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Interactions of quinine with polyacrylic and poly-L-glutamic acids in aqueous solutions

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Abstract

Drug-loaded polymers provide an attractive form for controlled drug delivery systems. A proper knowledge of polymer–drug interactions can aid in the designing of polymers for various drug-delivery applications. In this paper we have investigated the interaction of a drug such as quinine, with synthetic macromolecules such as poly(acrylic acid), PAA, and poly(L-glutamic acid), PGA, at pH 7 and 37 °C by fluorescence spectroscopy and viscometry. The analysis of the binding isotherms revealed that the association process is positive cooperative up to a threshold concentration and then it is negative. In addition, the thermodynamic parameters vary along the isotherm. Results also suggest that there is an optimum polymer to quinine ratio. Based on the viscometry results a mechanism of the interaction in which the polymer conformation plays a determinant role is proposed. Since the conformation depends on the molecular size, the architecture of the macromolecule, the effective charge and ergo the ionic strength, all these variables have been taken into account and their effect on the binding discussed.

Keywords: Polyelectrolytes; Drug-delivery systems; Interfaces; Fluorescence; Adsorption

1. Introduction

Polymers are a promising class of biomaterials that can be engineered to meet specific end-use requirements. Synthetic polymer-based drug-delivery systems have been applied in drug delivery for the past 50 years as they are capable of adjusting drug release rates in response to a physiological need. Since design and synthesis of novel combinations of polymers will expand the scope of new drug-delivery systems in the future [1], worldwide, there is currently considerable care for the design of novel supramolecular structures based on hydrogels for use as drug and cell carriers [2,3]. The research investigations into the synthetic methodology, physical and chemical properties of biocompatible or degradable dendrimers are also increasing exponentially with growing interest for a variety of medical applications, including the drug delivery [4–6].

Hoffman et al. have developed new mucoadhesive drug delivery formulations based on complexes of partially neutralized poly(acrylic acid) (PAA) and an anti-glaucoma beta blocker drug, levobetaxolol [7,8], as well as complexes of PAA or poly(methacrylic acid) (PMAA) with poly(ethylene glycol) (PEG), and a non-steroidal anti-inflammatory drug such as indomethacin, studying the effect of the molar mass on the release of the drug [9].

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Bo et al. [10] have shown that multi-layer polymer films based on PAA and hydroxyethylcellulose are useful for the controlled release of an antibiotic such as levomycetin. The same group has also studied the specific interactions of local anesthetic lidocaine hydrochloride with PAA and poly(2-hydroxyethyl vinyl ether), clarifying the mechanism of the drug binding to the polymers and the structures of the polycomplexes formed [11].

Our research focuses on quinine, a bitter crystalline alkaloid extracted from cinchona bark. Their salts are used as a tonic, antipyretic, analgesic, etc., and (usually in combination with chloroquine and similar drugs) in malaria therapy. Despite the development of more effective synthetic drugs, quinine is still the choice antimalarial drug due to the resistance developed by certain strains of the malarial parasite *Plasmodium falciparum* to these synthetic drugs. Currently the focal points of most of the studies is to obtain new therapies and different patterns of treatment for malaria to overcome the resistance [12,13] as well as to improve the efficacy and safety of quinine in treating nocturnal muscle cramps [14]. In previous works, we have investigated the quinine–membrane interactions over a wide range of experimental conditions, focusing in particular on the systems at different membrane phospholipid composition, pH and ionic strength of the media. The stoichiometry of the complexes formed was determined from the variation in the fluorescence intensity. The association isotherms were then quantitatively described by a combination of the Gouy–Chapman theory, which has been successfully used previously to examine the binding of many amphiphilic molecules to phospholipid bilayers [15] and a simple surface partition equilibrium [16]. The bilayer composition was proved to play a crucial role in the drug sensitivity [17], in a similar way than Shalmiev et al. revealed for interactions quinoline and erythrocyte membranes [18]. Results also indicated that monocationic quinine binds preferentially to negatively-charged membranes [19] and it penetrates more deeply in anionic bilayers than the dicationic ones [20]. In summary, we have shown that the association of quinine to liposomes is controlled primarily through electrostatic attractions, and, in a lesser extent, by hydrophobic forces. In the present paper, in order to gain further knowledge on the mechanism for a next therapeutic "action", we have extended the research to interactions between quinine and synthetic macromolecules such as PAA and PGA, two polymers of widespread use in drug delivery [8–11], by fluorescence spectroscopy and viscometry techniques. Since the polymer conformation depends on the molecular size, the architecture of the macromolecule, the effective charge and ergo the ionic strength, the effect of all these variables on the complex formation have been investigated and their effect discussed.

