

# Influence of chitosan characteristics on the properties of biopolymeric chitosan–montmorillonite

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**Abstract** Chitosan–montmorillonite is a modified montmorillonite in which the sodium ions in montmorillonite layers are replaced by biopolymeric chitosan. The effects of characteristics of chitosan (i.e. molecular weight and degree of deacetylation) and the chitosan/montmorillonite mass ratio on the properties of chitosan–montmorillonite were investigated. Thermogravimetric analysis, zeta potential and X-ray diffraction results confirmed intercalation of chitosan into montmorillonite layers. An interaction between chitosan and montmorillonite was revealed by FTIR and the zeta potential. The amount of chitosan intercalated into the montmorillonite layers depended on the characteristics of chitosan, with the largest amounts of intercalated chitosan achieved by addition of chitosan with a molecular weight of 71,000 g/mol or a degree of deacetylation of 80% at a fixed chitosan/montmorillonite mass ratio of 2:1. The resulting chitosan–montmorillonite had good adsorbent properties, especially for adsorption of cationic dyes, and also inhibited *E. coli* by almost 100%. The chitosan–montmorillonite may be useful as a functional material for dye adsorption and antibacterial applications.

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## 1. Introduction

Montmorillonite is a clay mineral consisting of silicate layers. Its chemical structure is composed of two fused silica tetrahedral sheets sandwiching an edge-shared octahedral sheet of either aluminum or magnesium hydroxide and an interlayer region containing  $\text{Na}^+$  or  $\text{Ca}^{2+}$  [1]. Montmorillonite is widely used in adsorption [2–4], catalysis [5], composites [1,6,7] and nanocomposites [8–11].

Several groups have tried to modify montmorillonite to make it more useful in applications requiring high selectivity toward monovalent anions [12], immobilization support for stability and activity of enzymes [13], antimicrobial activity [14], cationic dye adsorption [15], and electrochemical sensors [16]. Montmorillonite can be modified by cationic exchange reactions between the sodium ions in the montmorillonite layers and cations such as Ni, Co, Zn [17], cationic surfactants [18,19], salts of fatty acids [20], and polymers [21–25]. However, some cationic substances are difficult to degrade in nature and may be toxic to humans and the environment. Therefore, use of natural cationic substances such as chitosan has generated increasing interest.

Chitosan is a naturally occurring cationic polymer that is mainly extracted from shrimp biowaste [26–28]. Chitosan is composed of glucosamine and *N*-acetyl glucosamine units linked by  $\beta$ -(1–4)-glycosidic linkages [29]. Under slightly acidic conditions, most of the amino groups of chitosan are protonated and the resulting chitosan contains multiple cationic charges. Based on its properties of biodegradability, biocompatibility, antimicrobial effects, good film formation, and high positive charge [29,30], chitosan has been blended with montmorillonite for making composite films [31,32], sensors [12,33], absorbent material [34], enzyme immobilization supports [13] and nanocomposites [14]. In addition, Wang et al. [35] demonstrated that modification of montmorillonite with quaternized chitosan could enhance the antimicrobial activity of montmorillonite in a weak acidic or weak basic medium.

There are few reports on the effects of chitosan characteristics such as molecular weight and degree of deacetylation on the properties of chitosan–montmorillonite. In addition, the interaction between chitosan and montmorillonite has rarely been examined based on the characteristics of chitosan. Therefore, the aim of the present study was to investigate the effects of the molecular weight and degree of deacetylation of chitosan, and the chitosan/montmorillonite ratio, on the properties of chitosan–montmorillonite. The dye adsorption efficiency and antibacterial properties of various chitosan–montmorillonite materials were determined to identify the appropriate chitosan characteristics that allow for the best intercalation into montmorillonite layers.

