Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Polymers 65 (2006) 194–201

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Protonation constants of chitosan with different molecular weight and degree of deacetylation

Qi Zhao Wang, Xi Guang Chen *, Nan Liu, Shi Xi Wang, Cheng Sheng Liu, Xiang Hong Meng, Chen Guang Liu

College of Marine Life Sciences, Ocean University of China, 5# Yushan Road, Qingdao 266003, People's Republic of China

Received 19 May 2005; received in revised form 22 November 2005; accepted 5 January 2006
Available online 21 February 2006

Abstract

The protonation constants of chitosans with different molecular weight (M_w) and degree of deacetylation were demonstrated by the modified Henderson-Hasselbalch model and Högfeldt's three-parameter model. The protonation constants pK_a of chitosan showed a slightly decreasing from 6.51 to 6.39 when the molecular weight changing from 1370 to 60 kDa. The degree of deacetylation showed a greatly effect on pK_a values, which were increased from 6.17 to 6.51 with the degree of deacetylation decreasing from 94.6 to 73.3%. The degree of deacetylation influenced the balance of hydrophobic interactions and hydrogen bondings on chitsoan. The fitting of the titration data to both two methods showed that Högfeldt's three-parameter model provided a better agreement. The results obtained in this paper allowed to a better understanding of some physicochemical mechanisms and biological properties of chitosan.

Keywords: Chitosan; Protonation constant; Potentiometric titration; Molecular weight degree of deacetylation

---

1. Introduction

Chitosan (CT), a linear polysaccharide composed of β-(1 → 4)-linked 2-amino-2-deoxy D-glucose (GlcN) and 2-acetamido-2-deoxy- D-glucose (GlcNAc), is obtained on an industrial scale by the alkaline deacetylation of chitin. It has attracted great attention recently because of its biodegradable and biocompatible properties (Dodane & Vilivalam, 1998; Majeti & Ravi, 2000) and can also be shaped into several forms for its gelling ability and be further modified for its amino and hydroxyl groups.

Most applications of CT are based on the amine group of the macromolecule, such as lysozyme binding (Kristiansen, Varum, & Grasdalen, 1998), drug delivery (Van der Lubben, Verhoef, Borchard, & Junginger, 2001), solubility as a function of pH (Mao, et al., 2004), metal ion sorption (Guibal, 2004) and gene delivery (Sato, Ishii, & Okahata, 2001). In acidic solution, amine groups of CT are protonated to $–NH_3^+$. Therefore, the fraction of free amine groups is a key parameter to understand the mechanisms and then control the processing of materials. Actually, it would be better to consider the number of accessible-free amine groups rather than the fraction or number of free amine groups available for these applications. The parameters responsible for these amine groups are the degree of deacetylation (*DD*), molecule weight (M_w), and environmental parameters (ionic strength, pH, temperature, solvent, etc.). Domard's (1987) study on CT with different M_w and *DD* proposed to consider intrinsic dissociation constant (pK_{int}) as close to 6.5 and considered that pK_{int} of the ionizable groups was independent of the *DD*. Various authors working on a given CT, different in each study, contributed to give a large spectrum of pK_{int} values ranging from 6.1 to 7.0 (Anthonsen & Smidsrd, 1995; Park & Choi, 1983). Sorlier, Denuziere, Viton, and Domard (2001) and Sorlier, Viton, and Domard (2002) extensively studied the acid-base properties of CT in the function of the dissociation degree (α) and *DD* observed that pK_a values varied drastically between 6.3 and 7.2 at complete dissociation, corresponding to the intrinsic dissociation constant pK_0, while the change in the pK_a between extreme *DD* values considerably increased when dissociation degrees decreased.

In particular, it should be noted that it is not possible to determine the pK_a value for each amino group and therefore one must consider a variable protonation constant as a function of the degree of dissociation α. Different models have been

---

* Corresponding author. Tel.: +86 532 82032586; fax: +86 532 82032586.
E-mail address: xgchen@ouc.edu.cn (X.G. Chen).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.01.001

proposed to take into account the function pK_a *vs.* α for CT. The most popular model is the modified Henderson-Hasselbalch equation, used since 1949 to explain potentiometric data for titrations of polymethacrylic acid (Park et al., 1983; Saito & Tanioka, 1996). Domard (1987) first used the Katchalsky's equation to describe the variation of pK_a values of the $-NH_3^+$ groups during titration of CT. More recently, a simple three-parameter model has been proposed by Högfeldt based on Katchalsky equation to deal with the properties of ion exchangers (Högfeldt, Miyajima, Marinsky, & Muhammed, 1989), and Zentz, et al. (2001) used the three-parameter model to analyze the titration of CT. However, no papers dealt with pK_a of CT with both of the two models and compared with each other. The variability results obtained in previous reports may be due to different models.

In this paper, CT with different M_w (1370–60 kDa) and *DD* (94.6–73.3%) was prepared, the protonation of each samples and the relationship of pK_a with its M_w and *DD* was determined by the method of potentiometric titration. Glucosamine hydrochloride (GH) and *N*-acetylglucosamine (NAG), the monomer of CT and chitin, respectively, were used as controls to be described.

2. Materials and methods

2.1. Materials

CT (sample A) was made by our lab from the crab's shell. Hydrochloric acid, acetic anhydride, oxalic acid, Potassium hydroxide, sodium hydroxide, acetic acid, ethanol, methanol, aether were purchased from the Sigma Co. St Louis USA.

