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Further evidence of liquid-like correlations in polyelectrolyte solutions

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Abstract. — Elastic, quasi elastic light scattering and viscosity experiments were used to investigate polyelectrolytic polysaccharide succinoglycan solutions at low solute concentration C_p , and salt concentration C_s . The highest degree of « organization » in the solution necessary to describe the observations is a simple liquid type correlation, manifested by broad angular static and dynamic scattering maxima of visible light for solutions at very low ionic strength. Letting the solutions stand undisturbed for a few days did not lead to a sharpening of the broad maxima, nor did lowering the temperature. The positions of these maxima scale roughly as $C_p^{1/2}$. By adding salt, the maxima were found to maintain roughly the same position. The reciprocal diffusion coefficient $D^{-1}(q)$ corresponding to the liquid-like correlation state followed the intensity maxima, as has often been demonstrated for similar systems. No « slow mode » or « extraordinary regime » of diffusion was found associated with the static and dynamic light scattering maxima although extreme care in filtration of solution was necessary to avoid a spurious slow diffusional mode due to aggregates.

1. Introduction.

The solution properties of polyelectrolytes in media of different ionic strength are characterized by complex mechanisms involving interacting polyions, counterions and co-ions. Over the past decade there has been considerable interest in the static and dynamic properties of such systems from both theoretical and experimental points of view. Theoretical models include : crystal-solution of rods [1], notion of correlation hole [2-5], concept of electrostatic persistence length [6-8], counterion condensation [9], mode-mode coupling theory [10-12], entangled solution behavior [13, 14], phase transitions [15, 16] and weakly charged polyelectrolyte solutions based on the random phase approximation [17]. Various experimental techniques have been used to understand the behavior of such charged complex systems : small angle X-

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precipitation and subsequent reduced pressure room temperature drying, as has been already described [40, 41].

In this study, three different SG samples were studied. Virtually all the results presented here are from a fairly narrow molecular weight distribution of sonicated SG. Similar scattering behavior in all aspects was found also for the native SG sample. We concentrated on the sonicated sample because the lower mass solutions were easier to filter and handle.

Pure water was doubly distilled, de-ionized and filtered in a Millipore Alpha-Q; its conductivity was less than $0.05 \mu\text{S}$ corresponding to an equivalent NaCl concentration of around $3.3 \times 10^{-7} \text{ M}$. SG solutions were allowed to dissolve and equilibrate few days prior to filtration and measurements.

The characteristics of SG in its disordered conformation corresponds to one charge every $16.8 \text{ \AA}$, which gives a linear charge parameter $\lambda = 0.42$ as deduced from the chemical structure in the Na-salt form. The molar mass per unit length is $66.7 \text{ g/\AA.mol}$. These values were obtained from the ^1H NMR spectrum in D_2O in the presence of an internal standard which gave 0.98 pyruvate and 0.24 succinate groups/repeat unit. In this disordered form the contribution to the total salt concentration due to the SG counterions and bare polyelectrolyte is roughly $C_{s,c}[\text{equi/l}] = 0.864 \times C_p(\text{g/cm}^3)$. In excess of salt, the SG macromolecule adopts and extended helical conformation (stabilized by intramolecular H-bonds); the above values correspond to one charge every $15.65 \text{ \AA}$, $\lambda = 0.45$ and $73.7 \text{ g/\AA mol}$ for the molar mass per unit length. The helix-coil transition is induced by temperature increase or NaOH addition. Like Xanthan gum, the transition from disordered to helical state of SG is only 10 % of change in axial ratio. This explains why the molar mass/unit length and the charge parameter are slightly different in both conformations.

We give upper and lower estimates of the overlap concentration C^* based on the well-known formula $C^* = M_w / (N_A 4 \pi R^3 / 3)$, where R is a characteristic dimension of the molecule and N_A is Avogadro's number. We use the GPC results $M_w = 7.74 \times 10^5 \text{ g/mol}$. The lower limit is obtained by assuming the SG to be fully extended occupying a spherical volume whose radius R is half the rod length. With $R = 5800 \text{ \AA}$, this gives $C^* = 1.57 \times 10^{-6} \text{ g/cm}^3$. The upper limit makes the assumption that $R = \langle S^2 \rangle^{1/2}$ where $\langle S^2 \rangle^{1/2}$ is about $1100 \text{ \AA}$, the roughly constant value obtained at all C_s , as discussed below. This gives $C^* = 2.3 \times 10^{-4} \text{ g/cm}^3$.

To eliminate dust and other large particles, all samples were filtered. Both the pore size and material composition of the membrane filters used prior to light scattering experiments had large effects on the light scattering results. Due to the relative solvent affinity and the degree of swelling, the type of membrane (material) as well as the effective pore diameter indeed may play an important role on the filtration. All filters were 2.5 cm diameter membranes held in a stainless steel holder. The holders were attached *via* Luer lock fittings to glass syringes and steady manual pressure was used to filter the solutions. Three different types of filters were used: 1) all solutions not otherwise noted were filtered through $0.1 \mu\text{m}$ cellulose nitrate filters (CN) (Sartorius, W-3400, Germany). 2) Comparisons with filtration through $0.2 \mu\text{m}$ cellulose nitrate filters were also made (Sartorius), as well as comparisons with 3) $0.1 \mu\text{m}$ polyvinylidene difluoride (PVDF) filters from Millipore which are more hydrophobic than the CN membranes. Concentration losses upon filtering were estimated by different techniques (UV absorption and conductivity) and found to be roughly on the order of 10 %. All concentrations in this work are expressed in terms of weighed, dry material per ml of solvent, and thus represent an upper limit.

