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The aggregation behaviour of chitosan bioelectret in aqueous solution using a fluorescence probe

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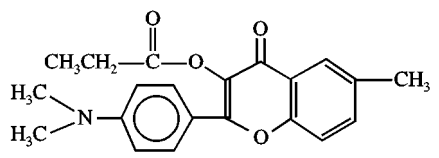
A kind of intramolecular charge-transfer compound, 3-hydroxy-6-methyl-4′-*N*,*N*-dimethylamino flavone derivatives (PF), was used to detect the self-association of chitosan bioelectret in aqueous solution. Results showed that the fluorescence intensity of PF at 430 nm increased with increasing chitosan concentration in 0.1 M acetic acid, and concomitantly, the emission at 515 nm decreased. However, no emission was observed at 515 nm after the polarization of chitosan solution and only an increase in the peak of PF at 430 nm could be found. When 0.1 M hydrochloric acid was used to dissolve chitosan and all other conditions were the same as above, the PF peak was weak and did not disappear after the chitosan solution was polarized. In addition, the effect of ionic strength on chitosan aggregation with a concentration of 5 mg ml^{-1} before and after polarization was examined. The results also showed that the fluorescence intensity of the probe increased remarkably at 430 nm with increasing ionic strength after polarization of the chitosan solution. All the results indicated that polarization can promote the aggregation behaviour of chitosan in aqueous solution. © *1998 Chapman & Hall*

1. Introduction

Chitosan, a kind of water-soluble polysaccharide, has found importance in various biomedical and pharmaceutical applications. Recent studies have shown that it could be used as support material for hydrophobic chromatography [1], as matrices for immobilization of enzymes and drugs, and as gel matrices for coating functionalized liposomes [2, 3] to improve the mucoadhesion of drug carriers and achieve both stability and slow release of the entrapped materials. As a kind of bioelectret [4], on the basis of molecular structure, chitosan's electret state is mainly ascribed to the high polarization store induced by the –OH group and bound water, which shows macroscopically that chitosan can retain the charge for a long time after the electric field is removed. A recent study has shown that chitosan/collagen compound bioelectret could effectively inhibit cancer cell growth after being positively polarized [5]. Interesting physicochemical properties of chitosan are directly related to the intra- and intermolecular association between the hydrophobic functionalities in aqueous solution. Amiji [6] reported a study of chitosan self-association in aqueous solution by using the peak III/I ratio of pyrene monomer fluorescence. To our best knowledge, there is no report relating to the self-association of the chitosan bioelectret before and after polarization.

A new kind of intramolecular charge-transfer compound, 3-hydroxy-6-methyl-4′-*N*,*N*-dimethylamino flavone derivatives (PF), was used in the present study to examine the aggregation behaviour of chitosan bioelectret before and after polarization. The molecular structure of PF is as follows



The fluorescence spectra of the PF shift to red and its intensity decreased gradually with increasing solvent polarity as shown in Fig. 1. In particular, only a very weak fluorescence could be found in water. Thus, a strong intramolecular charge transfer could be produced in PF after excitation, which resulted in

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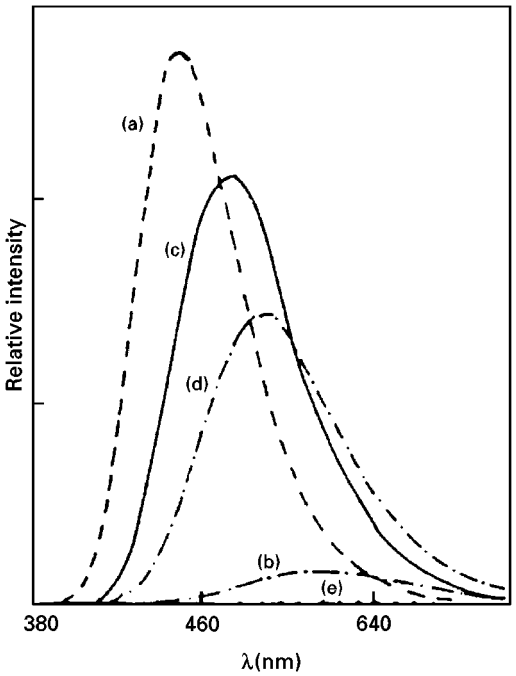


Figure 1 Fluorescence spectra of compound PF in different solvents: (a) tetrahydrofuran (THF); (b) ethanol; (c) acetone; (d) acetonitrile; (e) water.

