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A Small-Angle Neutron Scattering Study of the Structure of Gelatin/Polyelectrolyte Complexes

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ABSTRACT: Mixtures of a fractionated gelatin with very low concentrations of anionic polyelectrolytes have been studied by small-angle neutron scattering. The scattering pattern observed from the system is reminiscent of that from pure polyelectrolyte solutions, with a maximum in the scattering, but at a much greater intensity. The position of the scattering peak is independent of the polyelectrolyte molecular weight, but moves to larger momentum transfer, Q, with increasing concentration. The scattering intensity at higher Q reflects the extent of complexation of the gelatin and polyanion. The addition of salt removes the peak from the scattering pattern and high salt concentrations weaken the complexes. Raising the pH also weakens the complexes. At high pH (≥ 10), the scattering indicates that there is no interaction between gelatin and the polyanions. The extent of complex formation for similar polyelectrolytes depends on the charge density and distribution in the chain.

Introduction

The complexation of oppositely charged polyelectrolytes has been studied extensively.[1−5] When the molar ratios of two such components approaches unity, precipitation occurs due to charge neutralization. Similarly, interactions between polyelectrolytes and globular proteins with opposite charges have also been observed, again due to electrostatic attraction.[1,6−12] Interactions between globular proteins of the same net charge as the polyelectrolyte have also been reported.[6−9] This latter association is thought to be a result of association between the polyelectrolyte and patches of charge of the opposite sign to the net charge on the protein.[6,7]

The interactions of polyelectrolytes and random-coil polyampholytes have been much less studied than those of oppositely charged polyelectrolytes. One such system concerns the complexes formed between gelatin and anionic polyelectrolytes.[13,14] An attractive interaction between the polyanion and the polyampholyte is seen at pH values both below and above the isoelectric pH (IEP) of the gelatin, i.e., when the gelatin is either net positively or net *negatively* charged. This system is considerably different from those described above because the gelatin chain is not a globular protein, but has a random coil configuration under the conditions studied. Also, both the positive and negative groups on gelatin are randomly distributed along the length of the chain and not gathered together in significant clusters, as is the case for some globular proteins.

Bowman et al.[13,14] have used light scattering (LS) on dilute solutions to study how two strong polyacids, sodium poly(styrenesulfonate) and sodium poly(2-acrylamido-2-methylpropanesulfonate), referred to as NaPSS and NaPAMPS respectively, bind to gelatin above its isoelectric pH.[13] They have found that on adding gelatin to NaPSS (at pH 5.65 and 0.01 M sodium acetate), a complex is formed, whose molecular weight increases with increasing gelatin concentration. Beyond a ratio of 200:1 (gelatin:polyanion) the complex is saturated. At this point the complex had a M_w about 20 times greater than the bare polyelectrolyte chain, but the radius of gyration, R_g, was only about 10% larger. The NaPAMPS shows similar effects, but bound slightly less gelatin. These values were further supported by size exclusion chromatography[14] (apparent molecular weight) and rheology[15] (hydrodynamic complex size).

It has been postulated that the attraction between the net negatively charged gelatin and the negative polyelectrolytes is caused by the polarization of the polyampholyte by the electric field in the vicinity of the polyelectrolyte.[13] Bowman et al.[13,14] found that NaPAMPS binds fewer molecules of gelatin than does NaPSS. The reason for this is a complex balance of the more hydrophobic nature of the NaPSS, hence its higher affinity for gelatin, and the overall chain topology.

In this paper, small-angle neutron scattering (SANS) data from the gelatin-NaPSS complex and the gelatin−NaPAMPS complex are presented. SANS is sensitive to structures in the range $\sim 2\pi/Q$, which for the Q range available in this study gives 30−600 Å. The momentum transfer is given by $Q = 4\pi/\lambda\ (\sin \theta/2)$, where λ is the wavelength of the neutron and θ is the scattering angle. These results complement the earlier LS experiments, which are sensitive to much larger structures. In effect, LS gives the overall size of the complex whereas SANS gives information on the internal structure.

Measurements were made at two polyelectrolyte molecular weights as a function of gelatin concentration, polyelectrolyte concentration, added monovalent salt concentration, and pH.

I. Scattering from Polyelectrolyte Solutions. Nierlich et al.[16] were the first to report small-angle neutron scattering from semidilute salt-free sodium poly(styrenesulfonate) solutions in D_2O. The scattering was investigated as a function of polyelectrolyte concentration, c_p, salt concentration, c_s, and molecular weight. In the absence of salt, a maximum in the scattering intensity (at a momentum transfer of Q^*) was observed, which was shown to be independent of NaPSS molecular weight and to vary as $c_p^{1/2}$. This is in agreement with the predictions of both scaling theory[17−19] and

