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Changes in the Mark–Houwink hydrodynamic volume of chitosan molecules in solutions of different organic acids, at different temperatures and ionic strengths

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ABSTRACT

The objective of this study is to explore the cause(s) of changes in the hydrodynamic volume of chitosan molecules in solutions of different organic acids, at different temperatures and ionic strengths. Change in intrinsic viscosity is used as the parameter to elucidate the causes of changes in the hydrodynamic volume of chitosan molecules in these solutions. Results show that the intrinsic viscosity of chitosan decreases in acetic acid or in malic acid over storage time. These decreases are more pronounced in acetic acid solution than in malic acid solution, more significant in higher temperature than in lower temperature solutions, and greater in solutions without NaCl than in solutions containing higher NaCl. The decrease in intrinsic viscosity can perhaps be attributed to the compounded effects of compaction of the chitosan molecules and/or acidic degradation during storage.

1. Introduction

Chitosan is a high molecular weight polysaccharide linked by β-1,4 glucoside and is composed by *N*-acetyl-glucosamine and glucosamine. It is widely distributed biopolymer as it is readily available, with cationic polyelectrolyte in acid solution, and, as it is non-toxic and biodegradable, chitosan is considered to be both a versatile and an environmentally friendly raw material. Chitosan can be applied in food processing, agriculture, biomedicine, biochemistry, and wastewater treatment, and in preparing membranes, microcapsules, nanoparticles, cosmetics, textiles and liquid crystalline material (Chang, Chang, & Tsai, 2007; Rinaudo, 2006; Tsai, Bai, & Chen, 2008).

The hydrodynamics properties of chitinous materials in dilute solution are influenced by such factors as pH, ionic strength, temperature, concentration of urea, molecular weight and/or its distribution, degree of deacetylation (DD) and/or its distribution, and storage time (Anthonsen, Vårum, & Smidsrød, 1993; Chen, Lin, & Yang, 1994; Chen & Tsaih, 1998, 2000; Errington, Harding, Vårum, & Illum, 1993; Tsaih & Chen, 1997, 1999b; Wang, Bo, Li, & Qin, 1991).

Chitosan is susceptible to acid-catalyzed hydrolysis when it is stored in a strong acid solution, such as HCl. The hydrolysis of glycosidic linkage is assumed to be a S_N1 reaction where the rate-limiting step is the formation of the carbonium ion. The rate constant of chitosan acid hydrolysis increases in direct proportion to the HCl concentration (Vårum, Ottøy, & Smidsrød, 2001). Moreover, the initial rate constant for chitosan chloride at pH 4–6 increases with the H^+ concentration in the 0.8 power (Holme, Foros, Pettersen, Dornish, & Smidsrød, 2001).

Chen, Chang, and Shyur (1997) reported that after the chitosan–acetate buffer solution has been treated ultrasonically and then stored for 17 days at room temperature, both the molecular weight and polydispersity of the chitosan decreased with increasing storage time. This indicated that the degradation of chitosan in the acetic buffer occurred during storage. Zoldners, Kiseleva, and Kaiminsh (2005) reported that chitosan was soluble in 0.2% acetic acid at 20 °C and that the relative viscosity decreased very slowly with increasing storage time. It was thought that the chitosan was being oxidized and that hydrolytic splitting of the chains occurred during storing. The introduction of a small amount of ascorbic acid (0.1 g/l) into the solution induced a rapid reduction in the viscosity of the solution. Il'ina and Varlamov (2004) reported that chitosan–1% lactic acid solution was kept at 8, 22, and 37 °C over 180 days, with periodic sampling to measure the intrinsic viscosity. The results showed that chitosan hydrolysis in diluted lactic acid depended on the molecular weight and DD of the initial sample, i.e., the higher the values of both parameters, the quicker the decrease in intrinsic viscosity and viscosity-average molecular weight. No, Kim, Lee, Park, and Prinyawiwatkul (2006) reported that the viscosity of 1% chitosan–1% acetic and/or lactic acid solution decreased with increased storage time and temperature. After a 15-week storage period, the decrease in viscosity ranged from

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44% to 48% and 81% to 90% of the initial viscosity value at 4 and 25 °C, respectively. The decrease in viscosity observed over time was probably due to the partial degradation of chitosan by the organic acid solutions.

