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Complexes of chitosan and poly(methacrylic acid) studied by fluorescence techniques

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Summary

Fluorescence techniques have been used to study the interactions of poly(methacrylic acid) (PMAA) with chitosan(CS) in aqueous solution. These studies show that PMAA and CS form complexes within a pH range of 3 to 10 and along with complexing with CS, the conformation of PMAA at pH 4 changed from a hypercoiled to a loose coiled form. However, this conformational change is not complete since the optimal ratio for the complex formation is 0.5:1(CS:PMAA, in residue unit). PMAA/CS complexes at pH 4 were largely disrupted with the addition of NaCl, most likely due to screening of the electrostatic attraction between ionized carboxyl groups of PMAA and protonated amino groups of CS.

Introduction

In addition to studies of segmental mobility and conformational behaviour of polymers, fluorescence techniques, such as non-radiative energy transfer, fluorescence lifetime, fluorescence anisotropy measurements and fluorescence quenching have been used to investigate interpolymer complexation(1-9). The advantage of fluorescence techniques is that information about the behaviour of the polymers on the molecular level can be obtained, as opposed to the bulk properties determined by non-spectroscopic techniques. It is reported that a significant reduction in segmental mobility occurs upon complexation(6-8). Therefore, fluorescence anisotropy measurements should find increasing use in this respect.

Poly(methacrylic acid), PMAA, represents a particularly interesting case for study since the hydrophobic interactions introduced by the methyl groups in PMAA result in hypercoiling of the polymer in acid media(10-12). This conformation makes the aqueous solution of the polymer solubilize water insoluble organic compounds such as arenes and alkanes. It should be noted that the conformation of PMAA in aqueous solution is very dependent upon pH. Upon addition of base to PMAA solution, the carboxylic acid groups ionized and acquire negative charges. The increase in Coulombic repulsive forces results in a non-uniform, sudden conformational transition from the hypercoiled to the expanded form(13). This conformational change is reversible. It is expected that this conformational alteration may be taken as bases for the design and preparation of new smart hybrid materials, such as films and hydrogels.

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Chitosan(CS) is produced by partial deacetylation of chitin. The later is recovered from crustacean shells(crab, crawfish, etc.). It is, from a structural point, poly[β-(1-4)-2-acetamido-2-deoxy-D-glucopyranose]. Clearly, the structural residues of CS are glucosamine and N-acetylglucosamine. The idealised structures of CS and chitin are shown in Figures 1(a) and (b), respectively. CS-based materials, such as hydrogels, fibres, films, etc., have found increasing use in a variety of applications ranging from, for example, purification of drinking water to immobilization of enzymes, cells and drugs(14,15). The utilization of CS would be advantageous because (1) chitin is an abundant, inexpensive, renewable resource produced all over the world; (2) chitin is biodegradable and, hence, may be useful in applications where recovery or recycling would be difficult(14). In view of its strong propensity for H-bonding(15), we decided to investigate the interactions between PMAA and CS.

In the present report, we describe these preliminary investigations, using fluorescence techniques to study the effects of complexing CS upon the hypercoil conformation of PMAA and the nature of the resultant complexes.

Experimental

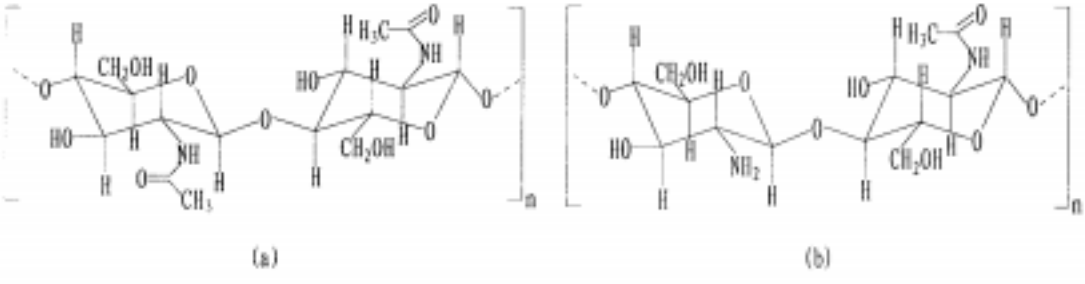
Materials.