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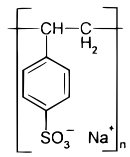
lattice models.[20] When the concentration of chains is greater than the critical chain overlap concentration, c_p*, the dependence of Q^* on the polymer concentration has been confirmed as $Q^* \sim c_p^{1/2}$ in several studies on other polyelectrolytes.[21−25] In addition, in the dilute regime, $c_p < c_p^*$, it has been shown that $Q^* \sim c_p^{1/3}$.[21,22,26]

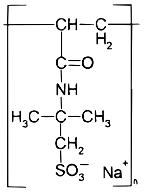
In their original study, Nierlich et al.[16] saw a monotonic decrease in the scattering intensity at $Q < Q^*$, when $c_s = 0$. Although some studies since then agree with this finding,[21,27−31] it is now more usual for a sharp upturn in the scattering at low Q to be observed.[22−24,32−35] This disparity is probably the result of a larger Q range being used in the more recent work. It has been suggested that this excess scattering at low Q is the result of microscopic phase separation into multichain, polymer-rich regions.[24]

Nierlich et al.[16] also studied the effect of increasing the salt concentration. It was found that as the ionic strength was increased, the scattering intensity at low Q increased, while remaining constant at $Q > Q^*$, thereby gradually "washing out" the maximum. However, other studies have shown that as well as Q^* remaining constant as salt is added,[23,35] in some cases Q^* moves to lower Q as the ionic strength increases.[28,30] Ermi and Amis have shown that both the peak in intensity and the upturn at low Q can be suppressed by the addition of salt.[24] These somewhat conflicting results are puzzling, but it is quite clear that electrostatic interactions play a key role in determining the scattering behavior. This was highlighted by the elegant experiment by Ermi and Amis who partially quaternized poly(2-vinylpyridine) (PVP) to obtain poly(*N*-methyl-2-vinylpyridinium chloride) (PMVP) and compared the scattering from the parent neutral PVP and the charged PVMP in 0.14 mol·dm^{-3} NaCl and found identical scattering curves.[24]

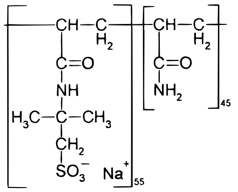
From a theoretical viewpoint, the peak in the scattering from polyelectrolyte solutions has been interpreted as either a correlation hole[36,37] or an ordered structure in the solution.[17,20,27,38] The characteristic size associated with the peak maximum is of order $2\pi/Q^*$. The idea of a correlation hole was first introduced by de Gennes.[39] In this model, each charged chain is surrounded by a tube from which other chains are excluded. Hayter et al.[36] and Koyama[37] have both suggested different, continuously increasing functions for the structure factor which, in conjunction with a form factor, which decreases as a function of Q (such as $P(Q) \sim Q^{-1}$ for a rigid rod), then gives a maximum in the $I(Q)$ plot. However, this model cannot account for the data of Nierlich et al.[29] and Ono et al.,[40] which both show that the structure factor itself displays a maximum. In 1988, Borue and Erukhimovich[41] (BE) calculated a structure factor for solutions of weakly charged polyelectrolytes with a hydrophobic backbone. Their model is based on the idea that, as the solvent quality for a weakly charged chain is decreased, the small number of charges on the backbone will prevent macroscopic phase separation since there is a large entropic penalty if counterions condense onto the polymer. It has been predicted that microscopic phase separation into polymer-rich and polymer-depleted regions may occur.[41] In their model, the peak in the scattering therefore reflects the average characteristic length scale of these polymer-rich microphases. The BE model has been used successfully to analyze SANS data on several systems above the low Q upturn.[24,30,42] This success is surprising, since some of the systems used were *strongly* charged and *hydrophilic* polymers.[24] The BE model has a major drawback in that it fails to account for the upturn in the scattering at low Q, and so in the cases where such scattering has been observed, these data have been arbitrarily removed before performing a fit to the remaining data.



sodium poly(styrene sulphonate) repeat unit



sodium poly(2-acrylamido-2-methylpropane sulphonate) repeat unit



random sodium poly(2-acrylamido-2-methylpropane sulphonate)-polyacrylamide copolymer: the number quoted is in monomers

Figure 1. Chemical structures of the polyelectrolytes.

Table 1. Molecular Weight and Polydispersity of the NaPSS Samples

NaPSS	M_p	M_w	M_w/M_n
82 kDa	82 800	77 400	<1.1
356 kDa	356 000	350 000	<1.1
800 kDa	800 000	650 000	<1.4

Experimental Section

All of the samples were prepared in D_2O (which has a relatively high scattering length density, ρ), to keep the proton content of the samples to a minimum, and to maximize the contrast between the gelatin ($\rho = 3.25 \times 10^{-6}$ Å^{-2} in D_2O) and the solvent ($\rho = 6.39 \times 10^{-6}$ Å^{-2}). All of the percentages quoted are weight-for-weight values. The gelatin was alkali-processed bovine ossein gelatin,[43] supplied at its IEP (4.9) and is polydisperse. The high molecular weight fractions were removed by fractional precipitation; as a result this sample contains a high fraction of α chains and is referred to as α-gelatin. The α-gelatin sample and its characterization data were supplied by T. H. Whitesides of the Eastman Kodak Co. and had a nominal weight-average molecular weight of 100 kDa.

