

On some original properties of dilute polyelectrolyte solutions at low salt content: sodium hyaluronate example

M. Rinaudo*, M. Milas, N. Jouon and R. Borsali

Centre de Recherches sur les Macromolécules Végétales, CNRS, and Joseph Fourier University, BP 53X, 38041 Grenoble cedex, France

(Received 25 February 1992; revised 21 December 1992)

Supramolecular order is established in dilute solutions of ionic polymers in the absence of salt or at low ionic strengths. This order is confirmed by the observation of a peak in light or neutron scattering and corresponds to the existence of an ordered phase. When a concentrated polyelectrolyte solution is diluted, a peak is also observed in the reduced viscosity. This behaviour could be attributed to a local transition from anisotropic to isotropic phase as a first hypothesis. The roles of the charge parameter and salt content are discussed.

(Keywords: supramolecular organization; dilute polyelectrolyte solution; hyaluronic acid)

INTRODUCTION

Different polyelectrolyte solutions show a peak in light scattering¹, X-ray diffusion²⁻⁵ or neutron scattering experiments⁶⁻⁹.

In the absence of external salt, the location of this peak depends directly on the polymer concentration (C). The position of the maximum of the peak, expressed as the momentum transfer q , varies as $C^{+1/2}$ (ref. 1). When salt is added, this peak is shifted to lower q values due to the screening of electrostatic interactions². At large salt excess, the system behaves like neutral polymers. The peak also disappears progressively when the degree of neutralization (charge density) decreases³. It is noted that the way in which the electrostatic interactions gradually slow down is different when a salt is added and when the degree of neutralization is decreased. It was shown experimentally that the position of this peak is independent of the molecular weight^{1,10}.

On the other hand, the variation of the reduced viscosity as a function of polyelectrolyte concentration presents a maximum^{11,12} and the interpretation of this peculiar behaviour is not yet clear. In fact, this peak was also interpreted as proof of an ordering in aqueous solutions of charged molecules. A description of the different regimes and structures of polyelectrolyte solutions was given by Odijk¹³ and discussed by Weill¹⁴ and Kaji *et al.*⁵. In semidilute regime, the solution is macroscopically isotropic but locally anisotropic corresponding to a lattice of polyions on the scale of the persistence length.

This locally anisotropic solution has also been described by Lin *et al.*¹⁵ as an 'extraordinary phase', which corresponds to long distance correlations in solution. This ordering could be related to the existence

of a slow motion as revealed by dynamic light scattering experiments¹⁶⁻²¹.

As previously discussed by Drifford and Dalbiez¹⁷, at low added salt concentrations, a large correlation exists due to interchain electrostatic interactions, as shown by the interactive peak in light scattering. Under the same conditions, the slow mode observed using dynamic light scattering experiments was attributed to dynamics of stable and anisotropic domains of chains. This mode disappears in the presence of an excess of external salt.

Whatever the technique used, the scattering peak is often attributed to a correlation hole¹⁴, an intermolecular ordering promoted by electrostatic repulsions¹ or less frequently by attractive forces²² inducing ordered regions coexisting with disordered ones⁴.

This paper presents evidence of order in very dilute solutions of polyelectrolytes having different charge parameters, and in particular the viscometric behaviour of Na-hyaluronate.

EXPERIMENTAL

Bacterial hyaluronic acid was kindly donated by ARD (Paris, France). It was carefully purified as the sodium salt. Its weight-average molecular weight was determined by light scattering and found to be 1.63×10^6 . Its charge parameter, λ , is 0.71 in the extended conformation. Dilute solutions of polymer were prepared by dilution, with the appropriate solvent, of a stock solution (1 g l^{-1}) obtained by direct dissolution of the polymer in freshly double-distilled water or in NaCl solutions.