However, Berkovich, Timofeyeva, Tsyurupa, and Davankov (1980) revealed that the anomalous low values of the Mark–Houwink exponent a (0.15–0.30) and the high values of intrinsic viscosity of 3.5–7.0 dl/g for a dilute acid solution of chitosan with a molecular weight of 12–170 kDa were accounted for on the basis of an assumed quasi-globular structure of macromolecules caused by a system of intramolecular hydrogen bonds and intermolecular hydrogen bridges.

The objective of this study was to explore the cause(s) of changes in the hydrodynamic volume of chitosan molecules in solutions of different organic acids, at different temperatures and ionic strengths.

2. Experimental section

2.1. Preparation of chitosan

β-Chitin was prepared from squid pens donated by Shin Dar Bio-Tech. Co. Ltd (Taoyuan, Taiwan). In brief, the squid pens were ground to a 40–60 mesh size. Each 100 g of powder was immersed in 500 ml of 1 M hydrochloric acid solution overnight. The powder was washed till neutral and drained before being soaked in 500 ml of 2 M sodium hydroxide at ambient temperature overnight. It was then soaked in 500 ml of 2 M sodium hydroxide solution at 100 °C for 4 h, then washed and dried to produce about 35 g β-chitin (Chang et al., 2007; Tsai et al., 2008).

β-Chitin was added to 50% (w/w) sodium hydroxide solution at a ratio of 1 (g solid): 20 (ml solution). The deacetylation reaction took place at 100 °C for 3 h. The sample was then washed till neutral and dried at 50 °C to get the chitosan product (Chang et al., 2007; Tsai et al., 2008). The DD of the prepared chitosan was determined by the colloid titration method (Toei & Kohara, 1976). Chitosan with a DD of 96% was used in this study.

2.2. Determination of intrinsic viscosity

Different concentrations (0.01–0.1%) of chitosan in 0.1 M acetic acid or 0.1 M malic acid solutions containing 0–0.5% sodium chloride were prepared. These solutions were passed through a 0.45 μm filter (Lida, St. Louis, MO, USA) to remove insoluble materials. The chitosan solutions were then stored at 25, 35, 45 °C for up to 30 days. The intrinsic viscosity of the chitosan solutions was determined by a capillary viscometer.

The capillary viscometer (No. 75, 100, Cannon–Fenske, State College, PA, USA) was filled with 5 ml of the sample and equilibrated in a water bath (Firstek B801, Taipei, Taiwan, ROC) with an extra thermostat (Firstek B403, Taipei, Taiwan, ROC) to maintain the temperature at 30 ± 0.1 °C. Each sample was measured three times. The running times of the solution and solvent were used to calculate the relative viscosity, specific viscosity, and reduced viscosity. The reduced viscosity was plotted against the concentration with the intercept being the intrinsic viscosity (Tsaih & Chen, 1999a).

2.3. Mathematical treatment

The measured intrinsic viscosity ($[\eta]$) was used to calculate the viscosity-average molecular weight (Mv), using the Mark–Houwink equation as follows:

$$[\eta] = k_{MH}\mathrm{Mv}^{a} \quad (1)$$

$$\mathrm{Mv} = ([\eta]/k_{MH})^{1/a} \quad (2)$$

where a and k_{MH} are constants for a given system, and the a and k_{MH} values depend on the solution conditions, such as temperature, pH level, and ionic strength. In this study, a and k_{MH} were 0.726, 5.11×10^{-4} for 0% sodium chloride, 0.703, 6.30×10^{-4} for 0.1% sodium chloride, 0.681, 7.50×10^{-4} for 0.2% sodium chloride, and 0.612, 1.11×10^{-3} for 0.5% sodium chloride (Tsaih & Chen, 1999a).

