

MAGNETIC RESONANCE DISTINCTION BETWEEN SITE BOUND AND ATMOSPHERICALLY BOUND PARAMAGNETIC COUNTERIONS IN POLYELECTROLYTE SOLUTIONS

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A combination of the water protons NMR chemical shifts, longitudinal and transversal relaxation rates and of the paramagnetic counterion EPR signal is shown to provide a clear distinction between site binding, atmospheric trapping and free counterions in solutions of polyelectrolyte TMA salts with increasing concentrations of the divalent counterions Co^{++} and Mn^{++} . Site binding is defined by the loss of water in the counterion first hydration shell while atmospheric binding results in a change in the counterion correlation time as compared to a free ion.

1. Introduction

Thermodynamic properties of polyelectrolyte solutions are generally well interpreted using a purely coulombic interaction, the polymer being represented by a linear or cylindrical distribution of charges characterized by a single charge density parameter λ [1]. For $\lambda > 1$ counterions are shown to bind or “condense” on the polyelectrolyte [2]. A number of evidences, particularly from binding specificity and volume changes [3] have led to distinguish among the condensed counterions between “site binding” and “atmospheric trapping”. This distinction is in many respects similar to that between contact ion pairs and solvent separated ion pairs in the case of small ions. Thermodynamic properties give no direct way to the physical state of bound counterions, particularly their state of hydration and their mobility. We have recently stressed [4] that in the case of paramagnetic counterions, the magnetic resonance techniques which have been widely developed for the study of ion binding on discrete sites of biological macromolecules [5], can be of much interest in the case of homopolyelectrolytes, with a regular distribution of charged groups along the macromolecule.

Three different types of experiments can be performed to probe the environment of a paramagnetic ion. Two use the water proton magnetic resonance, namely their chemical shift and relaxation times. The

third use the spin resonance of the counterion itself. Chemical shifts are induced in the protons of the first hydration shell as a result of the Fermi contact interaction. Fast exchange with the solvent water results in an averaged displacement of the proton's resonance. A paramagnetic ion with a short electronic relaxation time, like Co^{++} ($\tau_s \approx 10^{-12}$ s) has to be used to limit line broadening. Variations in the relaxation times T_1 and T_2 result both from changes in hydration, ligand fluctuation and rotational correlation time for the bound counterion. They require a paramagnetic counterion with an electronic relaxation time of the order of magnitude of the inverse of the nuclear resonance frequency. Mn^{++} ($\tau_s \approx 10^{-9}$ s) has the advantage that these same changes are reflected in the characteristic line shape of its ESR spectrum which results from the 5/2 value of both its electron and nuclear spin.

2. Materials and methods

Measurements have been performed on polyphosphate (PP) and on an alternating copolymer of maleic anhydride and methylvinylether (trademark Gantrez). The starting material was the tetramethylammonium (TMA) salt of the above polyacids. TMA was used as a reference counterion since its interaction with polyions give rise to a negligible volume change, owing to

its large ionic radius and weak hydrophobic and electrostrictive effects on surrounding water molecules. For each polysalt a series of solutions was prepared by adding aliquots of water and MCl_2 stock solution ($\text{M} = \text{Co}^{++}, \text{Mn}^{++}$) to the polysalt solution to keep the final polyelectrolyte concentration C constant for different ratios $r = C_{\text{M}^{++}}/C$ where $C_{\text{M}^{++}}$ is the concentration of paramagnetic ion in equivalent/liter.

2.1. Chemical shift measurements

Chemical shifts measurements with $\text{M} = \text{Co}^{++}$ have been performed on a Varian HA 100 spectrometer, locked on a benzene capillary. This signal corrected for the effect of the solution magnetic susceptibility — calibrated in pure Co^{++} solutions, versus the signal of 2—2-dimethyl-2-silapentane-5-sodium sulfonate (DSS) — is taken as a reference in all our measurements.

The chemical shift experienced by water protons in the presence of a paramagnetic ion can be directly related to the number of water molecules in its first hydration shell. If this number is changed due to site binding to a polyelectrolyte this change will be reflected in the change of chemical shift compared to a solution of free ion. The stoichiometry of binding can therefore be followed. A quantitative evaluation of the number of remaining exchangeable molecules in the hydration shell requires to postulate that its structure, and therefore the Fermi contact term is not largely perturbed due to replacement of a water molecule by another ligand. A study on several polyelectrolytes together with volume change measurements [6] seems to prove that this approximation is valid.