2.2. CT preparation

2.2.1. Degradation of CT

CT was degraded by the method of acetic acid hydrolysis referenced from Chen, Zheng, Wang, Lee and Park (2002). CT (10 g, 100 mesh power) was dissolved in 190 ml aqueous acetic acid solutions (5%, v/v), incubated at 50 °C for a given times, and then centrifuged (5000×g) for 20 min. The supernatant was added to 4 M aqueous NaOH to pH 7–9. The sediment was filtered and sequentially rinsed in water and ethanol and dried at 50 °C. Different CT samples are shown in Table 1.

2.2.2. Acetylation of CT

Acetylated CT was made by acetic acid and and chtiosan as reference method (Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998). The procedure in brief, acetic anhydride was added to methanol/aqueous acetic acid solution (1:1v/v) containing CT (0.667%, w/v), stirred 24 h, the polymer was fully precipitated by potassium hydroxide/ethanol solution (0.5 mol/L), washed with ethanol and dehydrated in aether, finally were vacuum dried over night. The samples are shown in Table 2.

2.2.3. Analysis of CT

The viscosity of CT was investigated by using an Ubbelohde viscosimeter in 25 °C. CT was dissolved in 0.2M CH_3COOH/0.1M CH_3COONa solution, and filtered through 0.45 μm membranes. The viscosity M_w was calculated based on Mark Houwink Eq. (Wang, Bo, Li, & Qin, 1991)

$$[\eta] = KM^a$$

Where $[\eta]$ is the intrinsic viscosity, K and a are constants the values of which depend on the nature of the polymer and solvent as well as on temperature. M is the relative molecular weight. In this paper, $K = 1.64 \times 10^{-30} \times DD^{14}$ (ml/g), $a = 1.02 \times 10^{-2} \times DD + 1.82$ and *DD* is the degree of the deacetylation of CT expressed as the percentage (Chen & Hwa, 1996). The FT-IR spectra were recorded using an IR spectrometer (Perkin–Elmer 1760×, USA) by KBr disc method.

2.3. Potentiometric measurement and DD determination

The two abrupt change potential titration method was employed (Jia & Li, 2001). CT (0.2 g) were dissolved under stirring in 20 ml of 0.1 M HCl for 4 h to prepare 1% m/v CT-HCl solution. Measurements were made in a 100 ml cell and performed with a solution of 0.1 M NaOH by using a pH meter (DELTA 320, METTLER TOLEDO). Potential concentrations were given as pH $-\log[H^+]$. The *DD* of CT was calculated as follows:

$$DD = \frac{\Delta V \times C_{NaOH} \times 10^{-3} \times 16}{M \times 0.0994}$$

Where ΔV is the volume of NaOH between two inflexion points, C_{NaOH} is the concentration of the NaOH solution, M is

Table 1
Physicochemical properties and protonation constants of chitosan with different M_w

Samples	M_w (kDa)	*DD* (%)	Katchalsky equation	Extended Henderson-Hasselbach equation		Three-parameter equation			
			pK_{int}	n	p$K_{1/2}$	pK_0	pK_1	pK_m	p$\bar{K}$
A	1370	82.4±0.05	6.71±0.02	1.37±0.04	6.49±0.02	6.14±0.02	6.51±0.02	6.54±0.04	6.51±0.01
B	500	80.1±0.05	6.70±0.02	1.25±0.01	6.46±0.03	6.12±0.02	6.67±0.02	6.54±0.03	6.44±0.01
C	150	80.7±0.09	6.66±0.05	1.17±0.02	6.43±0.02	6.10±0.03	6.58±0.03	6.56±0.03	6.41±0.02
D	90	79.8±0.02	6.65±0.02	1.09±0.03	6.40±0.04	6.09±0.02	6.49±0.01	6.56±0.01	6.38±0.02
E	60	84.6±0.04	6.68±0.04	1.09±0.04	6.39±0.01	6.06±0.02	6.47±0.01	6.58±0.04	6.37±0.01
GH		100	7.77±0.09	1.02±0.02	7.72±0.03	7.62±0.03	7.72±0.04	7.78±0.05	7.71±0.05

Table 2
Physicochemical properties and protonation constants of chitosan with different *DD*

DD (%)	pH		Katchalsy equation	Extended Henderson-Hasselbach equation		Three-parameter equation			
	$\alpha=0$	$\alpha=1$	pK_{int}	n	$pK_{1/2}$	pK_0	pK_1	pK_2	$p\bar{K}$
GH	4.72±0.20	9.75±0.21	7.77±0.09	1.02±0.02	7.72±0.03	7.62±0.03	7.72±0.04	7.78±0.05	7.71±0.05
94.6±1.00	3.86±0.10	9.12±0.20	6.31±0.04	1.20±0.02	6.17±0.01	6.03±0.01	6.25±0.03	6.26±0.05	6.18±0.04
86.4±0.80	3.93±0.12	9.17±0.12	6.57±0.02	1.15±0.03	6.30±0.02	6.04±0.04	6.4±0.01	6.53±0.02	6.32±0.02
80.5±0.70	4.00±0.15	9.18±0.15	6.68±0.03	1.09±0.03	6.42±0.02	6.12±0.02	6.47±0.02	6.58±0.01	6.39±0.02
73.3±0.20	4.21±0.12	9.21±0.10	6.83±0.05	1.04±0.02	6.51±0.05	6.15±0.01	6.51±0.02	6.76±0.05	6.47±0.03
13.7±0.50	4.82±0.18	9.07±0.08	7.27±0.05	1.54±0.05	6.84±0.02	6.38±0.04	6.89±0.02	7.25±0.06	6.84±0.05

the weight of CT sample, 16 and 0.0994 is the related molecular weight and theoretical amount of amino groups.