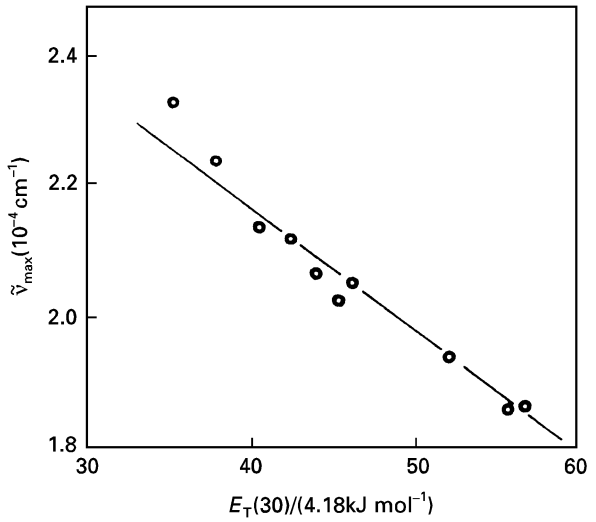


Figure 2 The change of maximum emission wave numbers of compound PF with the Dimroth $E_T(30)$ values.

solvatochromism effects and fluorescence-intensity quenching in different solvents.

Fig. 2 shows the change in wave numbers at the peak of the fluorescence spectra of PF with increasing polarity parameter Dimroth ET(30) [7] of a different solvent. A linear relationship could be found, which indicated that the fluorescence spectra of PF could change sensitively with change in the solvent polarity. Therefore, PF can be used as a fluorescence probe to detect the polarity of the surrounding medium.

2. Experimental procedure

Chitosan was prepared in our own laboratory. According to the normal method, chitosan was obtained from *N*-deacetylation of chitin in alkaline media. Chitin was purchased from Sigma Chemical Company. The degree of deacetylation of chitosan was 94% which was determined by acid–base titration [8], and its average molecular weight was 2.27×10^5 examined by viscometry [9]. All other chemicals were reagent grade. Deionized distilled water was used exclusively to prepare all aqueous solutions. Chitosan was dissolved in 0.1 M acetic acid and 0.1 M hydrochloric acid to prepare solutions with concentrations ranging from 0.01–10 mg ml^{-1}.

Chitosan solutions were polarized by a corona charge system whose electrode was composed of many needles and a plate. The polarization voltage was between 60 and 100 kV cm^{-1}, and the polarization time was 30–40 min.

The probe compound PF was synthesized according to the literature [10]. PF, dissolved in methanol, was added to chitosan solution to give a final concentration of 2.0 μM. PF emission spectra were obtained using a Hitachi MPF-4 fluorescence spectrophotometer. The probe was excited at 360 nm and the emission spectrum was collected in the range 380–590 nm. The excitation and emission slit openings were 14 and 8 nm, respectively.

3. Results and discussion

3.1. Influence of solvents on chitosan bioelectret association

Partially deacetylated chitosan, a linear polysaccharide, is composed of random repeating units of β-(1–4)-linked 2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acetamino-2-deoxy-D-glucopyranose (GlcNAc). On the basis of the degree of deacetylation, chitosan is expected to show the properties of an amphipathic molecule in aqueous solution.

Chitosan was dissolved in 0.1 M acetic acid and 0.1 M hydrochloric acid to give a concentration in the range 0.01–10 mg ml^{-1}. Fig. 3 shows the fluorescence spectra of PF in chitosan bioelectret solution in 0.1 M acetic acid before and after polarization. Fig. 3a shows that the peak of PF monomer fluorescence at 430 nm increased gradually with increasing chitosan concentration. When the chitosan concentration reached 1 mg ml^{-1}, it increased suddenly. This proved that there were hydrophobic microdomains in the solution which were detected by PF compound fluorescence when the chitosan concentration was increased to 1 mg ml^{-1} or above. Because the polarity of the environment where PF was localized gradually became weak, the fluorescence intensity of PF at 430 nm increased gradually.

After chitosan solutions were polarized, the peak of PF fluorescence at 515 nm disappeared at lower chitosan concentrations, as shown in Fig. 3b. Peaks of PF fluorescence were only observed at 430 nm. When the chitosan concentration was increased to 1 mg ml^{-1}, there still existed a sudden increase in the fluorescence peak. This showed that the aggregation behaviour of chitosan in 0.1 M acetic acid was strengthened after polarization. The polarity of the environment where PF was localized was weaker than that before polarization.