The viscosity measurements were performed at $25 \pm 0.01^\circ\text{C}$ using a Contraves LS30 viscometer for a range of shear rates from 10^{-2} to 128 s^{-1} . In order to improve the accuracy of the measurements, the LS30 is protected against draught by a Plexiglas enclosure. The

* To whom correspondence should be addressed

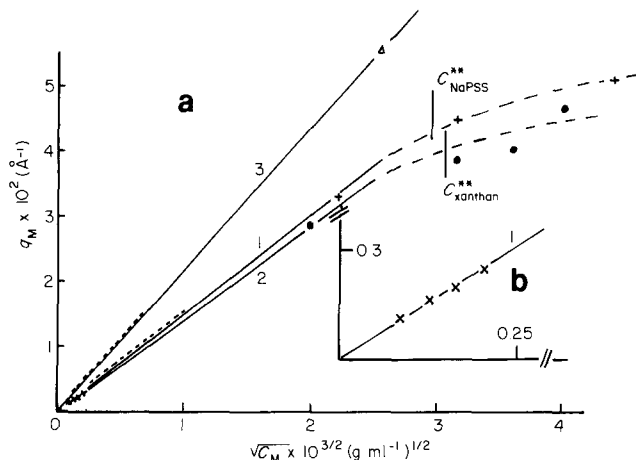


Figure 1 Dependence of the position of scattered peak (q_M) on the square root of the polymer concentration $\sqrt{C_M}$. Na-PSS (light scattering) (see insert (b))¹; +, Na-PSS (neutron scattering)⁶; ●, xanthan (neutron scattering)²³; △, Na-polyglutamate (neutron scattering)⁸. ---, domain covered by viscometry measurements for hyaluronate (line 3) and Na-PSS (line 1). Theoretical straight lines (relation (3)) for Na-PSS (line 1), xanthan (line 2) and polyglutamate and hyaluronate (line 3). C^{**} , critical concentration predicted by Odijk¹³

final relative viscosity is the average ratio $\eta_{\text{solution}}/\eta_{\text{solvent}}$, taken over all the speeds in the Newtonian plateau.

Fundamental approach on ordering of charged chains

The peak of scattered intensity observed in light or neutron scattering experiments is sometimes interpreted as a spacing corresponding to Bragg's law:

$$d = \frac{2\pi}{q_M} \quad (1)$$

where q_M is the critical momentum transfer ($q = (4\pi/\lambda) \sin(\theta/2)$; λ is the wavelength in solution and θ is the scattering angle) corresponding to the peak maximum. The position (q_M) depends on the polymer concentration (C_M).

Then, assuming stiff molecules on a scale of the order of the persistence length (L_T) and for a given momentum transfer, the polymer concentration C_M (expressed in g ml^{-1}) allows one to calculate an inter-rod distance as:

$$d = 2 \left(\frac{C_M \times 10^{-24} \pi N_a A}{m_0} \right)^{-1/2} = 2 \left(\frac{C_M \times 10^{-24} \pi N_a}{M_L} \right)^{-1/2} \quad (2)$$

where N_a is the Avogadro number, A is the length of the monomeric unit, m_0 is the corresponding monomolar mass (g) and $M_L = m_0/A$ is the mass per unit length; d is independent of the molecular weight.

According to relations (1) and (2), one deduces that:

$$q_M = C_M^{1/2} \times 10^{-12} \pi^{3/2} \left(\frac{N_a A}{m_0} \right)^{1/2} \quad (3)$$

From this relation, the position of the peak should be independent of the molecular weight. In fact this behaviour has already been shown experimentally⁸.

In *Figure 1*, the dependence of q_M (obtained from light and neutron scattering) as a function of $C_M^{1/2}$ is given for the different polyelectrolytes^{1,6,8,23}. The slope is characteristic of the chemical structure and reads:

$$\pi^{3/2} \left(\frac{N_a A}{m_0} \right) \text{ or } \pi^{3/2} \left(\frac{N_a}{M_L} \right)^{1/2}$$

In *Figure 1*, the values obtained using light¹ and neutron scattering⁶ are aligned as described by Drifford and Dalbiez¹ for sodium polystyrene sulfonate (Na-PSS). The slope depends only on M_L ; the straight line 1 is given for Na-PSS ($M_L = 82.4 \text{ g } \text{Å}^{-1}$), line 2 for xanthan ($M_L = 100 \text{ g } \text{Å}^{-1}$) and line 3 for polyglutamate and hyaluronate sodium salts ($M_L \approx 40 \text{ g } \text{Å}^{-1}$).