3. Models for the function pK_a vs. α

CT is soluble in acids on account of a protonation of free amine groups:

$$R\text{–}NH_3^+ + H_2O \Leftrightarrow R\text{–}NH_2 + H_3O^+$$

$$K_a = \frac{[R\text{–}NH_2][H_3O^+]}{[R\text{–}NH_3^+]} \quad \text{and} \quad pK_a = -\log K_a$$

Potentiometric data can be interpreted in terms of the protonation constant pK_a calculated at each α value. For CT, the true degree of dissociation α is calculated as follows:

$$\alpha = \alpha' + [H^+]/C \qquad (1)$$

Where α', the degree of dissociation, is deduced from the amount of added NaOH, and C is concentration in glucosamine residues.

The modified Henderson-Hasselbalch equation can be written as follows:

$$pH = pK_{1/2} - n\log[(1-\alpha)/\alpha] \qquad (2)$$

Or

$$pK_a = pK_{1/2} - (n-1)\log(1-\alpha)/\alpha \qquad (3)$$

In this equation, n is an empirical parameter related to the free energy change during titration ($n=1$, for GH; $n>1$, for CT). The presence of electrolyte in the solution influences the n parameter by reducing the electrostatic repulsions. A linear regression in the interval $-0.5 \leq \log[\alpha/(1-\alpha)] \leq 0.5$ allowed one to determine the values $pK_{1/2}$ and n, respectively, computed from the intercept ($\alpha=0.5$) and the slope of the plot of pH versus $\log[\alpha/(1-\alpha)]$.

The variation of pK_a values of the NH_3^+ groups during titration of chiosan was evaluated by using the Katchalsky Eq. (Domard, 1987), which described:

$$pK_a = pH + \log[(1-\alpha)/\alpha)] = pK_{int} - \frac{\varepsilon\Delta\varphi(\alpha)}{KT} \qquad (4)$$

For the rodlike model, $\Delta\phi(\alpha)$ represents the difference between the surface potential of the polyion and the reference state at a distance from the axis. The definitions of ε, K and T, are the unitary charge, Bootzmann's constant and the absolution temperature, respectively. In this equation, extrapolation of the pK_a value to $\alpha=1$ when the polymer becomes uncharged enabled the value of the intrinsic dissociation constant of the ionizable groups (pK_{int}) to be estimated. Eq. (4) can be further explained by the three-parameter equation proposed by Högfeldt:

$$pK\text{app} = \alpha^2 pK_1 + (1-\alpha)^2 pK_0 + 2\alpha(1-\alpha)pK_m \qquad (5)$$

Where pK_0 is the protonation constant at $\alpha \to 1$, pK_1 is the limit value for $\alpha \to 0$ and pK_m s an intermediate value. Eq. (5) is equivalent to the second-degree polynomial equation:

$$pK_a = A + B\alpha + C\alpha^2 \qquad (6)$$

With

$$A = pk_0$$

$$B = 2(pK_m - pk_0)$$

$$C = pk_0 + pk_1 - 2pK_m$$

The thermodynamic protonation constant, $\overline{K}$ can be obtained from:

$$p\bar{K} = \int_0^1 \log \bar{K}(\alpha)d\alpha = (pK_1 + pK_0 + pK_m)/3 \qquad (7)$$

The values of A, B, and C can be obtained and the parameters pK_1, pK_0 and pK_m can be easily calculated by multiple regression of Eq. (4). Then the value of $p\bar{K}$ can be determined from the Eq. (7). The titration curve can thus be calculated with Eq. (5).

4. Results and discussion

4.1. Characteristics of CT

Different M_w CT (sample *B* to *E*) was obtained by the acetic acid hydrolyzed from sample *A*. All the samples were white powders with the M_w ranged from 1370 to 60 kDa and without obvious difference of *DD* (shown in Table 1). On the FT-IR spectra of CT, there were strong amino characteristic peaks of CT at around 3420, 1655, and 1325^{-1}, and the peaks assigned to the saccharide structure were at 1152 cm^{-1} (C–H stretch), 1154 cm^{-1} (bridge-*o*-stretch) and 1094 cm^{-1} (C–O stretch). The spectrums of the CT samples *A*–*E* have no obvious difference with each other. The results showed that CT samples made from the method of acetic acid hydrolyze had no obvious

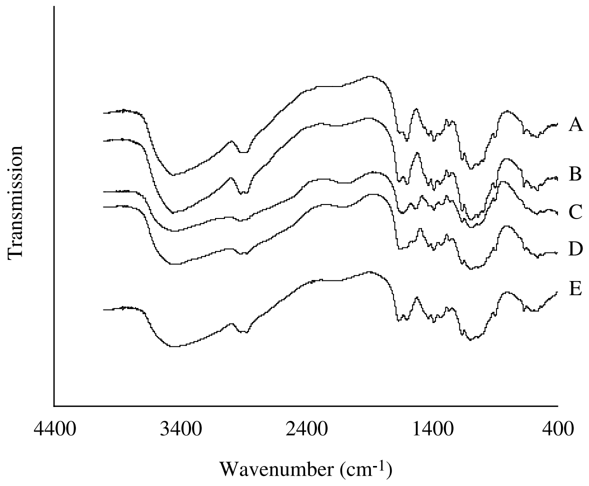

Fig. 1. FT-IR spectra of chitosan powders with different M_w.

change in the *DD*; the values of *DD* were about 80%. The similar result has been reported by Chen et al. (2002) (Fig. 1).