Acenaphthylene(ACE Aldrich-85%) was purified by triple recrystallization from ethanol followed by sublimation. Pyrene(Py, Aldrich-99%) was purified by multiple recrystallization(x3) from ethanol. Perylene(Pe, Aldrich-gold label) was used without further purification. ACE labeled PMAA(PMAA/ACE) was prepared by free radical polymerization using AIBN as initiator in benzene solution. The content of ACE in starting formulation is 0.7mol%[ACE/(ACE+MAA), in mole unit]. The sample was thoroughly degased, sealed under high vacuum (*ca.* 10^{-4}Torr) and polymerized at 60°C. Polymerization was terminated at less than 10% conversion in all cases. The polymer was purified by multiple dissolution(x5) in methanol followed by precipitation into diethyl ether. The same method was employed to prepare the unlabelled polymer, PMAA.

The content of ACE in PMAA/ACE was found to be 0.4mol% determined using UV spectroscopy. The number average molecular weights of PMAA and PMAA/ACE are 5.8×10^5 and 2.7×10^5, respectively. CS was prepared as described before(16). The degree of deacetylation was determined to be 52±3mol% by FTIR(15) and pH titration(17). The viscometry average molecular weight of CS was 7.9×10^5(18). Double distilled water was used through out. Nitromethane and other chemicals were of, at least, analytical grade.

Sample Preparation.

Polymer solutions were prepared from their stock solutions. The concentrations of the stock solutions for PMAA, PMAA/ACE and CS are all 0.1wt%. It is to be noted that the



(a) (b)

Figure 1. Molecular structures of (a) chitin and (b) chitosan

pH of the stock solution of the polyacid was about 10, whereas the pH of the stock solution of CS was around 4.

For the experiments involving occlusion of organic probe molecules into the water soluble polymer solutions, the probe, Py was initially dissolved in diethyl ether to obtain a stock solution of known concentration(*ca*. 10^{-3}mol/L). This solution was diluted to 10^{-5} mol/L just before using. One ml of the probe solution(10^{-5}mol/L) was injected into a 10ml volumetric flask. The ether was evaporated at room temperature. Subsequently, a polymer solution of known pH(10^{-3}wt%) was added to the flask. To ensure solubilization and equilibration, the polymer/probe solution was sonicated for 20mins, and then left at room temperature for more than 12hrs. Similar procedures were followed when using Pe as a probe, but the final concentration of the probe is 10^{-5}mol/L.

For complexation measurements, all samples of different CS to PMAA/ACE ratios(0:1; 0.01:1; 0.1:1; 0.5:1; 1:1; 3:1; 5:1; 10:1 and 50:1) were prepared in a similar manner. The method is described by using an example, a solution containing $2.8\text{x}10^{-6}$mol% of PMAA/ACE and $2.8\text{x}10^{-6}$mol% of CS(in residue unit, pH=4.0). To make this solution, 0.1ml of PMAA/ACE stock solution($2.8\text{x}10^{-4}$mol%) and 0.1ml of CS stock solution ($2.8\text{x}10^{-4}$mol%) were added, respectively, to a 10ml volumetric flask with shaking. The mixture was diluted to about 9ml and its pH was adjusted to 4.0 using 0.1M NaOH solution and/or 0.1M HCl solution. The solution obtained in this way was diluted to 10ml with water.

Analytical Methods

All fluorescence spectra were recorded on Perkin-Elmer LS 50B luminescence spectrometer. Fluorescence anisotropy measurements were performed on the same machine using the polarization accessory. This arrangement allowed estimation of I_{vv}, I_{vh}, I_{hh} and I_{hv}, where I_{vv} and I_{vh} stand for the fluorescence intensities observed parallel and perpendicular, respectively, to the plane of vertically polarized excitation. Similarly, I_{hv} and I_{vv} are the fluorescence intensities measured perpendicular and parallel, respectively, to the plane of horizontally polarized excitation. The instrumental correction factor, G, and the fluorescence anisotropy, *r*, can be calculated by using equation 1 and 2, respectively.

$$r = (I_{vv}-GI_{vh})/(I_{vv}+2GI_{vh}) \qquad (1)$$

where

$$G = I_{hv}/I_{hh} \qquad (2)$$

All the measurements and calculations were carried out automatically by the machine. Each measurement was repeated at least 10 times. All measurements of the polymer solutions were performed using quartz cuvette of path length 1 cm.