2.2 EQUIPMENT AND DATA ANALYSIS.

2.2.1 Static and dynamic light scattering. —

The elastic and quasi-elastic scattering measurements were performed using the ALV (Langen-FRG) apparatus with an automatic goniometer

table, a digital ratemeter and a temperature controlled sample cell. Temperature was 25 ± 0.1 °C unless otherwise noted.

The scattered light of a vertically polarized $\lambda_0 = 4880$ Å argon laser (Spectra-Physics 2020, 3 W, operating around 0.3 W) was measured at different angles in the range of 20-150° corresponding to $0.6 \times 10^{-3} < q/\text{Å} < 3.3 \times 10^{-3}$ where $q = (4\pi n/\lambda_0) \sin(\theta/2)$, θ the scattering angle, n the refractive index of the medium ($n = 1.33$). The reduced elastic scattering $I(q)/kC_p$, with $k = 4\pi^2 n_0^2 (dn/dc)^2 (I_0^{90^\circ}/R^{90^\circ})/\lambda_0^4 N_A$, was measured in steps of 5° in the scattering angle, where n_0 is the refractive index of standard (toluene), $I_0^{90^\circ}$ and R^{90° are respectively the intensity and the Rayleigh ratio of the standard at $\theta = 90^\circ$, (dn/dc) the increment of refractive index, C_p the polyelectrolyte concentration, expressed in g/cm³ and $I(q)$ the intensity scattered by the polymer. All elastic intensities were calculated according to standard procedures using toluene as reference with known absolute scattering intensity.

Because the total scattering intensity of the highly dilute SG in salt-free solution is scarcely above the scattering level of pure water the slightest amount of scattering impurities (e.g. « dust ») led to unacceptably noisy data. Consequently, an algorithm was written to extract the minimum intensity reading from a group of typically 50 separate intensity readings at each angle which are stored to disk by the ALV static data gathering program. The experimental and theoretical justification for this procedure has been previously given [24].

The full homodyne autocorrelation functions of the scattered intensity, also measured in steps of 5° in the scattering angle, were obtained using the ALV-5000 autocorrelator from ALV, Langen, FRG. The intermediate scattering function $S(q, t)$ is related to the measured homodyne intensity-intensity time correlation function by the Siegert-relation [42]

$$G^{(2)}(q, t) = B[1 + \alpha |S(q, t)|^2] \quad (1)$$

where B is the base line and α is the spatial coherence factor depending upon the geometry of the detection and the ratio of the intensity scattered by the polymer to that scattered by the solvent. The autocorrelation functions of the scattered intensity were analyzed by means of the cumulant method to yield the effective diffusion coefficient as a function of the scattering angle. Additionally, inverse Laplace transform (ILT) and constrained regularization method (Contin) developed by Provencher [43] were used to obtain the distribution $A(\tau)$ of decay times. For the latter, a statistical parameter « probability to reject » is calculated for each solution, and the suggested one is that closest to 0.5.

$$\left[\frac{G^{(2)}(q, t)}{B} - 1 \right]^{1/2} \approx \int_0^\infty A(\tau) e^{-t(\tau)} d\tau. \quad (2)$$

These methods are now routinely used to analyze the quasi elastic light scattering (QELS) data for polymer systems and allows the determination of the relaxation modes which characterize the dynamic of such systems.

Static experiments were performed with a wide aperture for the phototube (300 µm pinhole), whereas the dynamic experiments used the narrowest possible (typically 100 µm in this range of concentration).

2.2.2 Size exclusion chromatography. — Size Exclusion Chromatography (SEC) characterization was carried out using a Waters 150C ALC/GPC with multiple detectors ; a single capillary viscometer [44], a Wyatt Technology Dawn F multi-angle laser light scattering detector (MALLS) with a vertically polarized He-Ne laser at $\lambda_0 = 6320$ Å, and a refractive index detector built into the Waters unit. The simultaneous interfacing and operation of this system, as well as problems and procedures in data analysis will be presented in a

forthcoming paper. With specially written software the Dawn F was also used for « batch » measurements on the SG; i) Zimm plots, ii) determination of $\langle S^2 \rangle^{1/2}$ and second virial coefficients, A_2 , versus salt concentration C_s , and iii) dimensions and A_2 versus $[\text{NaOH}]$.