2.2. Relaxation enhancement

Water relaxation measurements have been performed using a Bruker spin echo spectrometer working in the range 4—90 MHz with home made probes of sufficiently small dimensions to work within the gap of the magnet of a HA 100 Varian spectrometer. T_1 was measured through 180—90° sequences and T_2 through Carr Purcell Meiboom Gill 90— n (180°) sequences.

In a paramagnetic salt solution, the water protons relaxation is essentially dominated by the nuclear spin—electron spin interaction, through the simple dipolar interaction as well as through the scalar hyperfine coupling. The spin—lattice $T_1(\text{M})$ and spin—spin

$T_2(\text{M})$ relaxation times of a proton coupled to the electron are given, assuming the nuclear Larmor frequency ω_I very small compared to that of the electron ω_S , by the Solomon—Bloembergen equations [7,10]:

$$\begin{aligned} T_1^{-1}(\text{M}) &= E \left(\frac{6\tau_c}{1+\omega_I^2\tau_c^2} + \frac{14\tau_c}{1+\omega_S^2\tau_c^2} \right) + D \frac{2\tau_e}{1+\omega_S^2\tau_e^2}, \\ T_2^{-1}(\text{M}) &= E \left(4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13\tau_c}{1+\omega_S^2\tau_c^2} \right) \\ &\quad + D \left(\tau_e + \frac{\tau_e}{1+\omega_S^2\tau_e^2} \right) \end{aligned} \quad (1)$$

with

$$E = \frac{1}{15} S(S+1) (\gamma g \beta)^2 / (4\pi \epsilon_0 c^2 d^3)^2$$

and

$$D = \frac{1}{3} S(S+1) A^2 / h^2.$$

The first term corresponds to the modulation of the dipolar interaction between spins S (electronic) and I (nuclear) with a correlation time τ_c . The second term corresponds to the modulation of the scalar coupling with a correlation time τ_e . γ is the proton gyromagnetic ratio, β Bohr's magneton, d the nuclear spin—electron spin distance, A/h the hyperfine coupling constant, c the light velocity and ϵ_0 the vacuum permittivity.

The correlation times are defined through

$$\tau_c^{-1} = \tau_s^{-1} + \tau_M^{-1} + \tau_R^{-1}, \quad \tau_e^{-1} = \tau_s^{-1} + \tau_M^{-1}, \quad (2)$$

where τ_M is the mean residence time of the water molecule in the hydration shell, τ_R the rotational correlation time of the paramagnetic ion and τ_s the electron spin relaxation time.

As a result of the ω dependent term and of the frequency dependence of τ_s , T_1 and T_2 are frequency dependent, even in simple water solutions [8]. A determination of the number of molecules of water in the first hydration shell, together with the parameters τ_e and τ_c permitting an estimation of the mobility of the counterions through τ_R , requires a detailed study of the frequency dependence of T_1 and T_2 in the presence of polyelectrolytes which is now in progress. Work at a single frequency as a function of the concentration of paramagnetic counterion will help to a qualitative distinction between different types of

counterions if one of these parameters varie sufficiently for "site bound", "trapped" and free ions. It is convenient to use the enhancement ratio defined by Eisinger [9]

$$\epsilon_i = \frac{T_i^{-1}(M,P) - T_i^{-1}(0,P)}{T_i^{-1}(M,0) - T_i^{-1}(0,0)} \quad (3)$$

where $i = 1$ or 2 for spin lattice and spin spin relaxation, M and P are the concentration in paramagnetic ion and polyelectrolytes. Its variation as a function of r will reveal the successive states of bound counterions.

2.3. Electron spin resonance

ESR measurements on Mn^{++} solutions in the presence and absence of polyelectrolytes have been performed on a Varian V 45000, X band spectrometer using a thin water cell and dual cavity measurements to correct for Q changes due to small variations in the positioning of the cell.

The ESR spectrum of the hexacoordinated free Mn^{++} in aqueous solutions is made of a set of five nearly coincident sets of six hyperfine lines. Upon binding this pattern may be profoundly changed due both to a loss of symmetry inducing large zero field splittings which remove the quasi-coincidence of the fine structure sets and a change in the electron spin relaxation which broadens the individual hyperfine lines. As a result in many cases, the spectrum of bound Mn^{++} becomes so broad that it is not observable at low concentration and a titration of "free" Mn^{++} can be performed on the basis of the intensity of the observed hyperfine structure. In more complicated cases a computer analysis of the signal has to be performed to separate signals with different line widths.

