Studying Flocculation Mechanism of Chitosan with Pyrene-fluorescence Probe Method

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A peak/ratio in pyrene-fluorescence spectrum was employed to measure the polarity of micro-environment of chitosan adsorbing pyrene molecules. The authors have in the first time detected the pyrene-fluorescence spectrum of chitosan with different degrees of deacetylation (D.D.), and found the relationship between the flocculation of bentonite colloid by chitosan and the peak/ratio values of different molecular weight (M.W.) and D.D. of chitosan. We find that M.W. plays a key role in the flocculation, but D.D. has limited effect on it. From micro-environmental structure of view, it can be proved that the inter-particle bridging rather than charge neutralization dominates the flocculation with chitosan.

Keywords pyrene-fluorescence spectrum, chitosan, flocculation mechanism

Introduction

Chitin found as a major component of the exoskeleton of many crustaceans is the second most abundant naturally occurring polymer after cellulose. Chitosan is a product from *N*-deacetylation of chitin in alkaline media. It is a linear polysaccharide and composed of randomly repeating units of β-(1,4)-linked 2-amino-2-deoxy-*D*-glucopyranose and 2-acetamido-2-deoxy-*D*-glucopyranose. Depending on its degree of deacetylation (D.D.) and molecular weight (M.W.), chitosan molecule in aqueous solution is expected to have the properties of an amphiphilic molecule. The number of NH_2 in the molecule is decided by D.D., and the molecule configuration in solution depends on M.W..

Chitosan is a non-toxic, biodegradable, linear cationic polymer with large M.W.. One of many applications of chitosan is as flocculants. Knorr found that chitosan is an effective flocculant for suspended solids from various food processing wastes.[1] Researchers reported that chitosan is a good chelating agent for chelating heavy metals, such as mercury, chromium *etc*.[2-4] The authors of the paper also found that chitosan, compared to other polymer flocculants, could improve dewatering of active sludge significantly.[5]

When developing more effective flocculants, researchers have strong interests in mechanism of flocculation. There are three well known mechanisms, double-charge-layer compression, charge neutralization, and interparticle bridging, to explain de-stabilization of a colloid system and flocculation of colloid particles.[6] The former two mechanisms are based on existence of charges on the colloid surface that is more likely to lead to polarization of colloidal particles or group in solution. The later happen because mainly of "van der Waals forces" affinity among the suspended particles or even of physical "bridging".

There are some methods available to study flocculation mechanisms of polymer flocculants, for example, using scan electronic-microscope to observe the structure of a flocculant,[7] measuring Zeta potential with a Zeta meter,[8] measuring (squared) end-to-end distance of a polymer molecule, *etc*. Huang, *et al*. conducted a series of flocculation tests of suspended bentonite solutions at different pH with chitosan.[9,10] They inferred that the charge neutralization has limited effect on formation of flocs while inter-particle bridging dominated the flocculation process. Other researches also assumed inter-particle bridging to be the main mechanism for chitosan flocculation.[11]

Recently, Mansoor and Kuen *et al*. applied so-called "fluorescence-probe" method to study the gathering and flocculating behaviors of chitosan in aqueous solution, and to study the gel formation of chitosan.[12-14] Pyrene is used as the fluorescence probe because of its high sensitivity to polarity of the surrounding micro-environment. Pyrene-fluorescence-probe method provides a spectrum with two characteristic peaks at 372 nm (peak I) and 384 nm (peak III), which are correspondingly related to electronic vibration of a pyrene molecule. Furthermore, the strength of the vibration is closely related to the polarization degree of the micro-environment around the pyrene molecules. Dualeh and Stainer found that in a polarized solution there exists an enhanced peak only at 372 nm, not at 384 nm.[15] Therefore, the peak III/I ratio can be used to represent a polarization change of the micro-environment of the solution in which chitosan is dissolved.

The aim of the paper is trying to explain the flocculation mechanism of chitosan by studying the relationship between the chitosan flocculation behavior and the polarity of micro-environment of the chitosan solution. The pyrene-fluorescence-probe method is adopted. The peak/ratio val-

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ue of pyrene-fluorescence spectrum is measured to evaluate the polarity of micro-environment of chitosan adsorbing pyrene molecules. And the explanation for the mechanism is drawn based on the obtained evidences.