Degradation of polymer is normally characterized by a series of parallel-proceeding reactions, since the original substance usually has a relatively broad molecular weight distribution. The exact rate constant of the degradation, therefore, is not obtainable directly from kinetic studies because of its dependence on molecular weight. Exact rate constants are only obtainable if the original substance is monodisperse, or if certain assumptions concerning the degradation mechanism are made (Basedow & Ebert, 1977). The degradation reaction of polymer by acid hydrolysis has demonstrated that the process can be adequately described by simple first-order kinetics. The rate constant (k) of degradation can be obtained from the plot of the reciprocal molecular weight against time, as follows (Holme et al., 2001; Singh & Jacobsson, 1994; Vårum et al., 2001):

$$1/M_t - 1/M_0 = (k/m)t \quad (3)$$

where M_0 and M_t are the molecular weights at time 0 and t, respectively; k (s^{-1}) is the first-order rate constant; t is the storage time; and m is the average molecular weight of a monosaccharide unit of chitosan, $m = 161 + 42$ (100% − DD).

Eq. (4) is derived from Eqs. (2) and (3), and k can be obtained from the slope ($k/(mk_{MH}{}^{1/a})$) of the plot of ($1/[\eta]_t{}^{1/a}$-$1/[\eta]_0{}^{1/a}$) *vs.* t, as follows:

$$1/[\eta]_t^{1/a} - 1/[\eta]_0^{1/a} = [k/(\mathrm{mk}_{\mathrm{MH}}{}^{1/a})]t \quad (4)$$

The temperature dependence of the k value can be obtained from the Arrhenius equation as follows (Lai, Lii, Hung, & Lu, 2000):

$$k = k_0 \exp(-E_a/RT) \quad (5)$$

$$\mathrm{Ln}k = (\mathrm{Ln}k_0) - E_a/RT \quad (6)$$

where k_0 (s^{-1}) is the Arrhenius frequency factor, E_a (J/mol) is the activation energy of depolymerization.

2.4. Size-exclusion high-performance liquid chromatography

The chitosan was stored in 0.1 M acetic acid, 0.1 M acetic acid/0.5% NaCl, or in 0.1 M malic acid at 45 °C for 7 days. The elution patterns of size-exclusion high-performance liquid chromatography (SE-HPLC) of this chitosan and the original chitosan were determined by the method of Tsaih and Chen (1999a) as follows. Columns (7.8 mm × 30 cm) packed with TSK gel G4000 PW_{XL} and G5000 PW_{XL} (Tosoh Co., Ltd., Tokyo, Japan) were used. The mobile phase consisted of 0.2 M acetic acid/0.1 M sodium acetate and 0.008 M sodium azide. A sample concentration of 0.1% (w/v) was loaded and eluted with a flow rate of 0.6 ml/min by an LDC Analytical ConstaMetric 3500 pump (Riviera Beach, FL). The elute peak was detected by an RI detector (Gilson, M132, Middleton, WI), and the data was analyzed with Chem-Lab software (Scientific Information Service Co., Taipei, Taiwan).

3. Results and discussion

3.1. Effect of storage time

The changes in the intrinsic viscosity of chitosan in 0.1 M acetic acid or in 0.1 M malic acid containing 0–0.5% sodium chloride at

25, 35, 45 °C for 0–30 days are shown in Fig. 1. The results show that the intrinsic viscosity of the chitosan decreased with increasing storage time in all the solutions studied. Stored at the same temperature and ionic strength, intrinsic viscosity decreases were more pronounced for the chitosan–acetic acid solutions than for the chitosan–malic acid solutions.