3. Results and discussion

Chemical shifts of water protons in the presence of Co^{++} and polyelectrolyte are given in fig. 1a and 1b. It is obvious that in the presence of polyelectrolytes there is no shift up to values of the order of $r \approx 0.4$. For values over $r \approx 0.6$ the shift closely parallels the one observed in the absence of polyelectrolyte. This means that the water of the first hydration shell, for the first added Co^{++} ions is either completely relaxed

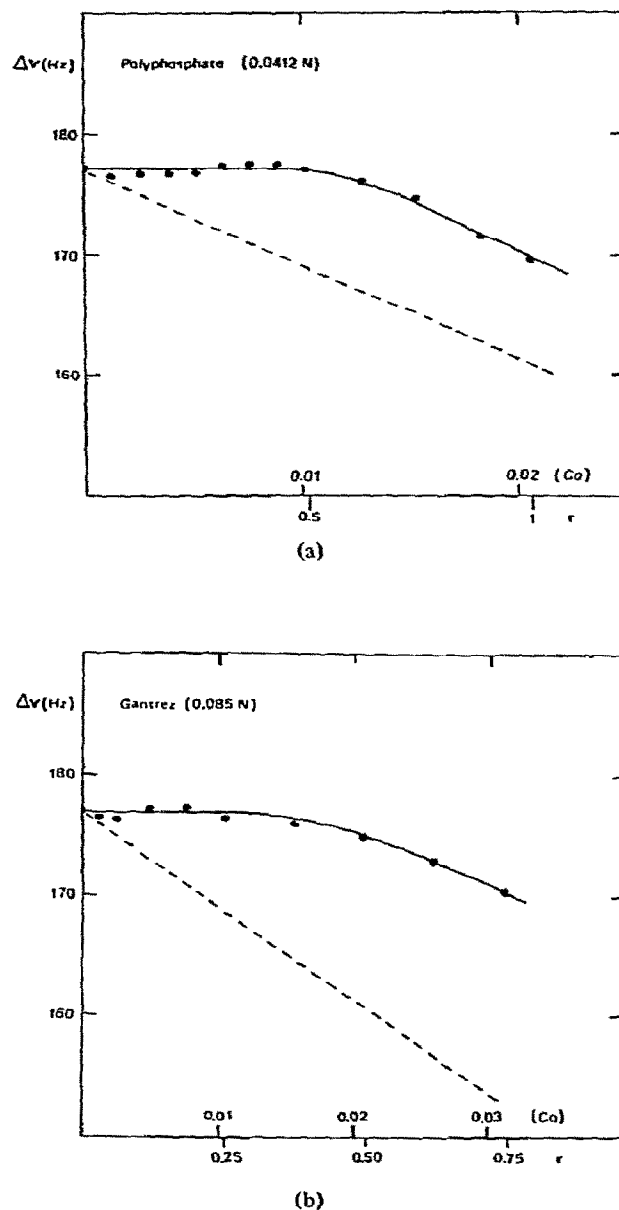
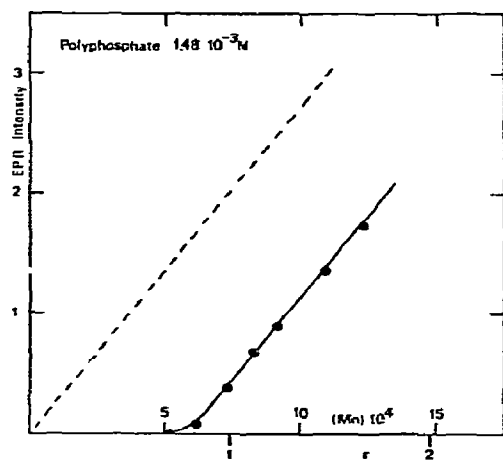
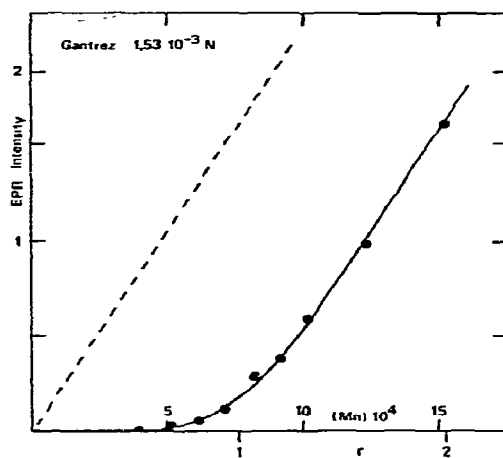