## 2. Experimental

### 2.1. Materials

Commercial sodium montmorillonite with a cation exchange capacity of 71 mequiv/100 g and a zeta potential of  $-39.3$  mV was purchased from Southern Clay (Perry Hall, MD, USA). Cationic dye and anionic dye were supplied by Ciba Specialty Chemicals (Bangkok, Thailand) and disperse dye was provided by Dystar (Bangkok, Thailand). Low molecular weight chitosan (LM-chitosan, average molecular weight ( $M_w$ )=71,000 g/mol) and high molecular weight chitosan (HM-chitosan,  $M_w$ =583,000 g/mol) were supplied by A.N. Laboratory (Samut Sakorn, Thailand), and medium molecular weight chitosan (MM-chitosan,  $M_w$ =220,000 g/mol) was provided by Ebase (Bangkok, Thailand). The degree of deacetylation (DD) of these chitosans was controlled at 85–90%. Chitosan with DD of 80% (chitosan-DD80) and that with DD of 90% (chitosan-DD90;  $M_w$ s of 310,000–330,000 g/mol) were prepared by alkaline deacetylation of

shrimp chitin [26]. Glacial acetic acid purchased from Carlo Erba was used as the solvent for chitosan. A 2% chitosan solution was prepared by dissolving 2 g of chitosan in 100 ml of 1% acetic acid and shaking at 150 rpm for 24 h. A 4% sodium montmorillonite colloid suspension was prepared by vigorously dispersing sodium montmorillonite in distilled water for at least 30 min.

### 2.2. Preparation of biopolymeric chitosan–montmorillonite

Chitosan–montmorillonite was prepared via an ion exchange reaction using a modified version of the method described by Wang et al. [32]. A 2% chitosan solution was slowly added to a 4% sodium montmorillonite colloid suspension to obtain an optimal ratio of chitosan/montmorillonite. The mixture was continuously stirred at 1000 rpm for 30 min following by dewatering. The resultant mixture was then washed with distilled water twice for the removal of excess chitosan and dried at 70 °C before powdering to obtain the chitosan–montmorillonite.

### 2.3. Variation of parameters

The effects of chitosan with different molecular weight and degree of deacetylation on the properties of chitosan–montmorillonite were examined at a fixed chitosan/montmorillonite mass ratio of 0.5:1. To study the influence of chitosan/montmorillonite mass ratio, the formulations were prepared at mass ratios of 0:1, 0.5:1, 1:1 and 2:1.

### 2.4. Characterization of chitosan–montmorillonite

The interaction of chitosan and montmorillonite was analyzed by Fourier transform infrared spectrometry (FT-IR, model Spectrum 400; Perkin Elmer, Spectrum One, USA) using a KBr disc. The determination was carried out under a transmittance mode at  $4000$ – $400$   $\text{cm}^{-1}$ , resolution of  $4$   $\text{cm}^{-1}$  and scan number of 16 times/sample. The interlayer expansion or *d*-spacing was determined by wide-angle X-ray diffraction (XRD, model PW 3710; Philips, the Netherlands). The detector was operated at a voltage of 40 kV and a current of 30 mA using Cu  $K_{\alpha}$  radiation (1.54 Å) with a scanning speed of  $0.2^{\circ}$   $2\theta$ /step at room temperature. The chitosan content and thermal property of chitosan–montmorillonite were measured by thermogravimetric analysis (model TGA/SDTA851<sup>e</sup>; Mettler Toledo, Switzerland). TGA was performed under a nitrogen gas flow from 30 °C to 1000 °C at a heating rate of 10 °C/min. The zeta potential of the chitosan–montmorillonite was determined using a Zetasizer (model Nano-ZS, Malvern Instruments, England). The specific surface area and average pore size were measured by the BET method.

### 2.5. Dye adsorption

Three types of dye were selected as adsorptives: cationic dye (Maxilon blue 5G GR 200%), anionic dye (Lanaset Red 2B) and disperse dye (Dianix Yellow brown SE-R). Aqueous dye solutions with a concentration of 10  $\mu\text{g/ml}$  were prepared. The dye adsorption experiment was carried out at room temperature. Adsorbent (chitosan–montmorillonite or sodium montmorillonite, 0.1 g) was added to 25 ml of aqueous dye solution and continuously stirred at 1000 rpm for 24 h. The adsorbents were

separated from the aqueous solutions by centrifugation at 10,000 rpm for 5 min and the concentration of each filtrate was measured using a UV spectrophotometer at wavelengths of 656 nm for the cationic dye and 255 nm for the anionic and disperse dyes. The dye adsorption efficiency was calculated from the difference in dye concentration before and after adsorption as a percentage of the initial concentration of dye solution.