2. Experimental part

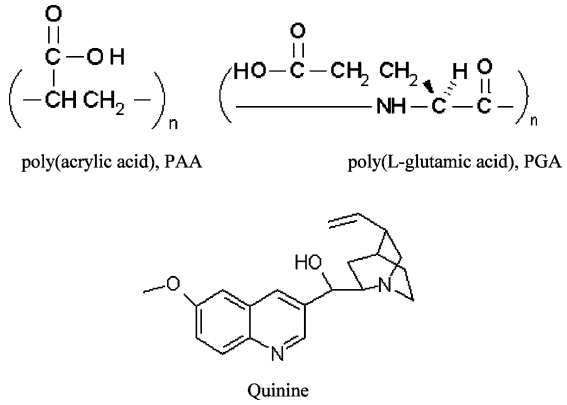
2.1. Materials

The polyelectrolytes tested were samples of poly(acrylic acid) (PAA) (pK_a 4.25) and poly(L-glutamic acid) (PGA) (pK_a 4.07–4.25) from Sigma–Aldrich (St Louis, MO, USA). Their nominal molar masses (in g mol^{-1}) were PAA 5000 (PAA-5k) and 90000 (PAA-90k) and PGA 13300 (PGA-13k), 43300 (PGA-43k) and 77800 (PGA-78k). They showed polydispersities lower than 1.1. Quinine (6′-methoxycinchonan-9-ol) (pK_a 5.07 and 9.7) was purchased from Sigma (St. Louis, MO, USA). All samples were used without further purification and their structures are shown in Scheme 1. Salts, buffers and reagents were of the highest purity available. Pure water was used as solvent in all experiments, distilled and deionized in a Milli-Q system (Millipore, Milford, MA). The water conductivity daily tested was $(2.7 \pm 0.5) \times 10^{-6}\ \Omega^{-1}\,\mathrm{cm}^{-1}$.

Phosphate buffer (14.75 mM Na_2HPO_4, 9.43 mM NaH_2PO_4) at pH 7 and ionic strength, μ, 0.045 M was prepared to dissolve the quinine and the macromolecules. Appropriate amounts of NaCl were added to this buffer in order to obtain the desired ionic strengths (0.145, 0.345 and 0.645 M).

2.2. Fluorescence

Emission fluorescence spectra were recorded at 30 °C using an AMINCO-Bowman Series 2 Luminescence Spectrometer (Spectronic Instruments Inc., New York, USA) equipped with an interface which enables high-speed computer-instrument communication. Throughout the experiments, samples were excited at 335 nm, being the maximum emission wavelength 383 nm, and the excitation and emission slits were both set at 5 nm. A



Scheme 1. Chemical structure of poly(acrylic acid), PAA, poly(L-glutamic acid), PGA, and quinine.

series of samples containing a fixed concentration of quinine (5 μM) and different concentrations of polymer (in order to achieve different polymer to quinine molar ratios, R_i, from 0 to 200) was annealed at 30 °C to assure equilibrium conditions for 10 min before the measurement was carried out. A previous kinetic experiment showed us that 10 min was enough to achieve the equilibrium (data not shown). All emission spectra were corrected for background fluorescence and polymer and solvent light scattering by subtraction of the appropriate blanks. Control experiments on the fluorescence of quinine at different pH and ionic strength were performed in previous works [19,20].

2.3. Viscometry

A conventional Ubbelohde capillary viscometer (Model AVS 440, from Schott-Gerate, Germany) was used to measure the viscosity of the polymer in aqueous buffered solvent in the presence of quinine at a given concentration. At least eight dilutions were automatically obtained by adding the appropriate aliquots of solvent, i.e., a quinine buffered solution. Under these conditions, the quinine concentration was kept constant whereas only the polymer concentration was diluted. Specifically, the quinine concentrations were 5 μM for all viscometric experiments dealing with PAA-5k, 0.5 μM for PAA-90k and 1 μM for PGA. Viscometry measurements of the polymer in the absence of the drug were also performed. Measurements were started after an equilibration time of 10 min. The flow time was always above 100 s at 30.0 ± 0.1 °C. The capillary sizes were selected so that kinetic energy corrections were minimal.

2.4. Circular dichroism

CD measurements were performed with a Jovin-Yvon CD6 spectropolarimeter, at a spectral bandwidth of 1 nm, with a time constant of 1 s (scan speed 50 nm/min) and a step resolution of 0.1 nm. Each spectrum was the average of three independent scans. Runs of polyelectrolyte in the presence and in the absence of quinine at different ionic strengths, polymer concentrations and pH were performed.