Materials and methods

Materials

Chitin made from Qingdao Chemical Co. was heated to 90 ℃ in an alkaline media to achieve different partially D.D. of chitosan, and was further oxidized with an oxidant to obtain different M.W.. Bentonite was laboratory grade. Pyrene purchased from Sigma Chemical Company was dissolved in pure methanol, and then mixed with a filtered chitosan solution to obtain a final concentration of 2.0 μg/L. All other agents used in the experiment were laboratory grade.

Methods

Preparation of colloid solution

The colloid solutions with desired turbidity were prepared by mixing 1 g of powdered bentonite into 1 L of deionized-water in a beaker. To ensure the bentonite particles be wetted, the mixture was strongly stirred with a magnetic stirrer for 3 h to homogenize the suspension and then stewed for 24 h prior to use. NaCl was added to maintain the ionic strength at 10^{-3} mol/L. The pH of system was adjusted and maintained at a range of neutral pH 6.5 to 7.5 with 0.75 mol/L HCl or 0.75 mol/L NaOH. The upper colloid solution in the beaker was taken out for the experiment and the settled particles at the bottom were abandoned. The particles size distribution of the colloid solution was measured by Mastersizer 2000 Particle Size Analyzer (Malnern Co. England), the result is 0.04—0.1 μm, 27.11%; 0.1—0.2 μm, 39.92%; 0.2—1 μm, 31.44%; 1—2 μm, 1.53%.

Determination of molecular weight and degree of deacetylation

The M.W. of chitosan was determined in terms of Mark-Houwink equation $[\eta] = k(\mathrm{M.W.})^{\alpha}$. Apparent viscosity was measured by Ubbelohde viscometer, and then intrinsic viscosity $[\eta]$ was calculated by extrapolation. k and α are the coefficients which are referred to the literature.[16]

The D.D. was determined by acid-base titration. 0.5 g of chitosan was accurately weighed and dissolved in 30 mL of 0.1 mol/L HCl solution. 2 drops of methyl orange indicator were added. The solution was titrated with a standard 0.1 mol/L NaOH solution to pH 3.4. The results of percentage of the number of NH_2 radical were calculated to obtain the D.D.. In parallel, water content in the chitosan was measured by drying it in an oven at 105 ℃. D.D. was calculated as follows:

$$\mathrm{NH_2\%} = \frac{(c_1V_1 - c_2V_2) \times 0.016}{G(100 - W)} \times 100 \qquad (1)$$

Where c_1 nd c_2 are the concentration of the standard HCl and NaOH solution (mol/L); V_1 is the volume of adding standard HCl solution (mL) and V_2 is the volume of titrating standard NaOH solution (mL); G is the weight of sample (g); W is the water content of sample (%); 0.016 represents the number of amine corresponding to 1 mL of 1 mol/L HCl solution (g).

$$\mathrm{D.D.\%} = \frac{\mathrm{NH_2\%}}{9.94\%} \times 100\% \qquad (2)$$

Pyrene-fluorescence spectrum

The prepared pyrene solution was added into chitosan solutions with different concentrations. Pyrene emitting spectra were detected by LS55 fluorescence spectra-photometer (Perkin-Elmer Co. England). The pyrene molecules as a probe is excited at 343 nm and the emitting spectrum was measured in the range of 360—500 nm at an integrating time of 1.0 s. The excitation and emission slit openings are 15 and 2.5 nm, respectively.

Flocculation test

A conventional jar test apparatus (6-Joint Mixer) was used for the flocculation test. The desired pH of the mixture solution was adjusted and maintained with 0.1 mol/L NaOH or 0.1 mol/L H_2SO_4. A planed dose of the chitosan was added to the bentonite colloid solution, then the solution was rapidly stirred at 200—300 r/min for 1 min, followed a slow mixing (60—80 r/min) for 5 min. Thereafter, the particles in the colloid solution were settled for 15 min, then the upper solution located at 1 cm were taken for turbidity measurement by Model SZD-1 turbidimeter (Shanghai Water Works Co., China) following the standard nephelometry method.

Results and discussion

Flocculation

In the experiment, bentonite solution was mixed with chitosan upon the parameters described as above. Applied four types of chitosan with four D.D. of 69%, 75%, 84% and 92% and a same M.W. of 300000. Fig. 1 provides the experimental results with the X-axis of the dose of chitosan in mg/L and the Y-axis of the residual turbidity in nephelometric turbidity unit (NTU).