Potentiometric titration of CT gave a curve (shown in Fig. 2) having two inflexion points, while the references (NAG and 0.1 M HCl) had only one inflexion point. The first and second inflexion points were the equivalence points of the titration of excessive HCl and the titration of protonated CT, respectively, and the accurate points could be obtained from Fig. 3. The titrated results of CT with different M_w and *DD* were quite agreed with the FT-IR spectra (Fig. 1), the values were shown in Tables 1 and 2, respectively. Acetylation of CT obtained the *DD* varied from 94.6 to 73.3% (Table 2). Potentiometric titration proposed by Broussignac (1968) was one of the simplest methods to determine the *DD* of CT. Park et al. in 1983 has already used this method to calculate the *DD* of CT, and more recently, Khan, Peh and Ch'ng (2002) used the similar method (HBr titrimetry) to obtained the values of *DD* for CT.

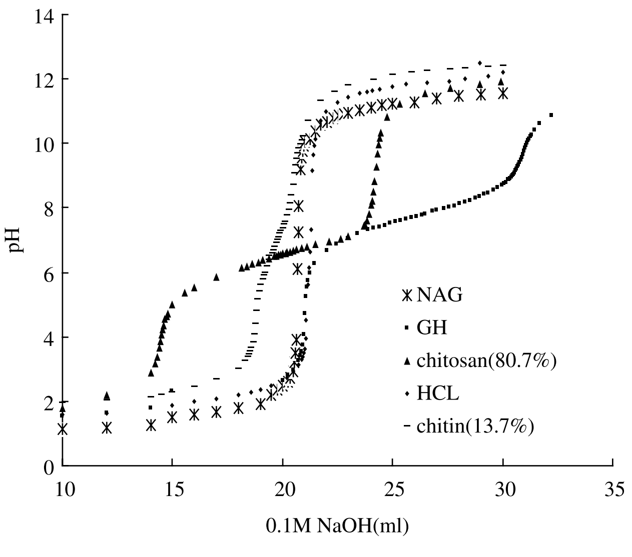

Fig. 2. Potentiometric titration of chitosan, GH, *N*-acetylg-lucosamine, and HCl.

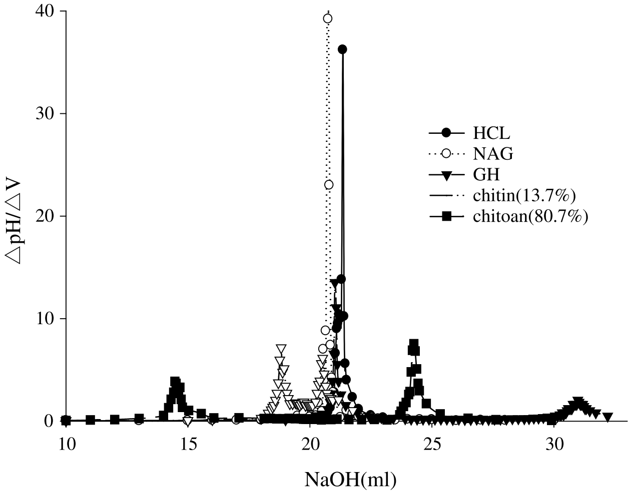

Fig. 3. Titration curves and two inflexion points of chitosan and controls.

4.2. Dependence of protonation constants on molecular weight

Apparent protonation constants were calculated, for various CT of different M_w in 0.1 M HCl, at each α value. All titration data were fitted to the modified Henderson-Hasselbalch model and Högfeldt's three-parameter model and smoothed values of pK_x,(pK_x=pK_0, pK_1, pK_m, p$K_{1/2}$, pK_{int}) and n are reported in Table 1.

Figs. 4 and 5 show the results of potentiometric titrations and pK_a curves calculated by Eq. (4) with various M_w of CT ranging from 1370 to 60 kDa. The pK_a vs. α titration curves showed an increase in the region of $0.1 \le \alpha \le 0.65$, and then a decrease until to $\alpha = 0.9$. At the regions close to $\alpha = 0$ and $\alpha = 1$, an increase could be seen but the accuracy of the measurement on those regions became quite poor. The curve trends for CT were the same as earlier reported by Zentz et.al. (2001). At $0.1 \le \alpha \le 0.65$, a straight line could be fitted by regression (see Fig. 5). Extrapolation of the data to zero charge density ($\alpha = 1$) gave the pK_{int} values as presented in Table 1. The pK_{int} of the CT with different M_w showed no obvious difference and had a value near 6.70, which was litter higher than the value of 6.5 obtained by Domard (1987). The pK_a value of the monomer, GH, was determined in the same way to

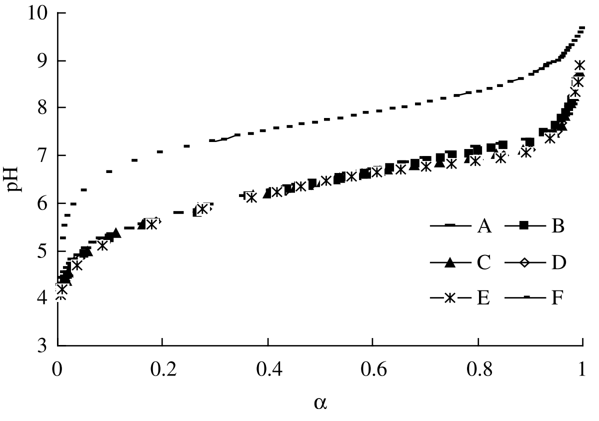

Fig. 4. Variation of pH as a function of α.