Results and Discussion

Fluorescence Anisotropy Studies

Our study toward the determination of the complexation between PMAA/ACE and CS is to focus on the segmental mobility of PMAA/ACE. Two sets of experiments were conducted to look at if there is any complexation between them. First, we performed steady-state fluorescence anisotropy measurements of the polymer as a function of pH. The results are shown in Figure 2. Clearly, there is a transition in *r* values between pH 5 and 7 which corresponds to the conformational transition of PMAA from hypercoiled to extended coil structure(11,12). It is not difficult to understand that the low segmental

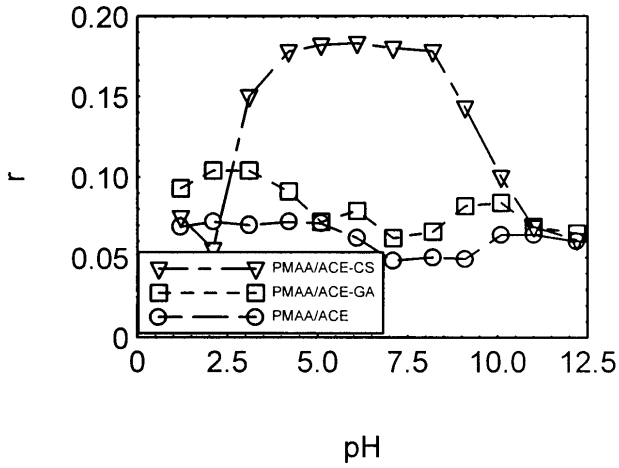


Figure 2. Plots of fluorescence anisotropy(r) against pH in PMAA/ACE, PMAA/ACE-CS and PMAA/ACE-GA aqueous solutions(2.8×10^{-6}mol%, in residue unit, for either PMAA/ACE, CS or GA).

mobility of the polymer at low pH is likely to be a consequence of steric restrictions. In contrast, at high pH the segmental mobility of the polymer would be large, and hence, *r* values should be smaller. Next, we performed similar measurements for the PMAA/ACE-CS system(1:1, in repeat unit). The pH dependence of the anisotropy values, for the complex system is also shown in Figure 2.

Clearly, the *r* data show qualitatively the effects of complexing with CS upon the segmental mobility of PMAA. At pH values less than 2.5, the *r* values of the PMAA/ACE-CS system are no much different from those of PMAA/ACE system indicating that there is no significant complexation between PMAA/ACE and CS. Similar argument applies to the pH values greater than 10. Substantial differences between the two sets of *r* values exist within a wide pH range of 3 to 10. The differences may be attributed to the complexation between PMAA/ACE and CS. In other words, complexation will reduce the flexibility of polymer chain, which has been reported in many publications(see, for example, 6-8). These findings may be understood in the following way. At pH less than 3, the amino groups along the CS chain are completely protonated and at the same time, PMAA adopts a compact coil conformation. Both factors

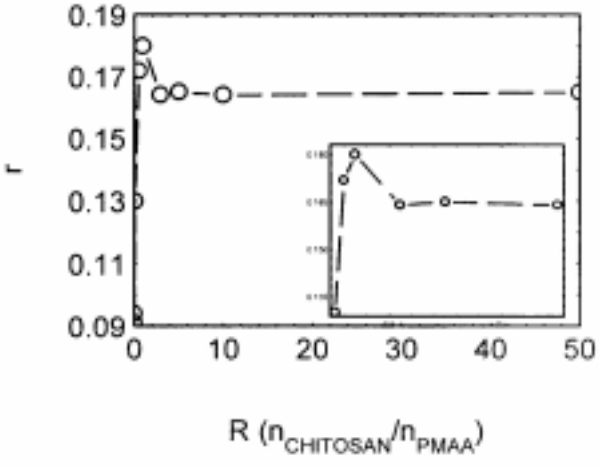


Figure 3. Plots of fluorescence anisotropy(r) against the ratio of chitosan to PMAA/ACE in aqueous solution(1:1, 2.8×10^{-6} mol%, in residue unit) at pH 4.0.