2.2.3 Viscosity measurements. — The reduced viscosity $\eta_r = (\eta - \eta_0)/\eta_0 C_p$, where η_0 is the solvent viscosity, measurements were made with a Low Shear 30 cylinder in cup viscometer. This allowed measurements to be made on the Newtonian plateau. The viscosity measurements made in pure water as solvent in the range of polymer concentration from 10^{-5} g/cm^3 to 10^{-3} g/cm^3 , which includes the domain over which the light scattering experiments were performed. These solutions were also filtered through $0.1 \mu\text{m}$ and $0.2 \mu\text{m}$ cellulose nitrate membranes.

3. Results and discussion.

3.1 SEC RESULTS. — Figure 1 shows SEC results for filtered through $0.1 \mu\text{m}$ cellulose nitrate. using the increment of refractive index $(dn/dc) = 0.154$ for SG in $100 \text{ mM NH}_3\text{NH}_4$ yielded number, weight and z-average molecular weights M_n , M_w , M_z respectively given in the figure. The slope of the root mean square radius of gyration $\langle S^2 \rangle^{1/2}$ is 0.584 ± 0.04 . The intrinsic viscosity was around $2.600 \text{ cm}^3/\text{g}$.

Using the coil limit of the worm-like chain formula $\langle S^2 \rangle = LL'_T/3$ where L is the contour length of the molecule, allows apparent persistence length L'_T to be estimated from the data in figure 1. One notes that this so-called apparent persistence length includes the electrostatic contribution as well as the excluded volume effect. It has been defined and discussed elsewhere [24-27, 45-47]. This value is around $300 \text{ \AA}$ at $C_s = 100 \text{ mM}$. This is in good agreement with earlier report [48] and shows that SG is a rather stiff polymer, in the class of xanthan, for example, and quite a bit stiffer than hyaluronate ($L_0 = 87 \text{ \AA}$, where L_0 is the intrinsic persistence length, estimated by extrapolation of L'_T to infinite ionic strength). With $L'_T = 300 \text{ \AA}$ for SG, the number of Kuhn segments is about 19. This value is not far from that of the coil limit for static dimensions, and certainly too many for the SG to be considered as a rod at this salt concentration.

Fig. 1. — SEC results for SG at $C_s = 0.1 \text{ M}$. M_n , M_w and M_z are given.

3.2 DIMENSIONS AND A_2 versus C_s . — Figure 2a shows the slope of $kC_p/I(q)$ versus q^2 for SG at 0.1 mg/ml for different values of C_s (from serial additions of small amounts of NaCl stocks). For reasons explained below, the slope is initially negative, and becomes flatter, and finally positive as the salt concentration C_s increases. By about 1 mM the slope is nearly zero and then becomes positive and remains constant thereafter. It is only after the slope has become positive, and electrostatic interactions between polymers sufficiently suppressed, at about 4 mM that one can use standard static light scattering techniques to estimate $\langle S^2 \rangle^{1/2}$ and A_2 .

Interestingly, the dimension of the SG in the accessible C_s range (after about 4 mM) is quite insensitive to C_s . The estimated values of $\langle S^2 \rangle$, calculated from the slopes (at $C_s > 4$ mM), are shown in figure 2b. The solid line shows the calculation of $\langle S^2 \rangle$ according to a combination of electrostatic persistence length (EPL) and electrostatic excluded volume (EEV) theories, which has given fairly good predictions of $\langle S^2 \rangle$ and A_2 versus $C_s^{-1/2}$ and C_s , respectively, with no adjustable parameters [24-27, 46, 47].

Although the calculated curve shows only a modest decrease of $\langle S^2 \rangle^{1/2}$ with increasing C_s , the experimental data show no change. The reason for this insensitivity of $\langle S^2 \rangle^{1/2}$ to C_s is unclear. Aside from experimental uncertainty, it may be due, for example, to residual electrostatic effects for the low C_s values which tend to depress the slope against the effect of coil expansion (Fig. 2a) which, contrarily, increases the slope.

Another possibility is that at the lower ionic strength the SG is in a mixture of helicoidal and disorganized states. The apparent intrinsic persistence length of SG in the disorganized forms is only about 50 Å [48]. According to EPL/EEV computations, SG dimensions in the disordered state at these ionic strengths would be smaller than those of the helical SG, the average radius of gyration being measured thus being less than that of the purely helical form.

In contrast, figure 2c shows how sensitive A_2 is to added salt concentration C_s , indicating appreciable intermolecular electrostatic excluded volume effects. The solid line in figure 2c shows the calculation of A_2 via the same combined EPL and EEV theories. It is emphasized that this calculation, which predicts A_2 quite well, involves no adjustable parameters. It is also noted that virtually the whole variation of $\langle S^2 \rangle$ over this range is due to EEV effects, the intrinsic stiffness of SG making the EPL contribution negligible over this range. Thus, it seems that while the interactions between polymer chains which are controlled by the electrostatic field are highly sensitive to C_s from zero added salt up to 0.1 M, as they should be, the dimensions are insensitive.