Experimental results obtained previously from neutron scattering on xanthan²³ and polyglutamate⁸ are also reported in *Figure 1*. Deviation from linearity is observed for the highest polymer concentrations in the range of C^{**} , as predicted by Odijk¹³ for melting of the electrostatic network; at the same time a large decrease in the degree of ordering is observed (see *Figure 3* in ref. 6). Under shear in a Couette geometry, better alignment of the molecules or segments of molecules is obtained with the appearance of a second order peak in the neutron scattered pattern²⁴. These observations provide evidence that the diffusion arises from the alignment of locally rigid molecules.

From these data it is clear that each polyelectrolyte is characterized by a single linear dependence, $q_M (\sqrt{C_M})$, corresponding to a preferential distance between locally rigid charged molecules, valid whatever the polymer concentration. For $C > C^{**}$, the experimental data show a deviation from this linearity.

The electrostatic repulsions between ionic chains cause this locally ordered structure of the solution. It was suggested²⁵ that there is an impenetrable shell of radius R equal to $2K^{-1}$ surrounding the polyion, where K^{-1} is the Debye length.

This causes an electrostatic excluded volume along a distance of the order of L_T . Then, the order exists for a given ratio d/K^{-1} .

The value of K^{-1} is derived from the ionic strength taking into account the concentration of counterions (C_p when $\lambda < 1$ or C_p/λ when $\lambda > 1$ with C_p in equiv. l^{-1}) and the external salt (C_s). The charge parameter λ characterizes the linear charge density of a polyelectrolyte; $\lambda = \gamma e^2/DhkT$, where γ is the number of ionic sites on a chain of length h , D is the dielectric constant, kT is the Boltzmann term and e is the electronic charge. K^{-1} (Å) is calculated from K^2 :

$$K^2 = 4\pi Q(C_p/\lambda + 2C_s)N_a 10^{-27} \\ = 4\pi A C_p(1 + 2C_s Q/C_p A)N_a 10^{-27} \quad (4)$$

where C_p is the concentration of counterions (equal to the monomolar concentration when there is one charge per repeating unit) and C_s is the salt concentration; Q is the Bjerrum length ($Q = e^2/DkT = 7.15 \text{ Å}$, $\lambda = Q/A$ with A the distance between two charged groups h/γ).

For $\lambda > 1$, and in the absence of salt excess it is easy to calculate the ratio d/K^{-1} which is related to the electrostatic repulsion.

Combining equations (2) and (4) yields:

$$\frac{d}{K^{-1}} = 4 \quad (5)$$

This relation justifies the previous suggestion of an impenetrable shell of radius $2K^{-1}$ surrounding the polyion.

Therefore, whatever the polymer concentration in the absence of external salt, a constant ratio exists between the inter-rod distance and the electrostatic screening length.

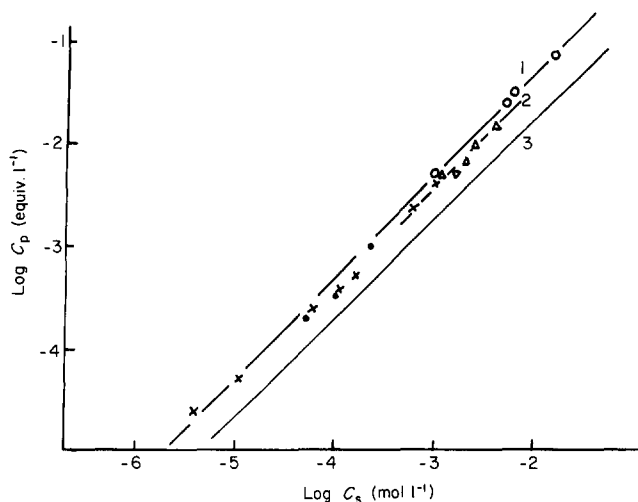


Figure 2 Plot of $\log C_p$ versus $\log C_s$. C_s is the added salt concentration that revealed the phase transitions at C_p^* , observed using quasi-elastic light scattering (LS) and viscometry (η) experiments. \circ , Na-PSS (LS)¹⁷; Δ , polylysine (LS)¹⁵; ∇ , polylysine (η)²⁹; \bullet , Na-hyaluronate (η) (this work); \times , Na-PSS (η)¹¹. Lines 1, 2 and 3 represent the theoretical predictions between C_p and C_s for Na-PSS, polylysine and hyaluronate, respectively