are not favorable for the complex formation. At pH greater than 11, PMAA exists as polyanions and CS is no longer polycations. Clearly, this situation is also not favorable for the complex formation since the basis for the H-bonding has lost. In contrast, only when the pH of the system is not too low or too high, the complexation can occur significantly.

To further illustrate that the differences in r values are caused by the polymeric effect of CS, glucosamine(GA) has been used as a model compound to instead CS. The result is shown in Figure 2. It can be seen that although addition of GA inhibits the segmental mobility of PMAA, the inhibiting effect is much weaker than that of CS. This indicates clearly that it is the polymeric effect of CS that regulating PMAA transformation.

Figure 3 presents results for the complex systems of different CS to PMAA ratios (in residue unit) at pH 4.0. With reference to the figure, it can be noted that the value of *r* for PMAA/ACE in the complex system increases from *ca*.0.09(in the absence of CS) to *ca*. 0.18 (in the presence of CS) (CS to PMAA/ACE ratio, *ca*.0.5:1). After that ratio, there is a reduction in *r* values, but *r* does not change very much with further increase in CS. Clearly, this is a result of complexation between PMAA/ACE and CS. When the ratio of CS to PMAA/ACE is low, the anisotropy data are dominated by the contribution of the free PMAA/ACE. As the concentration of CS is progressively increased, the segmental mobility of the PMAA/ACE is hindered due to complexing with CS. The maximum r value appears at a ratio of 0.5:1(CS:PMAA/ACE). These results would seem to imply that at this ratio, the PMAA/ACE segments experience the greatest restraint due to complexation. This is a rather surprising result since the ratio offering the greatest number of potential polymer-polymer "contact points" should be *ca*. 1:0.5 rather than 0.5:1 (CS:PMAA/ACE). This is because the degree of deacetylation of CS in this work is about 52mol%. At low CS content, PMAA molecules may compete for the binding sites (amino group) on CS and one complex molecule is likely to consist of a number of PMAA molecules bound to one CS molecule. The PMAA/ACE molecules present in the complex formed at a CS to PMAA/ACE ratio of, for example, 0.1:1, would be envisaged as having a greater characteristics of the conformation adopted by PMAA/ACE itself than those found in a 0.5:1 complex. Consequently, the apparent segmental mobility of PMAA/ACE would be expected to be greater in the former case. In the presence of excess CS, the PMAA/ACE chain appears to be a little bit more mobile than 0.5:1 complex. However, there is no further significant change in r value as the CS to PMAA/ACE ratio is greater than 3. These data suggest that CS-PMAA/ACE complex may have a relatively definite composition, that is 0.5:1 for CS to PMAA/ACE. The data would also seem to indicate that the conformation of PMAA/ACE changed a lot upon complexing with CS. The larger ratio of PMAA/ACE to CS(1:0.5) shows that only a part of the carboxyl groups in PMAA/ACE have taken part in the complexation, and the others may be still in the hydrophobic domains which might have been altered due to complexation. Furthermore, it is a little surprising to note that the CS seems too effective to fix PMAA conformation at very low ratios of CS to PMAA. This result may be understood from the fact that PMAA adopts a compact coil conformation at low pH. But at pH 4, some carboxyl groups on PMAA ionized. Therefore, some segments of the polymer chain will exist on the coil surface as loops or tails, which should be more flexible than the segments within the coil. Upon addition of CS, it is these segments that have the priority to complex with CS. Thus the anisotropy value of the ACE labeled PMAA increases dramatically with increasing

CS concentration at the very early stage.

