

# Study of Two Chitosan Derivatives Phosphorylated at Hydroxyl or Amino Groups for Application as Flocculants

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**ABSTRACT:** Two phosphorylated chitosan derivatives with a similar degree of substitution of about 0.5 were synthesized via either the esterification of chitosan's hydroxyl groups by  $P_2O_5$  in methanesulfonic acid or the selective incorporation of methylene phosphonic groups on chitosan's amino groups by formaldehyde/ $H_3PO_3$  in water. The performances of the resulting *O*-phosphorylated chitosan (OPC) and *N*-methylene phosphonic chitosan (NMPC) as amphoteric flocculants were assessed in comparison with that of unmodified chitosan. In turbidity removal experiments using kaolin suspensions in water, OPC removed about 90% turbidity, but NMPC only removed 60%. The

lower flocculation efficiency of NMPC was attributed to the decreased acid strength of the *N*-methylene phosphate groups in NMPC. These results revealed that the acidity of the phosphate groups introduced in the modified chitosans is an important factor with regard to the flocculation efficiency, and consequently that *O*-phosphorylation is more suitable than *N*-phosphorylation for preparing chitosan-based amphoteric flocculants. © 2012 Wiley Periodicals, Inc. *J Appl Polym Sci* 000: 000–000, 2012

**Key words:** chitosan; phosphorylation; wastewater treatment; flocculation

## INTRODUCTION

Chitosan is an amino-polysaccharide obtained by deacetylation of chitin, which is a biopolymer and can be easily manufactured from crab and prawn shells. Chitosan is a copolymer of glucosamine and *N*-acetyl glucosamine. The protonation of the amine groups in the polysaccharide backbones produces many  $NH_3^+$  groups, which is the reason why the polymer dissolves in aqueous solutions of organic acid such as acetic acid and formic acid. Indeed, with an intrinsic  $pK_a$  close to 6.5, pure chitosan is fully protonated at a pH close to 5.<sup>1</sup> Therefore, chitosan with a high degree of deacetylation (DDA) is a cationic polyelectrolyte when dissolved in acidic solutions, and it can be used for the removal of dissolved contaminants and suspended colloids by flocculation.<sup>2</sup> Flocculation of chitosan was reported since 1970s<sup>3</sup> and has recently been recognized to be an interesting subject.<sup>4</sup>

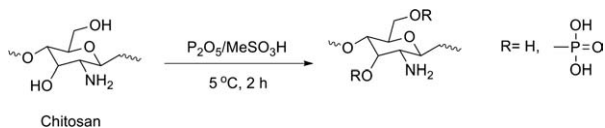
However, the poor solubility of chitosan when pH > 6.5 is a serious drawback in water-treatment applications. Thus, the chemical modification of chi-

tosan has been envisaged to overcome its limited solubility in aqueous media. Chitosan has three types of reactive functional groups: a primary amino group at the C2 position, and primary and secondary hydroxyl groups at the C3 and C6 positions, respectively. The reactions of chitosan are considerably more diverse than cellulose due to the presence of the amine groups. Apart from etherification and esterification which occur at the OH groups, chitosan undergoes chemical reactions such as acetylation, quaternization, and alkylation via the amino functionality. Therefore, there are considerable opportunities for the chemical modification of chitosan. Numerous efforts have been made to improve the flocculation efficiency of chitosan by its chemical modification. For example, quaternized chitosans<sup>5</sup> and polyelectrolyte-grafted chitosans<sup>6–8</sup> have been found to be efficient flocculants in water-treatment applications.

Among the chemical modification methods used for chitosan, phosphorylation (on  $NH_2$  and/or OH group) in particular has attracted much attention.<sup>9,10</sup> Phosphorylated chitosan was firstly prepared by the reaction of chitosan with phosphorous pentoxide in methanesulfonic acid (Scheme 1).<sup>11,12</sup> In the reaction mixture, methanesulfonic acid plays two roles: dissolving the chitosan and catalyzing the esterification of the OH groups. *O*-phosphorylated chitosan (OPC) is the main product in the modifying process. Recently, a novel *N*-methylene phosphonic chitosan (NMPC) was prepared by using phosphorous acid

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**Scheme 1** Syntheses of OPC.

and formaldehyde.<sup>13–15</sup> This is a simple way to phosphorylate amino groups of chitosan (N-phosphorylation). The amino phosphonate residues are formed by the attack of phosphonic acid on the C=N Schiff bases, which result from formaldehyde and the amino group of chitosan (Scheme 2).