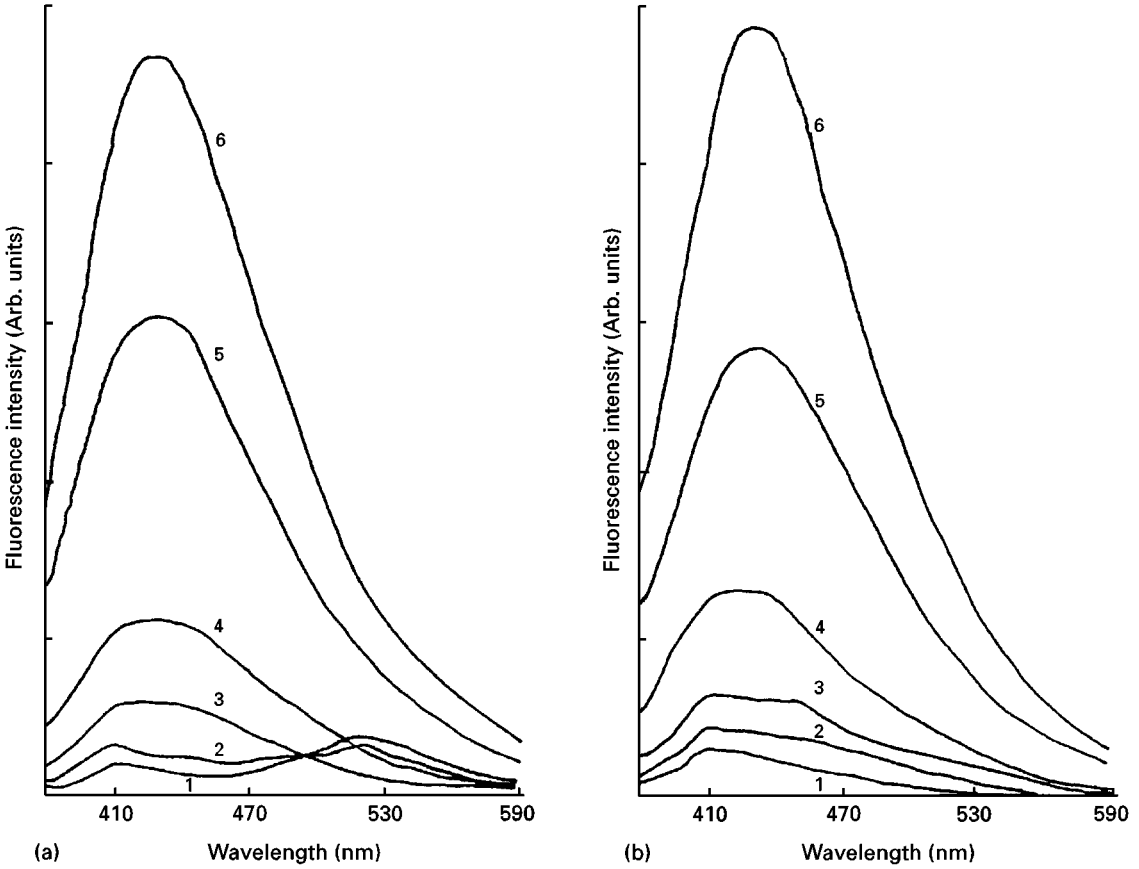
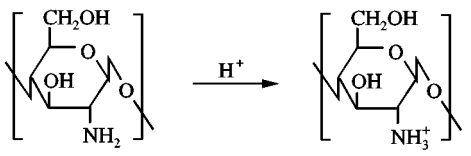


Figure 3 Fluorescence spectra of PF in chitosan solution (a) before and (b) after polarization with concentrations of (1) 0.01 mg ml^{-1}, (2) 0.1 mg ml^{-1}, (3) 0.5 mg ml^{-1}, (4) 1 mg ml^{-1}, (5) 5 mg ml^{-1}, (6) 10 mg ml^{-1} in 0.1 M acetic acid.