First observation from the figure is that the higher dose of chitosan produces better flocculation. For example, 2.5 mg/L of D.D. 84% chitosan produces a turbidity of 25 NTU and 5 mg/L of this chitosan generated only 5

NTU. Similarly, 5 mg/L of D.D. 69% results in 95 NTU, 10 mg/L of the chitosan in 63 NTU, 15 mg/L of the chitosan in 30 NTU and 20 mg/L of the chitosan in 10 NTU.

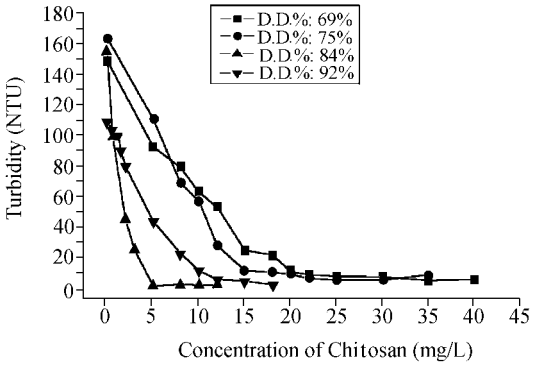

Fig. 1 Flocculation effect of chitosan with different D.D..

Secondly, comparing the D.D. and flocculation effect reveals that the higher D. D. chitosan generates higher flocculation efficiency. At the dose of 5 mg/L, chitosan of D.D. 69% and 75% produce a turbidity of 95 and 110 NTU, respectively, while chitosan of D. D. 92% and 84% yield 44 and 5 NTU, respectively. At a 10 mg/L dose, D.D. 69% and 75% yield 70 and 60 NTU, respectively, while D. D. 92% and 84% turn out 22 and 5 NTU, respectively. The results reveal that, generally, a chitosan of higher D.D. performs better than one with lower D.D.. However, the result difference among the four is not significant, particularly between the D.D. of 69% and 75%. Thus, a preliminary conclusion can be drawn that the D.D. of chitosan has limited effect on the flocculation.

On the other hand, Fig. 2 depicts a relation between chitosan M.W. and flocculation effect. The *X*-axis in the figure represents dose of added chitosan while the *Y*-axis as residual turbidity. The chitosan of four different M.W. have a same D.D. of 69%.

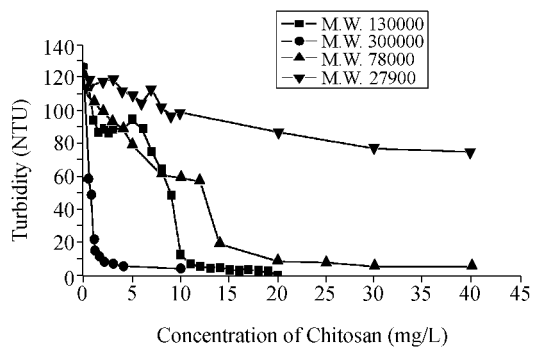

Fig. 2 Flocculation effect of chitosan with different M.W..

Fig. 2 reveals that larger M.W. of chitosan need lower minimum dose to achieve a good turbidity, *e.g.* 7 NTU. The highest M.W. of 300000 produces the good result at only a 2.5 mg/L dose, the second high M.W., of 130000 needs 12 mg/L, the third M.W. of 78000 achieves the same NTU at more than 25 mg/L. Unfortunately, the chitosan of the lowest M.W. of 27900, although at the highest dose of 40 mg/L, fails to achieve a good result with the worst of about 80 NTU, suggesting a minimum M.W. of chitosan required for flocculation.

All above results suggest that the M.W. of chitosan is a dominating parameter for flocculation, and the D.D. is a limited factor.

Pyrene fluorescence spectrum

Fig. 3 presents a fluorescence-emitting spectrum of pyrene solutions that dissolved in 1% acetic acid and different concentrations of chitosan with a M.W. of 300000 and a D.D. of 69%. The *X*-axis is wavelength, λ, in nm and the *Y*-axis the fluorescence density in percentage of transmit/incident. The concentrations of chitosan are (A) 0.01 g/L, (B) 0.1 g/L, (C) 1.0 g/L and (D) 2.0 g/L, respectively.