Although the structure, antibacterial activity, toxicity, and a number of biotechnological applications of the phosphorylated chitosan derivatives have been discussed elsewhere,<sup>16</sup> their application in water-treatment has not been reported until now. By introducing phosphate groups, chitosan became an amphoteric polyelectrolyte, having both cationic and anionic groups. They may be good flocculants over a wide pH range because they can coagulate both positive and negative particles. Clearly, the flocculation efficiency of phosphorylated chitosans is strongly dependent on their structural characteristics, mainly the degree of substitution (DS) and the locus, that is the amino or hydroxyl group.

In the present article, two phosphorylated chitosan flocculants with a similar DS values were prepared by the methods described in Schemes 1 and 2, respectively. Their flocculation efficiencies with regard to removal of turbidity from water were evaluated by using kaolin suspensions as the simulated wastewater. The relationship between the chemical structure and the flocculation efficiency was further analyzed.

## EXPERIMENTAL

### Materials

Chitosan (DDA, 91%; MW, 1000 kDa), polyacrylamide (PAM, MW, 8000 kDa) and kaolin particles (average size, 5  $\mu\text{m}$ ) were purchased from Excel Asia Enterprises (Shanghai, China). All deuterated reagents were obtained from Meryer Chemical Technology (Beijing, China). All other chemicals are of analytical grade and were purchased from Aladdin Reagent Co. and J&K Chemical Ltd (Shanghai, China).

### Characterizations

Infrared spectra were recorded on a Fourier transform infra-red (FTIR) spectrometer (Nicolet Avatar 370, Thermo Nicolet Corporation, Madison, Wisconsin) with a resolution of 4  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  spectra of

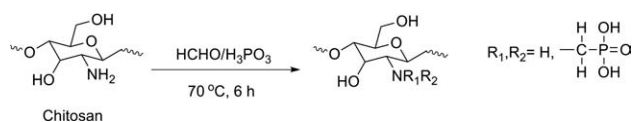
solutions in  $\text{D}_2\text{O}$  of chitosan derivatives were recorded at 400 MHz with a Digital FT-NMR Spectrometer (Avance AV, Bruker, Switzerland). Visible transmittance was measured with a visible spectrophotometer (SP-722E, Shanghai spectrum instruments co, Shanghai). Elemental analysis of P was carried out with an inductively coupled plasma optical emission spectrometer (ICP-OES, IRIS Intrepid II XSP, Thermo Electron Corporation, US). Viscometric measurements of the phosphorylated chitosan solutions (0.1 g in aqueous solution of acetic acid (100 mL, 1.0%, v/v)) were carried out with a rotary viscosimeter (NDJ-8S, Shanghai Yueping Scientific Instrument, Shanghai) at  $(25 \pm 0.1)^\circ\text{C}$ , and all viscosity measurements were repeated at least twice in order to check the reliability of the data which was within  $\pm 3\%$ . The  $M_w$  and  $M_n$  of the modified chitosans were measured by gel permeation chromatography (GPC) on a Waters-515 apparatus using water as eluent. The morphology of the kaolin particles and their flocs induced by the modified chitosans in kaolin suspension were observed by a scanning electron microscope (JSM-5610V, JEOL). Samples were coated with Au before SEM analysis. The zeta potential of kaolin suspensions in the presence and absence of flocculant was measured with a Zetasizer Nano-ZS (Malvern Instruments, Malvern, England). The reading of the supernatant zeta potential was performed after a settling time of 2 min.

### Synthesis of phosphorylated chitosan (OPC)

OPC was synthesized as reported earlier.<sup>11,12</sup> Chitosan powder (4.0 g,  $\text{NH}_2$ , 21.5 mmol) was mixed in methanesulfonic acid (32.0 mL), and added gradually with phosphorus pentoxide (7.2 g, 50.4 mmol). The heterogeneous mixture was mechanically stirred at  $0\text{--}5^\circ\text{C}$  for 2 h, poured into ethers for precipitation, and filtered. The solid product was washed with acetone (20 mL  $\times$  three times), methanol (20 mL  $\times$  three times), ethers (20 mL  $\times$  three times) and dried in vacuum at  $60^\circ\text{C}$  for 24 h.