### 2.6. Antibacterial properties

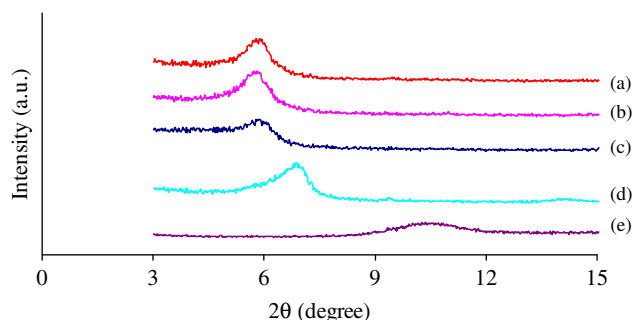
To study the antibacterial properties of chitosan–montmorillonite, *Escherichia coli* (*E. coli*) was used as a model pathogenic bacteria. *E. coli* was cultured and diluted to an appropriate concentration in phosphate buffer (pH 6.8). The culture suspension of 0.1 ml was then added into 5 ml nutrient broth containing different amounts of chitosan–montmorillonite and incubated at 37 °C for 24 h, followed by spreading on nutrient agar and incubation under the same conditions. The inhibition rate was calculated from the number of colonies after treatment with chitosan–montmorillonite as a percentage of the number of colonies in the control (without chitosan–montmorillonite addition).

## 3. Results and Discussion

### 3.1. Effects of molecular weight of chitosan

XRD patterns of chitosan, sodium montmorillonite and chitosan–montmorillonite with different molecular weight chitosan are shown in Fig. 1. Peaks for chitosan were observed at  $2\theta = 10.5^\circ$  and the diffraction peak of sodium montmorillonite was found at  $2\theta = 7.1^\circ$ . After mixing sodium montmorillonite with chitosan of different molecular weights, the diffraction peak of sodium montmorillonite disappeared and a new peak was observed at  $2\theta = 5.9\text{--}6.1^\circ$ . The movement of the diffraction peak of sodium montmorillonite to a lower angle indicated formation of an intercalated nanostructure, whereas the peak broadening and intensity decrease suggested the presence of a disordered intercalated or exfoliated structure [32].

The  $d$ -spacing of sodium montmorillonite was 12.5 Å, corresponding to montmorillonite intercalated with a monolayer of water, whereas the  $d$ -spacing of chitosan–montmorillonite at  $2\theta = 5.9\text{--}6.1^\circ$  was about 14.7 Å (Table 1).



**Fig. 1** XRD patterns showing the effect of the molecular weight of chitosan on the intercalation of chitosan into montmorillonite layers: (a) HM-chitosan–montmorillonite; (b) MM-chitosan–montmorillonite; (c) LM-chitosan–montmorillonite; (d) sodium montmorillonite and (e) chitosan.

It is possible that a chitosan monolayer was formed between the interlayer of montmorillonite, resulting in expansion of the montmorillonite layers. A similar result was also observed by Monvisade and Siriphannon [15], based on which it was proposed that the  $d$ -spacing of about 13.6 Å was due to monolayers of chitosan in montmorillonite, whereas the  $d$ -spacing around 22.5 Å was related to the intercalation of chitosan bilayers. Dader et al. [12] also suggested that the first chitosan layer is adsorbed in montmorillonite layers through a cationic exchange process.

The FTIR spectra in Fig. 2 show that the spectra of chitosan–montmorillonite with chitosan of different molecular weights is similar to the sodium montmorillonite spectrum, except for the peaks at 3430 and 1640  $\text{cm}^{-1}$  (N–H bands), the broadened peak at 2900  $\text{cm}^{-1}$  (aliphatic C–H band), and the peak at 1090  $\text{cm}^{-1}$  (C–O band). This phenomenon confirms that an ionic exchange reaction occurred between chitosan and sodium montmorillonite and that consequently chitosan was intercalated into the montmorillonite structure.