Three different polyelectrolytes have been used: poly(styrenesulfonate) or NaPSS, poly(2-acrylamido-2-methylpropanesulfonate) or NaPAMPS and a random copolymer synthesized from 55 mol of poly(2-acrylamido-2-methylpropanesulfonate) to 45 mol of polyacrylamide. The chemical structure of each of the polyelectrolytes is shown in Figure 1.

The three NaPSS samples were supplied by Polymer Standards Service. A description of the analytical data is provided in Table 1. Each NaPSS sample is referred to by its peak molecular mass in kDa (≡10^3 g·mol^{-1}). The degree of sulfonation of the NaPSS was greater than 90% in all cases.

Two different NaPAMPS samples were used; these samples and the copolymer sample were supplied by Kodak European

Research and Development as solutions which were dried in an oven at 60 °C before being used. The absolute molecular weights of these samples are not known but the qualitative interpretations given later are (at most) only weakly dependent on the chain size. The two NaPAMPS samples are designated “high” and “low” in terms of molecular weight to differentiate them.

The samples were prepared by mixing equal volumes of a gelatin solution (in D_2O) and a polyelectrolyte solution (in D_2O), both prepared at twice the final desired concentration. Adding polyelectrolyte to the gelatin solutions causes an increase in the pH of the solution, but the pH was not adjusted. In the case of salt additions, the gelatin sample was prepared in a sodium acetate solution and then mixed with the polyelectrolyte solution.

The pH was altered by adding small quantities of concentrated hydrochloric acid or sodium hydroxide to the gelatin/polyelectrolyte mixture.

Most of the SANS measurements were performed on the LOQ instrument at the ISIS facility, Didcot, Oxford, U.K. Some additional measurements were performed on the NG-3 instrument at the National Institute of Standards and Technology, Gaithersburg, MD.

ISIS is a spallation source, and produces neutrons with wavelengths between 2.2 and 10.0 Å for small-angle scattering. The neutrons are detected by a 64 cm × 64 cm position sensitive detector 4.1 m from the sample and sorted in wavelength by time-of-flight measurement. Data reduction procedures correct for wavelength, detector efficiency, and sample transmission. To obtain absolute scattering cross sections, the scattering from a standard polystyrene copolymer and polystyrene blend were measured and fitted. This procedure provides a scaling factor, which is applied to all of the data sets. Scattering spectra were collected over a Q range from 0.011 to 0.249 Å^{-1}. The samples were measured in 2 mm Helma quartz cells for 30 min at 45.0 ± 0.1 °C. The incoherent background was estimated by averaging over the data between $Q = 0.20$ and 0.249 Å^{-1}. These values have then been subtracted from the whole data set to leave only the coherent scattering.

The NG-3 instrument was set up with a 12 mm beam aperture, 700 cm sample–detector distance, beam stop number 2, no detector offset, and two neutron guides. Neutrons with a wavelength of 5 Å were used to give a total Q range from 0.0082 to 0.081 Å^{-1}. Transmissions were measured using 8 attenuators. The samples were loaded into 2 mm quartz “banjo” cells and the scattering measured for 20 min at 25.0 ± 0.1 °C. (No difference was observed in the scattering pattern over the available Q range on changing the temperature from 25 to 45 °C.) The data were corrected for sample transmissions, empty cell scattering, and background noise and put on an absolute scale using a previously calibrated silica standard (A1).

The data from NG-3 are not plotted with the incoherent background subtracted because the data were only obtained up to a value of $Q = 0.08$ Å^{-1}. In this region of the spectrum, the intensity is still decaying due to the presence of significant amounts of coherent scattering.

Results and Discussion

The scattering from two different molecular weight NaPSS samples is shown in Figure 2 at two different concentrations. In each case the scattering from the NaPSS solutions is small. At 0.5% the scattering is extremely small and has no distinctive features other than a slight upturn at very low Q. However, this is poorly resolved because of the low scattering intensity. At 5% (=54.7 mg·mL^{-1}), the data show the distinctive maxima characteristic of the scattering from salt-free polyelectrolyte solutions. The value of Q at which the maximum occurs is defined as Q^*. All of the maxima lie in the range $0.0868 < Q^* < 0.0893$ Å^{-1} *independent* of the polyelectrolyte molecular weight. Again there is an upturn in the scattering at low Q, which has been observed before, and this has been assigned to either the presence of dilute aggregates or a representation of the correlation length of local inhomogeneities.[35]

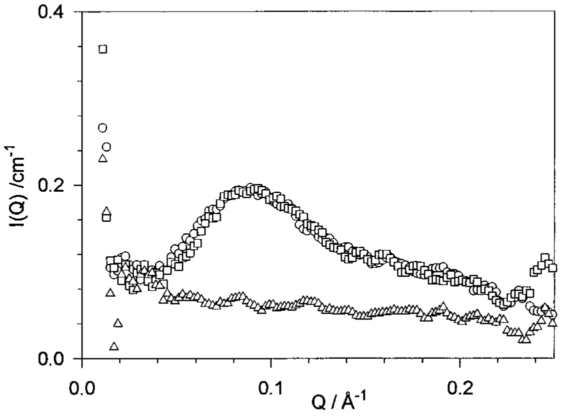


Figure 2. Scattering from (△) 0.5% and (□) 5% 82 kDa and (○) 5% 356 kDa NaPSS in D_2O, $T = 45$ °C.