3.3 CONTRACTION OF SG WITH SODIUM HYDROXYDE (NaOH). — In a preliminary effort to understand the high inherent rigidity of SG, the behavior of $\langle S^2 \rangle^{1/2}$ versus [NaOH] was measured. The solution was originally at $C_s = 0.1$ M NaCl before serial dilution with NaOH. This is given in figure 3, where it is seen that the SG undergoes a contraction of size as [NaOH] increases. The weight-average apparent persistence length L_T' decreases from about 318 to 100 Å as NaOH increases. This suggests that hydrogen bonds, which might stabilize helical segments of SG at lower pH, are being broken as the —OH groups on the saccharide rings are deprotonated to —O⁻ at high pH, leading to a loss of rigidity. The shape of the curve in figure 3 is remarkably similar to a titration curve, further strengthening the notion that it is the pH controlled deprotonation of —OH groups which control the inherent SG stiffness. All the same time (for [NaOH] > 0.1 M or pH > 13) the deprotonation will progressively increase the charge parameter without any effective role on the electrostatic expansion which is due to the high ionic concentration of the solution. Further evidence of this contraction comes from the behavior of A_2 versus NaOH, also shown in figure 3. Since A_2 essentially measures polymer volume per unit mass at high C_s , A_2 should decrease concomitantly with $\langle S^2 \rangle^{1/2}$, as is the

Fig. 2. — a) Slope of $kC_p/I(q)$ versus q^2 for different C_s , for $C_p = 0.1$ mg/ml. The negative slope at very low C_s is due to the range of q measured being on the increasing side of the I vs. q peak. The peak itself is at too high a q value to be measured with visible light. b) $\langle S^2 \rangle$ versus $(C_s)^{-1/2}$, calculated from the positive slopes in figure 2a. There is almost no dependence of the radius of gyration on C_s once the slope of $kC_p/I(q)$ becomes positive. The solid line shows the calculation of $\langle S^2 \rangle$ versus $C_s^{-1/2}$ according to combined EPL and EEV theories [24-27, 46]. c) Variation of A_2 as function of C_s , as obtained from the intercepts of kC_p/I versus q^2 . The solid line is the result of combined EPL and EEV theories with no adjustable parameters [24-27, 46].

Fig. 3. — Variation of $\langle S^2 \rangle^{1/2}$ and A_2 versus [NaOH]. The solution was originally at $C_s = 0.1$ M NaCl. It shows large contraction of the molecule with NaOH.

case in figure 3. These results are consistent with recent results for hyaluronate [49], which showed a similar loss of stiffness versus [NaOH] in a titration curve fashion. From $\langle S^2 \rangle^{1/2}$ and A_2 in figure 3, the pKa of the —OH group can be estimated to be roughly 13.7. The contraction of SG is not due to degradation. Solutions of SG at 1 M NaOH showed no degradation after 15 h.

3.4 STATIC AND DYNAMIC LIGHT SCATTERING.

3.4.1 Salt-free solutions. — In the absence of added salt, solutions of SG at extremely low concentrations, filtered through $0.1 \mu\text{m}$ cellulose nitrate (CN) filters manifested clear static angular scattering peaks. Broad but well defined peaks appear at certain q_m whose values depend on the polyelectrolyte concentration. Figure 4 shows typical examples of these peaks for several concentrations ranging from $0.65 \times 10^{-5} \text{ g/cm}^3$ to $4.53 \times 10^{-5} \text{ g/cm}^3$ where I/kC_p is plotted against q .

There is perhaps a hint of a second maximum, not very well defined, at the lowest investigated concentration $C_p = 0.65 \times 10^{-5} \text{ g/cm}^3$. Since this possible low peak falls within experimental error we do not pursue any further analysis.

In this range of concentration, the scattering vectors of the peak maxima scales as $C_p^{0.456 \pm 0.02}$ as displayed in figure 5 (curve 1). This exponent is consistent with the notion of correlation hole effect [2-5], and liquid-like correlations of cylindrical scatterers [27, 38]. Although the SG does not appear to be in the rod limit under these conditions it is perhaps locally stiff enough, and on the whole anisotropic enough that inter-molecular interactions resemble rod-rod encounters in the region of contact. Our above estimates lead us to believe that the range of concentrations for which peaks were observed may still be close to the semi-dilute regime, despite the extremely low concentrations. The fact that fits of q_m versus C_p always gave exponents slightly less than 0.5 may indicate, however, we are near the dilute to semi-dilute transition regime. A clear elbow of q_m versus C_p with a slope passing from 1/2

Fig. 4. — $I(q)/kC_p$ versus q for salt-free solutions of SG at various polyelectrolyte concentrations C_p : (○) 0.65×10^{-5} g/cm³; (●) 1.01×10^{-5} g/cm³; (▲) 1.59×10^{-5} g/cm³; (×) 2.38×10^{-5} g/cm³; (+) 3.03×10^{-5} g/cm³; (△) 3.84×10^{-5} g/cm³; (■) 4.53×10^{-5} g/cm³.

Fig. 5. — Variation of $\text{Log } [q_m]$ as a function of $\text{Log } [C_p]$ (curve 1). A 10 % concentration loss due to filtration is assumed. Curve 2 corresponds to rod limit, hexagonal 2D packing $q_m = 2 \pi [3(d/m) C_p(\text{g/cm}^3) N_A/4]^{1/2}$, and curve 3 corresponds to a cubic arrangement of scatterers in the dilute regime, $q_m = 2 \pi [C_p(\text{g/cm}^3) N_A/M]^{1/3}$.

in the semi-dilute regime to $1/3$ in the dilute regime has been demonstrated in several works for hard cylinders [38], DNA [50] and the semi-flexible polyelectrolyte sodium polystyrene sulfonate (NAPSS) [39].