In the presence of an excess of salt, the electrostatic repulsions decrease as well as the ordering and the stiffness of the molecule:

$$\frac{d}{K^{-1}} = 4 \left(1 + 2\lambda \frac{C_s}{C_p} \right)^{1/2} \quad (6)$$

For a given K^{-1} , the electrostatic persistence length L_e is imposed²⁵; it is inversely proportional to C_p in the absence of external salt:

$$L_e(\text{\AA}) = \frac{1}{4QK^2} = 0.675(C_p/\lambda + 2C_s)^{-1} \quad (7)$$

These relations are valid for Na-PSS but for Na-hyaluronate where $\lambda < 1$, we obtain:

$$K^2 = 4\pi Q(C_p + 2C_s)N_a 10^{-27} \quad (8)$$

and in the absence of external salt:

$$d/K^{-1} = 4\lambda^{1/2} \quad (9)$$

In the presence of monovalent external salt, relation (9) becomes:

$$\frac{d}{K^{-1}} = 4\lambda^{1/2} \left(1 + \frac{2C_s}{C_p} \right)^{1/2} \quad (10)$$

Under these conditions, the electrostatic length L_e is imposed and reads:

$$L_e(\text{\AA}) = \frac{\lambda^2}{4QK^2} = 0.675\lambda^2(C_p + 2C_s)^{-1} \quad (11)$$

It was found experimentally that in the presence of a salt excess, at a given polymer concentration, the neutron scattering peak disappears due to the screening of electrostatic repulsions⁶; this was observed for $C_s < 4.85 \times 10^{-2}$ N NaBr and $C_p = 0.140$ monomole l^{-1} , i.e. $d/K^{-1} < 6.85$ on Na-PSS. From dynamic light scattering on Na-PSS solutions, Drifford and Dalbiez¹⁷ claimed that the transition from extraordinary to

ordinary phase occurs at:

$$2\lambda \frac{C_s}{C_p} = 1 \quad \text{or} \quad \frac{C_p}{C_s} = 5.6 \quad (12)$$

in the presence of 1-1 electrolyte; then the transition corresponds to:

$$\frac{d}{K^{-1}} = 4\sqrt{2} = 5.65$$

The important role of C_p/C_s (and not the absolute ionic strength) is also pointed out by Förster *et al.*²⁶.

The experimental values of C_p and C_s found in the literature for this transition are given in Figure 2.

RESULTS AND DISCUSSION

Our experimental results concern the dependence of the reduced viscosity versus the polymer concentration in dilute solutions and at low salt excess. A maximum was observed as shown in Figure 3. All the measurements were performed in the Newtonian regime on the Na form of hyaluronic acid. The same type of curves were previously obtained on the Na form of polystyrene sulfonic acid¹².

The polymer concentration corresponding to the maximum in reduced viscosity was converted into a q_M value (relation (3)). The domain of q_M covers a range between light and neutron scattering data (Figure 1). This indicates that the range of polymer concentrations covered in this work by viscosity measurements implies the existence of interchain interactions.

In Table 1 are listed the experimental values of C_M obtained for different salt concentrations and the intermolecular distances, d , calculated using relation (2). Values of K^{-1} and L_e are also reported and compared with d . When a peak is observed in viscosity, d is usually larger than 400 Å and $L_e \geq d$. The question is to justify