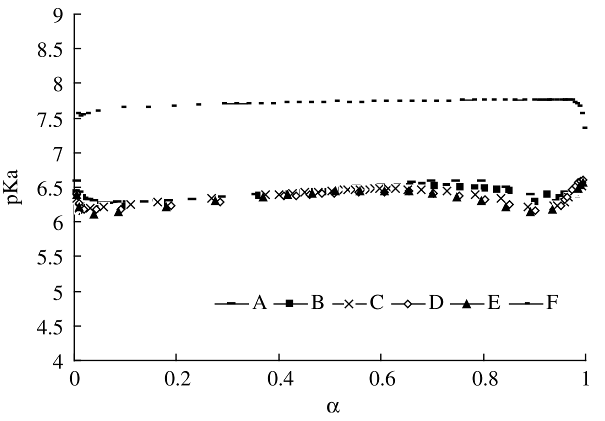

Fig. 5. Variation of pK_a as a function of α according to the Katchalsky equation.

be 7.77, which was in good agreement with earlier reported value of 7.8 (Park et al., 1983).

The modified Henderson-Hasselbalch equation involved two parameters, p$K_{1/2}$ ($\alpha=0.5$) and n. Variation of pH as a function of log$\alpha/(1-\alpha)$ was shown in Fig. 6 for different M_w of CT. For GH a linear increase in pH was observed, whereas the increase in CT had a break at $\alpha=0.67$ or pH 6.7. This suggested that molecular conformation of the polymers was changed at that point, which was in line with the previous finding of other anthors (Zentz et al., 2001 and Park et al.). At the point, the CT started to form precipitate and change of pK_a values (Fig. 4). No significant change in pH value could be found by changing the polymer molecular weight. In Eq. (3), n was a empirical parameter related to the free energy change during titration. The values of n which was calculated from the slope of modified Henderson-Hasselbach equation were shown in Table 1. The values varied from 1.4 to 1.09 when M_w changed from 1370 to 60 kDa. Moreover, n superior to 1 ($n=1$, for GH) revealed electrostatic repulsions among the neighboring $-NH_3^+$ groups in CT. This result could also be used to explain the phenomena that p$K_{1/2}$ of $-NH_3^+$ in CT found to be 6.4, while for GH monomer, 7.72. The influence of M_w on p$K_{1/2}$ was quite small, and a slight decreasing effect was observed with M_w decreasing.

Regardless of the actual process occurring throughout the titration, titration data of CT with different M_w could be analyzed by fitting them to empirical models such as the Högfeldt equation, which included three empirical parameters. The region $0.1\leq\alpha\leq0.65$ (Fig. 5) was also used to fit the second-degree polynomial regression for obtaining the values of pK_0, pK_1, pK_m and p$\bar{K}$ (shown in Table 1). The thermodynamic constants p$\bar{K}$ obtained by using this model also showed a slight decrease with the M_w, and the values of p$\bar{K}$ were smaller than the corresponding values of p$K_{1/2}$ obtained by the modified Henderson-Hasselbalch equation.

4.3. Dependence of protonation constants on the degree of deacetylayion

The *DD* and α played a major role in the apparent and intrinsic protonation constant values of the free amino groups (shown in Figs. 7, 8 and Table 2). GH and chitin with $DD=13.7\%$ were used as references. A linear relation between pH and *DD* at $\alpha=0$ could be deduced from the variations of pH as a function of α (shown in Table 2). Took $DD=13.7\%$ into account, the linearization was still well. The same phenomena had been observed by Sorlier (2001), but the value of pH extrapolated to $DD=0$ only 4.99, agreed with the value of GH at the same conditions. On the contrary, the value of pH 3.78, extrapolated at $DD=100\%$, agreeing with Sorlier's (2001) result. For $\alpha=1$, the pH vs. *DD* had no obvious differences (Fig. 9). Figs.7 and 8 showed the variation of pH and pK_a as a function of α. The titration curves were conformed to the well-known kinetic process of precipitation that progressively disappeared on the increasing *DD*.

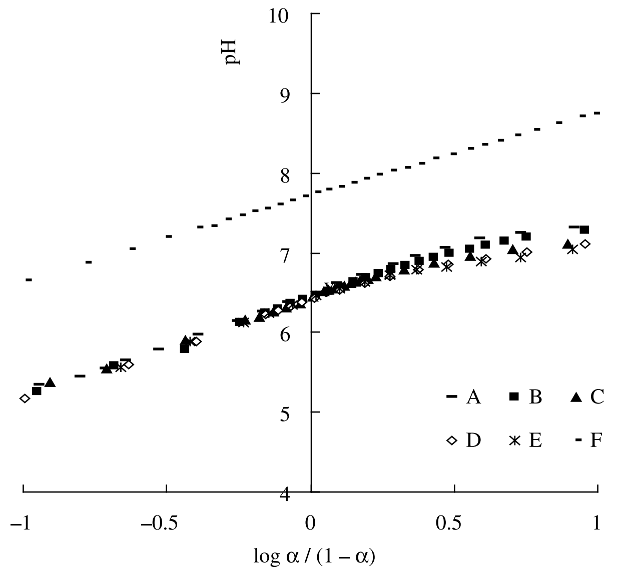

Fig. 6. Variation of pH as a function of log$\alpha/(1-\alpha)$.