### Synthesis of N-methylene phosphonic chitosan

NMPC was synthesized as reported earlier.<sup>13–15</sup> Chitosan (4.0 g,  $\text{NH}_2$ , 21.5 mmol) was mixed with aqueous solution of acetic acid (200 mL, 1.0%, v/v), stirred for 1 h at room temperature, and added dropwise a solution of phosphorous acid (2.1 g, 21.5



**Scheme 2** Syntheses of NMPC.

mmol) in water (1 : 1, v/v). Then the temperature of the mixture was raised to 70°C, and formaldehyde (21.5 mmol, 1.8 mL, 36.5%, v/v) was added. After heating for 6 h, clear pale yellow solution was obtained. The result solution was dialyzed against demineralized water for 48 h in a dialysis tube (cut-off value, 12.5 kDa) and freeze-dried.

### Flocculation processes

All the flocculation experiments were conducted at room temperature. Kaolin suspension (500 mL) in water (0.1%, w/v, pH was about 9.1) was added with a dilute HCl (0.01–0.1M) or sodium hydroxide (0.01–0.1M) solution to adjust the pH to a fixed value, then mixed with a solution (0.05–2.5 mL) of flocculant (1.0%, w/v). The mixture was agitated at a high velocity (180 rpm, 2 min), followed with a slow velocity (50 rpm, 10 min), and was settled down for 30 min. For the settling experiments, similar processes were carried out without the slow stirring step (50 rpm, 10 min). Then the upper clear liquid (the deepness was about 1.0 cm) was drawn for measuring visible transmittance at 550 nm. This flocculation process was repeated three times and the average transmittance was calculated. Residual turbidity (%) was calculated with the following equation [eq. (1)].

$$\text{Residual turbidity}(\%) = 100 \times (1 - T_f)/(1 - T_0) \quad (1)$$

where  $T_0$  and  $T_f$  are the transmittance values of the initial suspension and the supernatant solution, respectively. The value of  $T_0$  is  $0.212 \pm 0.008$ .

## RESULTS AND DISCUSSION

### Synthesis and characterization of chitosan derivatives

As shown in Scheme 1, OPC was synthesized by using phosphorous pentoxide ( $P_2O_5$ ) and methanesulfonic acid as has been reported previously.<sup>11,12</sup> It was reported that methanesulfonic acid works as a good solvent for chitosan and also acts as an efficient catalyst for the esterification reactions. Phosphorus pentoxide acts as an anhydride of phosphoric acid. Because the amino groups of chitosan are almost be protonated in the strongly acidic conditions and lose their nucleophilicity, the esterification reactions with the primary alcohols at the C6 position dominate in this case. NMPC was prepared through a selective N-phosphorylation strategy based on the Mannich-type reaction.<sup>13–15,17</sup> Chitosan was reacted with formaldehyde in an aqueous medium, and the resulting C=N Schiff bases were further reacted with phosphoric acid to give amino

**TABLE I**  
The Phosphorylation Details and Some Properties of the Two Phosphorylated Chitosans

Phosphorylated chitosan	OPC	NMPC
Reaction details		
Reaction	Scheme 1	Scheme 2
Dosage (P/N, mol/mol)	1.56	0.72
Reaction time (h)	2.0	6.0
Temperature (°C)	5	70
Medium acidity	Strong	Middle
Reaction phase	Solid and liquid heterogeneous	Liquid homogeneous
Analysis results		
Yield (%)	83	95
DS <sup>a</sup>	0.58	0.53
Viscosity <sup>b</sup> (mPa s)	82	224
$M_w$ (kDa) <sup>c</sup>	13	870
$M_n$ (kDa) <sup>c</sup>	10	580
Preferential positions	-OH groups C6, C2	-NH <sub>2</sub> groups C3