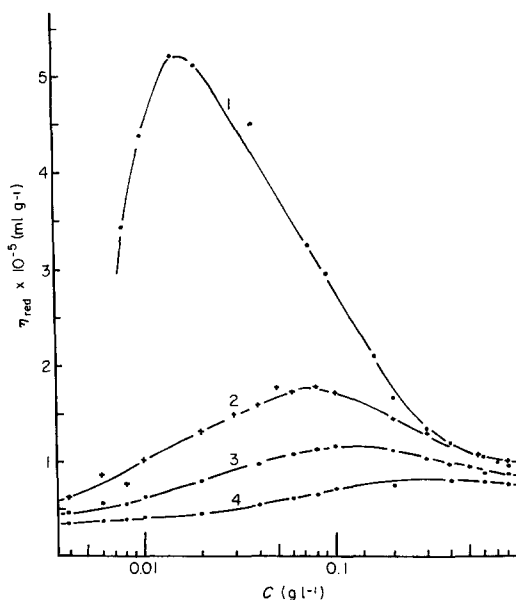


Figure 3 Variation of the reduced viscosity of Na-hyaluronate with the polymer concentration: 1, salt free; 2, 5×10^{-5} N NaCl; 3, 10^{-4} N NaCl; 4, 2×10^{-4} N NaCl. Values extrapolated to zero shear rate at 25°C. The solid lines are guides to the eye

Table 1 Characteristics of Na-hyaluronate solutions from viscosity measurements

C_s (N)	C_M (g l ⁻¹)	C_o (g l ⁻¹)	C_p^*/C_s (equiv. l ⁻¹)	L_e (Å) [rel. (11)]	d (Å) [rel. (2)]	K^{-1} (Å)	$(d/K^{-1})_{exp}$	$\left[\frac{(d/K^{-1})_{crit}}{(d/K^{-1})_o}\right]_{exp}$
0	0.016 ± 0.002	5 × 10 ⁻⁴	—	8630	2280	680	3.35	1
5 × 10 ⁻⁵	0.075 ± 0.01	8.86 × 10 ⁻²	3.74	1200	1063	254	4.18	1.247
10 ⁻⁴	0.14 ± 0.02	2.33 × 10 ⁻¹	3.48	619	786	185	4.24	1.26
2 × 10 ⁻⁴	0.4 ± 0.2	7.02 × 10 ⁻¹	4.98	247	460	115	4.0	1.19

Table 2 Critical concentrations for the different polymers investigated

	M_w	C^* (g l ⁻¹) (ref. 13)	C^{**} (g l ⁻¹) (ref. 13)
Xanthan	2 × 10 ⁶	3.3 × 10 ⁻²	10.3
Na-PSS	2 × 10 ⁶	1.60 × 10 ⁻²	8.9
Na-polyglutamate	—	—	4.3
Na-hyaluronate	1.6 × 10 ⁶	3.35 × 10 ⁻³	6.2

$C^* = 0.677 \text{ mol}\lambda^2/L$ when $\lambda < 1$; $C^* = 0.677 \text{ mol}\lambda/L$ when $\lambda > 1$; $C^{**} = 0.0154 \text{ mo}$ when $\lambda < 1$; $C^{**} = 0.108 \text{ mo}/A$ when $\lambda > 1$; A = distance between two ionic groups (Å); mo = equivalent mass (g); L = contour length of the molecule (Å); C^* and C^{**} are the polymer concentration for which the persistence length equals the contour length and that corresponding to the melting of the electrostatic network, respectively

the existence of a maximum in the reduced viscosity and its relation with the organization of the solution.

From a general point of view, when the concentration of the polyelectrolyte decreases within the range of polymer concentration $C^* < C < C^{**}$ (see Table 2 for the values of C^* and C^{**} ; C^* and C^{**} correspond to the limits of the domains introduced by Odijk¹³), the reduced viscosity decreases then passes through a minimum and increases due to the electrostatic expansion of the polymeric chain and long range correlation. Then, it passes through a maximum for a critical polymer concentration C_p^* (equiv. l⁻¹) independently of the overlap concentration (Table 1).

The overlap concentration (C_o) was calculated as a first approximation from the values of the radius of gyration R_G , and that of $L_T = L_p + L_e$ (L_T is the total persistence length and L_p the intrinsic persistence length of the corresponding neutral polymer):

$$C_o = \left(\frac{4}{3} \pi \frac{R_G^3 N_a}{M} \right)^{-1} \quad \text{and} \quad R_G^2 = \frac{L_T L}{3} \quad (13)$$

where L_p is taken as 80 Å (ref. 27).

Finally, the reduced viscosity decreases ($C < C_p^*$) reaching the characteristic of a single chain with a persistence length controlled by C_s .

