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Light Scattering and Small-Angle Neutron Scattering from Polyelectrolyte Solutions: The Succinoglycan

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ABSTRACT: The static properties of succinoglycan salt-free solutions are discussed in the semidilute range of concentration as revealed by light scattering and small-angle neutron scattering (SANS). As expected the results show the existence of a broad scattering intensity peak in the investigated range of polyelectrolyte concentration C_p and momentum transfer q in both light scattering and SANS. The positions of these peaks scale roughly as $C_p^{1/2}$ when using both techniques in different ranges of C_p. Contrary to flexible polyelectrolytes such as polystyrene sulfonate (NaPSS), the values of the peak positions obtained using light scattering and those obtained from SANS do not follow a master curve, although they have a $C_p^{1/2}$ behavior. This new and unexpected result could be explained on the basis of the stereoregularity and the semirigid nature of the succinoglycan polysaccharide which favors a change in linear mass density as the concentration increases.

I. Introduction

The presence of charges along a polyelectrolyte chain leads to different scattering properties with respect to neutral polymers.[1−11] The different interactions involved in the medium, inter- and intramolecular, unless they are screened out for example by adding external salt, are at the origin of certain peculiar behaviors observed in polyelectrolyte systems. For instance, a maximum in the scattered intensity is observed as function of the wave vector, and the reduced viscosity as a function of polyelectrolyte concentration also exhibits a peak. Various experimental techniques have been used to understand the behavior of such charged complex systems including:[12−24] small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), elastic and quasi-elastic light scattering, and viscosity measurements.

In this paper we shall present and discuss our experimental results obtained using light scattering and SANS in salt-free solutions where mainly electrostatic interactions govern the static properties of the system.

These experimental results concern a semirigid polysaccharide, the succinoglycan in salt-free solutions. New and unexpected information on the structure of the succinoglycan chain in solution as a function of polymer concentration are presented, and a possible explanation is given which is based on lattice model of aligned particles where the mass per unit length M_L is considered to be the important parameter.

II. Experimental Section

II.1. Materials, Methods, and Sample Preparation. Succinoglycan (SG) is an exocellular polysaccharide produced by the bacteria *Pseudomonas* sp. NCIB 11592. Its monomeric unit is composed of D-glucose/D-galactose/pyruvate/succinate in a molar ratio of 7:1:1:1; in some cases the content of pyruvate and succinate can be modified under specific chemical manipulation.[25,26] This sample was obtained by alcohol precipitation as has been already described[25,26] and the starting material was a bacterial broth supplied by Shell Research Limited (Sittingbourne Research Center, Sittingbourne, Kent England).

For light scattering experiments, pure water was doubly distilled, deionized, and filtered in a Millipore Alpha-Q; its conductivity was less than 0.05 μS corresponding to an equivalent NaCl concentration of nearly 3.3×10^{-7} M. Heavy water was used in SANS experiments. SG solutions were dissolved and allowed to equilibrate for a few days prior to the measurements.

The characteristics of the studied SG in its disordered conformation corresponds to one charge every 16.8 Å, which gives a linear charge parameter of $\lambda = 0.42$. These values were obtained from the ^{1}H NMR, spectrum in D_2O in the presence of an internal standard which gave 0.98 pyruvate and 0.24 succinate groups/monomeric unit. As it has been reported in our earlier work[21] on succinoglycan, in excess of salt, a molecular weight $M_w = 7.74 \times 10^5$, a radius of gyration R_g = 1100 Å and a second virial coefficient $A_2 = 2 \times 10^{-3}$ mol cm^3/g^2 were determined. Assuming wormlike chain behavior, $\langle R_g^2 \rangle \approx LL_p/3$ where L is the contour length of the macromolecule, the persistence length was estimated to be L_p = 300 Å. This value shows that SG is a rather semirigid polymer, such as xanthan for example, and more rigid than polystyrene (L_p = 15 Å). It is also important to recall that under certain conditions of ionic strength and temperature, the SG adopts an ordered single-helix chain conformation.[25,26]

II.2. Equipment and Data Analysis. (i) Static Light Scattering. The elastic light scattering measurements were performed using ALV (Langen-FRG) apparatus with an automatic goniometer table, a digital rate meter, and a temperature-controlled sample cell at 25 ± 0.1 °C. The experiments were performed with a wide aperture for the phototube (300 μm pinhole) in view of the low scattering level in the investigated range of polyelectrolyte concentration.