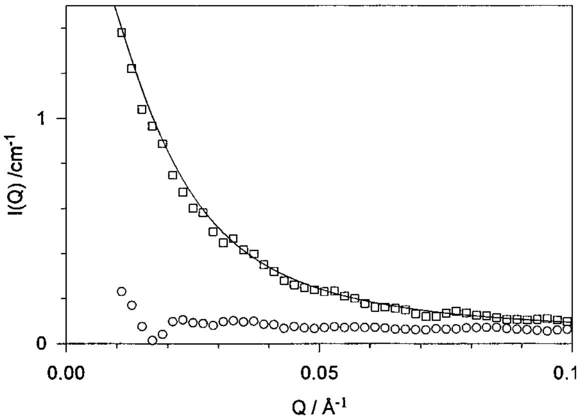


Figure 3. SANS from separate solutions of (○) 0.5% 82 kDa NaPSS in D_2O and (□) 5% α-gelatin solution. The solid line represents a fit using eq 1, giving $\xi = 58.9$ Å.

Figure 3 shows the scattering obtained from separate solutions of 0.5% 82 kDa NaPSS and 5% α-gelatin in D_2O. At such low concentrations the NaPSS hardly scatters at all, and the α-gelatin gives a monotonic decay. This has been fitted with de Gennes’ prediction for scattering from a semidilute polymer in a good solvent, given by

$$I_\xi(Q) = \frac{I_\xi(0)}{1 + Q^2\xi^2} + I_{\text{inc}} \tag{1}$$

where ξ is the average mesh size, $I_\xi(0)$ is the fitted scattering intensity at zero Q and I_{inc} is the incoherent scattering background. The fit to the data gives an average mesh size of 58.9 Å, which is larger than that found for an unfractionated gelatin from the same source of 51 Å and for a higher molecular weight sample of 35 Å.[44,45]

Figure 4 shows the effect on the scattering pattern when a NaPSS solution is mixed with α-gelatin solution in D_2O. There are two striking features: the scattering intensity has been increased significantly (in relation to both the pure gelatin and pure NaPSS scattering), and a peak in the scattering pattern develops at Q^*. The peak is at a much lower Q and much less broad than seen for the pure 5% NaPSS solutions (Figure 2), presumably because the peak position is controlled by

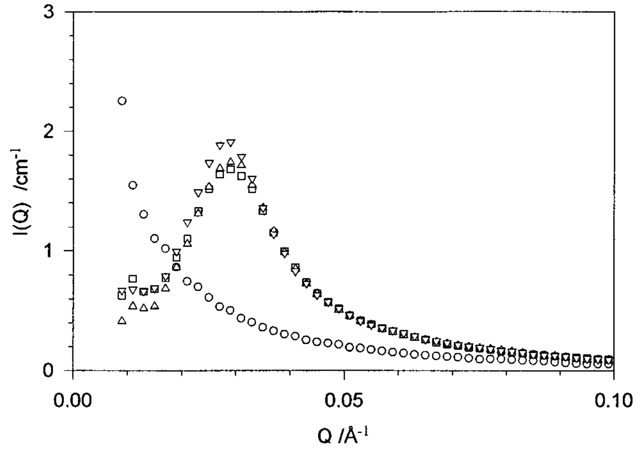


Figure 4. Effect of NaPSS molecular weight on 0.5% NaPSS/5% α-gelatin solutions: (○) no NaPSS; (□) 0.5% 82 kDa NaPSS; (△) 356 kDa PSS; (▽) 800 kDa PSS.

the NaPSS concentration. The increase in intensity indicates that there is a molecular interaction between the species in solution, and it can be ascribed to a large increase in the "density" of scattering bodies. This is due to the formation of a polyelectrolyte/gelatin complex. Bowman et al.[13] found that in dilute solution, the addition of gelatin to NaPSS and NaPAMPS increased the effective molecular weight of the polyelectrolyte by some 15−20 times, but the size of the coil only increased by around 15.

The NaPSS has not been contrast matched with the solvent, but the scattering from the polyelectrolyte is likely to be minimal, since its concentration in solution is very small (Figure 2): the scattering intensity is dominated by the gelatin. The shape of the curve is very similar to that observed for concentrated NaPSS solutions,[16] indicating that the gelatin has been structured by the polyanion (in the same way that "invisible" SDS causes gelatin to scatter like a solution of micelles[45−47]).

