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# Polyelectrolytes in mixed salts: Scatchard plots obtained by means of Poisson–Boltzmann calculations

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**Abstract**

The problem of the interaction between dilute polyelectrolytes and divalent counterions in the presence of a supporting uni-univalent salt is approached by means of Poisson–Boltzmann calculations. A very general definition of "bound" and "free" concentrations is given under limiting conditions, but it is shown that it can be retained also at finite polymer concentrations. With such definitions Scatchard plots can be obtained. Values of the Scatchard constant $K_2^0$ are given as a function of the reduced charge density, of the radius of the polymer and of the concentration of the supporting monovalent salt.

*Keywords:* Polyelectrolyte; Poisson–Boltzmann theory, Scatchard plot

## 1. Introduction

The application of Poisson–Boltzmann (P–B) equation to polyelectrolytes has been known for a long time [1–3], although only in the last decade an explicit evaluation of the approximations involved has been put forward [4,5]. It is generally acknowledged that the P–B equation is a valid tool to study polyelectrolytes. The solution of this equation provides information on the spatial behaviour of the electrostatic potential and therefore on the spatial distribution of the ions around the polymer.

In most cases this property cannot be experimentally investigated. Indeed, the quantities usually available from experiments are the concentrations of "free" and "bound" ions where a "free" and a "bound" state have to be defined (and are often defined by the experimental procedure).

In the past years a physically based schematization which has proved useful in understanding the behaviour of polyelectrolytes in solution has been proposed by Manning [6]. In his model the two above mentioned states are postulated to exist sharply distinct. This assumption has raised several criticisms among researchers, since such a

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clearcut separation of "free" and "bound" states is not predicted by the P–B theory. Nevertheless Manning's model allowed to compare experimental "binding" data with theoretical predictions.

Within the frame of P–B theory measureable quantities as the activity coefficient of the counterions in the presence of the polyelectrolyte and in the presence or absence of added salts have been compared with the results of the calculations. In particular the cell model firstly proposed by Alfrey et al. [1] and Fuoss et al. [2] was often employed and the quantities measured in solution were compared in several cases with the analogous quantities calculated from the model at the cell surface. However, useful concepts that rely on the existence of two different molecular states, like that of binding constant, appear to fall presently outside the P–B theory. In spite of the different theoretical interpretations of what *is not* free ion concentration (be it site binding, condensation, P–B accumulation, etc.), it should be noted that the outcome of experiments like equilibrium dialysis, potentiometry, dye indicators, etc., can always be expressed operatively (independently of their physical counterparts at the molecular level) in terms of "free" ion concentration (what is directly measured) and of its difference from the total concentration employed, i.e. the apparent "bound" concentration.

The aims of the present communication are: (i) to give a suitable definition of these quantities, i.e. the "free" and "bound" concentrations, in the limiting case of infinite dilution, within the frame of P–B theory; (ii) to see how far this definition can be retained also at finite concentrations of the polymer; and (iii) to calculate the extent of binding of divalent counterions in the presence of monovalent supporting salt, according to this definition, for different values of salt concentrations, polyelectrolyte reduced charge and radius.

## 2. Theory

The P–B approach to polyelectrolytes has been the subject of several research papers [1–3,7]. We will summarize here only the basic theory.

The polyelectrolyte is modelled as an infinite uniformly charged cylinder. We will consider explicitly the case where a uni-univalent ($A^+$ and $B^-$) supporting salt, a bi-univalent ($C^{2+}$ and $B^-$) salt and a negatively charged polymer are present. We can write the Poisson equation, in c.g.s. units, for the electrostatic potential (the variation of the dielectric constant of water around the cylinder is not taken into account):

$$\nabla^2 U = \frac{-4\pi}{\varepsilon} \sum_i \rho_i \tag{1}$$

and the Boltzmann equation for the distribution of any ionic species under the limiting condition of infinite dilution of the polymer:

$$\rho_i = \frac{N_{\mathrm{Av}}}{1000} c_i^\infty z_i q \exp\left(\frac{-z_i q U}{kT}\right) \tag{2}$$

where $c_i^\infty$ is the molar concentration of the $i$th species at an infinite distance from the cylinder (in the following we will simply write $c_1$ to mean $c_1^\infty$, the uni-univalent salt concentration, since we are not interested in the distribution of monovalent counterions around the polyelectrolyte). Substituting the second equation into the first we get the P–B equation which can be further specified in the present case by introducing the following reduced quantities:

$$\Psi = qU/kT \tag{3a}$$

$$\chi = k_D r = \left(\frac{8\pi q^2}{\varepsilon kT} \frac{N_{\mathrm{Av}}}{1000} c_1\right)^{1/2} r \tag{3b}$$

$$R = c_2^\infty / c_1 \tag{3c}$$

so that eq. (1) becomes:

$$\Psi'' = \frac{-\Psi'}{\chi} + \sinh \Psi + R[\exp(\Psi) - \exp(-2\Psi)] \tag{4}$$

The boundary conditions are given by the electric field at the surface of the polymer:

$$\Psi'(\chi_0) = 2\xi/\chi_0 \tag{4a}$$

where $\chi_0$ is the reduced radius of the cylinder (i.e. $\chi_0 = k_D r_0$), and

$$\xi = q^2/\varepsilon kTb \tag{4b}$$

and by the request that the potential becomes zero at infinite distance from the polymer:

$$\Psi \to 0 \quad \text{as} \quad \chi \to \infty \tag{5}$$

The P-B equation can be solved numerically (the Runge-Kutta-Nystrom fourth order method [8] was actually used) by a trial and error procedure. A tentative value for the potential at the polymer surface is chosen, then the solution is computed at increasing distances until either the potential or its derivative changes sign, then the new starting value is set halfway between the last two trial values which exhibited opposite divergencies. This procedure is applied until the new starting value differs from the old one by less than one hundred thousandth of its value. A strict convergence criterion has the advantage that the region where the integration procedure stops is already in the range where both (the exact) $\Psi$ and $\Psi'$ are close to zero, since we do not expect a slight modification of the starting value to bring up a large variation at the point of zero-crossing. The integration step size is decreased until no further changes are found in the solution.

Once the solution is obtained we will define as the number of "bound" divalent counterions per polymer charge ($c_2^b/c_p$), the integral of the excess concentration with respect to $c_2^\infty$ over the (infinite) volume pertaining to a monomer:

$$\frac{c_2^b}{c_p} = \frac{N_{Av}}{1000}\int_{r_0}^{\infty} 2\pi r c_2^\infty[\exp(-2\Psi) - 1] b \, dr \tag{6}$$

In practice, since in the presence of finite ionic strength the excess concentration decays rather fastly, it is sufficient to take the integral up to few Debye lengths. The integration procedure usually stops between four and six Debye lengths and the value of $\Psi$ is zero within few hundredths. (Recall the asymptotic behaviour of the solution of the equation:

$$\Psi \to \frac{\exp(-c\chi)}{\sqrt{\chi}}, \quad c > 1 \quad \text{as} \quad \chi \to \infty \tag{7}$$

in the presence of added salts). Obviously in the most natural way under the same limiting conditions the "free" concentration is assumed to be the bulk concentration ($c_2^\infty$).