2.5. Isotherm data

In polymer/quinine mixtures, the progressive addition of macromolecules modifies the original quinine spectrum by increasing the fluorescence emission intensity (see Fig. 1 as an example). The fraction of quinine bound to the polymer, α, for every R_i was obtained from the change of the quinine fluorescence intensity at 383 nm upon addition of polymer as [21] $\alpha = (I - I_o)/(I_{max} - I_o)$, being I, I_o and I_{max} the quinine fluorescence intensities

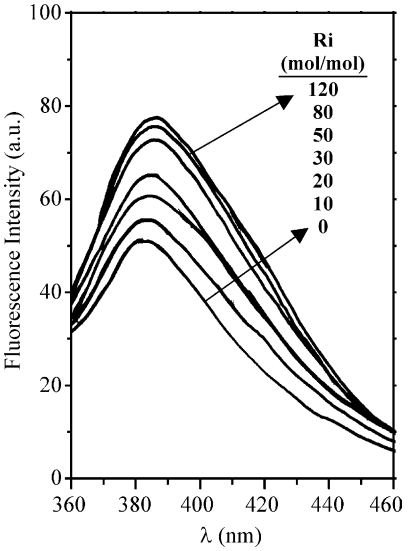


Fig. 1. Emission spectra of quinine (bottom) and in the presence of increasing amounts of PGA-13k at ionic strength 0.045 M. The polymer-to-quinine molar ratios are in the Figure. For the sake of clarity, only some spectra have been shown.

in the presence, in the absence of polymer and that corresponding to the quinine totally associated to the macromolecules, respectively. This later value was extrapolated from the double-reciprocal plot, $I_o/(I - I_o)$ versus $1/R_i$. Once the α value was known, the moles of quinine bound per mole of macromolecule, $\theta = [Q]_b/[P]$, as well as the bulk equilibrium concentration of the quinine, [Q], were calculated as follows: since α is the molar fraction of bound quinine per total quinine, $[Q] = (1 - \alpha) \times [Q]_{total}$ and $\theta = \alpha/R_i$, thus, the binding isotherms, θ versus [Q], were readily plotted.

Since the interaction has been monitored mainly by fluorescence spectroscopy, it is important to know if the variation of the experimental conditions can affect the quinine spectra. In this sense, the effect of the ionic strength and the pH on the quinine fluorescence has studied and reported previously [19,20]. Briefly, free quinine exhibits in buffer a pH-sensitive spectra. At pHs from 7 to 5 a single emission peak with a maximum centered at 383 nm was observed. At pH 4, however, a second peak appeared at 443 nm [20]. Regarding the influence of the ionic strength, the overall fluorescence profiles did not show a significant change in the intensity or the wavelength of the maximum by varying the ionic content [19].

2.6. Analysis of isotherm data

As a first approximation, the simplest model to describe the association of quinine to macromolecules is to assume a Langmuir adsorption isotherm [22,23] without

electrostatic interactions. To this aim, the binding model can be assumed. It proposes a simple binding equilibrium between the free quinine, Q, the unoccupied polymer sites containing N independent and equivalent binding points (or negative charges), S_N, and the quinine bound to polymer sites, QS_N, expressed as

$$Q + S_N \rightleftarrows QS_N$$

which is characterized by an association constant, K_A, given in terms of molar concentration by

$$K_A = \frac{[QS_N]}{[Q]_f[S_N]} \tag{1}$$

In this context, the fraction of quinine bound to the polymer, α, is defined by $\alpha = [QS_N]/[Q]_t$ and the total quinine concentration as $[Q]_t = [QS_N] + [Q]_f$. On the other hand, the total concentration of binding sites is given by $[S]_t = [QS_N] + [S_N]$. Recalling that every site consists of N points, it can also be expressed as $[S]_t = [P]_t/N$, where $[P]_t$ is the total polymer concentration. Then, the concentration of unoccupied sites at the equilibrium will be

$$[S_N] = [S]_t - [QS_N] = [S]_t - \alpha[Q]_t = \frac{[P]_t}{N} - \alpha[Q]_t \tag{2}$$

Taking into account the above expressions, the association constant given in Eq. (1) can be written as

$$K_A = \frac{\alpha[Q]_t}{[Q]_f([P]_t/N - \alpha[Q]_t)} = \frac{\theta N}{[Q]_f(1 - \theta N)} \tag{3}$$

A proper rearrangement of Eq. (3) leads to the relationship between θ and [Q], i.e., the Langmuir adsorption isotherm

$$\theta = \frac{K_A[Q]_f/N}{1 + K_A[Q]_f} = K_A[1/N - \theta][Q]_f \tag{4}$$

In order to obtain a more useful expression, the number of occupied polymer sites respect to those unoccupied can be neglected, so that $[QS_N] \ll [S]_t$. The extension of this argument to expressions (2) and (3) reduces Eq. (4) to

$$\theta = [K_A^{app}/N][Q]_f \tag{5}$$

where K_A^{app} is an apparent binding constant.