II. Effect of NaPSS Molecular Weight. Figure 4 also shows that there is no change in the peak position with the molecular weight of the polyanion. This indicates that the peak arises through interaction length scales shorter than the chain length of the NaPSS. Using the Bragg relation it is possible to use the position of Q^* to obtain an indication of this scale:

$$d^* \approx \frac{2\pi}{Q^*} \tag{2}$$

From the data in Figure 4 this gives values of d^* of 220 Å. Since this size (with no added salt) is significantly smaller than the size of the NaPSS coil ($2R_g \approx 1100$ Å, 780 kDa, 0.1 M NaCl, and 760 Å, 77 kDa, 0.1 M NaCl[48]) and of the α-gelatin ($2R_g \approx 500$ Å, 0.1 M NaCl approximated from literature data[44]). By way of comparison, we have obtained some rheological data on similar systems which give a hydrodynamic diameter for a 0.3% 780 kDa NaPSS/5% α-gelatin complex of around 1350 and 670 Å for a 0.3% 82 kDa NaPSS/5% α-gelatin complex.[49] It would appear that d^* represents a domain which is greater than the gelatin mesh size but is smaller than that of the complex. The domain size is also independent of the NaPSS chain length whereas the overall complex size is not.

III. Effect of NaPSS Concentration. Figures 5 and 6 show the effect on the scattering of changes in the ratio of NaPSS to gelatin. In Figure 5, as the concentration of 82 kDa NaPSS is increased at 5% α-gelatin, the peak in the data moves to higher Q. At $Q > Q^*$ the intensity increases sharply from 0.19% to 0.3% NaPSS, but less so on increasing the polyanion concentration to 0.5%. The increase in Q^* with increasing NaPSS indicates that the domain size is decreasing as more gelatin is complexed.

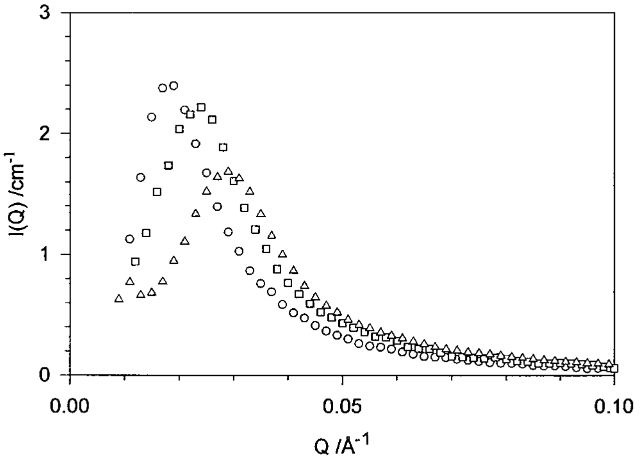


Figure 5. Effect of 82 kDa NaPSS concentration on 5% α-gelatin solutions, at $T = 45$ °C: (○) 0.19% NaPSS; (□) 0.3% NaPSS; (△) 0.5% NaPSS.

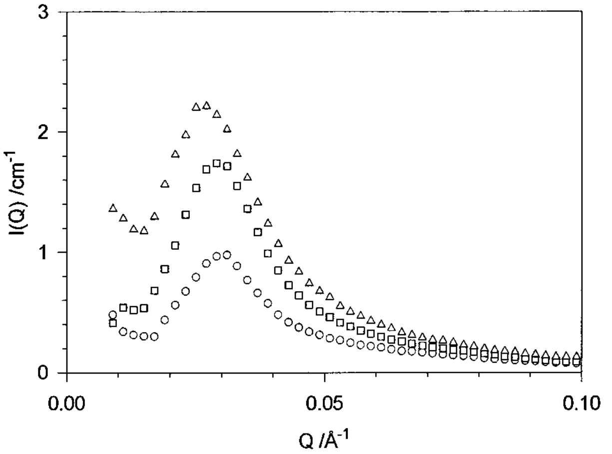


Figure 6. Effect of α-gelatin concentration on 0.5% 356 kDa NaPSS solutions, at $T = 45$ °C: (○) 2.5%; (□) 5.0%; (△) 10% gelatin.

The data in Figure 6 are for 0.5% 356 kDa NaPSS with α-gelatin concentrations 2.5, 5.0, and 10%. On moving from 2.5 to 5% α-gelatin, the peak position is unchanged and the intensity nearly doubles. At 10% α-gelatin, the peak has moved to slightly lower Q and the intensity increased by less than 30% over that at 5% α-gelatin, suggesting that the NaPSS has been saturated. The domain size is clearly independent of the gelatin concentration and only relies of the NaPSS concentration. These data taken with the earlier graphs highlight that the controlling factor in determining Q^* is the NaPSS monomer concentration, which determines the solution ionic strength at least for the range of polymer molecular weights examined here. However, the intensity of the scattering is directly related to the gelatin scattering and must be related to the amount of complex formed. Using the scattering data of others and that presented in Figures 5 and 6, it can be shown (Figure 7) that the position of the maximum in the data varies as, $Q^* \sim c_p^{1/2}$, as predicted by scaling theory[17−19] and lattice models.[20]