It is worth to comment on the above definitions. When we consider the cell model in the limit $D \to \infty$ ($D$ is the radius of the cell) the perturbation effect on the local concentration due the polymer is negligible over most of the cell volume. The concentration of the divalent counterion would be $c_2^\infty$ over the whole volume except for a very limited portion of it around the polyelectrolyte where the electrostatic potential fastly decays to zero. When $D \to \infty$ any measurement would sample therefore regions where $c_2$ is equal to $c_2^\infty$. However, the total number of divalent counterions ($c_2^{\text{tot}}$) present in the cell pertaining to a single monomer would be different from:

$$\frac{N_{Av}}{1000}\int_{r_0}^{D} 2\pi r c_2^\infty b \, dr \tag{8}$$

being equal to:

$$\frac{N_{Av}}{1000}\int_{r_0}^{D} 2\pi r c_2^\infty[\exp(-2\Psi)] b \, dr \tag{9}$$

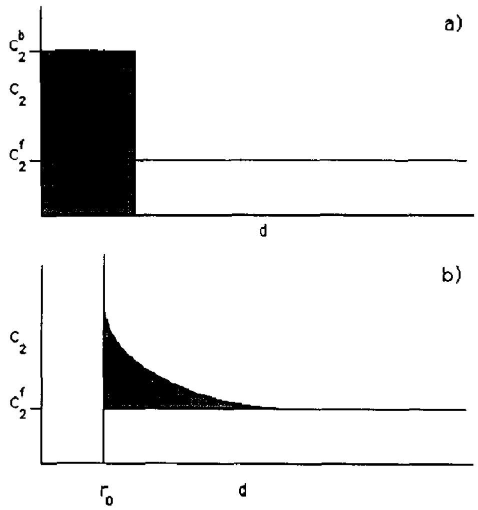

Fig. 1. Pictorial representation of (a) Manning's definition of "free" and "bound" divalent counterion concentration and (b) the present one. $c_2$ is plotted against $d$ which is the distance from the polymer. In both cases the integral (over the whole volume competing to one monomer) represents the number of "bound" ions per unit charge on the polymer.

While both these quantities diverge as $D \to \infty$, their difference remains finite and is defined in eq. (6) as $(c_2^b/c_p)$ since the same difference expresses the number of apparently bound counterions per unit charge. Though not assuming two real different states these definitions are able to match exactly under this limiting condition what would be measured as "free" and "bound" concentrations in several experiments like equilibrium dyalisis, or potentiometric measurements, in the absence of any hypothesis on the physical process involved. For the sake of clarity we have compared in a pictorial way the present definitions with those of Manning in Fig. 1.

With these definitions we can calculate points in a Scatchard plot for several different situations. Scatchard variables [9] are defined, as usual, in the following way:

$$c_2^b/c_p = r_2, \quad r_2/c_2^\infty = K_2 \tag{10}$$

A plot of $K_2$ vs. $r_2$ leads to a straightforward visualization of the extent and of the possible cooperativity of the interaction.

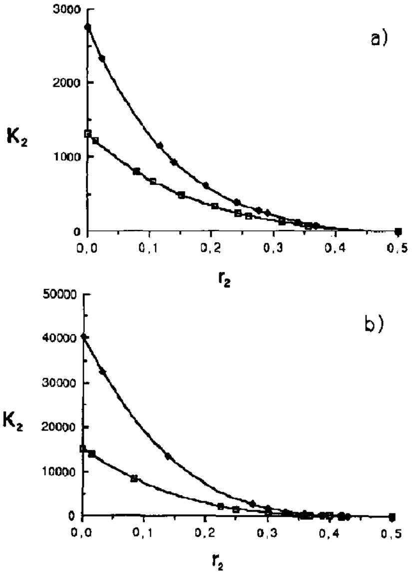

Fig. 2. Scatchard plots ($K_2$ expressed in $M^{-1}$) obtained for $c_1 = 0.01$ $M$ and $\xi = 2$ (a), and $\xi = 5$ (b). The higher curves refer to $r_0 = 6$ Å and the lower ones to $r_0 = 10$ Å.