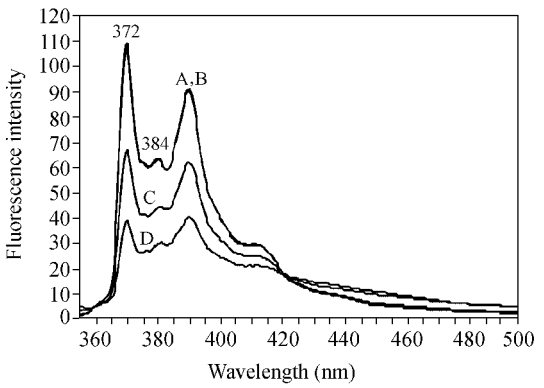

Fig. 3 Fluorescence spectra of pyrene in chitosan solution with concentrations of (A) 0.01 g/L; (B) 0.1 g/L; (C) 1.0 g/L; (D) 2.0 g/L in 1% acetic acid. Pyrene is dissolved in chitosan solution at a final concentration of 2×10^{-6} mol/L.

Comparing four curves in Fig. 3 clearly shows that the intensity of the spectrum is being enhanced with decreasing of the chitosan concentration. However, there is no further substantial enhancement in the spectrum when the concentration of chitosan becomes low enough, *i.e.* 0.1 g/L or lower 0.01 g/L. From the two concentrations of curve A and B, it can be calculated that the peak III/I ratio value is about 0.60, equivalent to the peak/ratio value from a water or other types of strong polarizing solutions. This result can be explained as that the solution is a very polarized system in which chitosan molecules are not gathering at the low concentration and pyrene molecules added that are partially hydrophobic spread evenly in the polarized environment. With increasing of the chitosan concentration, the pyrene fluorescence peak at 372 nm is decreasing faster than the peak at 384 nm. This phenomenon is due to gathering of chitosan molecules at high-

er concentration that leads to creating some hydrophobic micro-pockets where pyrene molecules could be gathered due to its hydrophobic nature.

For the tendency of gathering of chitosan in water solution, it can be explained at molecular level. Partially D.D. chitosan is composed of multi-2-amino-2-deoxy-*D*-glucopyranose and 2-acetamido-2-deoxy-*D*-glucopyranose. Many potentially radical amino-groups in a chitosan molecule are able to combine with protons in a diluted acidic solution, which leads chitosan to a polyhydrolyte with positive charges. In a weak acidic solution, most of free-amino-group (NH_2) are easily changed to (NH_3^+). These groups with the same positive charges repulse each other, which results in linearly expanding chitosan molecules in the solution.

The added pyrene molecules can act as probes to polarization of the micro-environment in dilute acidic solution.[17-19] With increasing of the chitosan concentration, more chitosan molecules capture the protons (H^+), leading to a weak repulsive force among the NH_3^+ groups on the molecule chain of chitosan and consequently a weak polarization within and around the chitosan molecules. All these factors combine together and result in gathering and, finally, flocculating of chitosan molecules.

Fig. 4a and 4b illustrate the relations between the peak/ratio value of pyrene-fluorescence spectrum and the concentration of chitosan. The *X*-axis represents concentration of chitosan in log (g/L) and the *Y*-axis of the peak/ratio. In Fig. 4a, the two chitosan with the same M.W. of 300000 but different D.D. of 84% and 69% are used, and in Fig. 4b with the same D.D. of 69% but different M.W. of 27900, 125000 and 300000.

From the curves in Fig. 4a, it can be seen that the higher chitosan concentration results in higher peak/ratio value, or the weaker polarization in the micro-environment of the solution. When the concentration of chitosan increases from lg (g/L) − 2.0 (= 0.01 g/L) to 0, the peak III/I ratio value increases from 0.56 to 0.66. When the concentration increases further to lg (g/L) 0.25, the corresponding peak III/I ratio value is 0.76. However, the difference of D.D. shows little effect on the polarization of the micro-environment in terms of the peak/ratio value. From Fig. 4b, it is observed that, under high concentrations, the chitosan M.W. has an effect on the polarization. For the smaller M.W. of chitosan, its pyrene-fluorescence peak/ratio value increases (weaker polarized micro-environment) when the chitosan concentration increases. With larger M.W. chitosan when the chitosan concentration is changing, the peak/ratio value has slight changes between 0.58—0.76. The reason is that the chitosan molecule is semirigid and not flexible. The effect of space impediment is obviously exist and the molecules are not easy to get closer and together, so it has little effect on the polarization of solution. But the smaller chitosan molecules are easy to get closer and together, to gather through integrating of glucopyranose chains, to weaken the polarization of micro-environment around pyrene probe.