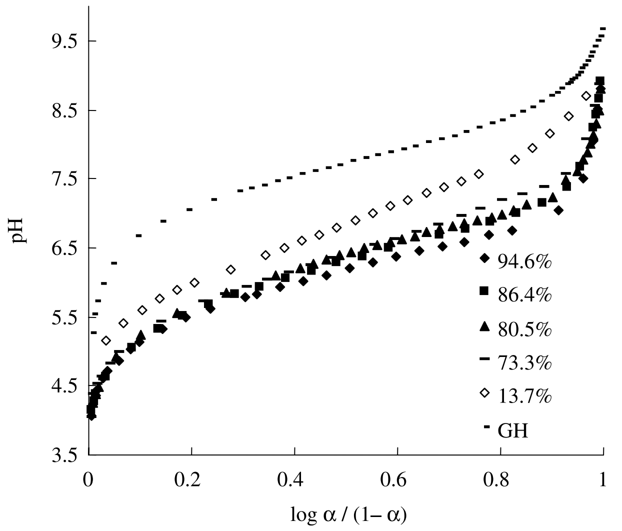

Fig. 7. Variation of pH as a function of log$\alpha/(1-\alpha)$ according to the Katchalsky equation.

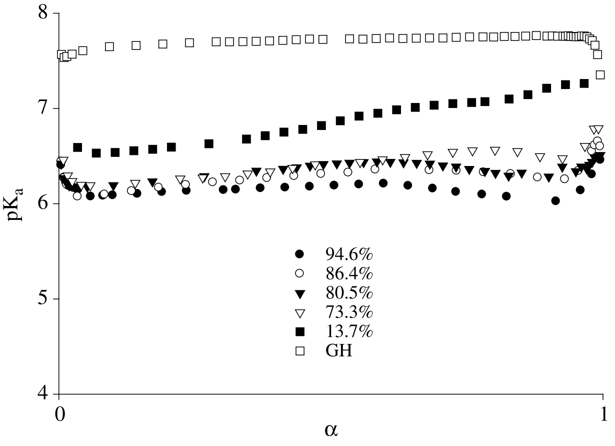

Fig. 8. Variation of pK_a as a function of α according to the Katchalsky equation.

pK_{int}, p$K_{1/2}$, pK_0, pK_1, pK_m and p$\bar{K}$ deduced from two models are presented in Table 2. Fig. 9 show that pK (pK_{int}, p$K_{1/2}$, pK_0, pK_1, pK_m and p$\bar{K}$) increased from 6.17 to 6.51 with a linear manner as a function of *DD* decreasing from 94.6 to 73.3%, the values of pK_{int} were litter higher than others, which increased from 6.31 to 6.82. These results were opposite to the Domard's result which considered that pK_{int} of the ionizable amino groups was independent of *DD*. The increasing of pK_a with the decreasing of *DD* allowed the interpretation of some behaviors already published. Various values of pK_a could be obtained from the extrapolation of pK_a at the two ends of the curves. For $DD = 100\%$, all values of pK_a were about 6.1, while for $DD = 0$, pK_a values were 8.61, 7.71, 6.60, 7.43, 8.41 and 7.47, respectively. For $DD = 100\%$, glucosamine is the only units on CT chain, thus the high charge density responsible for strong electrostatic repulsions between CT chain segments (Montembault, Viton, & Domard, 2004) that favors for the protonation of amino groups. For $DD = 0$, $pK_{1/2}$ and p$\bar{K}$ had the similar values with GH. At this point, CT must be regarded as a polymer bearing isolated charges which hydrophobic environment increases with *DD*. These behavior related to the progressive involvement of chain associations corresponding to the formation of aggregates (Ottøy, Vårum, Christensen, Anthonsen, & Smidsrød, 1996).

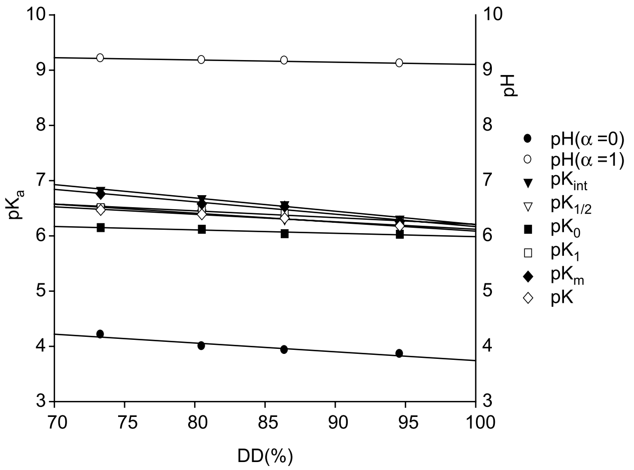

Fig. 9. Variation of pK_a and pH at $\alpha = 0$ and 1 with *DD*.