The scattered light of a vertically polarized λ_o = 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 $k = 4\pi^2 n_o^2 (\mathrm{d}n/\mathrm{d}c)^2 (I_o^{90°}/R^{90°})/\lambda_o^4 N_A$, was measured in steps of 5 deg of the scattering angle, where n_o is the refractive index of standard (toluene), $I_o^{90°}$ and $R^{90°}$ are respectively the intensity and the Rayleigh ratio of the standard at θ = 90°, (dn/dc) the increment of refractive index, C_p the polyelectrolyte concentration, expressed in g/cm^3, 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.

To eliminate dust and other larger particles, all samples were filtered through 0.1 μm cellulose nitrate filter (Sartorius, W-3400, Germany). Concentration losses upon filtering were estimated by different techniques (UV absorption and conduc-

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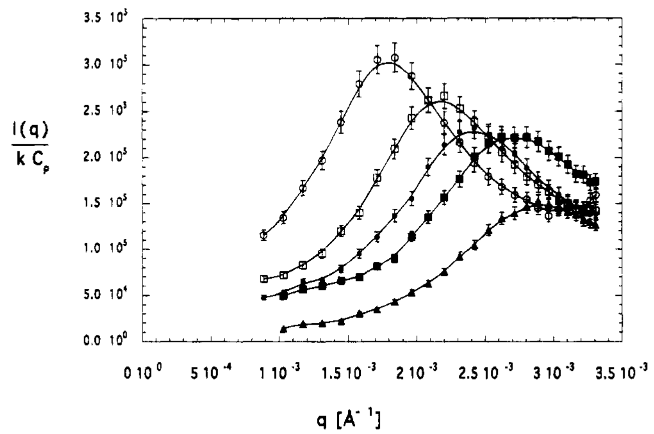

Figure 1. Effect of concentration, light scattering data. $I(q)/kC_P$ versus q for salt-free solutions of SG at various polyelectrolyte concentrations C_P: (▲) 1.59 × 10^{-5} g/cm^3; (■) 2.38 × 10^{-5} g/cm^3; (●) 3.03 × 10^{-5} g/cm^3; (□) 3.84 × 10^{-5} g/cm^3; (O) 4.53 × 10^{-5} g/cm^3. The solid lines are guides to the eyes.

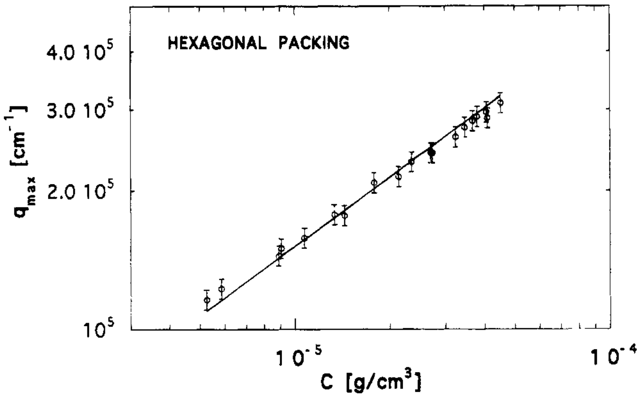

Figure 2. Variation of log[q_m] as a function of log[C_P]. The solid line corresponds to rod limit, hexagonal packing.

tivity) and found to be roughly on the order of 10%. Other details regarding the characterization of the polymer and the method of sample preparation are presented in ref 21.

(ii) SANS Experiments. The SANS experiments were carried out on PACE spectrometer using the circular ring detector at LLB (Saclay), France. The scattering wave vector $q = (4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle and λ is the wavelength of the neutrons, ranges from 0.02 to 0.14 Å^{-1}. All the data were treated according to standard Saclay procedures (correction of transmission and sample thickness) for small angle isotropic scattering.

III. Results and Discussion

III.1. Light Scattering. In the absence of added salt, solutions of SG at extremely low concentrations, but still in semidilute regime, manifested clear angular scattering peaks. Broad but well-defined peaks appear at certain q_m whose values depend on the polyelectrolyte concentration. Figure 1 shows typical examples of these peaks for several concentrations ranging from 1.59 × 10^{-5} to 4.53 × 10^{-5} g/cm^3 where $I(q)/kC_p$ is plotted against the wave vector q.