The decreases in intrinsic viscosity may be attributed to the chitosan being degraded by acetic acid or malic acid during storage. In addition, based on the Mark–Houwink equation of $[\eta] = k_{MH}Mv^a$, intrinsic viscosity is in proportion to the molecular weight of a molecule. Exponent a is a parameter used to indicate the molecule's conformation which is independent of the narrow molecular weight range, but constant for a molecule at constant media pH, ionic strength, and temperature (Launay, Doublier, & Cuvelier, 1986). For the a values higher than 1.0, in 0.5–0.8, and below 0.5, the molecule is a rod-like shape, a random coil, or a very compact globular shape, respectively (Launay et al., 1986). However, the decreases in intrinsic viscosity, as shown in Fig. 1, may also have been due to the compounded effect of compaction of the chitosan molecules and acidic degradation during storage. Sklyar et al. (1981) reported that the normalized viscosity (viscosity ratio of aged solution to fresh solution) of 2% chitosan in 10% acetic acid was much higher than that in 2% acetic acid after 2 days storage. More pronounced decreases in viscosity in higher pH solutions than in lower pH solutions were attributed to the compression of chitosan molecules, rather than to the breakdown process during storage. Berkovich et al. (1980) revealed the anomalous low values of the Mark–Houwink exponent a (0.15–0.30) and the high values of intrinsic viscosity of 3.5–7.0 dl/g for a dilute acid solution of chitosan with a molecular weight of 12–170 kDa. The anomalous values were accounted for on the basis of an assumed quasi-globular structure of macromolecules caused by a system of intramolecular hydrogen bonds and intermolecular hydrogen bridges. The intramolecular hydrogen bonds and intermolecular hydrogen bridges may have taken place during storage due to the low dissociation constant of acetic acid and malic acid in water (1.76×10^{-5} for acetic acid, 3.9×10^{-4} and 7.8×10^{-6} for malic acid at the 1st and 2nd steps, respectively). A low H^+ concentration would restrict the polyelectrolyte expansion (third electroviscous effect) and diminish the coulombic repulsion between molecules (second electroviscous effect), which would in turn decrease the intrinsic viscosity leading to the lower Mark–Houwink exponent a values. Another reason may be due to that malic acid contains two carboxylic groups. These carboxylic groups act as crosslink agents and lead to stronger intermolecular interactions (Bodnár, Hartmann, & Borbély, 2005; Chang et al., 2007), and thus hinder the hydrolysis process. Therefore, the results, as shown in Fig. 1, may have been due to the acid hydrolysis of the chitosan molecules, as well as to the shape of the chitosan molecules changing from that of a random coil to a more compact shape.

3.2. Elution patterns of SE-HPLC of chitosan in different organic acid solutions

Fig. 2 shows the elution patterns of SE-HPLC of chitosan after aging in 0.1 M acetic acid, 0.1 M acetic acid/0.5% NaCl, or in