Fig. 1. Water protons chemical shift as a function of the molar concentration of Co^{++} ions: (a) in the presence of polyphosphate, 0.0412 N; (b) in the presence of Gantrez, 0.085 N. The dotted line gives the chemical shift in the absence of polyelectrolyte. The r scale give the corresponding Co^{++} /polymer ratio.



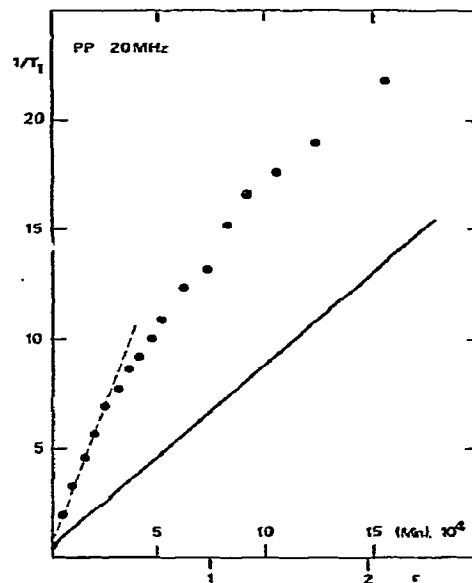
(a)



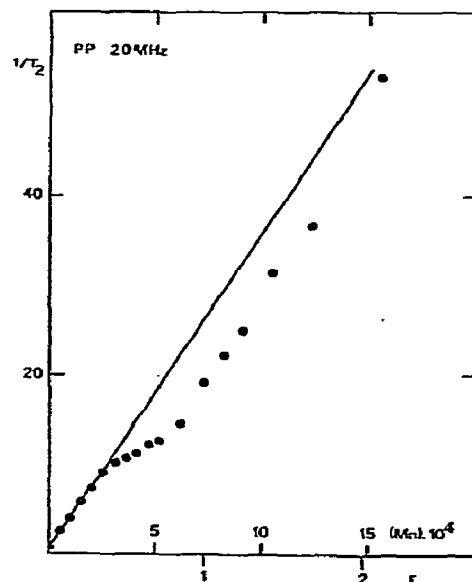
(b)

Fig. 2. Intensity of the hyperfine EPR signal of Mn^{++} as a function of the molar concentration of Mn^{++} ions: (a) in the presence of polyphosphate, (b) in the presence of Gantrez. The dotted lines gives the intensity in the absence of polyelectrolyte. The r scale gives the corresponding Mn^{++} /polymer ratio.

or not any more in fast exchange with the free solvent molecules. Volume changes upon binding [6] are in favour of a total relaxation of the first hydration shell.



(a)



(b)

Fig. 3. Relaxation rates of water in a polyphosphate solution as a function of the concentration of added Mn^{++} : (a) T_1^{-1} ; (b) T_2^{-1} . The straight line gives the relaxation rates in the absence of polyelectrolyte.