Assuming a hexagonal 2D arrangement of infinitely long cylinders (although the SG is not expected to be in the rod limit) leads to a prediction of $q_m = 2 \pi [3(d/m) C_p (\text{g/cm}^3) N_A/4]^{1/2}$ where (d/m) is the distance/molar mass (cm.mol/g) of the polymer, taken as $(1.45 \times 10^{-10} \text{ cm.mol/g})$ in this case. This corresponds to curve (2) in figure 5. It is stressed that this simple picture is a means of estimating average distances between idealized rods in the semi-dilute regime and in no way is meant to imply that liquid crystals or other types of static, organized domains exist. Assuming a cubic arrangement of scatterers in the dilute regime gives $q_m = 2 \pi [C_p (\text{g/cm}^3) N_A/M]^{1/3}$, where M is the molecular weight of the polyelectrolyte. This function for the SG with $M = 7.74 \times 10^5$ is also shown in figure 5. Taking account of possible concentration losses on the order of ten percent yields curve (3) in figure 5 for the cylindrical packing estimate. In this respect, the effect of the molecular weight on the slope is in progress in our laboratory.

At this level one notes that the negative values of the slopes reported in figure 2a are due to C_p being high enough and the $I(q)$ peak occurs above the highest accessible C_p ($q_m = 4.47 \times 10^5 \text{ cm}^{-1}$) and the monotonically increasing $I(q)$ gives a negative slope for $kC_p/I(q)$.

The shape and values of $I(q)/kC_p$ versus q were insensitive to time and only slightly sensitive to the temperature (over the range 12°C to 50°C). This behavior is illustrated in figure 6. It seems that there is a slight broadening and a decrease in peak height with increasing temperature. This is consistent with a slight increase of thermal motion between scatterers. One notes that earlier reports have indicated that scattering peaks associated with latex spheres evolved and sharpened over a time scale of many hours or even days to crystallize into lattices with long range order [51]. The dashed curve in figure 6 shows the scattering peak remained

Fig. 6. — Variation of $I(q)/kC_p$ as a function of q for $C_p = 3 \times 10^{-5} \text{ g/cm}^3$ SG with no added salt. The symbols ($\square$, $\circ$, $\circ$) show I/kC_p at $t = 50^\circ\text{C}$, $t = 25^\circ\text{C}$, $t = 12^\circ\text{C}$, respectively and ($\bullet$) shows the same solution after being left for 3 days at room temperature. No peak narrowing or other changes in the scattering suggest the liquid-like correlated state is an equilibrium state.

unchanged after 4 days of remaining undisturbed at $t = 25^\circ\text{C}$. Thus, there is no evidence that the SG solutions « crystallize » into systems with long range order over the time and temperature scales studied here.

QELS experiments were also performed on salt-free SG solutions. The autocorrelation functions for solutions filtered through $0.1\ \mu\text{m}$ (CN) filters were described by a single exponential decay (see the discussion in the section below, « the problem of aggregates »).

Figure 7 shows the angular variation of the reciprocal diffusion coefficient $1/D(q)$ deduced from the standard second order cumulant analysis of the autocorrelation functions.

$$\ln |S(q, t)| = - \langle \Gamma \rangle \tau + \frac{\mu_2}{2!} \tau^2 - \frac{\mu_3}{3!} \tau^3 + \dots$$

where μ_i are the moments about the mean of the distribution $A(z)$.

Fig. 7. — Angular variation of the reciprocal diffusion coefficient D^{-1} computed from the standard second order cumulant analysis of the autocorrelation functions and $I(q)/kC_p$ at $C_p = 3.01 \times 10^{-5}\ \text{g/cm}^3$, filtered through $0.1\ \mu\text{m}$ CN.

The solution was salt-free SG at $C_p = 3.01 \times 10^{-5}\ \text{g/cm}^3$, filtered through $0.1\ \mu\text{m}$ CN. Below $\theta = 50^\circ$, the autocorrelation functions were too noisy due to the weak scattering signal and occasional dust particles which scatter more strongly at low angles. On the same figure is also reported $I(q)/kC_p$ for the same concentration. Both $I(q)$ and $1/D(q)$ have qualitatively the same shape and approximately the maximum at the same q -position. This result shows that the mobility $M(q)$ is independent of q according to the general Ferrel-Kawasaki expression for the apparent diffusion coefficient $D \approx M(q)/S(q)$, as has often been observed [22, 27, 52]. Depending on the range of concentration (dilute or semi-dilute) the difference between the detailed shapes of the peaks has been attributed to hydrodynamic effects [53].