## 3. Results and discussion

We have extensively calculated Scatchard plots for various values of $\xi$, $r_0$ and $c_1$ at 25°C; we show here only few of these plots (Fig. 2) while we give an exhaustive table (Table 1) of values of $K_2^0$, i.e. the value of $K_2$ for $r_2 = 0$. The dependence of this constant on $\xi$ and $r_0$, is illustrated in Figs. 3 and 4; as can be easily gleaned by the figures, $K_2^0$ does not depend too heavily on these parameters; therefore the often observed affinity change, following intramolecular conformational transitions involving only minor changes in the radius and reduced charge of the polymer (as is the case, for instance, of the B–Z transition in DNA), cannot be explained within this approach. This does not imply that the mere electrostatics cannot account for the transition, since the global free energy change (including monovalent counterions and coions contributions) should be calculated. There is instead a marked dependence on the ionic strength and the equation given by Manning [6]:

$$\frac{\mathrm{d} \log K_2^0}{\mathrm{d} \log c_1} = -2 \tag{11}$$

in the present case, agrees satisfactorily with the results of P–B model obtained for high values of $\xi$ (Fig. 5). However care must be used since $K_2^0$, which is in Manning's approach depends only on $\xi$, is found to depend also on $r_0$ and is different from Manning's calculated values for cases like B-DNA ($\xi = 4.2$, $r_0 = 10$ Å), Z-DNA ($\xi = 3.9$, $r_0 = 9$ Å) and A-RNA ($\xi = 5.1$ and $r_0 = 11$ Å). Moreover a straightforward manipulation of the formulae leads to the following scaling equations for the Scatchard variables:

$$r_2 = f(R, \xi, \chi_0) \tag{12a}$$

$$K_2 = \frac{1}{c_1} g(R, \xi, \chi_0) \tag{12b}$$

Though we will discuss in the following the validity of maintaining these results under non-limiting conditions, it is worth mentioning that the predicted absolute levels of binding satisfactorily

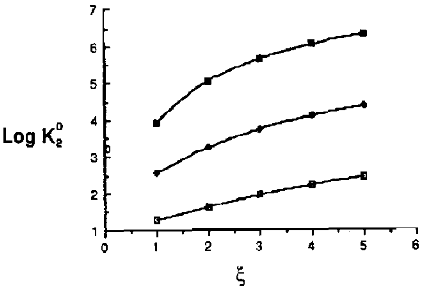

Fig. 3. The decimal logarithm of the constant $K_2^0$ (expressed in $M^{-1}$) plotted against the reduced charge density $\xi$ for the case $r_0 = 8$ Å and, starting from the lower curve, $c_1 = 0.1$ $M$, $c_1 = 0.01$ $M$ and $c_1 = 0.001$ $M$.

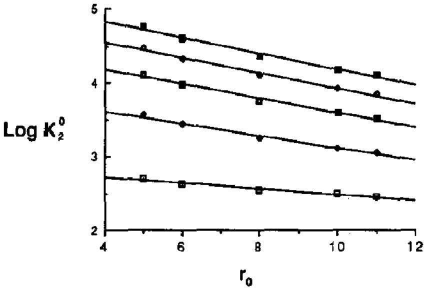

Fig. 4. The decimal logarithm of the constant $K_2^0$ (expressed in $M^{-1}$) plotted against the radius of the polymer (in Å) for different reduced charged densities ($\xi$ is 1, 2, 3, 4 and 5 starting from the lower curve) and for the case of $c_1 = 0.01$ $M$.

Table 1

Values of the constant $K_2^0$ as a function of the reduced charge density $\xi$, radius (in Å) and molar concentration of the supporting salt.