The intercalation of chitosan into montmorillonite layers of chitosan–montmorillonite was also shown by TGA. The weight loss at the decomposition temperature range of chitosan (250–450 °C) implies intercalation of chitosan into montmorillonite layers. The results in Table 1 show that chitosan could intercalate into montmorillonite layers, as indicated by an increase of weight loss and  $d$ -spacing of chitosan–montmorillonite compared to sodium montmorillonite. The amount of chitosan intercalated into montmorillonite increased with the decrease of Mw of chitosan. For example, weight loss of LM-chitosan–montmorillonite was higher than that for MM- and HM-chitosan–montmorillonite by about 23% and 29%, respectively. Regardless of the Mw of chitosan, the  $d$ -spacing of chitosan–montmorillonite increased by about 18% compared to that of sodium montmorillonite. Thus, the ability of chitosan to expand montmorillonite layers was independent of the Mw of chitosan.

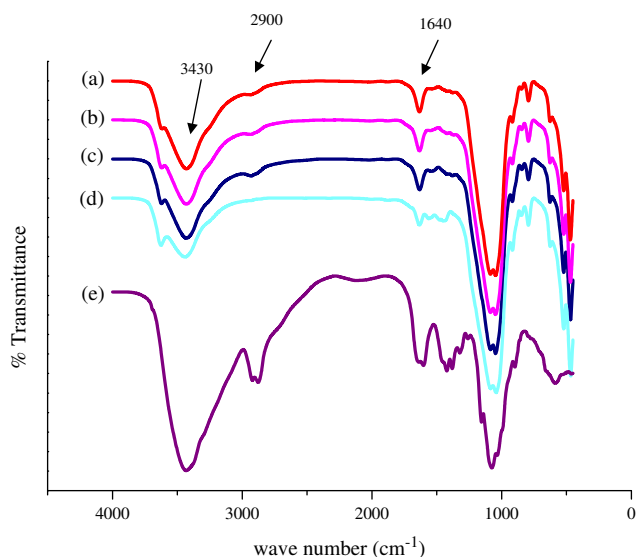
The zeta potential of chitosan–montmorillonite with chitosan of various molecular weights was less negative than that of sodium montmorillonite (Table 1). Greater amounts of low molecular weight chitosan could intercalate into montmorillonite layers and fill the pores of the montmorillonite structure, resulting in a lower specific surface area and pore volume compared to high molecular weight chitosan. This phenomenon might occur due to the compact packing of chitosan in the interlayer space resulting in pore blocking [15].

### 3.2. Effects of degree of deacetylation of chitosan

XRD patterns of chitosan, sodium montmorillonite, chitosan–montmorillonite with different degrees of deacetylation (DD) of chitosan are shown in Fig. 3. Chitosan with DD 80% or 90% could be intercalated into montmorillonite layers because the diffraction peak of sodium montmorillonite moved from  $2\theta = 7.1^\circ$  to  $6.0^\circ$  after incorporation of chitosan. The influence of DD on the properties of chitosan–montmorillonite is shown in Table 1. The  $d$ -spacing of chitosan–montmorillonite with DD 80% or DD 90% was larger than that of sodium montmorillonite, at about 18%. The weight loss of chitosan–montmorillonite with chitosan DD 80% (chitosan–montmorillonite-DD80) was higher than that of chitosan–montmorillonite-DD90 by about 55% at

**Table 1** Effects of molecular weight and degree of deacetylation of chitosan on the characteristics of chitosan–montmorillonite.