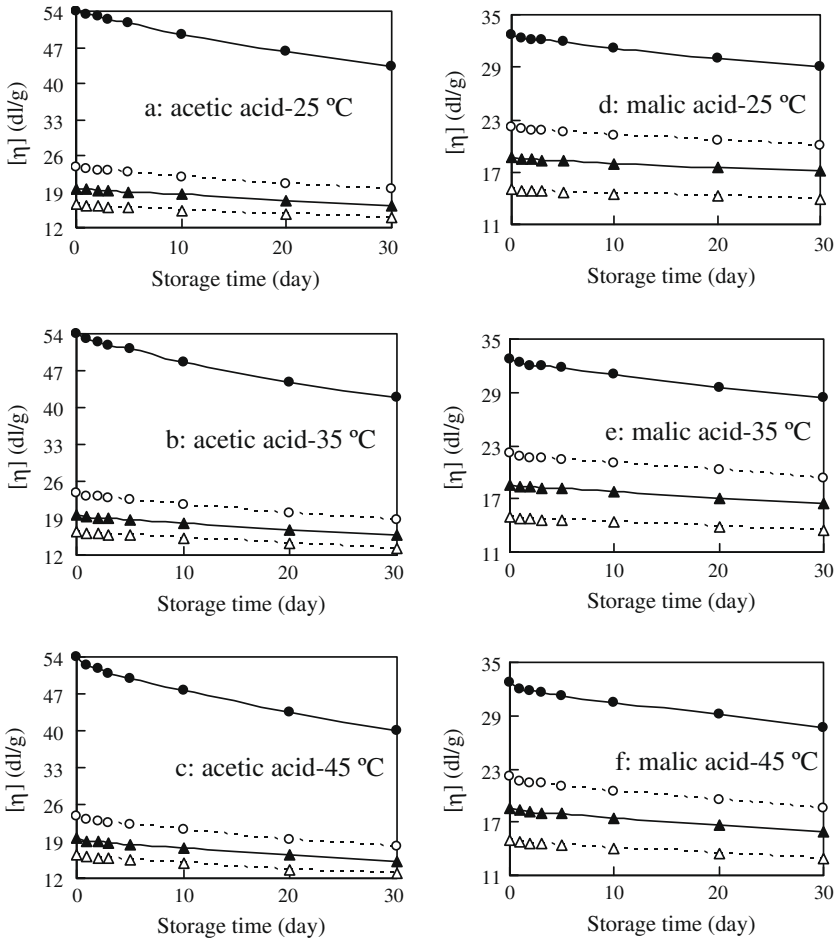

Fig. 1. Changes in intrinsic viscosity ([η]) of chitosan in 0.1 M acetic acid or 0.1 M malic acid solutions during storage at 25, 35, and 45 °C (●, 0% NaCl; ○, 0.1% NaCl; ▲, 0.2% NaCl; △, 0.5% NaCl).

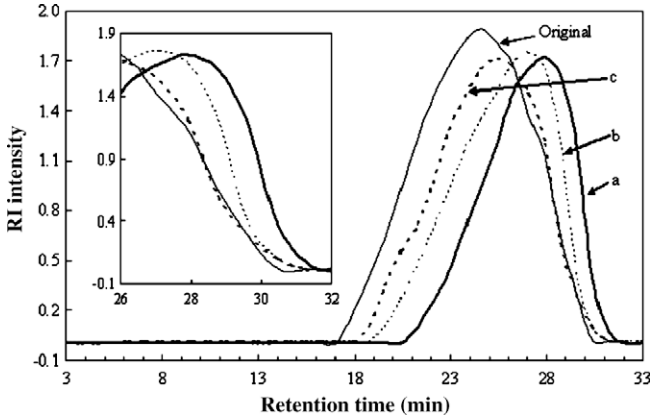

Fig. 2. Elution patterns of size-exclusion high-performance liquid chromatography (SE-HPLC) of chitosan stored in 0.1 M acetic acid (a), 0.1 M acetic acid/0.5% NaCl (b), and 0.1 M malic acid solutions (c) at 45 °C for 7 days. Elution patterns between retention time 26–32 min were inserted.

0.1 M malic acid at 45 °C for 7 days. The elution patterns indicate that, after 7 days storage, the molecular weight of the stored chitosan decreased. The average molecular weight of chitosan stored in 0.1 M acetic acid (a) was lower than for that stored in 0.1 M acetic/0.5% NaCl (b), which in turn was lower than for the chitosan stored in 0.1 M malic acid (c).

In comparing the distribution of different molecular weight fractions, the lower molecular weight fractions of the chitosan/malic acid system did not change significantly (almost overlapping with the original chitosan); however, decreases in the higher molecular weight fractions were pronounced (Fig. 2c). It indicated that the changes in the hydrodynamic diameter or hydrodynamic volume in term of retention time of chitosan in 0.1 M malic acid are mainly due to the compaction of chitosan molecules (Berkovich et al., 1980; Sklyar et al., 1981), because there are no smaller molecular species generated during stored at 45 °C for 7 days. However, for the chitosan/0.1 M acetic acid system, the high molecular weight fractions decreased to the same degree that the lower molecular weight fractions increased (Fig. 2a). The results indicated that the changes in the retention time of chitosan in 0.1 M acetic acid system was mainly due to acid hydrolysis (Chen et al., 1997; Il'ina & Varlamov, 2004; No et al., 2006; Zoldners et al., 2005). The generated smaller molecular weight species are derived from the higher molecular weight ones. The distribution of different molecular weight fractions pattern for the chitosan/0.1 M acetic acid/0.5% NaCl system were in between the two above-mentioned systems. The results indicated that the change in hydrodynamic volume of chitosan in 0.1 M acetic acid/0.5% NaCl system were due to the compound effects of compaction and acidic hydrolysis.