| $\xi$ | $r_0$ | | | | | $c_1$ |
|---|---|---|---|---|---|---|
| | 5 | 6 | 8 | 10 | 11 | |
| 1 | 24.20 | 22.13 | 19.48 | 17.76 | 17.19 | 0.1 |
| 1 | 105.8 | 94.63 | 80.87 | 72.27 | 69.08 | 0.0316 |
| 1 | 495.8 | 434.1 | 357.3 | 311.1 | 294.3 | 0.01 |
| 1 | 2493.2 | 2142.5 | 1699.0 | 1438.0 | 1344.3 | 0.0032 |
| 1 | 13470 | 11300 | 8658.7 | 7126.1 | 6579.6 | 0.001 |
| 1 | 367174 | 315768 | 244049 | 198132 | 181129 | 0.0001 |
| 2 | 72.48 | 58.48 | 43.31 | 35.10 | 32.34 | 0.1 |
| 2 | 488.4 | 379.2 | 259.9 | 199.8 | 179.4 | 0.0316 |
| 2 | 3673.0 | 2671.9 | 1795.2 | 1308.7 | 1148.0 | 0.01 |
| 2 | 29949 | 22039 | 13735 | 9661 | 8341.8 | 0.0032 |
| 2 | 255756 | 185654 | 112939 | 77284 | 66008 | 0.001 |
| 2 | 195 $10^7$ | 1.40 $10^7$ | 8340282 | 5600920 | 4731378 | 0.0001 |
| 3 | 182.7 | 137.8 | 90.66 | 67.46 | 59.83 | 0.1 |
| 3 | 1501.2 | 1099.0 | 684.5 | 482.0 | 417.2 | 0.0316 |
| 3 | 13196 | 9487.6 | 5696.2 | 3875.7 | 3299.1 | 0.01 |
| 3 | 121260 | 86164 | 50609 | 33756 | 38410 | 0.0032 |
| 3 | 1136665 | 803650 | 466139 | 306630 | 256661 | 0.001 |
| 3 | 1.17 $10^8$ | 7.95 $10^7$ | 4.37 $10^7$ | 2.79 $10^7$ | 2.31 $10^7$ | 0.0001 |
| 4 | 371.6 | 271.4 | 168.4 | 118.6 | 102.7 | 0.1 |
| 4 | 3324.4 | 2375.4 | 1416.3 | 960.2 | 816.1 | 0.0316 |
| 4 | 30891 | 21863 | 12761 | 8451.2 | 7103.0 | 0.01 |
| 4 | 295853 | 207938 | 119737 | 78305 | 65442 | 0.0032 |
| 4 | 2862643 | 2003492 | 1145063 | 743612 | 618806 | 0.001 |
| 4 | 3.57 $10^8$ | 2.33 $10^8$ | 1.22 $10^8$ | 7.56 $10^7$ | 6.19 $10^7$ | 0.0001 |
| 5 | 647.3 | 464.8 | 279.2 | 190.7 | 162.7 | 0.1 |
| 5 | 6034.4 | 4272.4 | 2492.2 | 1655.7 | 1392.1 | 0.0316 |
| 5 | 57599 | 40476 | 23295 | 15241 | 12729 | 0.01 |
| 5 | 560498 | 392485 | 224036 | 145220 | 120868 | 0.0032 |
| 5 | 5512631 | 3843783 | 2180080 | 1406133 | 1167827 | 0.001 |
| 5 | 7.99 $10^8$ | 5.11 $10^8$ | 2.58 $10^8$ | 1.56 $10^8$ | 1.26 $10^8$ | 0.0001 |

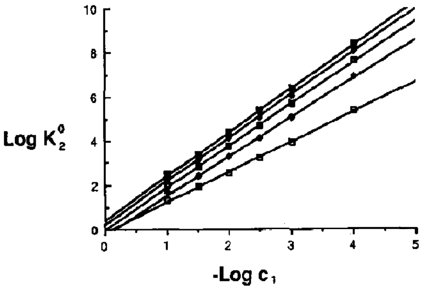

Fig. 5. The decimal logarithm of the constant $K_2^0$ (expressed in $M^{-1}$) plotted against the decimal logarithm of $c_1$ (in $M$) for different reduced charge densities ($\xi$ is 1, 2, 3, 4 and 5 starting from the lower curve) and for the case of $r_0 = 8$ Å.

reproduce experimental data obtained previously in our laboratory [10,11] (Fig. 6).