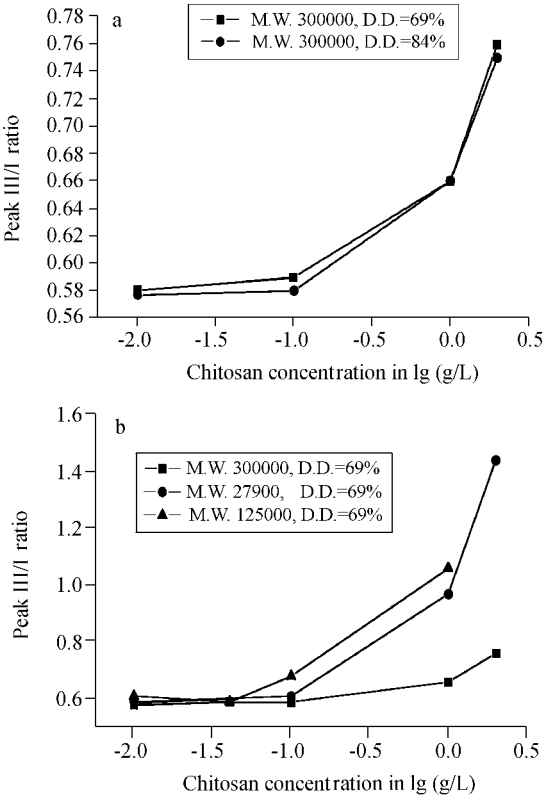

Fig. 4 Peak III/I ratio of pyrene fluorescence as a function of chitosan concentration. a: the same molecule but different D.D.%; b: the same D.D.% but different molecules.

On the other hand from Fig. 4b, it is noticed that the peak/ratio value in a low concentration of chitosan solution has almost no change when chitosan M.W. changes and stays at 0.6, a polarized micro-environment. It should be emphasized that this concentration of chitosan is just equivalent to the dose for chitosan to be used as flocculant.

It is known that the pyrene-fluorescence emitting spectrum method is very sensitive to the polarization of a micro-environment in a solution. And the density of charges on the molecular chain of the dissolved polymer determines the strength of the polarization of the micro-environment. It is also known that the density of charges on the polymer molecules determines the neutralization capability of the opposite charges in the solution. It is clear, the stronger the polarization of micro-environment is, the more capable of the charge neutralization.

When the concentrations of two chitosan solutions, one with a larger M.W. and the other with a smaller M.W., are equal, the total number of monomers in each solution should, by stoichiometry, equal each other although the numbers of the polymer molecules respectively in the two solutions are not equal. Under the circumstance, if the D.D. of two chitosan with different M.W. equals each other, the number of total free NH_2 groups for the two should be same, thus their capacities of charge neutralization are same.

Recalling what is observed in Fig. 2 is helpful. In

the figure, the four chitosan are with a same D.D. of 69%, which means all four has a same capacity of charge neutralization under a same concentration. Obviously, the chitosan of the largest M.W. of 300000 produces the best flocculation result at the lowest concentration that is within a very polarized micro-environment found by the pyrene-fluorescence spectrum, while the chitosan of the smallest M.W. of 27900 generates the poorest flocculation performance also at a low concentration found as the polarized micro-environment, or with large capacity of charge neutralization. Comparing the two results reveals that the very polarized micro-environment, or a large capacity of charge neutralization helped little for flocculation, but the large M.W. does. This comparison leads to a conclusion that the inter-particle bridging capability of high M.W. chitosan should be the main flocculation mechanism for chitosan.

Conclusion

From the above analysis, some conclusions can be drawn:

(1) The D.D. of chitosan has limited effect on the flocculation. Instead, the M.W. of chitosan dominates the flocculation process. The larger the M.W. is, the better the flocculation.

(2) Based on the pyrene-fluorescence spectrum, the D.D. of chitosan has little effect on the polarization of micro-environment, and at low concentrations, the micro-environment of chitosan with larger M.W. is very close to that with smaller M.W..

(3) For the same D.D. and M.W., the concentration of chitosan does affect the characteristic peak/ratio value or the polarization of micro-environment. The higher the concentration is, the higher peak/ratio value or the weaker polarization of micro-environment.

(4) It has been proved by the pyrene-fluorescence spectrum that among molecular structure factors, the inter-particle bridging rather than charge neutralization plays a key role in flocculation mechanism of chitosan.

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