3.3. Effect of solution temperature

The results in Fig. 1 show that the chitosan molecule degraded faster in higher temperature solutions. Table 1 also shows that the k values increased with increasing temperature based on the Arrhenius equation. Our results were consistent with those of Holme et al. (2001), Jia and Shen (2002), and Xing et al. (2004).

Table 1
Effect of concentration of sodium chloride, type of acid, and solution temperature on the rate constant (s^{-1}) of chitosan stored up to 30 days.

NaCl (%)	0.1 M acetic acid			0.1 M malic acid		
	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
0	2.24E−11	2.63E−11	3.09E−11	1.13E−11	1.41E−11	1.72E−11
0.1	2.12E−11	2.59E−11	3.01E−11	1.07E−11	1.36E−11	1.65E−11
0.2	1.98E−11	2.41E−11	2.85E−11	9.77E−12	1.24E−11	1.58E−11
0.5	1.89E−11	2.34E−11	2.76E−11	9.31E−12	1.21E−11	1.52E−11

The enthalpy–entropy compensation effect and the Arrhenius law can be described as the change in frequency factor (k_0) and activation energy (E_a) of acid hydrolysis (Lai et al., 2000). Fig. 3 shows the relationship of the natural logarithm k value and the reciprocal of the reaction temperature of chitosan aged under different solution conditions. The correlation equations of k value and solution temperature have been included in Fig. 3. E_a and k_0 were calculated from these equations and have been listed in Table 2. The results have shown that E_a increased with an increasing concentration of sodium chloride, and the E_a of the chitosan molecules stored in 0.1 M malic acid was higher than for those stored in 0.1 M acetic acid. Chitosan molecules were more expanded (higher intrinsic viscosity) in acetic acid solutions than in malic acid solutions of the same NaCl concentration, and were also more expanded in either acetic acid or malic acid solutions of lower NaCl concentrations than those of higher NaCl concentrations. The expanded molecules facilitated acid hydrolysis because the exposed glycosidic linkage was easier to attack by H^+ and, thus, resulted in a lower E_a value. In contrast, chitosan molecules were contracted in higher NaCl solutions or in malic acid solution, the acid hydrolysis was hindered due to the enclosed glycosidic linkages rendered the difficult to access by H^+, therefore the E_a value was higher. The E_a values (12.67–19.36 kJ/mol), shown in Table 2, were lower than those of 109–114 kJ/mol for thermal degradation of chitosan chloride (Holme et al., 2001) and also lower than the E_a of 130–158 kJ/mol for 0.4 M HCl hydrolysis of chitosan (Vårum et al., 2001). The lower E_a values reported in Table 2 indicate that the degradation reaction of chitosan molecules stored in acetic acid or malic acid was relatively temperature insensitive (Levenspiel, 1972). This may have been because the acid hydrolysis rate of glycosidic linkage is dependent on the concentration of H^+ (Holme et al., 2001;