The experimental data can also be analysed in terms of the partition model, which has been previously applied for characterising the association of macromolecules [24,25] or drugs [17] to vesicles. Such a model considers two phases where the quinine can be found: the macromolecular coil domain forms one phase and the other is the aqueous solution. It allows calculating the partition coefficient of quinine between the two phases, K_r, which is defined as

$$K_r = \frac{a_Q^P}{a_Q^A} = \frac{\gamma_Q^P[Q]_b}{\gamma_Q^A[Q]_f} \tag{6}$$

where a_Q^P and a_Q^A are the activities of the drug in the polymer domain and in the aqueous media, respectively, and γ_Q^P and γ_Q^A are the respective activity coefficients of the drug bound to polymer with a concentration $[Q]_b$ and of the free drug with a concentration $[Q]_f$. These coefficients take into account the electrostatic interactions between the cationic molecules of quinine in the two phases. Since the volume of the polymer $\bar{v}_p$ can be neglected respect of the aqueous media [26] we can consider that

$$\frac{[Q]_b}{[Q]_f} = \frac{[QS_N]}{\bar{v}_p[P]_t[Q]_f} = \frac{\theta}{\bar{v}_p[Q]_f} \tag{7}$$

and hence, by substituting Eq. (7) into Eq. (6) it holds that

$$\frac{\theta}{[Q]_f} = \frac{K_r\bar{v}_p}{\gamma} = \frac{\Gamma}{\gamma} \tag{8}$$

where $\gamma = \gamma_Q^P/\gamma_Q^A$ and reflects the non-ideality of the system [27,28].

At low concentrations of free quinine, $\gamma_Q^A \to 1$ and then $\gamma = \gamma_Q^P$, as generally assumed for small interacting probes. Γ is proportional to the partition coefficient K_r. It must be independent on the quinine concentration and determined by the difference in the free energy of the probe between macromolecular and aqueous media. When $\theta \to 0$ the electrostatic repulsions between the associated and free quinine can be neglected, $\gamma = \gamma_Q^P \approx 1$, and Eq. (8) can be rewritten as

$$\theta/[Q]_f = \Gamma^{app} \quad (\theta \to 0) \tag{9}$$

where the partition coefficient Γ^{app} must be considered as an apparent partition parameter to emphasize that the electrostatic interactions are not specifically taken into account.

Concerning the binding model, when $\theta \to 0$, i.e., in absence of electrostatic interactions, $N\theta$ can be neglected with respect to the unity in Eq. (3), then such an equation is converted to

$$K_A = N\theta/[Q]_f \quad (\theta \to 0) \tag{10}$$

By comparing Eqs. (9) and (10) it yields

$$\Gamma^{app} = K_A/N \quad (\theta \to 0) \tag{11}$$

expression that relates the characteristic parameters of the two models in absence of electrostatic interactions. By substituting Eq. (11) into Eq. (3), a conventional Scatchard Eq. [29] is obtained.

$$\theta/[Q]_f = \Gamma^{app} - N\Gamma^{app}\theta \quad (\theta \to 0) \tag{12}$$

Therefore, by plotting $\theta/[Q]_f$ versus θ, the partition coefficient obtained from the intercept ($\theta \to 0$) is only an

apparent constant since the activity coefficient γ when $\theta \neq 0$ is ignored.

3. Results and discussion

The values of quinine bound to poly(acrylic acid) of molar masses 5000 and 90000 have been plotted in Fig. 2A against R_i at the ionic strengths 0.045, 0.145 and 0.345 M. These plots, that represent the extent of the association between the drug and the macromolecule, appeared to be similar in shape. Two trends can be clearly distinguished. Namely, after an initial rapid increase of α at low R_i values, the subsequent binding becomes more and more difficult. The slope decreases progressively up to a plateau observed at higher R_i values, indicating saturation. Comparison of these curves at a fixed polymer-to-quinine ratio reveals that the fraction of drug bound to PAA increases with de molar mass of the polymer. On its hand, the effect of the ionic strength depends on the molar mass: whereas for PAA-5k α is enhanced with the ionic strength, in the case of PAA-90k α diminishes. Nevertheless, as previously described, the analysis of the association is based on equations relating the ratio of associated quinine to the macromolecule, θ, with the free quinine concentration. Therefore, it seems more reasonable to use the association isotherms, θ versus $[Q]_f$, to interpret the experimental data. The corresponding binding isotherms are shown in Fig. 2B. Examination of these curves reveals that, although the Eq. (4) is fulfilled, it is obvious that the binding does not follow the classical pattern of the Langmuir adsorption isotherm. It is noteworthy that Langmuir is a quantitative theory for the distribution of material between the surface and the gas phase and it is based on various assumptions. The principal assumptions underlying the deviation of the Langmuir isotherm are independence and equivalence as well as a fixed number of the adsorption sites. Intuition suggests that there is likely to be a variety of kinds of sites for adsorption at any site depends on whether or not its neighbours are already occupied. Deviations from the Langmuir isotherms are widely observed, typically a bending of the curve, and the discrepancy can normally be traced to the failure of these assumptions. The unusual results on Fig. 2B are the deviation of the curves from the origin and it is a sign of that in our systems, the values of K_A and N parameters do not remain constant along the isotherm. Indeed, an increase of K_A and N with the concentration of free quinine up to a threshold concentration and a subsequent decrease are responsible for the distance from the origin. Fig. 2B also shows a crossing of the different curves of PAA-5k that can be caused by a lower variation of N with $[Q]_f$ at the highest ionic strength. The divergences with the assumptions of the Langmuir isotherm may either pre-exist in the different adsorption sites, or be caused by the repulsive forces between adsorbed molecules, especially if the surface-to-adsorbate bond is partially ionic and electrostatic forces govern the association. The screening of PAA-5k charges will be more important at a highest ionic content and then the variation on N will be reduced. The fact that PAA-90k does not exhibit such an intersection is probably due to the different macromolecular conformation as a consequence of their different molar masses. Accordingly, the random coil structure of PAA-90k, in contrast with the worm-like chain of PAA-5K, would expose less charges to the media, doing the effect of the ionic strength less apparent.