Chitosan self-association in aqueous solution could be explained from its molecular structure. Much free $-NH_2$ in the chitosan chain can combine H^+ from the acid solution, which induces the chitosan molecule to become a polyelectrolyte bearing a positive charge



(1)

At lower chitosan concentrations, all the free $-NH_2$ in the chitosan chain changed to $-NH_3^+$ due to the action of acid. They repelled one another, which made chitosan in aqueous solution become a linear polysaccharide. PF fluorescence could mainly detect the polarity of 0.1 M acetic acid. When the chitosan concentration was increased to 1 mg ml^{-1} or above, the free $-NH_2$ in the chitosan chain which can combine H^+ in an acid solution increased, which led to an increase in the pH value. Thus, the repelling action of $-NH_3^+$ in the chitosan chain became weaker, and correspondingly, the action of the intra- and intermolecular hydrogen bond increased, which resulted in the formation of hydrophobic microdomains in the solution. The hydrophobic functionalities of PF could enter the microdomains which had a relatively weak polarity, so the PF fluorescence peak became strong at 430 nm.

After polarization, the aggregation behaviour of chitosan in aqueous solution was strengthened. Because the high polarization storage induced by the –OH dipole group and bound water in the chitosan molecule, restrained the repelling action of $-NH_3^+$ in the chitosan chain, correspondingly there were no fluorescence peaks of PF at 515 nm at lower chitosan concentration, and only at 430 nm were there weak peaks of PF fluorescence. With increasing of chitosan concentration, strong peaks of PF fluorescence were also observed at 430 nm, which was similar to that before polarization. This proved that polarization can strengthen chitosan self-association in an aqueous solution.

Fig. 4 shows the fluorescence spectra of PF in chitosan bioelectret solution in 0.1 M hydrochloric acid before and after polarization. At lower chitosan concentrations, the PF fluorescence peak at 515 nm was stronger than that in 0.1 M acetic acid under the same conditions. When the chitosan concentration was increased to 1 mg ml^{-1} or above, the PF fluorescence peak at 430 nm rose suddenly. After polarization, the PF fluorescence peak at 515 nm became weaker than that before polarization. There was little difference from the chitosan solution in 0.1 M acetic acid. Because the ability to supply H^+ in 0.1 M hydrochloric acid was stronger than in 0.1 M acetic acid, the repelling action of $-NH_3^+$ in the chitosan chain was accordingly stronger at lower chitosan concentrations, which made the chitosan molecule more linear