Probe Studies

To inlustrate the effect of complexation upon the conformation of PMAA at pH 4, two sets of dye solubilization experiments were carried out. In the first experiment, Py was used as a probe because the fine structure of its fluorescence emission spectrum is sensitive to the changes in the polarity of its microenvironment(19,20). In particular, the intensity ratio of two specific vibronic bands(the I_3/I_1 ratio) is a measure of the polarity of its microenvironment. The larger values of I_3/I_1 indicate a more hydrophobic environment. This property has been widely used to monitor the conformational behaviour of water-soluble polymers in aqueous phase(21).

The effect of complexation upon the fine structures of the fluorescence emission spectra of Py solubilized in PMAA and PMAA/CS(1:1, in residue unit) solutions, respectively, at pH 4 and at a polymer concentration of $2.8x10^{-5}$mol% and probe concentration of 10^{-6}mol/L is depicted in Figure 4(a). Reference to the figure, it reveals that complexation of PMAA with CS was accompanied, as expected, by a conformational change as evidenced by the decrease in the hydrophobicity of the environment which was characterized by a decrease in the hydrophobic microdomain number or reduction in the domain size. This is because the I_3/I_1 ratio decreases from *ca*. 1.1 for PMAA to *ca*. 0.7 for PMAA/CS(1:1) system.

The effect of complexation upon the PMAA conformation at pH 4 was also probed using Pe as a probe. The result is shown in Figure 4(b). Clearly, complex formation is accompanied by a decrease in the solubilizing ability of the polymer. Unlike Py, Pe is almost insoluble in water. Therefore, decrease in the solubilizing capacity of the PMAA for Pe is an indication of decrease in the number of hydrophobic domains or reduction of the domain size. The tentative result about the conformational change of PMAA upon complexation is in support of the result from anisotropy measurements.

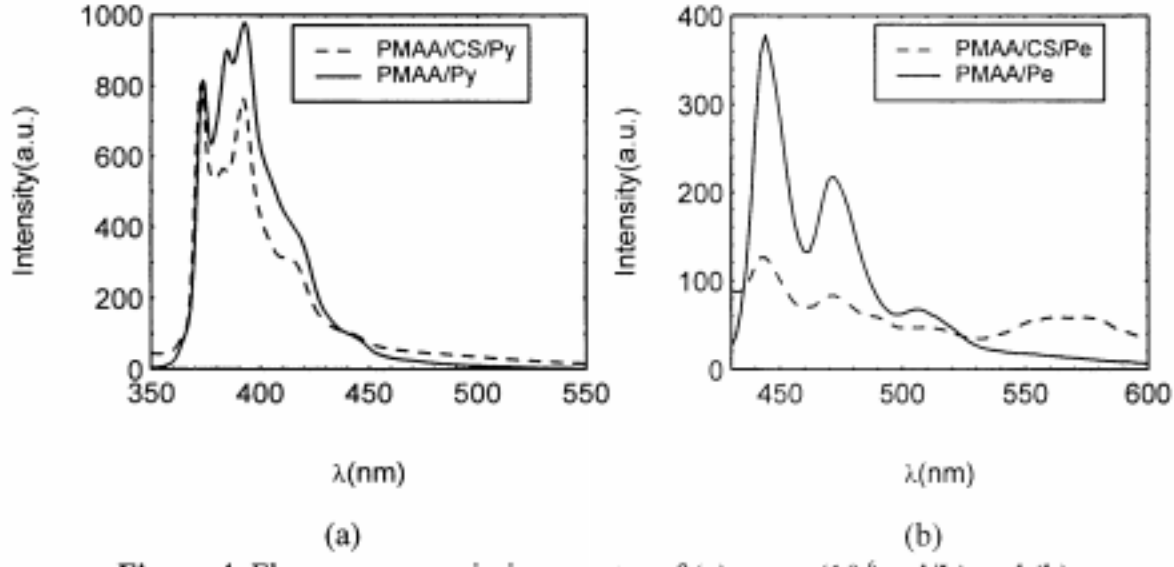


Figure 4. Fluorescence emission spectra of (a)pyrene(10^{-6}mol/L) and (b) perylene (10^{-5}mol/L) dispersed in PMAA (2.8×10^{-5}mol%, in residue unit) and in PMAA/CS(1:1, 2.8×10^{-5}mol%, in residue unit) aqueous solution at pH 4.0.

