyelectrolyte occurs until $(1 - \xi^{-1})$ of the charge is neutralized. For COP3 and COP4 this condensation could also occur by a more specific interaction between the Tl(I) ions and the carboxylate groups on the chain. Support for such an interaction comes from studies by Morishima et al. [14], who showed that Tl(I) ions condensed on acrylic acid–acrylamide copolymers, are not expelled by the addition of Li^+, Na^+ or K^+. Furthermore, it may also be expected that Tl^+ binds to the anionic atmosphere around the polyion by non-specific Debye–Hückel interactions. However, although in the cases of COP1 and COP2 the theory predicted that around 10% and 40%, respectively, of the chain charge would be compensated by the condensation of thallium ions, the experimental values of the association constants show that the binding is even greater, reaching about 35% and 60%, respectively. For these copolymers, the actual charge density may be larger than that calculated according to the Manning theory, as the greater amount of hydrophobic units on the backbone of the polymer would lead to closer structures. This will reduce the spacing between the charged groups and increase the effective charge density ξ, allowing a greater condensation of Tl(I) than predicted by the theory, which only considers rod-like structures.

The results with Ca(II) show that this ion is bound more strongly than Tl(I), and also leads to an increased emission yield of the bound anthracene, implying that there is a conformational change in the polymer chain which creates a more hydrophobic environment for the probe. This emission yield increases with the addition of Ca(II), up to the point when the ratio $[Ca^{2+}]/[COO^-]$ is around 0.5, after which there is no further increase of the fluorescence intensity. That concentration can be considered to be the saturation point of the polyelectrolyte. The value 0.5 means that there are about two carboxylate groups per Ca^{2+} ion, indicating a 2:1 ratio corresponding to a mainly electrostatic interaction. The same value is obtained from the Klotz plots, supporting this idea. The binding constants for Ca^{2+} are about 40 times larger than those for Tl^{+}. The weaker binding of the monovalent ion is possibly due both to the smaller charge as well as less specific cation–carboxylate interactions in the Tl(I) case.

Morishima proved recently [28] that thallium ions condensed on acrylamide–acrylic acid copolymers can be almost completely exchanged by calcium(II) ions. The estimation of calcium bound to the polyelectrolytes is larger than that predicted by the theory of counter-ion condensation [29], that assumes that the bonds are purely electrostatic and independent of the nature of the anionic sites. It is well known that calcium ions interact quite strongly with polymers and polysaccharides containing carboxylic groups, like heparin [30], carboxymethylcellulose, poly(galacturonic acid) [31], and others [32], Malovíková and Rinaudo [33] showed that the binding of Mg^{2+} ions to poly(galacturonic acid) is very strong, inducing conformational changes and inter-chain interactions. These results suggest specific interactions with the carboxylate groups, as already proposed by Mattai and Kwak [34].

The interaction of the Tb^{3+} ion is comparatively stronger than that with the other ions. The increase of the fluorescence quantum yield induced by this ion is about three times that for Ca^{2+}. On the other hand, for ions the ratio $[Tb^{3+}]/[COO^{-}]$ at which the plateau is reached around 1.12–0.15, which indicates that these ions will form six bonds (three due to electrostatic interactions and the other three to coordination to carboxylate groups). This is consistent with the findings of Kido et al. [35], who found from fluorescence studies that six water molecules were expelled when the Tb^{3+}/PAA complex is formed. Thus, assuming that the Tb^{3+} aquo-complex is hydrated by nine water molecules [36] six bonds will be involved in the interaction with the PAA carboxylic acid groups.

Estimation of the complexation constants of terbium(III), and other lanthanides, with poly(acrylic acid) and its copolymers prove that the association is favored by entropy, and is accompanied by the dehydration of the polymers [37]. Therefore, in addition to the decrease of the charge density on the polymeric chain, which is compensated more readily than with Ca^{2+}, the coordination to carboxylic groups will lead to closer and more hydrophobic environments for the anthracene moiety, and, hence, the observed effect on the fluorescence intensity.

Acknowledgements Support for the collaboration between São Carlos and Coimbra from JNICT and CNPq is gratefully acknowledged. M.G.N. thanks FAPESP (94/3505) and FINEP (65/92.0063.00) for financial support. Two of us (H.D.B. and M.G.M.) also thank PRAXIS XXI (project 2/2.1/QUI/411/94) and Chymiotechnon for financial assistance. M.J.T. and V.A.D.O. thank CNPq for postdoctoral fellowships.