3.4.2 Effect of added salt. — Addition of a simple electrolyte (NaCl) to the solutions screens the electrostatic interactions and reduces the osmotic pressure. Figure 8 indicates the behavior of the peaks with C_s at fixed polyelectrolyte concentration $C_p = 3 \times 10^{-5}\ \text{g/cm}^3$. The peak height decreases with C_s , and its position seems to remain constant. One observes that the

Fig. 8. — Variation of $I(q)/kC_p$ as a function of q for $C_p = 3.0 \times 10^{-5} \text{ g/cm}^3$ SG at different added salt concentrations. (O) $C_s = 0$; (●) $C_s = 3.0 \times 10^{-5} \text{ M}$; (▲) $C_s = 4.0 \times 10^{-5} \text{ M}$; (x) $C_s = 8.0 \times 10^{-5} \text{ M}$; (Δ) $C_s = 12 \times 10^{-5} \text{ M}$; (+) $C_s = 40 \times 10^{-5} \text{ M}$.

region $q < q_m$ is very sensitive to the addition of salt and that of $q > q_m$ preserves a level of scattering close to the value in the absence of salt as expected by most theoretical models for electrostatic interaction in the semi-dilute range of concentration. However, one notes that as C_s increases this behavior is progressively lost and the reason is that the addition of salt shifts the system from semi-dilute to dilute according to the change of the molecule conformation.

When enough salt is added the scattering behavior is closer to that of a neutral polymer. For example this neutral type behavior is reached for $C_p = 3.0 \times 10^{-5} \text{ g/cm}^3$ at $C_s = 4 \times 10^{-4} \text{ mol/l}$ (see Fig. 8) and corresponds to the slopes in figure 2a above $C_s = 4 \text{ mM}$ for $C_p = 10^{-4} \text{ g/cm}^3$. The same behavior has been observed for proteoglycan monomer [27].

The effect of added salt concentration C_s on the dynamic behavior of the SG sample at $C_p = 3 \times 10^{-5} \text{ g/cm}^3$ is represented in figure 9. The equivalent apparent hydrodynamic radius computed by the Stokes-Einstein equation from the second cumulant analysis is plotted as a function of C_s , where C_s is the sum of roughly 0.026 mM contribution of the SG counterion and the added salt NaCl. No added salt corresponds to $C_s = 0.026 \text{ mM}$ in the figure. Two angles were chosen; $\theta = 90^\circ$, which is the scattering peak, and 150° which is far from the peak. For convenience the D^{-1} value at the peak is converted to an hypothetical value of R_h and is seen to fall from 450 Å at no added salt, to about 350 Å at 0.014 mM of added salt, and then to quickly fall to a constant value of around 200 Å, which converges with the value at $\theta = 150^\circ$. The value at $\theta = 150^\circ$ is fairly constant falling from about 235 Å at no added salt to about 200 Å at high C_s . Since $D^{-1}(q)$ basically follows $I(q)$, the quick fall of R_h with C_s is consistent with the loss of the static peak as C_s increases in figure 8.

R_h far away from the peak may be close to a true measure of the equivalent hydrodynamic radius, which scarcely varies with C_s . The magnitudes of R_h are not very close to what would be expected for a wormlike chain close to the coil limit: with an $R_g = \langle S^2 \rangle^{1/2}$ of about 1 100 Å

Fig. 9. — Variation of R_h as a function of C_s for $C_p = 3 \times 10^{-5}$ g/cm³ SG at two scattering angles 90° (the peak of R_h) and 150°.

an ideal coil would have an R_h of about 730 Å. The fact that $R_h = 210$ Å is lower than this value is consistent with similar hydrodynamic behavior for many linear polyelectrolytes [24-26], where the ratio of R_h/R_g is often observed to be much smaller than the ratio of 0.67 predicted for the non-draining coil limit. In this case $(R_h/R_g) = 0.2$ which is close to the value for another semi-flexible polymer: xanthan $((R_h/R_g) = 0.35)$ [54]. This difference is suspected to be due to the rigidity of the chain which is probably at the origin of partial draining effect. In the case of the SG it is seen that R_h/R_g remains constant for all measured values of C_s . It should be pointed out that in the case of other linear polyelectrolytes such as hyaluronic acid [24], and variably ionized polyacrylate (polyacrylic acid) [25] there were large, measurable changes in R_g with C_s , but none in R_h , again perhaps attributable to some type of draining effect.

At any rate it is clear that the $R_h = 450$ Å at the peak and at zero added salt does not correspond to any structure of unusual dimensions, such as temporal aggregates, domains, etc. [55]. In fact, the magnitude and behavior of R_h with C_s does not resemble the « slow mode » of diffusion often reported for polyelectrolytes at low C_s [29, 31-33, 55]. Only incomplete elimination of aggregates led to a « slow mode » in SG solutions, as is now discussed.

3.5 THE PROBLEM OF AGGREGATES. — The succinoglycan contained a relatively large amount of aggregates, probably already existing or nascent in the dry material, perhaps due to the method in which it was prepared. When filtering SG solutions through 0.2 μm cellulose nitrate membranes the aggregate population dominated the scattering; there was high background scattering from the solution and the peak was far less pronounced. Only upon filtering through a 0.1 μm cellulose nitrate filter was a clean solution obtained. Surprisingly, when the same stock solutions were filtered through 0.1 μm PVDF filters from Millipore, there was still a large dominant aggregate population, which gave even less peak definition than when the 0.2 μm cellulose nitrate filter was used. The I/kC_p data from the different filtrations are shown in figure 10. This result shows that extreme care must be taken both in choosing membrane pore diameters and the type of material.