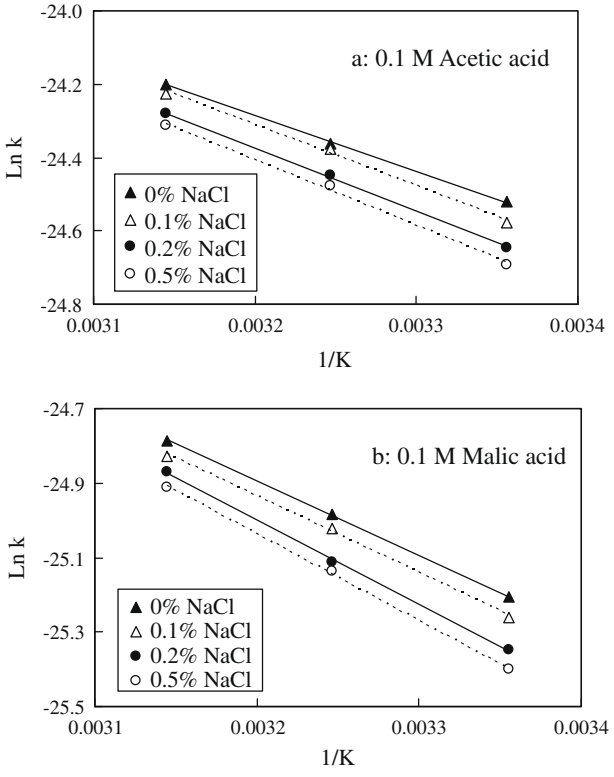

Fig. 3. Plots of natural logarithmic hydrolysis rate constant *vs.* reciprocal of absolute temperature (K) of chitosan solutions stored in various conditions.

Table 2
Hydrolysis activation energy (E_a, kJ/mol) and Arrhenius frequency factor (k_0, s^{-1}) of chitosan stored in various conditions.

NaCl (%)	0.1 M acetic acid		0.1 M malic acid	
	E_a	k_0	E_a	k_0
0	12.67	3.71E−09	16.55	9.02E−09
0.1	13.82	5.65E−09	17.08	1.06E−08
0.2	14.35	6.52E−09	18.81	1.93E−08
0.5	14.93	7.88E−09	19.36	2.31E−08

Vårum et al., 2001). The concentration of H^+ in 0.1 M acetic acid or in 0.1 M malic acid solution is much smaller than in a strong acid such as HCl. So, the hydrolysis rate constant (k) in 0.1 M acetic acid or in 0.1 M malic acid solution was lower than in HCl acid. Another reason may be that the dissociation constant of acetic or malic acid decreased with increasing temperature. The dissociation constants of acetic acid are 1.754×10^{-5}, 1.728×10^{-5} and 1.670×10^{-5} at 25, 35 and 45 °C (Weast, 1973–1974). Thus, the concentration of H^+ decreased with temperature increases between 25 and 45 °C. The inverse dependence of the dissociation constant of both acids on the solution temperature not only decreased the reaction rate with increases in temperature for the acid-catalyzed hydrolysis of chitosan in acetic or malic acid, but also nullified the effect of increasing reaction temperature to promote the formation of activated intermediate of the acid hydrolysis reaction (Levenspiel, 1972), which in turn increased the rate constant with increasing solution temperature. Therefore, the increase of rate constant with increasing solution temperature was low and led to a low E_a value. Furthermore, by increasing the solution temperature, the effect of compaction on decreasing acid hydrolysis should be less for malic acid than for acetic acid because the conformation of chitosan molecules in acetic acid is more expanded than in malic acid. Indeed, the effect of elevated temperature on molecular expansion was less pronounced for expanded molecules than for contracted ones, and the E_a value of the chitosan/0.1 M acetic acid system was lower than that for the chitosan/0.1 M malic acid system.

3.4. Effect of concentration of sodium chloride

Chitosan is a cationic polyelectrolyte in acidic solutions. The intrinsic viscosity of chitosan is dependent on the molecular weight, DD, distribution of the acetyl group, ionic strength and pH of the solution (Anthonsen et al., 1993; Chen & Tsaih, 1998; Tsaih & Chen, 1997, 1999b). Fig. 1 shows that the intrinsic viscosity of chitosan solutions containing NaCl was much lower than for those containing no NaCl: the higher the NaCl concentration, the lower the intrinsic viscosity. This was due to the shielding effect of counterion Cl^- on the $-NH_3^+$ group of chitosan which caused the contraction of the chitosan molecules. So, the intrinsic viscosity of chitosan was lower and rendered the decreases in the access of H^+ to hydrolyze the chitosan in solution (Chen et al., 1994; Errington et al., 1993; Tsaih & Chen, 1997, 1999b).