Sorlier, Denuziere, Viton, and Domard (2001) has discussed the variation of pK_a at $\alpha = 0$ and 1 as a function of *DD* using Eq. (2) in details. The role of *DD* influenced the pK_a through the participation of hydrophobic interaction and hydrogen bondings on the electrostatic parameters. In this paper, the value of p$K_{1/2}$ and p$\bar{K}$ were near the points at $\alpha = 0.5$. It may be influenced by both hydrophobic interaction and hydrogen bondings. The decrease of *DD* brought out a number of acetyl groups contributed to favor the formation of hydrophobic interactions (Amiji, 1995). For the range of *DD*: between 94.6 and 73.3%, CT behaved a polycationic polyelectrolyte, the charge density of which decreases with *DD* and then electrostatic repulsions decreased favors the formation of hydrogen bondings between chain segments, and thus pK_a increasing. This could be demonstrated by the values of n decreased from 1.20 to 1.05 when *DD* decreased from 94.6 to 73.3% (Table 2).

4.4. Comparison between different models expressing pK_a vs. α

The comparison of two models was taken three main factors into account: (a) fitting ability; (b) significance of the parameters obtained; (c) simplicity and usefulness of their use. Fig. 10 presented the fitting of the titration data (sample C) by two models. Eqs. (2) and (3) were used for fitting by modified Henderson-Hasselbalch model.The fitting of data to Högfeldt's model was done by applying Eqs. (4) and (5). The standard deviations calculated between the experimental data and calculated data shown in Table 3. These results showed that Högfeldt's three-parameter models have a better fitting to the experimental data than the modified Henderson-Hasselbalch model. Various values of pK_a obtained by using the two models. For $DD = 100\%$, all pK_a values were about 6.1. This meant that, at this point, the modified Henderson-Hasselbalch model and Högfeldt's three-parameter model could be considered equivalent. For $DD = 0$, different pK values showed the difference between two models. The modified Henderson-Hasselbalch model fitted well with the titration data of CT only with lower M_w and higher *DD*, while Högfeldt's three-

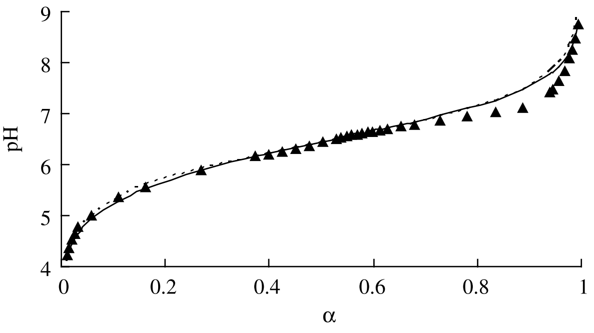

Fig. 10. Dependency of pH on α fitted by two models (▲, experimental data; continuous line, Högfeldt model; dashed line, modified Henderson-Hasselbalch model).

Table 3
Standard deviation for the experimental pH fitting by the modified Henderson-Hasselbalch model (HHM) and Högfeldt's Three-parameter model (HM)

Models	Standard deviations									
	Chitosan with different M_w					Chitosan with different *DD* (%)				
	A	B	C	D	E	94.6 ± 1.00	86.4 ± 0.80	80.5 ± 0.70	73.3 ± 0.20	13.7 ± 0.50
HHM	0.421 ± 0.024	0.277 ± 0.012	0.163 ± 0.005	0.121 ± 0.009	0.138 ± 0.010	0.085 ± 0.005	0.129 ± 0.006	0.140 ± 0.012	0.323 ± 0.015	0.443 ± 0.014
HM	0.150 ± 0.005	0.184 ± 0.002	0.131 ± 0.008	0.134 ± 0.002	0.149 ± 0.010	0.106 ± 0.005	0.142 ± 0.006	0.151 ± 0.008	0.208 ± 0.012	0.146 ± 0.011

parameter model fitted all titration data well. The more favorable fitness of the three-parameter model may be due to its higher flexibility. As regards point (b), Högfeldt's three-parameter model could pointed out the values of pK_0 and pK_1. In many cases, these values were necessary to analyse the protonational conditions at the regions $\alpha \rightarrow 0$ and $\alpha \rightarrow 1$.

Sorlier et al. (2001) tried to use a new mathematical method for further treatment the Katchalsky's equation. Unfortunately, there was no uniform equation can be found and the process was very complex. The parameters of the modified Henderson-Hasselbalch model could be easily calculated from the intercept ($\alpha = 0.5$) and the slope of the plot of pH vs. $\log[\alpha/(1-\alpha)]$ and Högfeldt's three-parameter model could be uniformly and easily used to further deal with the Katchalsky's equation.

5. Conclusions

The protonation constants of CT with different M_w and *DD* were successfully determined by potentiometric titrations. Different pK_a values were obtained by using the modified Henderson-Hasselbalch model and Högfeldt's three-parameter model, and the results had no obvious differences. The protonation constants pK_a of CT showed a slightly decreasing from 6.51 to 6.39 with the M_w changing from 1370 to 60 kDa. The *DD* showed a greatly effect on pK_a values, which increased from 6.17 to 6.51 when *DD* decread from 94.6 to 73.3%. The fitting of the data to both two methods showed that Högfeldt's three-parameter model provided a better agreement than the modified Henderson-Hasselbalch model. The obtained results allowed to a better understanding of some physicochemical mechanisms and biological properties of CT.