In this range of concentration, the scattering vectors of the peak maxima scales as $C_P^{0.46\pm0.02}$ as displayed in Figure 2. This exponent, very close to $^1/_2$, is consistent with the notion of correlation hole effect and liquidlike correlations of cylindrical scatterers.[1-12] The solid line in Figure 2 corresponds to the calculated q values when assuming a hexagonal packing of locally stretched rodlike particle (although the SG is not expected to be in the rod limit). Under this geometrical assumption the corresponding wave vector q_m is equal to $2\pi[C_P(\mathrm{g/cm^3})N_A \sin 60°/M_L]^{1/2}$, where M_L is the mass per unit length and N_A Avogadro's number. One notes that this simple picture does not necessarily imply that a sort of organized domains exist such as liquid crystals or other sophisticated organization.

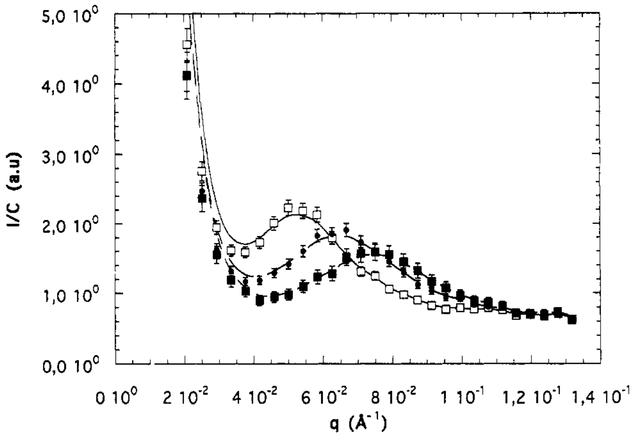

Figure 3. Effect of concentration, SANS data. $I(q)/C_P$ versus q for salt-free solutions of SG at various polyelectrolyte concentrations C_P: (□) 1.95 × 10^{-2} g/cm^3; (●) 2.97 × 10^{-2} g/cm^3; (■) 3.975 × 10^{-2} g/cm^3. The solid lines are guides to the eyes.

It is also important to recall that the theoretical slope 0.5 for a geometrical model of hexagonally packed rods assumes the rods are infinitely long. Finite length effects of the rods, for example, may lower the exponent below 0.5, as may other physical effects. Furthermore, the scaling of q_m vs C_P is $^1/_3$ for infinitely diluted particles and there is a transition region from $^1/_3$ to $^1/_2$ domain as the concentration increases for both flexible polyelectrolytes and hard cylinders.[23,24] Those two reasons may be at the origin of the slight difference between the exponents, i.e. 0.46 instead of 0.5.

III.2. Small-Angle Neutron Scattering. Four concentrations were prepared independently and ranged from 1 × 10^{-2} to 4 × 10^{-2} g/cm^3. The polymer was dissolved in D_2O and allowed to equilibrate for few days prior to the measurements. According to the high range of concentration and to the fact that the neutrons are not sensitive to dust, the solutions were neither filtered nor centrifuged.

As in the case of light scattering experiments, the scattering intensity curves $I(q)$ as a function of the wave vector q presents a very distinct and broad peak for all investigated polyelectrolyte concentrations. The position of the peak q_{max} is shifted toward higher q values with increasing concentration as is shown on Figure 3. The exact maximum position q_{max} with an uncertainty of $\pm10^{-4}$ Å^{-1} has been obtained using a Lorentzian function to fit the data. The scattering vectors of the peak maxima scales with the polyelectrolyte concentration as $C_P^{0.50\pm0.02}$ (see Figure 4). Although the expected scaling law is obtained in this range of concentration, in absolute value, however, the SANS results do not match with those of light scattering experiments (see the discussion on the next section).