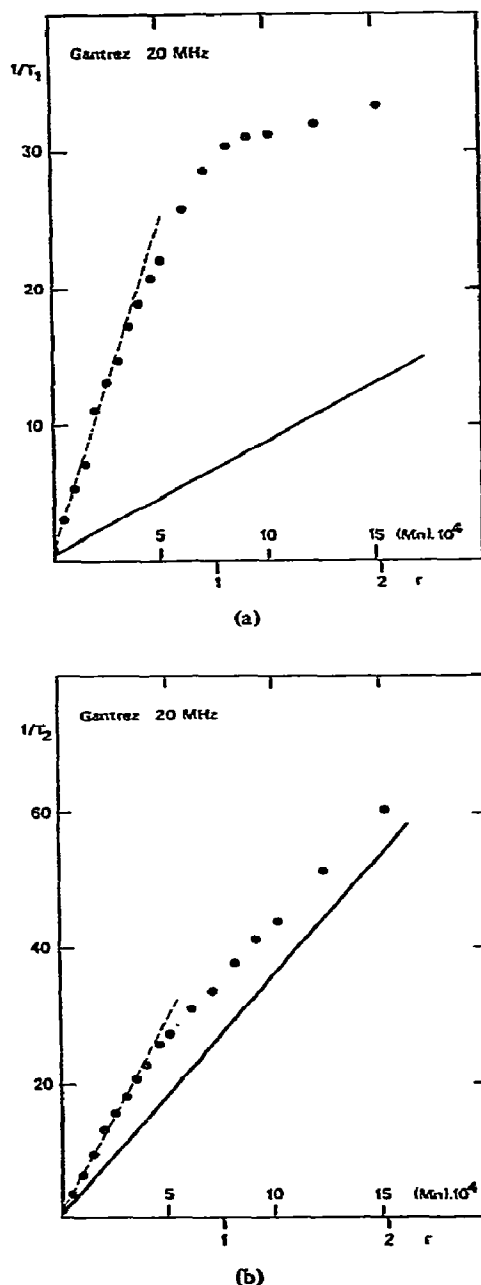


Fig. 4. Relaxation rates of water in a *Gantrez* solution as a function of the concentration of added Mn^{2+} : (a) T_1^{-1} ; (b) T_2^{-1} . The straight line gives the relaxation rates in the absence of polyelectrolyte.

The fraction of such bound ions is much smaller than the total fraction of condensed divalent ions which from Manning theory should be between 0.8 and 0.9 for the two polymers. Measurements of the intensity of the EPR signal reveal however that in the range $0.4 < r < 0.8$ there is still a considerable broadening of the line. The intensities of the small unbroadened hyperfine signal in the presence of polyelectrolytes have been plotted in figs. 2a and 2b.

No detailed line shape analysis have been performed so that this signal may be due either to the presence of a small fraction of free ions, due to the competition of Mn^{2+} and TMA^+ for atmospheric trapping or to the residual hyperfine structure of atmospherically bound counterions. In any case atmospheric trapping is shown to be reflected in the Mn^{2+} EPR spectrum. The total fraction of perturbed Mn^{2+} ions as obtained from an extrapolation of the curves at $r > 1$ indicates a value of 0.8 to 0.9 as the fraction of condensed counterions in good agreement with Manning's theory.

The distinction between the two types of binding appears more clearly in the relaxation measurements as shown in figs. 3 and 4 where T_1^{-1} and T_2^{-1} have been plotted as a function of r .

They all show an initial linear behaviour up to $r \approx 0.4$ and a more or less marked break at $r \approx 0.4$ and $r \approx 0.8$ to 0.9 . The first one corresponds to the transition from site binding to atmospheric trapping the second one to free counterions. A quantitative evaluation of the exaltation factor ϵ associated with the two types of bound counterion can be performed. In the fast exchange limit one has

$$M_t \epsilon = M_F + \epsilon_{b1} M_{b1} + \epsilon_{b2} M_{b2}, \quad (4)$$

where M_t , M_F , M_{b1} , M_{b2} are the total concentration in paramagnetic ions and the concentrations of free, site bound and atmospherically bound ions respectively, ϵ_{b1} and ϵ_{b2} being the exaltation factors for the two types of ion binding. Since