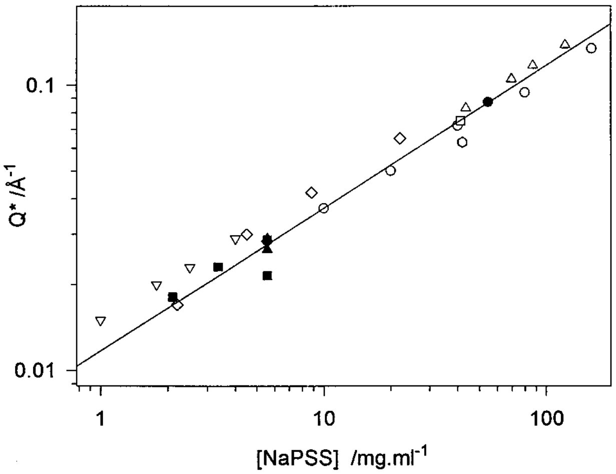


Figure 7. Q^* vs [polyelectrolyte] from various references. Included are the following: NaPSS solutions measured using SAXS by Kaji et al.[27] (□) and Ise et al.[28] (□), SANS by Nierlich et al.[29] (△) and light scattering by Drifford and Dalbiez[50] (▽); PMVP measured using SANS by Ermi and Amis[24] (▼) and DNA measured using SANS by Borsali et (○)al.[35] Also included are our measurements for pure NaPSS (●) and for the 82 kDa (■), 356 kDa (▲), and 800 kDa (◆) NaPSS/α-gelatin complexes (note this point is included in the overlapping points at 6.6 mg·mL^{-1}). The line is a fit to the pure NaPSS data, $Q^* = 0.0117c_p^{1/2}$.

The values of Q^* for the 82 kDa, 356 kDa, and 800 kDa NaPSS/α-gelatin complex fit in extremely well with the data in the literature, showing both the correct trend and, rather surprisingly, an absolute value which is in reasonable agreement with the data from pure NaPSS solutions. Clearly the change in Q^* with concentration is independent of molecular weight and therefore independent of polydispersity and again reflects that the controlling factor is the monomer concentration above some critical molecular weight of NaPSS and also possibly above a critical concentration. These two effects will be addressed in a later publication.

IV. Effect of Polyelectrolyte Structure. Bowman et al.[13] studied the interactions of NaPSS and of sodium poly(2-acrylamido-2-methylpropane sulfonate) (or NaPAMPS) with deionized gelatin in dilute solution. Their data indicated that the polyelectrolyte with the more hydrophilic backbone (NaPAMPS) does not bind to gelatin as strongly as the NaPSS. Less gelatin binds to the NaPAMPS (~64 NaPAMPS monomers per gelatin chain against ~57 NaPSS monomers per gelatin chain;[13] i.e., the NaPAMPS binds 15 times its own mass of gelatin whereas NaPSS binds 18.7 times its own mass of gelatin).

As well as the NaPAMPS, a further polyelectrolyte was used in this study. This polymer was prepared by the co-polymerization of 80% (by weight) charged AMPS monomers and 20% uncharged hydrophilic acrylamide monomers. The resulting random copolymer was therefore similar to NaPAMPS, but with a lower charge density on the backbone.

The form of the scattering for the three polyanions with gelatin is similar (see Figure 8). However, there are two significant differences on changing the type of polymer from NaPSS through NaPAMPS to the copolymer: the overall scattering intensity falls and the position of the peak moves to lower Q (see Table 2).

The two different molecular weight NaPAMPS samples (high and low) give the same scattering pattern. This molecular weight independence also lends weight to the argument that the peak is due to the complex structure rather than inter-complex interactions. It might be expected that the copolymer binds less gelatin than NaPSS since it has fewer charges along a given section of chain than the NaPAMPS. Also, it is known[13] that NaPSS binds more gelatin than NaPAMPS. It could therefore be supposed that the greater the amount of gelatin bound to a given length of polyelectrolyte, the more intense the scattering at $Q > 0.03$ Å^{-1} (since it is the α-gelatin itself which is giving rise to the scattering), with no effect on the peak position.

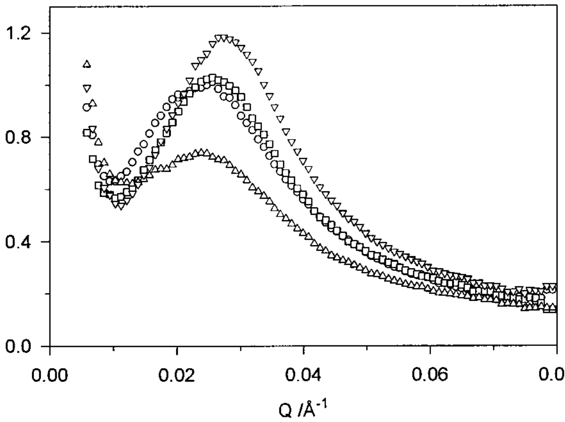


Figure 8. Effect of 0.5% polyelectrolyte on the scattering from 5% α-gelatin solutions, at $T = 25$ °C: (○) NaPAMPS low M_w; (□) NaPAMPS high M_w; (△) NaPAMPS−polyacrylamide; (▽) 82 kDa NaPSS.