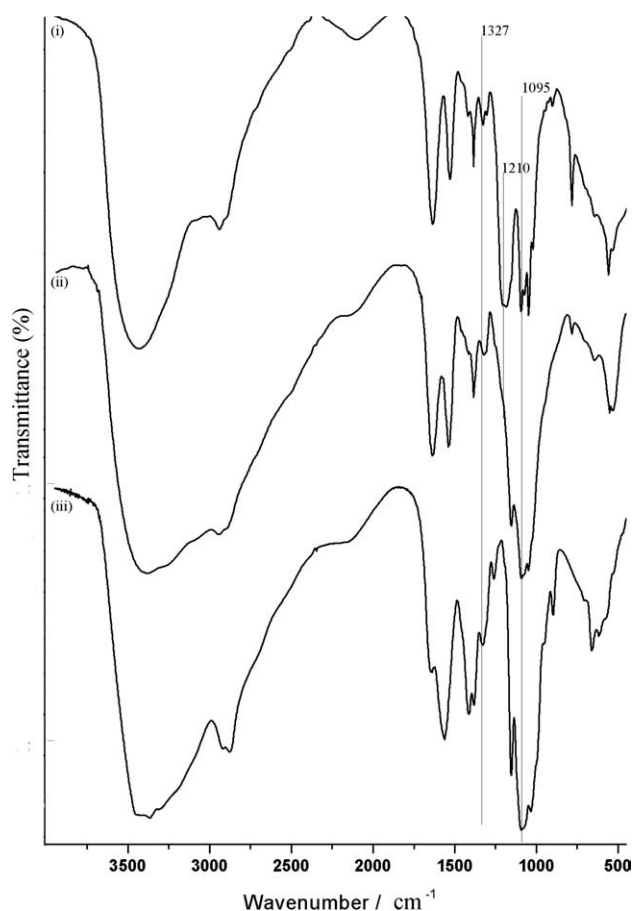
<sup>a</sup> DS value was calculated from elemental analysis of P in the phosphorylated chitosans.

<sup>b</sup> The viscosity data were measured from the aqueous solutions of the phosphorylated chitosans (0.1 g in aqueous solution of acetic acid (100 mL, 1.0%, v/v)).

<sup>c</sup>  $M_w$  and  $M_n$  were obtained from GPC method.

phosphorylated chitosan derivatives. Therefore, the two phosphorylation methods used in this study are selective, respectively, for the hydroxyl and amino groups, and they result in chitosan derivatives with different chemical structures. The reaction conditions of the two methods are compared in Table I. As in other reactions of anhydride with alcohols, an excessive dosage of the phosphorylation reagent is needed in the case of OPC. But for the NMPC with a similar DS value, only half the amount of the phosphorylation reagent was used. The DS values of the chitosan derivatives were calculated from the results of P element analysis of the phosphorylated chitosans, and it was found that the DS values (Table I) were in good agreement with the previous reports.<sup>12,13</sup> In contrast with the homogeneous aqueous medium of the reaction for NMPC, the OPC was produced in a heterogeneous environment that contained a solid phase (chitosan and phosphorus pentoxide) and a liquid phase (methanesulfonic acid). Thus, the degradation of chitosan in the liquid phase of almost pure methanesulfonic acid was unavoidable. Although the temperature of the reaction system was controlled at less than 5°C, as shown in Table I, the viscosity of OPC was significantly lower than that of NMPC. The GPC analysis results also showed that the average molecular weights of NMPC are about 60 times more than those of OPC.

Figure 1 shows the FTIR spectra of OPC (i), NMPC (ii), and chitosan (iii). The different chemical structures between OPC and NMPC were confirmed.