III.3. Comparison between Light Scattering and SANS Results. Figure 4 represents the log–log variation of q_{max} as a function of polyelectrolyte concentration per mass per unit length C_P/M_L for light scattering and SANS data. As is seen both results show a $C_P^{1/2}$ behavior but in absolute values they are different. On the other hand as reported by Nierlich et al.[14] and Drifford and Dalbiez[16] when investigating salt-free

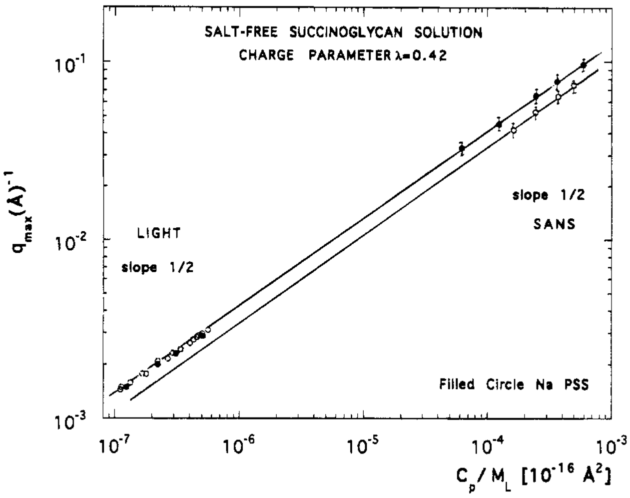

Figure 4. Variation of log[q_m] as a function of log[C_P/M_L] for all studied concentrations using both techniques, light scattering and SANS. The two solid lines correspond to hexagonal packing for single chain (light scattering data, low concentrations) and locally double linear mass density, (SANS data, high concentrations). For comparison the NaPSS result (●) are also reported (refs 14 and 16).

solution of NaPSS, a good agreement was found between light and neutron scattering data with regard to the C_P dependence of q_{max}. (See Figure 4; the filled circles represent the data for NaPSS from refs 14 and 16.) Our experimental results show a rather different and unexpected result. According to light scattering data where the calculated q_{max} values seem to favor hexagonal packing, the solid lines which best fit the light scattering and SANS data for SG seems to be reached for $(M_L)_{SANS} = 2(M_L)_{light}$ with $(M_L)_{light} = 69.6$ Å^{-1}. In the case of NaPSS, $(M_L)_{light} = (M_L)_{SANS} = 82.4$ Å^{-1}.

On the basis of this comparison, one finds that at relatively low concentration, but still in semidilute system, the succinoglycan has single-chain behavior. As the concentration is increased, the chains are organized in double chains or segments at least at a local level. This structure was never observed nor described before and we believe that the chemical structure of this stereoregular SG polysaccharide[27] and the semirigid nature of the chain favor such an organization. In contrast to the NaPSS case, this kind of organization does not exist because it is a very flexible polymer (the intrinsic persistence length is about 15 Å).

It is very interesting that a unique dependence of q_{max} as a function of C_P/M_L is confirmed for NaPSS and SG in the low concentration range.[21,28] This implies that the chains are locally extended and exclude the blob chain model.[4] This model would apply for very weakly charged polyelectrolytes. Such investigations have been performed on SG having different charge parameters[29] and the results in the framework of the blob model will be presented in a forthcoming paper.[30]

In orther words, for the investigated range of concentration and when using both techniques of light scattering and SANS, the system presents all the semidilute polyelectrolyte features. Namely a scaling law $q_m \approx C_P^{1/2}$ and a rodlike hexagonal packing behavior of single chains at extremely low concentration $C_P < 5 \times 10^{-5}$ g/cm^3 and a double chain or segment, at least locally, for $C_P > 10^{-2}$ g/cm^3 or even below.

It would be interesting to follow this organization or this change in the structure as a function of the concentration if the gap, between light and neutrons, in the momentum transfer could be covered. This could be achieved by the use of synchrotron radiation. Such studies are under investigation.

IV. Conclusion

In this paper we have investigated the structure via the polyelectrolyte behavior of succinoglycan solutions in the semidilute range of concentrations in salt-free solution using light scattering and SANS. The results show the existence of a single, pronounced peak, in the scattered intensity when using both techniques. The simplest explanation consistent with all the observations is interpreted in terms of strong repulsive interactions between nearest chains (low concentrations) or segments at the intermolecular level (high concentrations). A scaling law, $q_{max} \approx C_P^{1/2}$, previously predicted[1-4] and observed by many authors in other systems[14,16,21-24] is again confirmed in our observation and supports a hexagonal packing of the chains. The new result is the disagreement between light scattering and SANS data regarding the absolute values of the peak position q_{max} as a function of the polyelectrolyte concentration. This new and unexpected result may be explained on the basis of the chemical structure change of this stereoregular SG polysaccharide and the semirigid nature of the chain which seems to favor such organization and changes the linear mass density as the concentration increases.

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