Table 2. pH and Q^* for Various Polyelectrolytes (0.5%) Mixed with 5% α-Gelatin

5% α-gelatin plus	Q^*/Å^{-1}	pH
0.5% 82 kDa NaPSS	0.0275	5.90
0.5% NaPAMPS, IV = 1.23 dL·g^{-1}	0.0239	5.60
0.5% NaPAMPS, IV = 1.98 dL·g^{-1}	0.0258	5.63
0.5% copolymer	0.0233	5.51
no added polyelectrolyte		5.18

It was shown in Figure 7 that the position of Q^* is controlled by the polyelectrolyte concentration. Figure 8 shows that Q^* for the NaPAMPS sample is slightly lower than for NaPSS. This change may be due to the slight reduction in pH relative to the NaPSS sample (see Table 2 and section V). The copolymer has its Q^* shifted to slightly lower Q. This may again be a result of a small reduction in pH, or may be due to a reduction in the density of the charges on each chain. The lower scattering intensity at $Q > Q^*$ for the copolymer-gelatin solution supports the hypothesis that the homopolyanions complex more gelatin.

V. Effect of Added Electrolyte. The effect of increasing salt on the scattering from the α-gelatin is to both decrease the scattering intensity at low Q and reduce the rate of decay of the scattering, which corresponds to a reduction in the mesh size.[51]

Figures 9 and 10 show the effect of added salt to the NaPSS/α-gelatin complex for molecular weights of 82 kDa and 356 kDa, respectively. In general, the peak in the scattering is "washed out" by the addition of electrolyte. As 6 mM salt is added the peaks move to slightly lower Q and the scattering intensity at low Q increases until the maxima vanish at high salt concentrations. This behavior is very similar to that observed when salt is added to pure polyelectrolyte solutions, where both a movement of the peak to lower Q[28,30] and a constant value of Q^* [23,35] have been observed. It was

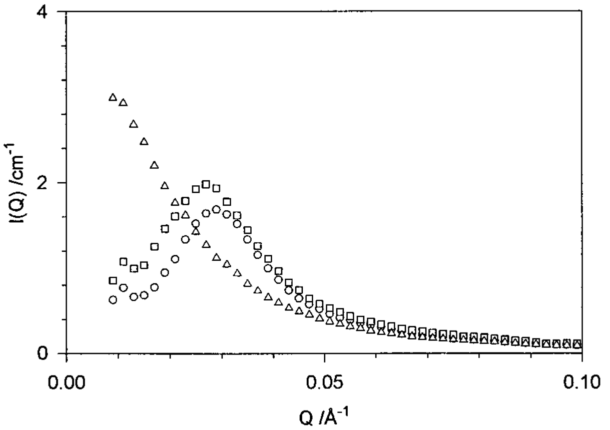


Figure 9. Effect of added sodium acetate on 0.5% 82 kDa NaPSS/5% α-gelatin, at T = 45 °C: (○) no salt; (□) 6 mM sodium acetate; (△) 60 mM sodium acetate.

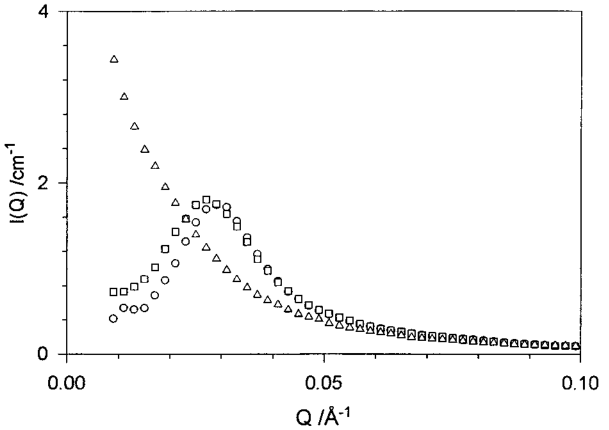


Figure 10. Effect of added sodium acetate on 0.5% 356 kDa NaPSS/5% α-gelatin, at T = 45 °C: (○) no salt; (□) 6 mM sodium acetate; (△) 60 mM sodium acetate.

shown by Bowman et al.[13] that the addition of salt up to 10 mM reduces the repulsion between the negatively charged gelatin and the anionic polyelectrolyte, allowing slightly more gelatin to bind. At higher ionic strengths (>10 mM), the increase in screening reduces the attraction between the polyanion and gelatin resulting in a decrease in the stoichiometry of the complex. These trends can be seen in the scattering data at high Q values. Increasing the salt concentration to 6 mM, Figure 9 shows that there is a small increase in the scattering at $Q > 0.03$ Å^{-1} (the scattering at high Q should be dependent on the density of scattering bodies while being unaffected by the peak in the data). However, Figure 10 shows little change in the scattering on adding 6 mM salt. At high ionic strength, both figures show a decrease in intensity at $Q > 0.03$ Å^{-1} in comparison to the low ionic strength samples, as a result of a decrease in the amount of gelatin bound to the polyanion chains.