We have considered so far the limiting case of polymer infinite dilution; we are left with three questions. First of all, how does a finite concentration of the polyelectrolyte affect the solution of the equation, i.e. which is the effect of moving the boundary condition from infinity to a finite distance corresponding to the radius of the cell enclosing the polyelectrolyte. The answer is straightforward: nothing will happen as long as the cell radius is maintained in a range where the P–B equation solution is sufficiently close to zero, i.e. at least four to six Debye lengths away from the center of the cylinder. For the case of B-DNA this condition is met roughly at polymer concentrations in the range of 10 m$M$ at 10 m$M$ supporting salt.

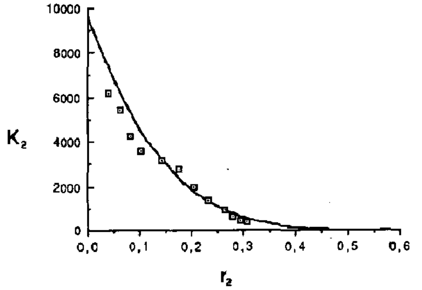

Fig. 6. Experimental data (⊡) (taken after ref. [10]) for the calcium induced B–Z transition in poly(dG–dm$^5$C) in the presence of NaCl 0.01 $M$ plotted together with the calculated Scatchard curve for B-DNA ($\xi = 4.2$, $r_0 = 10$ Å) ($K_2$ is expressed in ($M^{-1}$). The analogous curve for Z-DNA, almost completely overlapping the other, is not shown.

The second and more subtle point regards the adherence of the formal definition of "bound" and "free" counterion concentrations to the experimentally obtainable quantities and this is connected to the experimental procedure used. In cases where a measure of free ions involves regions not too near to the polyelectrolyte as for equilibrium dialysis, potentiometry, dye indicators, this adherence is fulfilled. On the contrary this is not the case of quadrupolar NMR experiments where a more restrictive definition of "bound" ions has to be used (and has actually been used [12,13]). However, we think that having a reference for the extent of binding due to electrostatics can help in discriminating among different type of interaction. The third question is what kind of process is associated to the free energy change $-RT \ln K_2(r_2)$. Let us consider three baths containing AB at a common concentration and, respectively, (i) $CB_2$ at a concentration $c_2^\infty$, (ii) polyelectrolyte (at concentration $c_p = 1$ $M$) and $CB_2$ at a concentration such to produce the given value of $r_2$ and the free counterion concentration $c_2^\infty$ (i.e. $c_{2,ii} = c_2^\infty + r_2 c_p$), and (iii) $CB_2$ at concentration $c_2 = r_2 M$. The polymer concentration is taken as 1 $M$ (in repeating units), to accomplish with the conventional units, keeping in mind that this is a purely idealized reference situation. $\Delta G_{iii \to i}$ is equal to $-RT \ln r_2/c_2^\infty$, as far as the activity coefficient can be assumed independent of concentration, hence, by definition, it is equal to $-RT \ln K_2(r_2)$. Therefore the same $\Delta G_{iii \to i}$ value is associated to the process of transferring one mole of counterions from iii to ii. From Table 1 values of $K_2^0$ at different temperatures, and therefore values of the entropy and enthalpy of the process described above, can be extracted after suitable interpolation, since a change in the temperature is reflected by a change in $\xi$ and in $\chi_0$.

The approach to polyelectrolyte–counterion interaction described in the present paper aims to be the P–B analogue of Manning's solution to the same problem [6]. Unfortunately, opposite to that case, no analytical formula is available, so numerical interpolation of the values reported in Table 1 is necessary. Fair agreement between theoretical prediction with experimental data (see references [10,11] and also the data reported in reference [6]), which holds true over a large range of $\xi$ and ionic strength values, proves that this treatment can be useful in common experimental situations.

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