In the absence of external salt, the maximum is caused by the presence of ions in the water used for dilution (in the range 10⁻⁷ to 10⁻⁵ M) or due to the hydrolysis phenomenon²⁸.

Then, the maximum in the reduced viscosity is moved to larger polymer concentration in the presence of an excess of salt and disappears (Figure 3, or ref. 11). Above a give ionic content, the electrostatic potential is ineffective in inducing electrostatic repulsions between the chains at a given distance d .

To demonstrate that the maximum of the reduced viscosity is related to the transition observed earlier using other techniques, the data given in literature for Na-PSS

obtained from dynamic light scattering¹⁷ are compared with those of viscosity¹¹ in Figure 2. Drifford and Dalbiez claimed that the transition occurs for $2\lambda C_s/C_p = 1$ or $C_p/C_s = 5.6$. In fact the maximum reduced viscosity data of Na-PSS from Cohen *et al.*¹¹ fit quite well on the line $\log C_p = \log 5.6 C_s$, as well as with the critical concentration of the transition determined from the diffusion coefficient measurements by Drifford and Dalbiez¹⁷. Moreover, the data for polylysine are shifted with respect to those of Na-PSS; this is due to the modification of its charge parameter from which one can predict that $C_p/C_s = 2\lambda = 3.8$. It is lower than the ratio obtained for Na-PSS¹⁵ but in harmony with the experimental data. These results seem to indicate that the maximum in the reduced viscosity and the transition in diffusion coefficients have the same origin.

Schurr and co-workers also showed the existence of a critical peak in viscosity²⁹ and a transition in conductivity³⁰ corresponding to the phase transition. Their experimental data are also in good agreement with the results obtained from light scattering (see Figure 2). They attributed the enhanced viscosity at low shear rate to the expansion of individual polyions or to enhanced intermolecular interactions. From conductivity measurements, they estimated only a very small change in counterion interactions corresponding to a small change in the conformation. They concluded that the phase transition at low salt concentration is induced primarily by the increasing strength and range of intermolecular repulsions.

We suggest that the same interpretation can be applied to the hyaluronate results. The polymer concentrations (C_p^*) at the maximum in reduced viscosity are plotted as a function of salt concentration in Figure 2.

On the other hand, Cohen *et al.*¹¹ discussed the existence of a maximum in the reduced viscosity based on the weak-coupling approximation (WCA) model originally developed by Hess and Klein for highly charged spherical particles³¹; from this treatment we obtain:

$$\frac{\eta_{sp}}{C_p} \propto R_H \frac{C_p}{K^3} \propto \frac{C_p}{(C_p + 2C_s)^{3/2}} \quad \text{for } \lambda < 1 \quad (14)$$

or

$$\propto \frac{C_p}{(C_p/\lambda + 2C_s)^{3/2}} \quad \text{for } \lambda > 1 \quad (15)$$

where R_H is the hydrodynamic radius of the macromolecule.

In fact, Cohen *et al.*¹¹ estimated K without taking into account the charge parameter λ for Na-PSS. Nevertheless, this approach is interesting since it predicts that the reduced viscosity varies linearly with C_p for constant K (isoionic dilution) and with $C_p^{-1/2}$ in the absence of external salt. This last dependence is reached for