References

1. Polynucleotide–ligand interactions (1988) In Encyclopedia of Polymer Science and Engineering. New York, Vol 12, p 764
2. Holliday L (1975) Ionic Polymers. Applied Science, London; Eisenberg A, King M (1977) Ion Containing Polymers. Academic, New York
3. Morishima Y, Itoh Y, Nozakura SI, Ohno T, Kato S (1984) Macromolecules 17:2264–2269
4. Morawetz H, Taha AI (1971) J Am Chem Soc 93:829–833
5. Meisel P, Matheson M (1977) J Am Chem Soc 99:6577–6581
6. Calvert J, Casper JV, Bunstead RA, Westmoreland TD, Meyer TJ (1982) J Am Chem Soc 104:6620–6627
7. Chu DY, Thomas JK (1987) Macromolecules 20:2133–2138
8. Laschewsky A (1995) Adv Polym Sci 124:1–86
9. Miller ML (1966) The Structure of Polymers, Ch 12. Reinhold, New York
10. Tsuchida E, Nishide H (1977) Adv Polym Sci 24:1–87
11. Kotliar AM, Morawetz H (1955) J Am Chem Soc 77:3692–3695
12. Okamoto S, Vypracxhticky D, Furoya H, Abe A, Okamoto Y (1996) Macromolecules 29:3511–3514
13. Elridge RJ, Treloar FI (1970) J Phys Chem 74:1466–1449
14. Morishima Y, Sato T, Kamachi M (1966) Macromolecules 29:1633–1637
15. Eisinger T, Estrup FF, Shulman RG (1965) J Chem Phys 42:43–53
16. Neumann MG, Rodrigues MR (1994) J Photochem Photobiol A 83:161–164; Neumann MG, Tiberti L, Rodrigues, MR (1995) J Braz Chem Soc 6: 179–183
17. Tiera MJ, Neumann MG (1995) J Braz Chem Soc 6:191–197
18. Conio G, Patrone E, Russo S, Trefiletti V (1976) Makromol Chem 177:49–63
19. Delaire JA, Rodgers MAJ, Webber SE (1984) J Phys Chem 88:6219–6227
20. Morrinson ME, Dorfman RC, Clendening MD, Kiserow DJ, Rossky PJ, Webber SE (1994) J Phys Chem 98: 5534–5540
21. Morishima Y, Ohgi H, Kamachi M (1993) Macromolecules 26:4293–4297
22. Lissi EA, Encinas MV, Alvarez J, Borsarelli C (1993) J Colloid Interface Sci 160:472–474
23. Morishima Y, Higuchi Y, Kamachi M (1993) Macromolecules 31:373–379
24. Soutar I, Swanson L (1994) Macromolecules 27:4304–4311
25. Molyneux P (1982) Water Soluble Synthetic Polymers: Properties and Behavior, Vol II. CRC Press, Florida, p 119
26. Mattai J, Kwak JCT (1981) Biophys Chem 14:55–64
27. Ise N, Okubo T, Kunugi S (1982) Acc Chem Res 15:171–177
28. Morishima Y, Sato T, Kamachi M (1996) Macromolecules 29:3960–3964
29. Manning GS (1978) Rev Biophys 2:179–246
30. Mattai J, Kwak JCT (1980) Biochim Biophys Acta 677:303–312
31. Newman JK, McCormick CL (1994) Macromolecules 27:5114–5122
32. Satoh M, Hayashi M, Komiyama J, Iijima T (1990) Polymer 31:501–505; Satoh M, Kawashima T, Komiyama J (1991) Polymer 32:892–896
33. Malovikova A, Rinaudo M, Milas M (1994) Biopolymers 34:1059–1064
34. Mattai J, Kwak JCT (1986) Macromolecules 19:1663–1667
35. Kido J, Brittain HG, Okamoto Y (1988) Macromolecules 21:1872–1875
36. Richardson FS (1982) Chem Rev 82: 541–552; Amis EJ, Janmey PA, Ferry JD, Yu H (1983) Macromolecules 16:441–446
37. Nishide H, Izushi T, Arai H, Yoshioka N, Tsuchida E (1987) J Macromol Sci Chem A 24:343–351