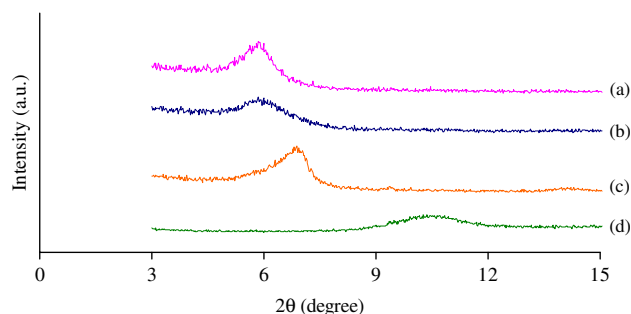
Samples	<i>d</i> -Spacing (Å)	Weight loss at 250–450 °C (%)	Zeta potential (mV)	Specific surface area (m <sup>2</sup> /g)	Pore volume (ml/g)
Montmorillonite (control)	12.5	0.0	−31.6	nd	nd
LM-chitosan–montmorillonite	14.4	10.5	−28.0	55.8	0.1974
MM-chitosan–montmorillonite	15.0	8.1	−23.3	68.4	0.2064
HM-chitosan–montmorillonite	14.8	7.5	−24.7	71.6	0.2281
Chitosan–montmorillonite-DD80	14.5	11.7	−16.0	51.0	0.1467
Chitosan–montmorillonite-DD90	14.8	7.5	−16.1	68.6	0.2022

**Fig. 2** FTIR spectra of sodium montmorillonite, chitosan and chitosan–montmorillonite with chitosan of different molecular weights: (a) HM-chitosan–montmorillonite; (b) MM-chitosan–montmorillonite; (c) LM-chitosan–montmorillonite; (d) sodium montmorillonite and (e) chitosan.

250–450 °C. This indicates that a greater amount of chitosan with DD 80% intercalated into the montmorillonite layers and resulted in a lower specific surface area and pore volume compared to these parameters with DD 90%. The intercalation of chitosan into montmorillonite layers was also confirmed by the zeta potential, which was less negative when chitosan with DD 80% or 90% was included in the formulation. Wang et al. [32] suggested that a single amino group and two hydroxyl groups in one chitosan unit can form hydrogen bonds with hydroxylated silicates in montmorillonite, leading to a strong interaction between chitosan and the silicate layers of montmorillonite.

### 3.3. Effect of chitosan/montmorillonite ratio

The effect of the chitosan/montmorillonite mass ratio on the properties of the resulting chitosan–montmorillonite is shown in Table 2. The *d*-spacing of sodium montmorillonite was expanded by addition of chitosan to the sodium montmorillonite colloidal

**Fig. 3** Effect of the degree of deacetylation of chitosan on the intercalation of chitosan into montmorillonite layers: (a) chitosan–montmorillonite-DD90; (b) chitosan–montmorillonite-DD80; (c) sodium montmorillonite and (d) chitosan.

suspension, with a resulting less negative zeta potential. This might be explained by the electrostatic interaction between the cationic charges of chitosan and the anionic charges of montmorillonite. Higher amounts of chitosan in the ratio provide more cationic charges and consequently a stronger electrostatic interaction with the anionic silicate layers of montmorillonite.

The amount of chitosan in chitosan–montmorillonite was about 9–11%. An and Dultz [34] also found that a monolayer of chitosan was formed in the montmorillonite at low loading of chitosan (<50%). Darder et al. [12] suggested that adsorption as monolayer or bilayers of chitosan chains depended on the relative amounts of chitosan with respect to the cationic exchange capacity of clay and that the first chitosan layer was adsorbed through a cationic exchange procedure. Greater chitosan content in the chitosan–montmorillonite, as shown by the weight loss at 250–450 °C, also resulted in a lower specific surface area (Table 2).

### 3.4. Dye adsorption

The results in Table 3 indicate that sodium montmorillonite and chitosan–montmorillonite with different ratios could adsorb cationic, anionic and disperse dyes with different efficiencies. Chitosan–montmorillonite has better adsorbent properties, especially with the cationic dye. For example, the sample containing chitosan/montmorillonite ratio of 2:1 adsorbed cationic dye at a level of almost 99%, whereas sodium montmorillonite had only 65% adsorption efficiency.