Fig. 3 shows the Scatchard plot for the systems quinine/PAA-5k and quinine/PAA-90k at the three ionic strengths. As can be seen, from extremely low bound quinine concentrations, the partitioning of quinine between the polymeric and the aqueous phases, $\theta/[Q]_f$,

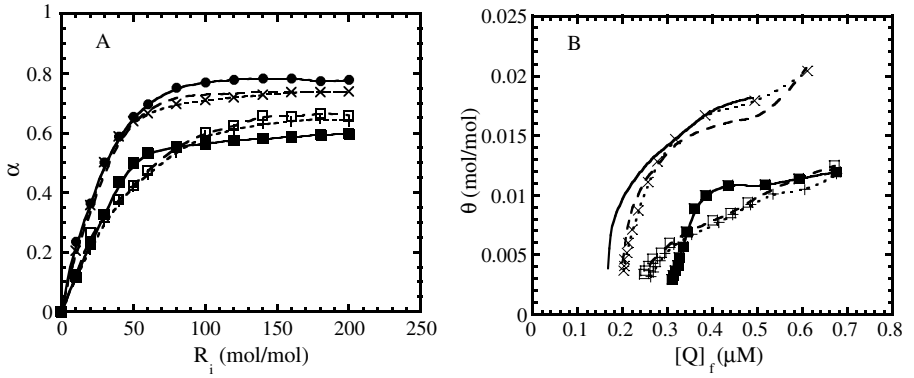


Fig. 2. Association of PAA to quinine. (A) Variation of the fraction of bound quinine to PAA, α, of two molar masses with quinine-to-polymer molar ratio, R_i, as a function of the ionic strength. (B) Binding isotherms for the PAA/quinine system. Symbols stands for: PAA-90k (●) 0.045 M, (○) 0.145 M, (×) 0.345 M, and for PAA-5k (■) 0.045 M, (□) 0.145 M and (+) 0.345 M.

increases with the concentration of bound quinine θ. Such a behaviour reflects the cooperative character of the association, like in the case of binding of ligands [30] or polymers [31] onto proteins. Beyond this threshold concentration, $\theta/[Q]_f$ begins to decrease revealing an anticooperative character of the association as observed for the binding of peptides to vesicles [32]. The bell overall shape of the curves has also been described in the case of ligand–protein interactions [30]. It has usually been attributed to a cooperative association where the decrease would be due to the saturation of the protein binding centers. Since the structure of synthetic polymers, such as PAA, is more flexible than that of a protein, in our systems, the presence of increasing quantities of quinine could induce changes in the form and size of the macromolecule [33]. These changes would explain the coincident positive and negative cooperativities of the association revealed by the Scatchard plots. It is well known that the polyelectrolytes, such as PAA, change their form, size and effective charge as a function of their own concentration as well as of the ionic strength of the solvent [34–36]. Because of its size, quinine can behave as a counter-ion, involving the ionic strength, therefore a variation in the polymer-to-quinine ratio implies that the free quinine modifies the effective hydrodynamic volume (EHV) of PAA.

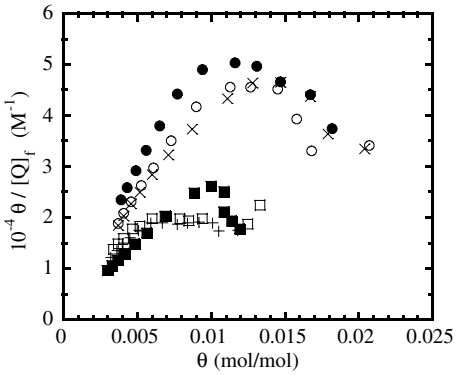


Fig. 3. Scatchard analysis for the association of PAA to quinine at different ionic strengths. Symbols as in Fig. 2.