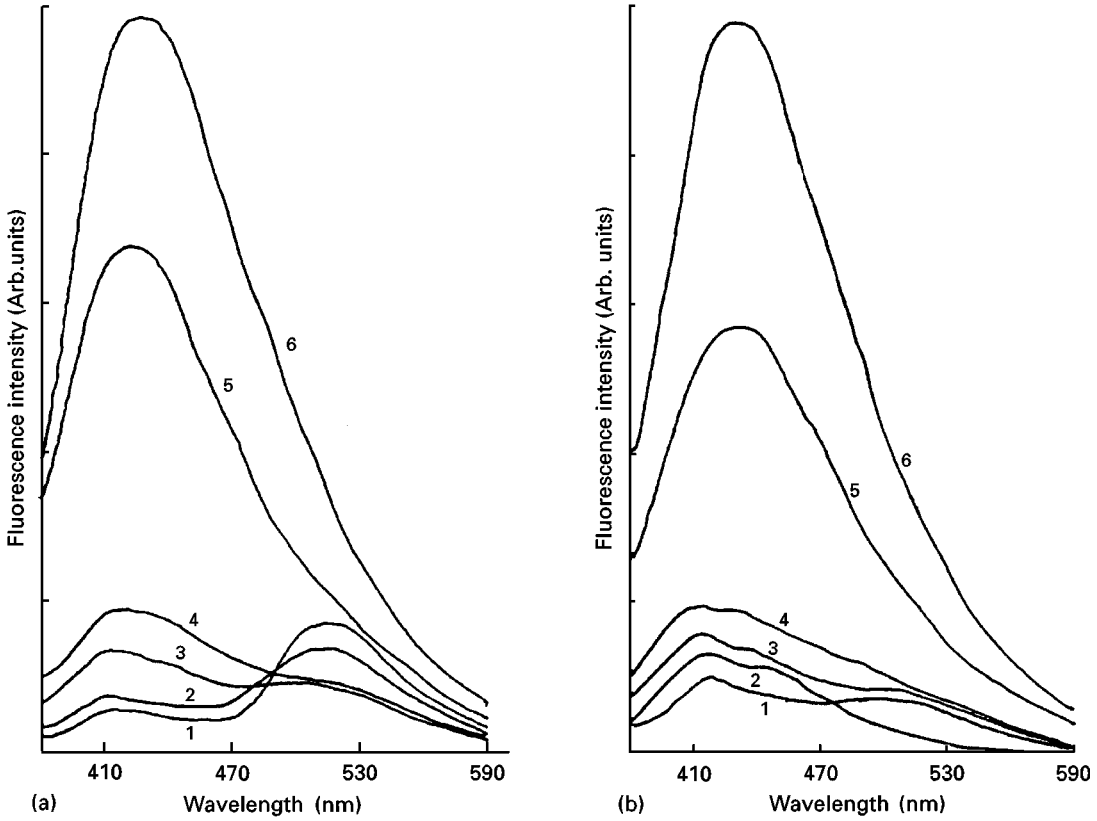


Figure 4 Fluorescence spectra of PF in chitosan solution (a) before and (b) after polarization with concentrations of (1) 0.01 mg ml^{-1}, (2) 0.1 mg ml^{-1}, (3) 0.5 mg ml^{-1}, (4) 1 mg ml^{-1}, (5) 5 mg ml^{-1}, (6) 10 mg ml^{-1} in 0.1 M hydrochloric acid.

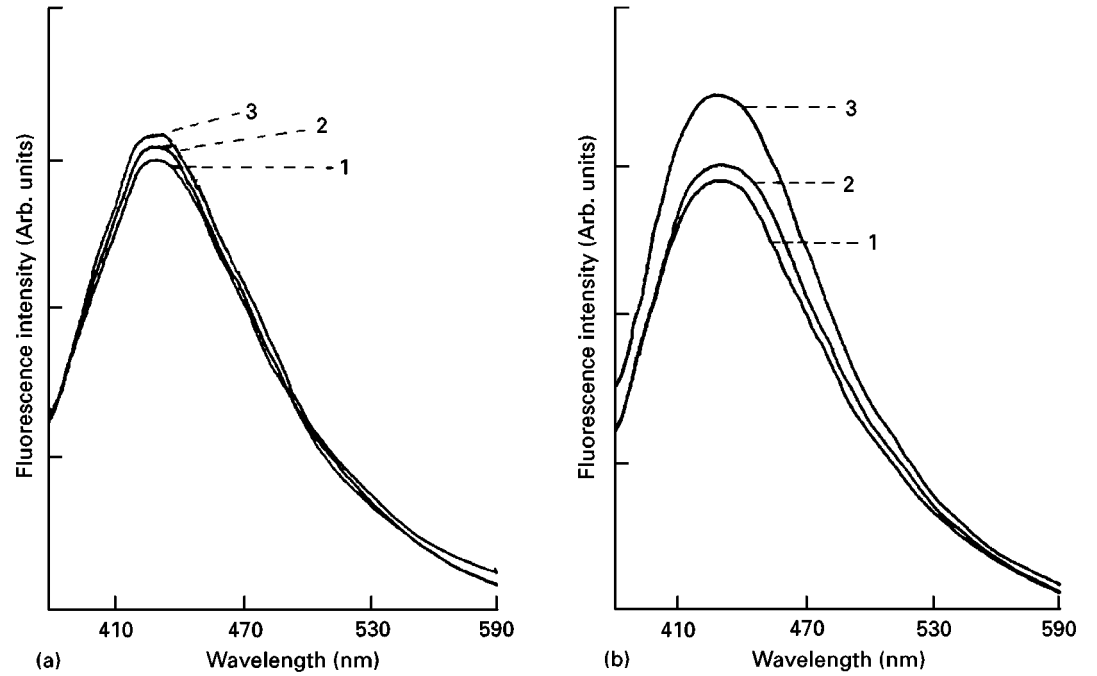


Figure 5 Fluorescence spectra of PF in chitosan solution (a) before and (b) after polarization with concentrations of 5 mg ml^{-1} at the NaCl concentrations of (1) 0 M, (2) 0.01 M, (3) 0.1 M in 0.1 M acetic acid.

than in 0.1 M acetic acid. The probe compound PF could only detect the polarity of 0.1 M hydrochloric acid solution. The results showed that at 515 nm, the PF fluorescence peak was stronger than in acetic acid at the same chitosan concentration. After polarization, the high polarization store induced by the –OH dipole group and bound water in the chitosan molecule reduced the repelling action of $-NH_3^+$ in the chitosan chain, so the PF fluorescence peak at 515 nm became weaker than before polarization at lower chitosan concentrations. This also proved that polarization can enhance the aggregation behaviour of chitosan in an