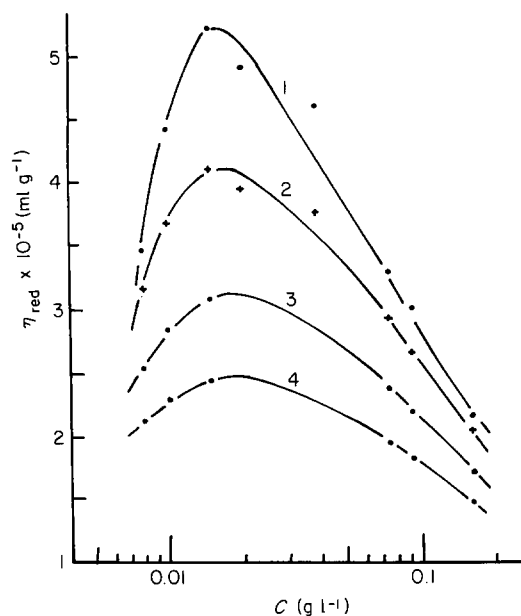


Figure 4 Influence of the shear rate ($\dot{\gamma}$) on the reduced viscosity of Na-hyaluronate at 25°C. 1, $\dot{\gamma} = 0.51 \text{ s}^{-1}$; 2, $\dot{\gamma} = 2.37 \text{ s}^{-1}$; 3, $\dot{\gamma} = 6 \text{ s}^{-1}$; 4, $\dot{\gamma} = 11 \text{ s}^{-1}$. The solid lines are guides to the eye

sodium hyaluronate, for polymer concentrations between 3×10^{-2} and $8 \times 10^{-1} \text{ g l}^{-1}$.

The derivation of relations (14) and (15) gives a direct relation between the maximum C_p^* of η_{sp}/C_p for a given C_s and is given by:

$$C_p^* = 4\lambda C_s \text{ for } \lambda > 1$$

or

$$C_p^* = 4 C_s \text{ for } \lambda < 1 \quad (16)$$

allowing the prediction of the critical polymer concentration at the maximum reduced viscosity for a given salt content assuming R_H to be constant.

The ratio C_p^*/C_s is double that predicted by Drifford and Dalbiez¹⁷ (for $\lambda > 1$, relation (12)); additionally there is no good agreement between predicted values (relation (15)) and the experimental data of viscosity obtained by Cohen *et al.*¹¹.

For $\lambda < 1$, from Table 1, the average ratio C_p^*/C_s is found to be around 4 for sodium hyaluronate, in agreement with the prediction (relation (16)). Considering the critical value of C_p^*/C_s , one can predict a corresponding value for d/K^{-1} over which the electrostatic repulsions are screened and where the electrostatic networks melt (this prediction has no relation with Odijk's model¹³). From relation (10) we obtain:

$$\left(\frac{d}{K^{-1}}\right)_{\text{crit}} = 4\lambda^{1/2}(1.5)^{1/2}$$

or

$$(d/K^{-1})_{\text{crit}}/(d/K^{-1})_0 = 1.22$$

for the ratio between the critical value of (d/K^{-1}) and the value of $(d/K^{-1})_0$ in the absence of salt. The experimental values are in good agreement with this prediction (see Table 1).

Therefore, the existence of a maximum in the reduced viscosity is probably related to the phase transition in connection with a critical value of d/K^{-1} . From this discussion it emerges that the maximum in the reduced viscosity has the same origin as the phase transition

observed from the light scattering correlation peak when the polymer concentration decreases.

Additionally, the role of the shear rate was investigated and the results are plotted in Figure 4. The shear rate effect on the maximum reduced viscosity is less significant for hyaluronic acid than for Na-PSS¹². This is probably related to their respective intrinsic flexibilities. The dynamics of the domains formed by ordered chains was previously discussed by Schurr and co-workers²⁹.

CONCLUSION

This paper discusses the organization of water-soluble polyelectrolyte solutions in the dilute concentration range. In addition to the existence of a peak in the scattered intensity (light and neutron) localized at the wavevector q_M , such as $q_M \approx C^{1/2}$, the variation of the reduced viscosity with concentration also exhibits a peak. This peak is sensitive to salt concentration and is independent of the molecular weight as shown for Na-PSS. A hypothesis of an electrostatic ordering based on electrostatic repulsion between locally stiff chains is adopted. Excess salt progressively cancels this maximum as well as the ordering of ionic molecules and corresponds to the transition to an isotropic phase. The existence of anisotropic domains of locally ordered chains and consequently high electrostatic persistence length L_e may be at the origin of the increase of the reduced viscosity as the concentration decreases to C_p^* . This behaviour can be quantified by a critical value of C_p^*/C_s for a given polyelectrolyte. Complementary experiments are in progress on different polyelectrolyte systems having different charge parameters using static and dynamic light scattering to give a better understanding of the mechanism observed.