The change in the scattering from showing a peak (typical of a polyelectrolyte solution) to a smooth monotonic decay (indicative of a neutral homopolymer solution) suggests that at high salt concentrations, charges on the polyelectrolyte backbone are totally screened. Again this is a very similar behavior to that of the SDS–gelatin complex discussed earlier.[45] Consequently, we surmise that the complex has a structure more like that of a neutral polymer than that of a polyelectrolyte, as seen for pure polyelectrolyte solutions by Ermi and Amis at high salt.[24]

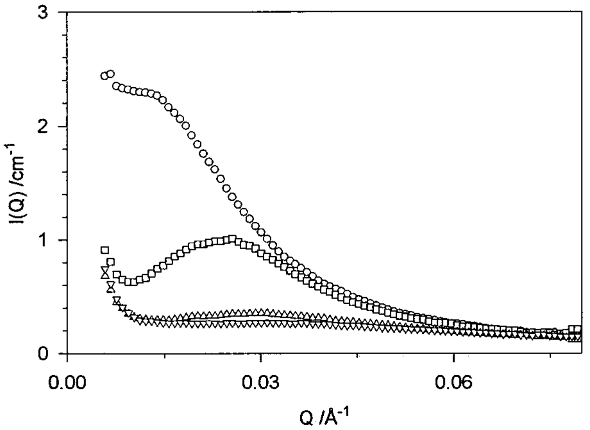


Figure 11. Effect of pH on 0.5% NaPAMPS (low M_w)/5% α-gelatin solutions, at T = 25 °C: (○) pH 5.26; (□) pH 5.60; (△) pH 7.35; (▽) pH 9.37.

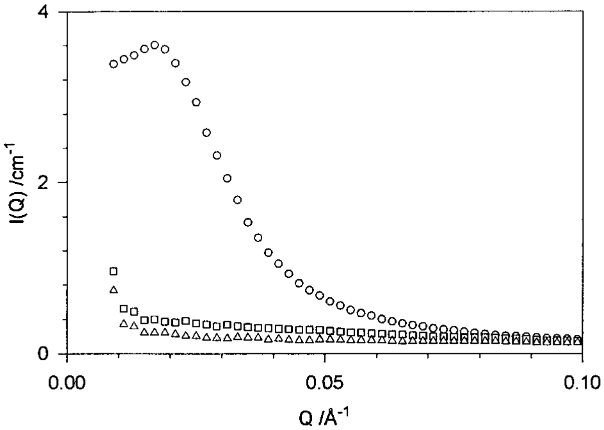


Figure 12. Effect of pH on 0.5% 780 kDa NaPSS/5% α-gelatin solutions in 6 mM sodium acetate, at T = 45 °C: (○) pH 5.2; (□) pH 10.1; (△) pH 12.3.

VI. Effect of Varying the pH. Bowman et al.[13] have shown from previous light scattering data that the stoichiometry of the gelatin/NaPSS and gelatin/NaPAMPS complexes decreases with increasing pH. At pH > 10, the complex fails to form at all. It is reasonable to assume that as the pH rises and the net negative charge on the gelatin increases, there is a decrease in the attraction between the polyelectrolyte and the polyampholyte. In particular, the binding will weaken as the amine groups deprotonate.

Figures 11 and 12 show the effect of increasing the pH (by adding concentrated NaOH) on the NaPAMPS/gelatin and NaPSS/gelatin complexes. It is apparent that as the pH rises the scattering intensity decreases. This is because the complex progressively dissociates and probably does not exist at all at pH > 10.[13] As a consequence, the pattern observed at high pH is similar to that seen previously for deionized gelatin at pH 10.[46]

Figure 11 also shows the effect of decreasing the pH (by adding concentrated HCl) below the (unaltered) pH of the NaPAMPS/gelatin system (pH 5.60). Here, the scattering intensity increases as more gelatin binds to the polyelectrolyte chains (because of a reduced repulsion between the two polymers), and the peak moves to lower Q.

Conclusions

In this paper we have used SANS to explore the complexes formed by gelatin with polyelectrolytes. The results show that the gelatin is strongly structured by the polyelectrolytes and this accounts for the large increase in bulk viscosity that has been observed. The addition of salt breaks up these complexes, as does an increase in pH. In the former case the attractive charge interactions are screened and in the latter the repulsion between the components is strongly enhanced.

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