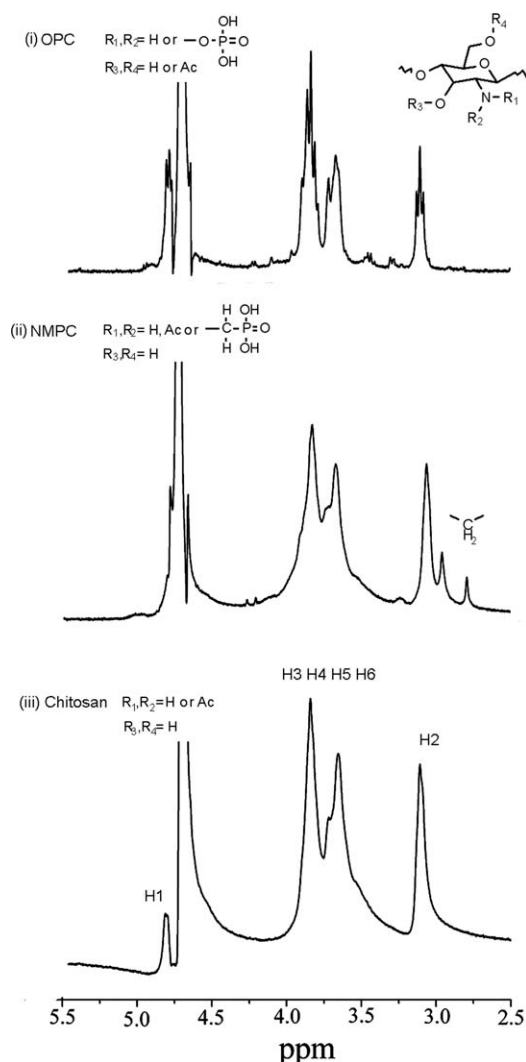


**Figure 1** FTIR spectra of OPC (i), NMPC (ii), and chitosan (iii).

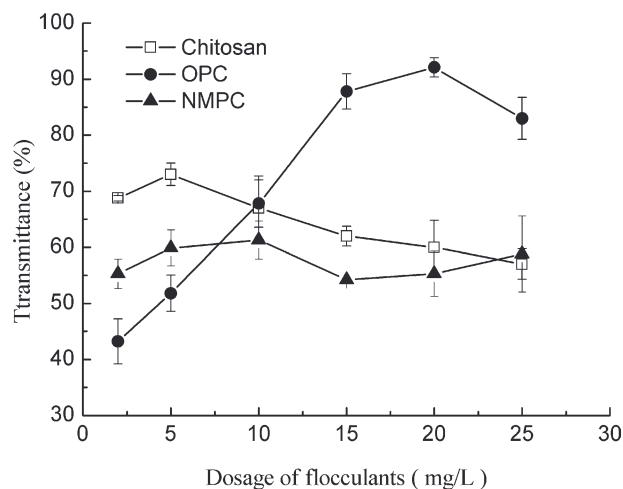
The spectrum of unmodified chitosan showed characteristic peaks of amide II at  $1575\text{ cm}^{-1}$  (N—H in plane deformation coupled with C—N stretching),  $1327\text{ cm}^{-1}$  (C—N stretching coupled with NH in plane deformation),  $1157\text{ cm}^{-1}$  (bridge O stretching), and  $1095\text{ cm}^{-1}$  (C—O stretching). The broad peak observed at  $3200\text{--}3500\text{ cm}^{-1}$  was attributed to the inter- and intra-molecular hydrogen bonding of  $\text{—NH}_2$  and the  $\text{—OH}$  stretching vibration of chitosan molecules, namely the hydrogen-bonded OH stretching at  $3455\text{ cm}^{-1}$ , the  $\text{NH}_2$  asymmetric stretching at  $3390\text{ cm}^{-1}$ , and the NH stretching (in hydrogen bond) at  $3290\text{ cm}^{-1}$ . For the two phosphorylated chitosan derivatives, the peaks at  $1645\text{ cm}^{-1}$  and  $1535\text{ cm}^{-1}$  were attributed to  $\text{NH}_3^+$  groups, (antisymmetrical deformation and symmetric deformation). The initial amide I and II bands were possibly overlapped by these vibrations. The phosphorylations also led to the appearance of a new peak at  $1210\text{ cm}^{-1}$ , which was attributed to the  $\text{P=O}$  asymmetric stretching in the phosphates. Moreover, a clear decrease in the absorbance of the peak at  $1095\text{ cm}^{-1}$ , which was due to the hydroxyl group of chitosan, and the appearance of new peaks at  $1075\text{ cm}^{-1}$ , which was assigned to the C—O—P stretching in

phosphate esters, were observed in the FTIR spectrum of OPC (i). These results showed that the phosphorylation mainly occurred at the OH groups in chitosan (C3 and C6), which is in good agreement with previous reports.<sup>11,12</sup> As shown in Scheme 2, NMPC was prepared through a selective N-phosphorylation strategy based on the C=N Schiff reaction.<sup>13–15,17</sup> Chitosan was reacted with formaldehyde in an aqueous medium and the resulting C=N Schiff bases were further reacted with phosphoric acid to give amino phosphorylated chitosan derivatives. The peak at  $1327\text{ cm}^{-1}$  (C—N—H bending) for NMPC was clearly smaller than that of OPC, suggesting that lots of the amino groups were substituted by phosphoric acid.