REFERENCES

- 1 Drifford, M. and Dalbiez, J. P. *J. Phys. Chem.* 1984, **88**, 5368
- 2 Ise, N., Okubo, T., Yamamoto, K., Matsuoka, H., Kawai, H., Hasimoto, T. and Fujimura, M. *J. Chem. Phys.* 1983, **78**, 541
- 3 Ise, N., Okubo, T., Hiragi, Y., Kawai, H., Hasimoto, T., Fujimura, M., Nakajima, A. and Hayashi, H. *J. Am. Chem. Soc.* 1979, **101**, 5836
- 4 Ise, N. and Okubo, T. *Accounts Chem. Res.* 1980, **13**, 303
- 5 Kaji, K., Urakawa, H., Kanaya, T. and Kitamura, R. *J. Phys. (France)* 1988, **49**, 993
- 6 Nierlich, M., Williams, C., Boué, F., Cotton, J. P., Daoud, M., Farnoux, B., Jannink, G., Picot, C., Moan, M., Wolff, C., Rinaudo, M. and de Gennes, P. G. *J. Phys. (France)* 1979, **40**, 701
- 7 Williams, C., Nierlich, M., Cotton, J. P., Jannink, G., Boué, F., Daoud, M., Farnoux, F., Picot, C., de Gennes, P. G., Rinaudo, M., Moan, M. and Wolf, C. *J. Polym. Sci. Lett.* 1979, **17**, 379
- 8 Rinaudo, M. and Domard, A. *J. Polym. Sci., Polym. Lett. Edn* 1977, **15**, 411
- 9 Cotton, J. P. and Moan, M. *J. Phys. Lett. (Paris)* 1976, **37**, L75
- 10 Rinaudo, M. in 'Ions in Macromolecular and Biological Systems' (Eds D. H. Everett and B. Vincent), Colston Papers no. 29, Scientechica, Bristol, 1978, pp. 79–80
- 11 Cohen, J., Priel, Z. and Rabin, Y. *J. Chem. Phys.* 1988, **88**, 7111
- 12 Ganter, J. L. M. S., Milas, M. and Rinaudo, M. *Polymer* 1992, **33**, 113
- 13 Odijk, T. *Macromolecules* 1979, **12**, 688
- 14 Weill, G. *J. Phys. (France)* 1988, **49**, 1049
- 15 Lin, S. C., Lee, W. I. and Schurr, J. M. *Biopolymers* 1978, **17**, 1041
- 16 Drifford, M., Dalbiez, J. P., Tabti, K. and Tivant, P. *J. Chim. Phys.* 1985, **82**, 571
- 17 Drifford, M. and Dalbiez, J. P. *Biopolymers* 1985, **24**, 1501
- 18 Koene, K. S. and Mandel, M. *Macromolecules* 1983, **16**, 973

- 19 Drifford, M. and Dalbiez, J. P. *J. Phys. Lett.* 1985, **46**, L311
20 Fulmer, A. W., Benbasat, J. A. and Bloomfield, V. A. *Biopolymers* 1981, **20**, 1147
21 Schmitz, K. S. and Lu, M. *Biopolymers* 1984, **23**, 797
22 Oosawa, F. *Biopolymers* 1968, **6**, 1633
23 Milas, M., Rinaudo, M. and Duplessix, R. ILL Experimental Report, Annex to the Annual Report of ILL, Grenoble, 1982
24 Milas, M., Rinaudo, M., Linder, P. and Oberthur, R. ILL Experimental Reports, Annex to the Annual Report of ILL, Grenoble, 1985
25 Odijk, T. and Houwaart, A. C. *J. Polym. Sci., Polym. Phys. Edn* 1978, **16**, 627
26 Förster, S., Schmidt, M. and Antonietti, M. *Polymer* 1990, **31**, 781
27 Fouissac, E., Milas, M., Rinaudo, M. and Borsali, R. *Macromolecules* 1992, **25**, 5613
28 Vink, H. *Polymer* 1992, **33**, 3711
29 Martin, N. B., Tripp, J. B., Shibata, J. H. and Schurr, J. M. *Biopolymers* 1979, **18**, 2127
30 Shibata, J. and Schurr, J. M. *Biopolymers* 1979, **18**, 1831
31 Hess, W. and Klein, R. *Adv. Phys.* 1983, **32**, 173