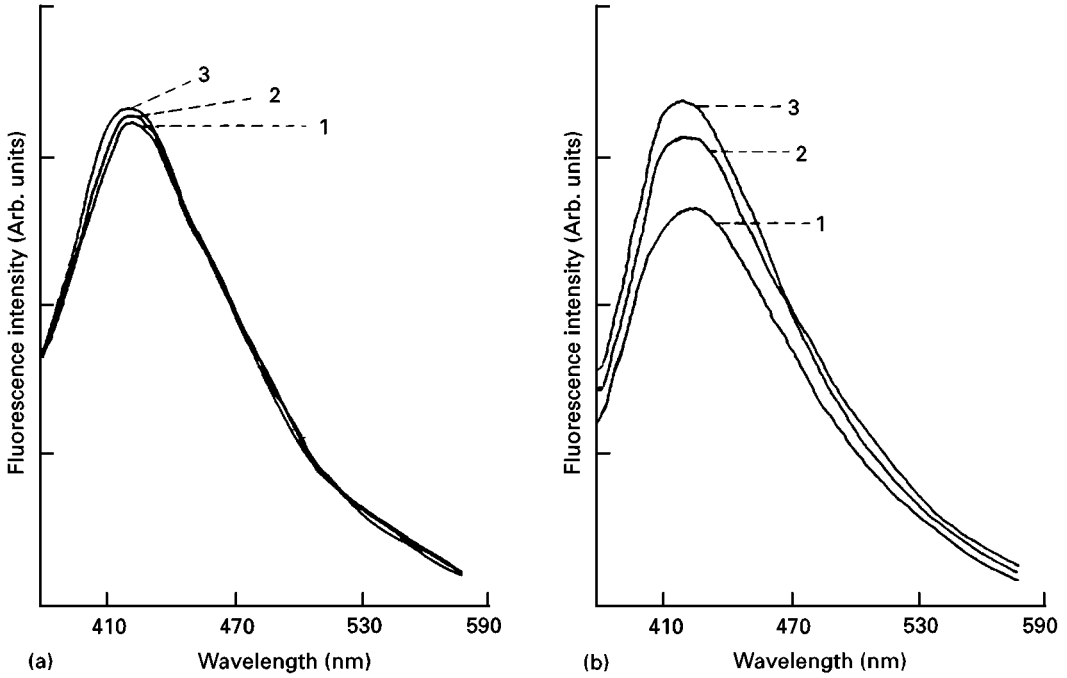


Figure 6 Fluorescence spectra of PF in chitosan solution (a) before polarization and (b) after polarization with concentrations of 5 mg ml^{-1} at the NaCl concentrations of (1) 0 M, (2) 0.01 M, (3) 0.1 M in 0.1 M hydrochloric acid.

aqueous solution, but the effect on 0.1 M acetic acid was more remarkable than in 0.1 M hydrochloric acid.

3.2. Influence of ionic strength on chitosan bioelectret association

To further investigate the aggregation behaviour of chitosan in an aqueous solution, the effect of ionic strength on chitosan aggregation was examined. Figs 5 and 6 show PF fluorescence spectra in 0.1 M acetic acid and 0.1 M hydrochloric acid with different ionic strengths at the chitosan concentration of 5 mg ml^{-1} before and after polarization, respectively. There was a slight increase in the peak of PF fluorescence at 430 nm when the concentration of NaCl was increased from 0 M to 0.1 M. However, after polarization, the PF fluorescence peak at 430 nm increased remarkably with increasing NaCl concentration.

Generally, for chitosan polyelectrolyte bearing a positive charge, the electric double layer was compressed in the solution when the ionic strength was increased [11], which results in a reduction of the repelling action of $-NH_3^+$ in the chitosan chain. So the aggregation behaviour of chitosan could be strengthened slightly. However, after polarization, there were two factors which enhanced the aggregation of chitosan in an aqueous solution: (1) the effect of ionic strength was the same as that before polarization; (2) the electret state of chitosan solution produced by polarization could also restrain the repelling action of $-NH_3^+$ in the chitosan chain. Thus, after polarization the increase in ionic strength could remarkably enhance the aggregation behaviour of chitosan in an aqueous solution.

4. Conclusion

The results of this study clearly show that chitosan self-associates in aqueous solution by intermolecular hydrophobic interactions, and after polarization, the association behaviour is remarkably strengthened. As a bioelectret, the self-association behaviour of chitosan will have more applications in the biomedical and pharmaceutical fields.

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