The 400 MHz  $^1\text{H-NMR}$  spectra of NMPC, OPC, and chitosan are shown in Figure 2. The basic assignment of the chitosan resonance is that: signal of 4.8 ppm is the resonance of H1, the peaks in the range of 3.5–4.0 ppm are H3–H6 protons, and the



**Figure 2**  $^1\text{H-NMR}$  spectra of OPC (i), NMPC (ii), and chitosan (iii).



**Figure 3** The flocculant dosage of the flocculation effect for OPC, NMPC, and chitosan (pH = 9.0).

signal of 3.1 ppm is H<sub>2</sub> proton resonance. These resonances can be found in the <sup>1</sup>H-NMR spectrum of chitosan described in previous reports.<sup>13,18</sup> In the case of NMPC, two additional peaks at 3.0 and 2.8 ppm are appeared in the NMR spectrum. They are attributed to the methylene structures in the mono-phosphonic secondary amine (–NH–CH<sub>2</sub>–PO<sub>3</sub>H<sub>2</sub>) and the tertiary diphosphonic amine (–NH(–CH<sub>2</sub>–PO<sub>3</sub>H<sub>2</sub>)<sub>2</sub>). In contrast with the NMR spectrum of chitosan, the NMR spectrum of OPC has very separated peaks at the positions assignable to the protons in the chitosan units. This result suggests that the introduction of the phosphonic acid function in the chitosan macromolecule mainly occurred at the hydroxyl groups.

As a result, the two phosphorylation methods compared in this study gave two phosphorylated chitosan derivatives. They had similar DS values and water-solubility, but a different degree of degradation and different chemical structures. The flocculation effects of the phosphorylated chitosans were therefore of interest and were assessed by comparing them with the unmodified chitosan.

#### Flocculation effects of the phosphorylated chitosans

Turbidity removal tests were carried out by using three flocculants: OPC, NMPC, and chitosan. Two commercial flocculants, PAM and aluminum sulfate (AS) were also used as the references in parallel flocculation experiments. As shown in the Figure 3 and Table II, the flocculation efficiency of chitosan was significantly improved by the O-phosphorylation, but NMPC showed an ordinary flocculation behavior. The transmittance value of the kaolin suspension treated with NMPC was obviously lower than that treated with OPC and even than that treated with

**TABLE II**  
The Optimized Dosage and the Maximum Turbidity Removal Results of the Flocculants<sup>a</sup>

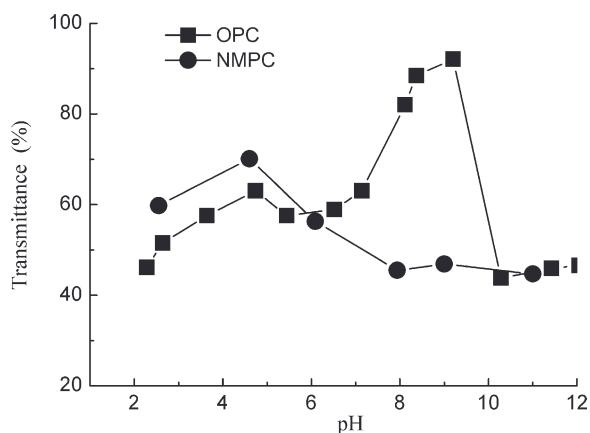
Flocculant	Dosage (mg/L)	Residual turbidity (%)
Chitosan	5.0	34.3
OPC	20.0	10.1
NMPC	10.0	40.1
PAM	5.0	16.5
Aluminum sulfate	5.0	21.6

<sup>a</sup> Residual turbidity (%) was calculated from transmittance with the eq. (1); the experiments were carried out at pH 9.0.