These results are supported by the conclusions drawn from the viscometry technique. Indeed, the EHV of the macromolecule in the presence of quinine has been evaluated. The reduced viscosity, η_{sp}/c_p, (in our case, η_{sp} refers to the specific viscosity of the polymer in the presence of quinine and c_p to the total polymer concentration) is proportional to the EHV of the macromolecule [37–39]. In this way, Fig. 4 displays the dependence of η_{sp}/c_p of PAA-5k (Fig. 4A) and PAA-90k (Fig. 4B) in the presence of quinine at a given concentration (5 and 0.5 μM, respectively) on c_p at different ionic strengths, covering the range from 0.045 to 0.345 M. The analysis of these figures reveals that for a given ionic strength and macromolecular molar mass, the curves show a maximum of the reduced viscosity value, in a similar way than the Scatchard plots shown in Fig. 3. To explain this feature it might be speculated that in the presence of counter-ions in the milieu PAA adopts a random coil structure, more or less straighten depending on the ionic strength, concentration, ... (Fig. 5A). Such an assumption is supported by the shape of the plot η_{sp}/c of PAA in the absence of quinine versus c at 0.045 M (Fig. 4), similar to that encountered with uncharged polymers [33]. Under the experimental conditions, a decrease on c_p leads to a decrease on R_i. Such a diminution means that the quinine concentration increases with respect to the macromolecular one. Due to its size and charge, quinine goes in the coil of the polymer, unfolding the PAA chain and the negative charges that remained hidden inside the coil lay open to the solvent (Fig. 5B). Therefore, the hydrodynamic volume of the coil swells resulting in an increase of the reduced viscosity, as Fig. 4 shows. In regard to the extent of the

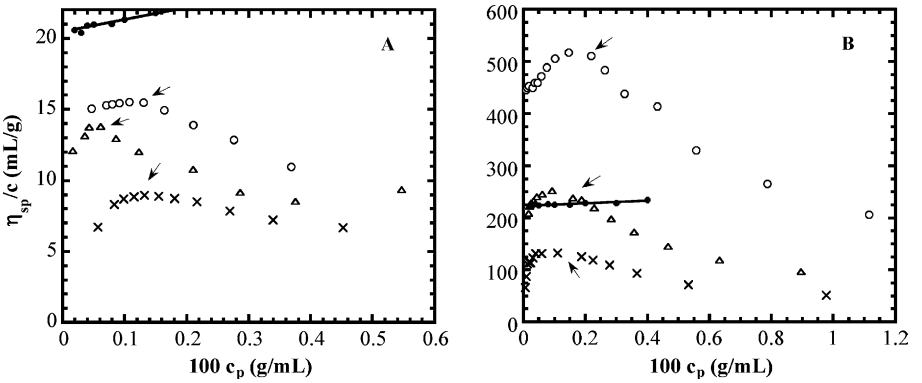


Fig. 4. Reduced viscosity of PAA in buffered solutions of a given quinine concentration as a function of the polymer concentration at different ionic strengths (○) 0.045 M, (△) 0.145 M and (×) 0.345 M. (A) PAA-5k, quinine concentration: 5 μM (B) PAA-90k, Quinine concentration: 0.5 μM. (●) PAA alone in a buffered solution of ionic strength 0.045 M.

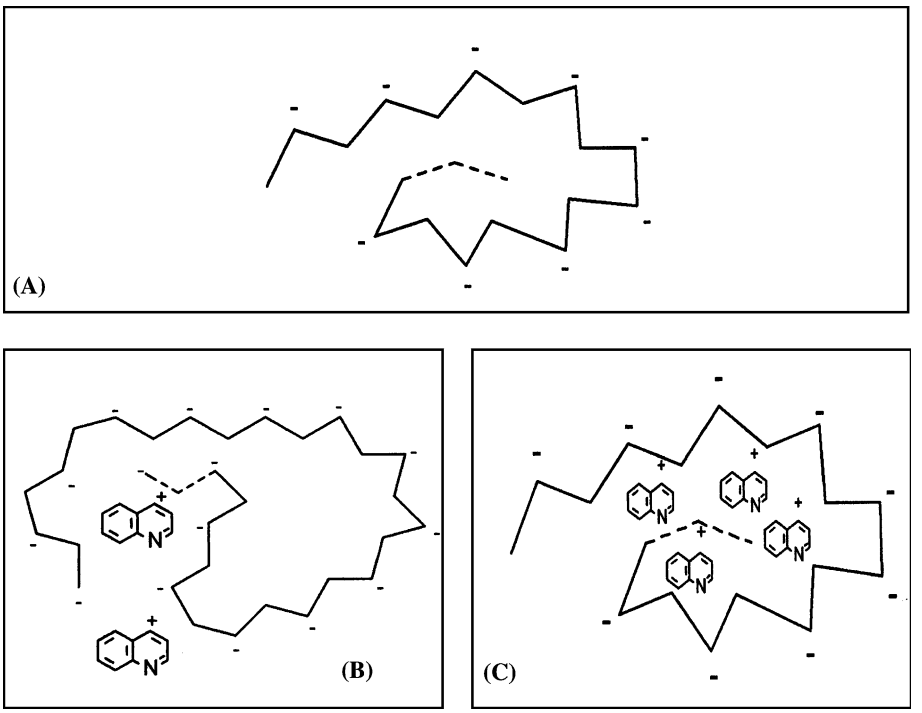


Fig. 5. Schematic illustration of the association between the macromolecule and quinine.

association, the concentration of quinine bound to the macromolecule increases, showing a positive cooperativity, up to a threshold drug concentration. When the relative amount of bound quinine with respect to the polymer concentration is high enough (maximum $\theta/[Q]_f$ and reduced viscosity in Scatchard and viscometry plots, respectively), the screening effect of quinine overcomes the effect explained in Fig. 5B. Thus, the negative charges of PAA are less exposed again and the macromolecule begins to enfold, decreasing $\theta/[Q]_f$, as well as the EHV, and showing a negative cooperativity (Fig. 5C).