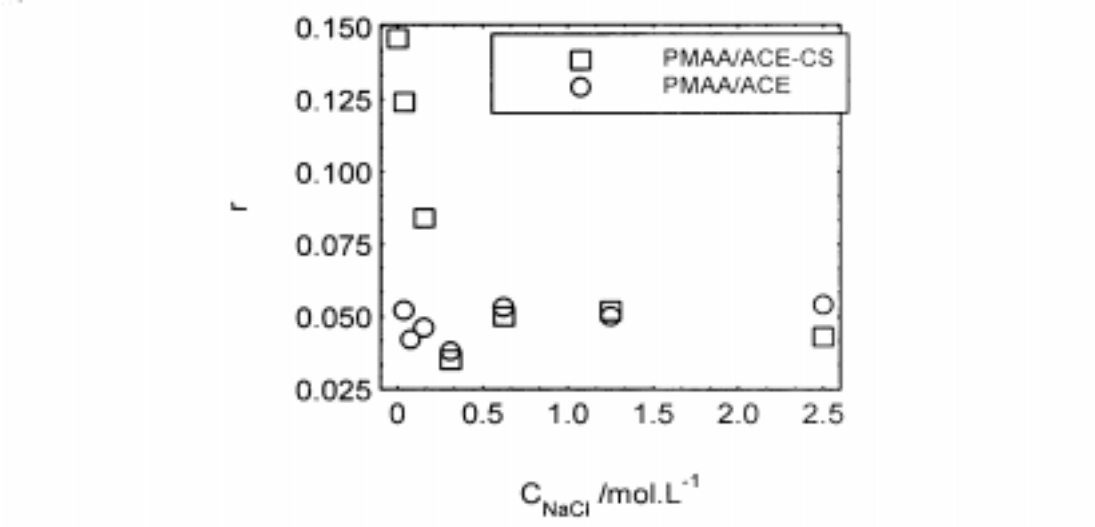


Figure 5. Plots of fluorescence anisotropy (r) against NaCl concentration in PMAA/ACE (2.8×10^{-5}mol%, in residue unit) and PMAA/ACE-CS(1:1, 2.8×10^{-5} mol%, in residue unit, for either PMAA/ACE or CS) aqueous solution at pH 4.0.

Salt Effect Studies

In order to gain further understanding of the nature of the complexation between PMAA and CS, work was undertaken to study the effect of NaCl on the interaction. Figure 5 depicts the fluorescence anisotropy data of PMAA/ACE in the absence and presence of CS at pH 4 as a function of NaCl concentration.

Inspection of the figure reveals that the anisotropy data for the PMAA/ACE-CS system are very sensitive to the addition of salt. Less than 0.4 mol/L of NaCl disrupts the complex completely. However, addition of salt has little effect upon the *r* values for polyacid system. These results indicate that addition of NaCl may screen the charge effect and therefore disrupts the complexation between the two polymers. In other words, the main driving force for the complexation is electrostatic attraction.

Conclusion

Fluorescence studies show that interpolymer complexation between PMAA and CS is both pH and molar ratio dependent and a major conformational change occurs when PMAA is mixed with CS in aqueous phase at pH 4. This conformational change is most likely resulted from the hypercoiled (for PMAA) to less coiled conformation as evidenced by the decrease in the hydrophobic microdomain size or domain number. Solutions at pH range between 3 and 10 are necessary for the complex formation. At pH 4, the complexation occurs most efficiently at a molar ratio of 0.5:1(CS:PMAA, in residue unit). This suggests that only some of the carboxyl groups in PMAA have taken part in the complexation. Introduction of NaCl disrupted PMAA/ACE-CS complexes, likely due to screening of the electrostatic attraction between ionized PMAA/ACE carboxyl and protonated CS amino groups.

These findings should be helpful for the design and preparation of new kinds of "smart" polymeric materials.

Acknowledgement

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