The degradation rate constant of chitosan decreased with an increasing concentration of sodium chloride (Table 1), i.e., increasing the concentration of sodium chloride alleviated the hydrolysis of chitosan molecules. By adding sodium chloride to the chitosan solution, the chitosan molecules contracted as the $-NH_3^+$ groups of chains were neutralized to $-NH_2$ by counterions. In contrast, in the solutions containing lower concentrations of sodium chloride, the chitosan molecule has an expanded conformation (Chen et al., 1994; Errington et al., 1993; Tsaih & Chen, 1997, 1999b). It has been assumed that when the chitosan molecule has extended contour, the k value is higher as the glycosidic linkage is easier to access by H^+ and the hydrolysis reaction proceeds (Fig. 4) (Kasaai, Charlet, Paquin, & Arul, 2003; Tsaih & Chen, 2003; Tsaih, Tseng, & Chen, 2004). Furthermore, the interaction force of polyelectrolyte segments increases with increasing ionic strength, which leads to a retarded hydrolysis reaction (Singh & Jacobsson, 1994).

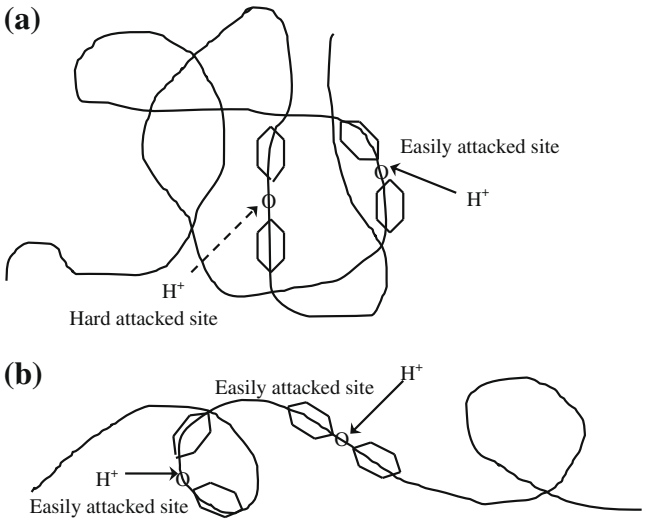

Fig. 4. Schematic diagrams illustrate the acid hydrolysis reaction of chitosan with a contracted conformation (a), and with an extended conformation (b).

3.5. Effect of type of acid

Fig. 2 shows that the average molecular weight of chitosan in 0.1 M acetic acid solution was lower than in 0.1 M malic acid solution, and that the k values (Table 1) of chitosan in acetic acid were higher than in malic acid. These results indicate that the chitosan molecule degraded faster in acetic acid than in malic acid, possibly because malic acid contains two carboxylic groups. These carboxylic groups act as crosslink agents and lead to stronger intermolecular interactions (Bodnár et al., 2005; Chang et al., 2007), and thus hinder the hydrolysis process. The pH levels of the chitosan solutions in 0.1 M acetic acid or in 0.1 M malic acid were 3.12 and 2.27, respectively. However, the intrinsic viscosity of chitosan in 0.1 M acetic acid was higher than in 0.1 M malic acid (Fig. 1). This indicated that the chitosan molecules in 0.1 M acetic acid were more expanded than in 0.1 M malic acid (owing to stronger intermolecular interaction) and the expanded molecules facilitated the degradation reaction (Fig. 4) (Kasaai et al., 2003; Tsaih & Chen, 2003; Tsaih et al., 2004). Therefore, chitosan molecules degraded faster in acetic acid than that in malic acid.

4. Conclusions

The changes in the hydrodynamic volume of chitosan molecules in solutions of different organic acids, at different temperatures and intrinsic ionic strengths may have been due to acid hydrolysis and/or compaction. The decreases were more pronounced in acetic acid solutions than in malic acid solutions, more significant in higher temperature solutions than in lower temperature ones, and greater in solutions without NaCl than in solutions containing higher NaCl. Decreases in the hydrodynamic volume of chitosan in the 0.1 M malic acid system were mainly due to the compaction of chitosan molecules; however, in the 0.1 M acetic acid system the decreases were mainly due to acid hydrolysis, while in the 0.1 M acetic acid plus 0.5% NaCl system the decreases were the result of the compounded effect of compaction and acidic hydrolysis.

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