Concerning the effect of the ionic strength, the reduced viscosity diminishes with the salt content for the two molar masses of PAA. Such a decrease reflects the screening of charges on PAA, then resulting in a more flexible chain that progressively changes from a stiff chain to a random coil [33,40]. With respect to the molar mass, as drawn in Fig. 4, PAA-90k reaches values of the reduced viscosity higher than PAA-5k. Such a feature is consistent with the correlation between the viscosity data and the EHV or the size of the macromolecule. On the other hand, a more detailed comparison of Fig. 3A and B reveals that the EHV maximum is reached at similar polymer-to-quinine ratio for all three ionic strengths and polymer molar mass. In addition such a R_i value falls in the same range than the maximum obtained in the Scatchard plots (the arrows in Fig. 4). It is worth mentioning that, in order to achieve similar polymer-to-quinine ratios in the two kind of experiments, in the case of PAA-90k the quinine concentration is not the same for fluorescent and viscometric measurements due to the experimental limitations in the latter technique. Nevertheless, the mechanism proposed and the discussion are supported by the similarity in shape of the curves in Figs. 3 and 4 and, irrespective of the different quinine concentration in Fig. 3A and B, they also show similar shape.

On the other hand, the different shape of the plots η_{sp}/c versus c shown in Fig. 4 for PAA in absence and in the presence of quinine reveals that the effects on reduced viscosity observed are related to the interaction with the drug and not caused by the polyelectrolyte dilution. Although, for the sake of clarity only the results for PAA at 0.045 M have been plotted, the shape of the curves for the other two ionic strengths are identical. Thus, the intrinsic viscosity values $[\eta]$ obtained from the intercept of the plot for PAA-5k are 16 and 14 at 0.145 and 0.345 M, respectively. For PAA-90k $[\eta]$ are 141 and 105, respectively.

3.1. PGA

Fig. 6 shows the Scatchard plots of the interactions between quinine and poly(l-glutamic acid) of molar masses 13300 (PGA-13k) (Fig. 6A), 43300 (PGA-43k) (Fig. 6B) and 77800 (PGA-78k) (Fig. 6C) at two ionic strengths 0.045 and 0.645 M. For all three systems, the

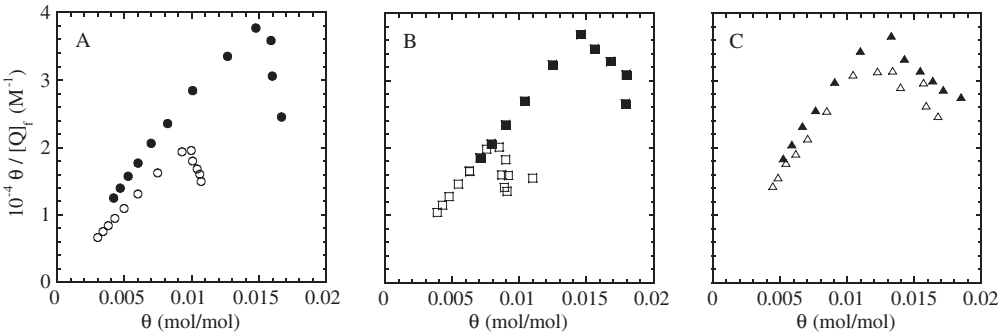


Fig. 6. Scatchard analysis for the association of PGA to quinine at different ionic strengths for three different polymer molar masses: (A) PGA-13k, (B) PGA-43k and (C) PGA-78k. Open symbols stands for 0.045 M, closed symbols for 0.645 M.

curves appears similar in shape to observations with PAA, namely, a practically linear increase of $\theta/[Q]_f$ with θ. Beyond a threshold value, $\theta/[Q]_f$ decreases. Hence, like PAA at low θ values, the association of PGA to Quinine is cooperative whereas at higher θ values is anticooperative. With regard to the effect of the ionic strength, for a given PGA the association results reduced when the salt content is supplemented, being this effect less important at larger molar mass. Moreover, the threshold θ value, which is smaller at the highest ionic strength, increases with the molar mass. This feature is a consequence of the dominant electrostatic character of the association, i.e., higher the ionic content greater the screening lower the association. This is in agreement with the diminution of θ that implies a decrease of the content of bound quinine. Besides, as typically for polyelectrolytes, higher the molar mass, more folded over the macromolecule and, obviously, the effect of the ionic strength is less significant.

It is noteworthy that the ionic strength has been increased up to 0.645 M in order to observe an effect of the ionic content on the association of PGA to quinine (intermediate data not shown).