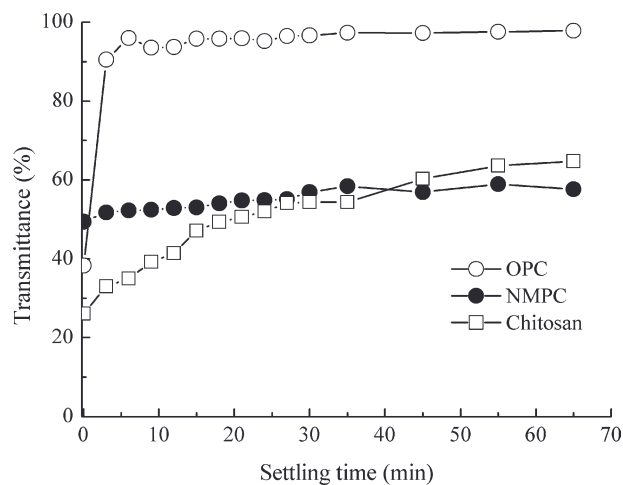
chitosan. Because excessive flocculant would make the colloidal particles steady and difficult to precipitate, there was an optimum dosage for each flocculant, as shown in Figure 3. The lowest residual turbidity rates for each flocculant are displayed in Table II. OPC removed almost 90% turbidity from the kaolin suspension with an optimum dosage of 20.0 mg/L, but NMPC only 60%. These results indicate that the flocculation efficiency of OPC was far superior to that of chitosan and NMPC. High-molecular weight PAM and AS are the flocculants that have been used traditionally in water-treatment. OPC also showed better flocculation efficiency than these substances.

The pH dependence of the flocculation effect for the phosphorylated chitosans was examined using their optimum dosages (Fig. 4) in a wide pH range. As presented in Figure 4, OPC showed an outstanding effect in removing turbidity between pH 8 and 10. The maximum efficiency of OPC was observed at pH 9.2, when about 90% turbidity was removed. In the case of NMPC, the flocculation efficiency did not alter significantly with increasing pH, and the maximum removal of turbidity was obtained at pH 4.6.

Figure 5 displays the impact of settling time on the flocculation of a kaolin suspension in the



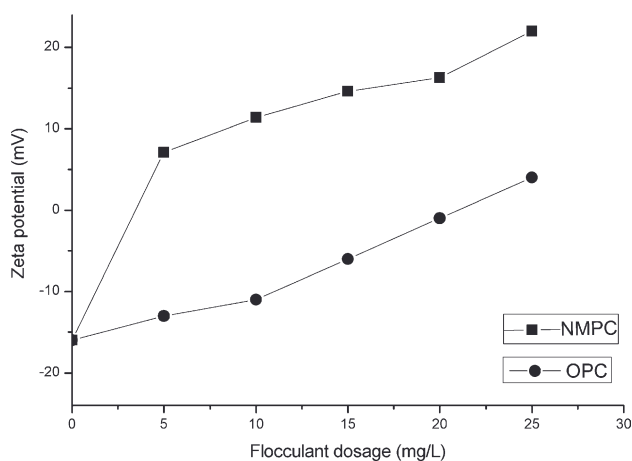
**Figure 4** The pH dependence of the flocculation effect for OPC and NMPC.



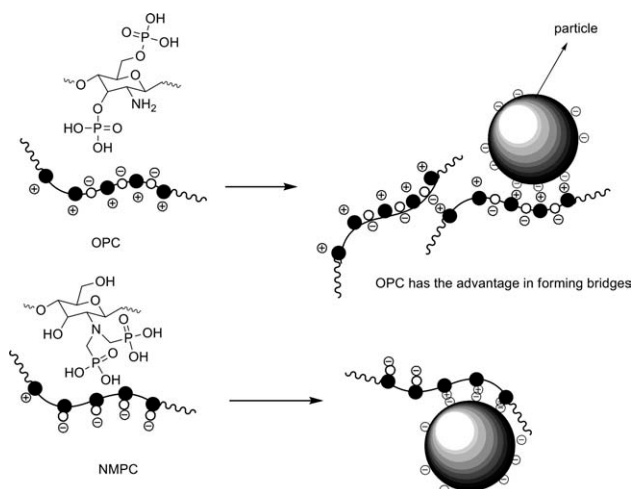
**Figure 5** The transmittance vs. the settling time for the kaolin suspensions in the presence of OPC, NMPC, or chitosan.

presence of the flocculants, at their optimum dosages. With chitosan the natural decantation of the kaolin suspension was very slow, and the transmittance value of the supernatant solution did not exceed 65% even after 60 min of settling. The settling time was significantly reduced by replacing chitosan with OPC. It was found that the increase in transmittance was more pronounced in the first 5 min, and that transmittance reached 95%. However, for NMPC, the turbidity removal effect became constant after the first 3 min, and the transmittance value did not reach more than 60%.