**Table 2** Effect of chitosan/montmorillonite ratios on the characteristics of chitosan–montmorillonite.

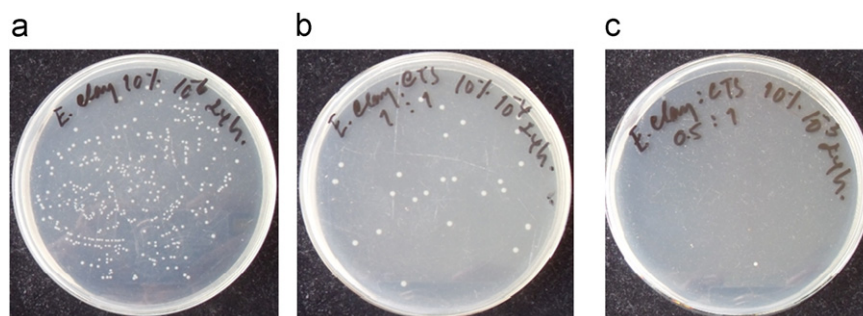
Chitosan/Montmorillonite ratio	<i>d</i> -Spacing (Å)	Weight loss at 250–450 °C (%)	Zeta potential (mV)	Specific surface area (m <sup>2</sup> /g)	Pore volume (ml/g)
0:1	12.6	–	–31.6	nd	nd
0.5:1	14.6	9.19	–8.8	62.0	0.2183
1:1	14.7	11.1	–16.3	54.0	0.1921
2:1	14.6	10.5	–8.5	51.7	0.2020

**Table 3** Dye adsorption efficiency of chitosan–montmorillonite with different chitosan/montmorillonite ratios.

Chitosan/Montmorillonite ratio	Dye adsorption efficiency (%)		
	Cationic dye	Anionic dye	Disperse dye
0:1	65.7	38.2	22.7
0.5:1	97.4	39.7	40.1
1:1	98.9	72.9	71.1
2:1	99.3	67.4	68.6

**Table 4** Effect of chitosan–montmorillonite on the inhibition of *E. coli*.

Chitosan/montmorillonite ratio	Weight of chitosan–MMT in nutrient broth (%)	Inhibition rate (%)
0:1	5	0
1:1	5	0
2:1	5	0
0:1	10	0
1:1	10	99.63
2:1	10	99.99

**Fig. 4** Appearance of colonies of *E. coli* after treatment with chitosan–montmorillonite with various chitosan/montmorillonite ratios: (a) 0:1; (b) 1:1 and (c) 2:1.

These results may be ascribed to the charges of the dyes and chitosan–montmorillonite. Chitosan–montmorillonite has anionic charges, which facilitates an electrostatic interaction with cationic dye. However, chitosan–montmorillonite could also adsorb anionic and disperse dyes because of the chitosan content. These results show that chitosan enhances the dye adsorption efficiency of montmorillonite and that the efficiency depends on the amount of chitosan in the material and the type of dye.

### 3.5. Antibacterial properties

The results in Table 4 show that sodium montmorillonite could not inhibit *E. coli* growth, even at a level of 10% of the nutrient broth. In contrast, chitosan–montmorillonite inhibited *E. coli* to an extent that depended on the chitosan/montmorillonite ratio. For example, a chitosan/montmorillonite material of ratio 2:1 inhibited *E. coli* by almost 100% when applied at 10% of the nutrient broth. The morphology

of *E. coli* colonies after treatment with chitosan–montmorillonite compared with control (sodium montmorillonite) is shown in Fig. 4. Based on the antibacterial properties of chitosan [36–38], it is possible that chitosan may have been released from chitosan–montmorillonite into the nutrient broth and consequently inhibited the growth of *E. coli*.

#### 4. Conclusions

Chitosan–montmorillonite can be prepared by a cationic exchange reaction and its properties depend on the molecular weight and degree of deacetylation of chitosan, and on the chitosan/montmorillonite ratio. These results suggest that chitosan can be used as an enhancer for improvement of the dye adsorption and antibacterial properties of montmorillonite.

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