In contrast with PAA, as shown in Fig. 7, the viscometry results from the experiments performed over the same range of R_i do not show the maximum observed in the Scatchard plots. In effect, the plateau of the reduced viscosity reached after an initial increase implies that the EHV of PGA does not vary beyond a polymer concentration. The different shape of the curves shown by PGA in the presence and in the absence of quinine indicates that such a feature does not arise from the variation of the polymer concentration. Alternatively, it could be an indication of two concomitant phenomena, i.e., the presence of quinine could induce a modification on the PGA structure that would organize the chain. This arrangement would be in opposition to the swelling of the macromolecule, keeping constant its hydrodynamic volume. Such an explanation is supported by the fact that, as reported elsewhere [41], the PGA structure

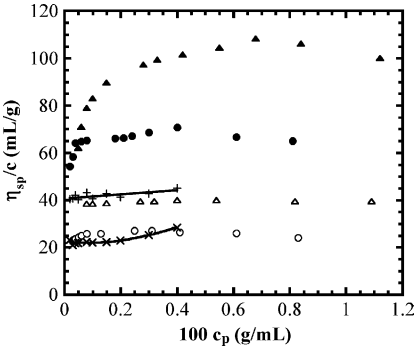


Fig. 7. Reduced viscosity of PGA in buffered solutions as a function of the polymer concentration, at different ionic strengths. In the presence of quinine at 1 μM concentration: (●) PGA-43k, $\mu = 0.045$ M; (○) PGA-43k, $\mu = 0.645$ M; (▲) PGA-78k, $\mu = 0.045$ M; (△) PGA-78k, $\mu = 0.645$ M. In the absence of quinine (×) PGA-43k, $\mu = 0.045$ M; (+) PGA-78k, $\mu = 0.045$ M.

bears changes with the pH, forming an helix at low pH values. We have examined the role of quinine in the conformational rearrangement of PGA by circular dichroism spectroscopy. Thus, in order to complement the viscometric and fluorescence information, samples containing PGA in the absence and presence of quinine at different ratios under identical conditions of ionic strength and pH were prepared for CD spectroscopy experiments. These preliminary CD tests (data not shown) reveal that quinine induces changes in the CD spectra of PGA, i.e., at a fixed polymer concentration, as quinine amount is raised, the CD spectra display a new band of negative ellipticity with increasing amplitude and its position is red shifted. The distinctive features of the CD spectra provide strong evidence of a conformational rearrangement from random coil to another conformational state that does not correspond to the classical α-helix, β-sheet, ... whose CD patterns are well established. Careful examination of the microenviron-

ment of polymer–quinine conformation is currently being investigated.

It is also worth mentioning the feasible existence of a plateau at higher PAA concentrations.

Fig. 7 also shows that, as in the case of PAA, the reduced viscosity is increased with the molar mass of PGA and decreasing the ionic strength. By increasing the ionic content, the screening of the counter-ions promotes the progressive change from a stiff chain to a random coil, inducing a diminution of the EHV of PGA and, therefore, the reduced viscosity is decreased. Obviously, since the viscosity reflects the size, higher the molar mass of the macromolecule, higher the reduced viscosity.

4. Conclusions

In the present study we have used a physical–chemical approach to provide insight into the association between a drug such as quinine and two macromolecules like poly(acrylic acid) and poly(L-glutamic acid) of different molar masses in diverse ionic environments. The conventional Scatchard analysis of the binding isotherms led to non-linear plots deviated from the origin indicating a more complex binding behaviour than anticipated on the basis of a Langmuir adsorption model. The deviation of the curves from the origin is explained by a variation of the thermodynamic parameters along the isotherm; hence, an apparent binding constant from the initial part of the isotherm cannot be obtained. From a qualitative point of view, in a similar way than earlier described for the interaction of proteins with ligands [30], the bell shape of the Scatchard plots is a sign of coincident positive and negative cooperativities.

The conformation of the polyelectrolytes, the form, size and effective charge of which change depending on their own concentration as well as on the ionic strength of the solvent, appears to play a determinant role in the association. Since, owing to its small size, quinine can behave as a counter-ion, a variation in the polymer-to-quinine ratio implies that the free quinine modifies the conformation of the macromolecule, i.e., its effective hydrodynamic volume, as shown by viscometry results. In addition, the polymer-to-quinine ratio for which the maxima of the reduced viscosity as well as of the extent of the association in the Scatchard plot are obtained, is similar for all three ionic strengths and polymer molar mass. Consequently, it is likely to there is an optimum R_i for the partitioning.

The differences between poly(acrylic acid) and poly(L-glutamic acid) regarding the viscometry results can be interpreted by two concomitant and opposed phenomena that take place in the case of PGA. Hence, the presence of quinine could induce a modification organizing the PGA chain, in opposition to the swelling of the macromolecule, which would keep constant the hydrodynamic volume. Although some circular dichroism experiments performed seems to be in agreement, assessment of these possible structural changes deserves further investigation, currently in course.

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