Figure 6 shows the zeta potential of kaolin suspension as a function of flocculant dose. Prior to flocculant addition, the kaolin suspension has a zeta potential of  $-16.0$  mV. With increasing the dosage of both NMPC and OPC, the zeta potential values dramatically increased as an outcome of the electrostatic attraction forces between the charges on the chains of



**Figure 6** Zeta potential dependence on the flocculant dosage for OPC and NMPC (pH = 9.0).



**Figure 7** Schematic representation of the flocculation mechanism of the amphoteric flocculants.

the chitosan derivatives and the particle surface. It is worth noting that the negative zeta potential value of the kaolin particles decreased with an increase in the OPC dosage and close to zero at optimal dosage value, but NMPC can not act in this way.

The flocculation mechanism of an amphoteric polyelectrolyte usually involves the formation of net-like bridges between the polymers and between the particles and the polymers, including the electrostatic neutralization between the particles and the flocculant molecules and the ionic bonding between the flocculant molecules (Fig. 7). When the anionic group and cationic group of flocculants cooperated with each other, the apparent molecular weight of the polymer increases rapidly, causing the particles to bridge and flocculate.<sup>19</sup> Based on this flocculation mechanism, The acidity of phosphate groups in the modified chitosans is an important factor in the flocculation efficiency as the anionic phosphate groups can link the  $\text{NH}_3^+$  groups produced from the amino groups. Although the OPC has a small molecular weight, it showed much higher flocculation efficiency than NMPC, and the turbidity removal rate dramatically increases with increasing dosage of OPC (Fig. 3) until reached the maximum that roughly corresponded to charge neutrality (Fig. 6). These analysis results indicate that both charge neutralization and polymer bridging mechanisms play important roles in the OPC system. Although NMPC advantaged at both molecular weight (Table I) and charge neutralization capability (Fig. 6), its flocculation effect is totally inferior as compared with OPC. The difference in the flocculation behaviors of OPC and NMPC can be explained by the chemical structures of the two chitosan derivatives (Fig. 6). Because of the electron-donating effect of the methylene structure, the acidity of the  $-\text{O}-\text{PO}_3\text{H}_2$  groups in OPC are more strong than that of the

$-\text{CH}_2-\text{PO}_3\text{H}_2$  groups in NMPC. Therefore, the phosphorylated position of the modified chitosan, i.e., the hydroxyl or amino groups in the glucosamine structures, is an important factor in controlling the flocculation effects. As described above, OPC was obtained mainly through esterification reactions between phosphorus pentoxide and the primary alcohols at the C6 position. Thus the phosphorylation mainly produces phosphate groups having a structure like  $-\text{O}-\text{PO}_3\text{H}_2$ . However, in the case of NMPC, amino groups in the chitosan molecular chains were phosphorylated by the selective N-phosphorylation resulting  $-\text{CH}_2-\text{PO}_3\text{H}_2$  groups. Despite of the excellent electrostatic neutralization behavior of NMPC for kaolin particles as shown in Figure 6, the decreased acid strength of NMPC reduce the ionic bonds between NMPC chains, and lead the obvious decreases in flocculation efficiency in contrast with OPC. On the other hand, because the phosphate groups of OPC have been partly introduced to the adjoining position (C2) of the amino group in the chitosan units, the neutralization effect of OPC for kaolin particles can be weak as the negative charge repulsion. This may be the main reason why the optimal dosage of OPC is more than that of NMPC. However, the  $-\text{O}-\text{PO}_3\text{H}_2$  phosphate groups make OPC more favorable to link each other and form net-like bridges, this can directly result in the high flocculation efficiency. The explanation above was verified by the other findings on the pH effect on flocculation and the settling behaviors. As a high pH value is favorable for producing electrovalent bonds between phosphate group and amino group in the OPC chains, its optimal pH for removing turbidity was shifted to the basic range. Because OPC has the advantage in forming net-like bridges between the polymer chains, then it caused the flocs to quickly sedimentate from the suspension.

### CONCLUSIONS

Modifying chitosan with  $\text{P}_2\text{O}_5$  in methanesulfonic acid gave an OPC. Its flocculation effect in terms of

the removal of turbidity was significantly better than that of chitosan. However, the N-phosphorylated chitosan, NMPC, performed worse in removing turbidity. These results indicated that the acidity of the phosphate groups introduced in the modified chitosans is an important factor in their flocculation efficiency, and consequently suggested that the O-phosphorylation is an effective way to prepare chitosan-based flocculants.

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