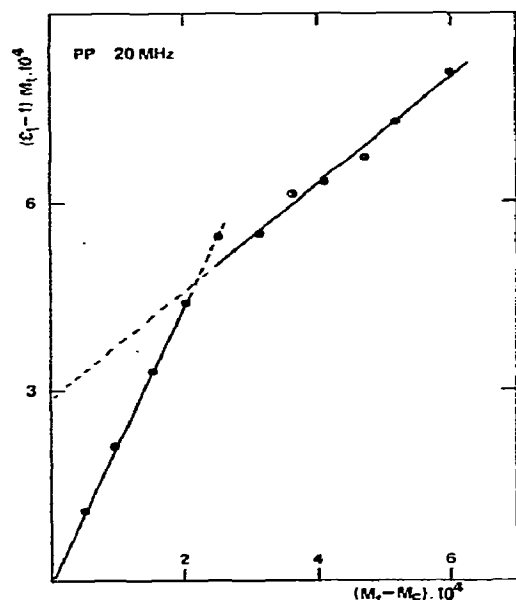
$$M_t - M_F = M_{b1} + M_{b2},$$

eq. (4) can be rewritten as

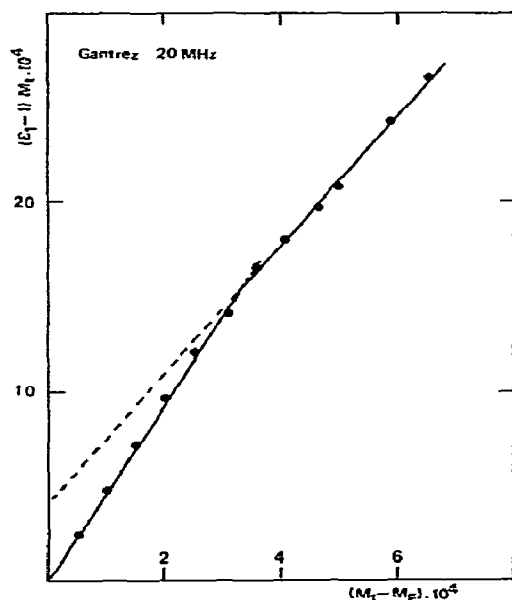
$$(\epsilon - 1)M_t = (\epsilon_{b1} - \epsilon_{b2})M_{b1} + (\epsilon_{b2} - 1)(M_t - M_F). \quad (5)$$

For the case of a single binding site it becomes

$$(\epsilon - 1)M_t = (\epsilon_b - 1)(M_t - M_F). \quad (6)$$



(a)



(b)

Fig. 5. Determination of the enhancement ratios following relation (5): (a) polyphosphate; (b) Gantrez.

Using M_F as calculated from the intensity of the EPR hyperfine structure such relations have been plotted in figs. 5a and 5b using the T_1 measurements of figs. 3 and 4 for polyphosphate and Gantrez. The breaking point at $r \approx 0.4$ corresponds well to what has been observed in the chemical shifts measurements and the two slopes give directly ϵ_{b1} and ϵ_{b2} . From the extrapolation of the second segment to $(M_t - M_F) = 0$ one gets a value of the maximum value of M_{b1} in very good agreement with the abscissa of the breaking point. The following values have been obtained from the exaltation of T_1 :

Polyphosphate: $(\epsilon_{b1})_1 = 3.1$ $(\epsilon_{b2})_1 = 1.8$,

Gantrez: $(\epsilon_{b1})_1 = 5.6$, $(\epsilon_{b2})_1 = 4.4$.

The abrupt break in the ϵ plot indicates that site binding and atmospheric binding do not compete and can be considered as successive processes upon addition of divalent ions to the TMA salts. The same analysis performed with T_2 measurements leads to $\epsilon_2 \approx 1$.

ϵ_1 and ϵ_2 are dependent on several factors: A decrease in hydration number at constant geometry in the first hydration shell should decrease the relaxation rate ($\epsilon < 1$) as well as a complete dehydration which leads to the loss of the proton electron scalar interaction. An increase in relaxation rate for the site bound counterions can only arise from an increase in the correlation time τ_c . For Gantrez at 20 MHz the value of $T_1/T_2 = 1.2$ arising from site bound Mn^{++} is very close to the theoretical value $7/6$ given by eq. (1) for $D = 0$, $\omega_S \tau_c \gg 1$, $\omega_I \tau_c \ll 1$ leading to an order of magnitude for $\tau_c \approx 10^{-9}$ s. Assignment of changes of τ_c to changes in τ_e or τ_R (see eq. (2)) can only arise from the analysis of the frequency dependence of ϵ_1 and ϵ_2 .

Such a work is now in progress on these polyelectrolytes as well as with other where site binding involves only a partial dehydration [6] or where there is no site binding at all.

4. Conclusion

Magnetic relaxation methods developed for the study of paramagnetic counterion binding on discrete sites of biopolymers have been adapted to the case of homopolyelectrolytes with a continuous distribution

of charges along the macromolecular chains. The combination of chemical shift, nuclear relaxation and EPR spectrum of the paramagnetic ion has led to a clear experimental distinction between site binding and atmospheric trapping on the basis of the counterion hydration and correlation times. A quantitative evaluation of the dehydration accompanying site binding has been performed. A quantitative interpretation of the exaltation of the relaxation rates as a function of frequency now in progress should lead to an evaluation of the restriction in the counterion rotational mobility.

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