Acknowledgements

The authors are indebted to the financial support from National Natural Science Foundation of China (No. 30370344) and the Scientist Encouragement Foundation of Shandong Province (2004BS7001).

References

Amiji, M. M. (1995). Pyrene fluorescence study of chitosan self-association in aqueous solution. *Carbohydrate Polymers*, *26*, 211–213.

Anthonsen, M. W., & Smidsrd, O. (1995). Hydrogen ion titration of chitosans with varying degrees of N-acetylation by monitoring induced H-NMR chemical shifts. *Carbohydrate Polymers*, *26*, 303–305.

Broussignac, P. (1968). Haut Polymère Naturel Connu dans l'Industrie: Le. Chitosane. *Chimie Industriel Genie, Chimie*, *99*, 1241–1247.

Chen, R. H., & Hwa, H. D. (1996). Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane. *Carbohydrate Polymers*, *29*, 353–358.

Chen, X. G., Zheng, L., Wang, Z., Lee, C. Y., & Park, H. J. (2002). Molecular affinity and permeability of different molecular weight chitosan membranes. *Journal of Agricultural and Food Chemistry*, *50*, 5915–5918.

Dodane, V., & Vilivalam, V. D. (1998). Pharmaceutical applications of chitosan. *Pharmaceutical Science and Technology Today*, *1*, 246–253.

Domard, A. (1987). pH and c.d. measurements on a fully deacetylated chitosan: Application to Cu(II)—polymer interactions. *International Journal of Biological Macromolecules*, *9*, 98–104.

Guibal, E. (2004). Interactions of metal ions with chitosan based sorbents: A review. *Separation and Purification Technology*, *38*, 43–74.

Högfeldt, E., Miyajima, A., Marinsky, J. A., & Muhammed, M. (1989). Application of a simple three-parameter model to titration data for some linear polyelectrolytes. *Acta Chemica Scandinavica*, *43*, 496–499.

Jia, Z. S., & Li, Q. B. (2001). Determination of the deacetylation degree of chitosan by two abrupt change potential titration method. *Chemical World*, *5*, 240–241.

Khan, T. A., Peh, K. K., & Ch'eg, H. S. (2002). Reporting degree of deacetylation values of chitosan: The influence of analytical methods. *Journal of Pharmacy and Pharmaceutical Sciences*, *5*, 205–212.

Kristiansen, A., Varum, K. M., & Grasdalen, H. (1998). Quantitative studies of the non-productive binding of lysozyme to partially *N*-acetylated chitosans: Binding of large ligands to a one-dimensional binary lattice studied by a modified McGhee and von Hippel model. *Biochimica et Biophysica Acta*, *1425*, 137–150.

Majeti, N. V., & Ravi, K. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers*, *46*, 1–27.

Mao, S., Shuai, X., Unger, F., Simon, M., Bi, D., & Kissel, T. (2004). The depolymerization of chitosan: Effect on physicochemical and biological properties. *International Journal of Pharmaceutics*, *281*, 45–54.

Montembault, A., Viton, A., & Domard, A. (2004) Physico-chemical studies of the gelation of chitosan in hydroalcoholic medium. *Biomaterials*, 26, 933–943.

Ottøy, M. H., Vårum, K. M., Christensen, B. E., Anthonsen, M. W., & Smidsrød, O. (1996). Preparative and analytical size-exclusion chromatography of chitosans. *Carbohydrate Polymers*, *31*, 253–261.

Park, J. W., & Choi, K. H. (1983). Acid-base equilibria and related properties of chitosan. *Bulletin of Korean Chemical Society*, *4*, 68–72.

Saito, K., & Tanioka, A. (1996). Polyamphoteric membrane study: 1. Potentiometric behaviour of succinyl chitosan aqueous solution. *Polymer*, *6*, 2493–2498.

Sato, T., Ishii, T., & Okahata, Y. (2001). In vitro gene delivery mediated by chitosan effect of pH, serum, and molecular mass of chitosan on the transfection efficiency. *Biomaterials*, *22*, 2075–2080.

Sorlier, P., Denuziere, A., Viton, C., & Domard, A. (2001). Relation between the degree of acetylation and the electrostatic properties of chitin and chitosan. *Biomacromolecules*, *2*, 765–772.

Sorlier, P., Viton, C., & Domard, A. (2002). Relation between solution properties and degree of acetylation of chitosan: Role of aging. *Biomacromolecules*, *3*, 1336–1342.

Sugimoto, M., Morimoto, M., Sashiwa, H., Saimoto, H., & Shigemasa, Y. (1998). Preparation and characterization of water-soluble chitin and chitosan derivatives. *Carbohydrate Polymers*, *36*, 49–59.

Van der Lubben, I. M., Verhoef, J. C., Borchard, G., & Junginger, H. E. (2001). Chitosan and its derivatives in mucosal drug and vaccine delivery. *European Journal of Pharmaceutical Sciences*, *14*, 201–207.

Wang, W., Bo, S. Q., Li, S. Q., & Qin, W. (1991). Determination of the Mark–Houwink equation for chitosans with different degrees of deacetylation. *International Journal of Biological Macromolecules*, *13*, 281–285.

Zentz, F., Bédouet, L., Schuller, M. J., Milet, C., Lopez, E., & Giraud, M. (2001). Characterization and quantification of chitosan extracted from nacre of the abalone *Haliotis tuberculata* and the oyster *Pinctada maxima*. *Marine Biotechnology*, *3*, 36–44.