Fig. 10. — Variation of I/kC_p as function of q for SG filtered through different types of membranes ($C_p = 3 \times 10^{-5} \text{ g/cm}^3$). Top to bottom ; 0.1 μm PVDF, 0.2 μm cellulose nitrate and 0.1 μm cellulose nitrate.

As to the fact that cellulose nitrate cleans up the systems better than PVDF of the same « pore size », we are not sure exactly why. We now mention, however, that the manufactures quoted « pore size » for most polymeric membranes does not refer to clean, cylindrical round holes in the membranes, actually refers to a sort of average porosity, the « holes » being torturous, complex paths in the membrane. (Exceptions to this are the nucleopore series of membranes, for which the pores are actually close to cylindrical, but which we did not use). Added to this are possible effects of chemical specificity and retention in the membranes.

The possibility that there is only « one characteristic length » in « such complex systems » is understandable in the context of cleaning up the solutions with a particular membrane type and nominal pore size : basically the system is not so complex. In terms of filtrable, large molecules, there are the well-dispersed polymers, which constitute the vast weight percentage of the solute and lead to the interesting physical properties, and a small population of aggregates of entanglements which lead to a « slow » diffusive mode and reduced scattering peak definition, whose average dimensions are larger than the well-dispersed polymers. Using the proper membrane preferentially filters out a large percentage of these large aggregates, allowing the remaining well-dispersed polyelectrolytes to dominate the solution scattering.

Figure 11a shows autocorrelation functions for SG solutions at $C_p = 3 \times 10^{-5} \text{ g/cm}^3$ and $\theta = 70^\circ$, filtered through 0.1 μm cellulose nitrate (curve a) and 0.1 μm PVDF filters (curve b), with monoexponential fits to each shown by the solid lines. As the scattered intensity by polymer changes with the filtration (see Fig. 10) the parameter α (in Eq. (1)) changes. The CN autocorrelation function (curve a), corresponding to the best defined I/kC_p peak in figure 10 (symbol O) is well fit by a single exponential (solid line), whose equivalent $R_h = 320 \text{ \AA}$. The

Fig. 11. — a) Typical autocorrelation functions for SG solutions, filtered through 0.1 μm cellulose nitrate (curve a) and 0.1 μm polyvinylidene fluoride filters (curve b) at $C_p = 3.0 \times 10^{-5} \text{ g/cm}^3$ and $\theta = 70^\circ$. The solid lines represent monoexponential functions. b) CONTIN and ILT analysis of the autocorrelation function for SG at $C_p = 3 \times 10^{-5} \text{ g/cm}^3$ and $\theta = 70^\circ$ filtered through a 0.1 μm cellulose nitrate membrane, the filtration method which gives the highest peak contrast and definition. There is a single dominant peak around $R_h \approx 300 \text{ \AA}$. c) CONTIN and ILT analysis of the autocorrelation function for the same concentration ($C_p = 3 \times 10^{-5} \text{ g/cm}^3$ and $\theta = 70^\circ$) SG filtered through a 0.1 μm polyvinylidene fluoride membrane. In addition to the peak near $R_h \approx 300 \text{ \AA}$ there is a « slow mode » with $R_h \approx 1000 \text{ \AA}$, due to the aggregate population. The dashed lines are from figure 11b, to illustrate that the single mode population for the 0.1 μm CN filtered solution corresponds to the faster mode of the 0.1 μm PVDF filtered solution.

Fig. 11 (continued).

function for the 0.1 μm PVDF filtered solution corresponding to top curve (●) in figure 10, is not well fit by the single exponential as is readily seen. The same behavior for the other autocorrelation functions and their fits was observed for the angles ranging from 50° to 150° . One notes that the extremely low values of the intercepts of autocorrelation curves is due to the fact that $I_{\text{poly}}/I_{\text{solv}}$ is extremely low. For the range of concentration studied in this work, the value of the ratio $I_{\text{poly}}/I_{\text{solv}}$ was about 1.6.

As shown in figures 11b and 11c these results are confirmed by CONTIN and ILT analyses of the same autocorrelation functions. The autocorrelation function for a solution filtered with 0.1 μm CN filter, which corresponds to the cleanest solutions with the highest contrast and definition peaks, shows a single main CONTIN/ILT peak at $R_h = 300 \text{ \AA}$ (Fig. 11b). There is no « slow mode », indicating that virtually all the aggregates have been successfully removed by the filtration. The CONTIN/ILT analysis in figure 11c shows that the solution is full of aggregates, as reflected by the « slow mode » in the distribution function $A(\tau)$, corresponding to the dominant peak with R_h around 1 000 $\text{\AA}$. The smaller peak is from the non-aggregated SG and has an R_h around 200-300 $\text{\AA}$, which is close to the value of the single peak in figure 11b. It should be noted that multimodal decay functions have commonly been observed for salt-free solutions even at such low concentrations as reported here [Refs. 22, 31 and 33].

Thus, the static peaks are most strong and well-defined when there is no slow mode of diffusion. As seen in figure 10, the existence of the slow mode actually decreases the amplitude and contrast of the static scattering peaks ; the static scattering peaks and the slow mode are antagonistic to each other.

3.6 VISCOSITY MEASUREMENTS. — Reduced viscosity η_r versus C_p taken on the low shear rate Newtonian plateau for SG in pure water is shown in figure 12.

η_r increases monotonically over the studied C_p range for the two curves. Thus, over the regime where the pronounced static and dynamic scattering peaks occur, the reduced viscosity behavior is similar to that of most neutral polymers. Conspicuously absent is any trend towards increasing η_r with decreasing C_p , as is found for dilution with pure water (or low

Fig. 12. — Variation of the reduced viscosity η_r as function of C_p for SG in pure water. It increases monotonically with C_p . The symbol (O) is for the fractionated sonicated sample filtered through $0.1 \mu\text{m}$ CN. The (●) curve is for the same sample filtered through $0.2 \mu\text{m}$ CN.

C_p solutions) for such semi-flexible polyelectrolytes as polystyrene sulfonate [35], hyaluronic acid [56], gellan, polyvinyl sulphate [57] and charged inextensible latex spheres [58]. Such an effect is traced to the fact that ionic concentration decreases as dilution proceeds. This decrease of ionic strength leads to effects such as: polyelectrolyte expansion, increase in the electrostatic screening length, increase in polyelectrolyte-polyelectrolyte friction, etc. There is no general agreement currently on the exact mechanism of the effects in different situations, although abundant models exist.

Absence of the polyelectrolyte effect — i.e. monotonic increase of η_r with C_p — has been reported long time ago for thymonucleic acid TNA [59].

We do not speculate here on whether the monotonic decrease of η_r with decreasing C_p is due to the more rigid nature of SG compared to these other polyelectrolytes, or possibly to such an effect lying at concentrations lower than the sensitivity of the viscosimeters permitted to measure. In the absence of external salt, the investigated domain of concentration remains in the semi-dilute. The polyelectrolyte effect is usually observed on flexible molecules, short rigid molecules [37] or charged spheres [18]. Moreover, for low charged spheres, it was shown [60] that the behavior mimics that of neutral one (monotonic increase of η_r with increasing C_p).

Succinoglycan has relatively a low charge density ($\lambda = 0.45$) as compared to other polysaccharides. However, the viscosity peak exists for a more flexible polyelectrolyte, hyaluronate ($\lambda = 0.7$) having a charge parameter in the same order as SG. To draw a definitive conclusion on this peculiar behavior, further experiments should be done on different semi-rigid polyelectrolytes having different molecular weights and different charge densities. What is clear is that the existence of the static and dynamic scattering peaks do not depend on their being any reduced viscosity peak *versus* C_p , nor even a simple increase in η_r with decreasing C_p in the concentration ranges where the scattering peaks are observed. Furthermore, the fact that the viscosity behavior of the SG when filtered through 0.1 and $0.2 \mu\text{m}$ CN filters is nearly the same illustrates the fact that the viscosity is closer to a number averaging technique, and is

not nearly as sensitive to aggregates as light scattering, which combines weight and z -averaging of the particle populations, and is hence extremely sensitive to even small populations of aggregates and other large particles.

4. Conclusion.

In this paper we have investigated the structure and dynamic properties of succinoglycan solutions at very low concentrations and ionic strength. Static and dynamic light scattering measurements show the existence of single, pronounced peaks, which are rapidly lost with the addition of salt. While clearly electrostatic in origin, there may yet be some debate about the exact origin of the peaks. The simplest explanation consistent with all the observations is that the electrostatic repulsions at very low C_s produce enhanced effective volumes for SG which lead to liquid like correlations. This mechanism has been discussed previously [27, 38]. Liquid-like correlations generally imply strong neighbor-neighbor correlations, with very quickly damped second and more distant neighbor interactions.

It has been demonstrated that the static and dynamic light scattering peaks are unrelated to any slow mode of diffusion (also previously demonstrated for PG [27]), and that in fact such a slow mode, which corresponds to incompletely eliminated aggregates, actually decreases the contrast and definition of the peak when present. Fortunately, these aggregates can be virtually totally eliminated by proper filtration (0.1 μm cellulose nitrate membrane diameter in this case). Such a « slow mode », commonly observed in many polyelectrolyte solutions at low added salt concentration C_s , has recently been traced to the presence of aggregates and/or other particles for a variety of polyelectrolyte solutions studied [28].

It has been further demonstrated that the scattering peaks in this case are not related to any peaked behavior of the reduced viscosity η_r as a function of C_p , nor to any Fuoss-type increase of η_r with decreasing C_p . The lack of such an effect is perhaps related in this case to the stiffness and to the low charge parameter of this polysaccharide. The effects of charge parameter λ , preliminary results are presented elsewhere [61].

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