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Kinetics and efficiency of chitosan reacetylation

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1. Introduction

Chitosan is a cationic polysaccharide prepared by alkaline deacetylation of chitin that is found in the shells of crustaceans. It is a linear polyelectrolyte composed of glucosamine and Nacetyl-glucosamine units linked by β -(1 \rightarrow 4) glycosidic bonds, where the fraction of units that are glucosamine is defined as the degree of deacetylation (DDA). Chitosan is usually obtained by alkaline deacetylation of chitin, where particulate chitin is suspended in a hot alkaline solution (typically 40–50%, w/w, NaOH). Although acetyl content can be targeted by alkaline deacetylation, this heterogeneous reaction is thought to result in a block distribution of acetyl groups (Aiba, 1991; Kurita, Sannan, & Iwakura, 1977). Using ¹H NMR, Varum, Anthonsen, Grasdalen, and Smidsrod (1991) confirmed that heterogeneously prepared chitosans have a slightly more blockwise distribution and Ottoy, Varum, and Smidsrod (1996) showed that such chitosans often comprise a highly acetylated acid-insoluble fraction. On the other hand, using nitrous acid depolymerisation and steric exclusion chromatography, Sashiwa, Saimoto, Shigemasa, Ogawa, and Tokura (1991) and Sashiwa, Saimoto, Shigemasa, and Tokura (1993) obtained results suggesting a random distribution of N-acetyl group in both heterogeneously and homogeneously prepared chitosans.

ABSTRACT

Chitosan reacetylation kinetics and efficiency were studied in water–methanol (MeOH) mixtures. The polymer was dissolved using acetic acid and acetic anhydride was used for reacetylation. Combining second-order kinetics and acid–base dissociation equations of chitosan, and using acetic anhydride hydrolysis rates determined by conductivity measurements, reacetylation reaction rate constants of 187, 108, 46 min⁻¹ M⁻¹ were found in 0, 50 and 80% MeOH (v/v), respectively. Contrary to previous literature, it was found that improvement in reacetylation efficiency in the presence of MeOH is mainly due to an increase of acetic acid pK_a by MeOH that limits the ionization of the polymer in the course of the reaction rather than to a decreased acetic anhydride hydrolysis rate, as previously thought. Based on these insights, the model developed in this study was able to predict the significantly reduced efficiency of the reaction for a large extent of reacetylation, without requiring any steric hindrance from the acetyl group. Conditions to maximize the reaction efficiency for a large extent of reacetylation efficiency for a large extent of reacetylation were identified.

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Reacetylation is a previously applied alternative which produces chitosan with a more homogeneous and random distribution of acetyl groups compared to heterogeneously deacetylated chitosan (Aiba, 1992, 1994). Reacetylation is useful to prepare chitosan samples with similar molecular weight but different DDA (Knaul, Kasaai, Bui, & Creber, 1998; Maghami & Roberts, 1988) and to obtain soluble chitosan at physiological pH values (Dal Pozzo et al., 2000). Homogeneously reacetylated chitosans display different enzymatic degradation properties compared to heterogeneously prepared chitosans, and are typically less degradable (Shigemasa, Saito, Sashiwa, & Saimoto, 1994), due to the random distribution of acetyl groups on the polymer backbone (Aiba, 1992). Hydrogels may be prepared by reacetylating chitosan to a sufficient extent so that it becomes insoluble (Hirano, Kondo, & Ohe, 1975; Moore & Roberts, 1980; Vachoud, Zydowicz, & Domard, 1997).

Reacetylation of chitosan is performed by adding acetic anhydride to chitosan solutions. The reaction involves a nucleophilic attack of an unprotonated primary amino group of glucosamine on one carboxyl function of the acetic anhydride. Since acetic anhydride is hydrolysed in water, methanol (MeOH)/water mixtures are typically used for efficient reacetylation. It is worth noting that chitosan is not soluble in 100% MeOH so that the reaction cannot be performed under conditions where hydrolysis of acetic anhydride could be completely eliminated. Acetic acid is typically used to solubilise chitosan for reacetylation, as it is preferred to HCl since dissociation equilibrium of the amine of chitosan can be displaced during the course of the reaction (Vachoud et al., 1997).

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Previous studies report the reacetylation of chitosan using different conditions (% MeOH, chitosan and acid concentrations, etc.) and report variable efficiencies (Aiba, 1994; Berger et al., 2005; Knaul et al., 1998; Qun, Ajun, & Yong, 2007; Vachoud et al., 1997). A reduced efficiency of the reaction for larger extent of reacetylation has been observed and has been attributed, at least partly, to increased steric hindrance at high acetyl content (Qun et al., 2007; Vachoud et al., 1997). Although this reacetylation process has been widely used, the kinetics of the reaction has not been examined in detail (Vachoud et al., 1997).

In this paper, we have characterized the kinetics and efficiency of chitosan reacetylation in water–methanol mixtures of 0, 50 and 80% MeOH (v/v). A model and solution to the set of differential equations describing the kinetics of reacetylation is described, including the calculation of the degree of ionization of glucosamine unit during the course of the reaction and the hydrolysis kinetics of acetic anhydride. Using appropriate dielectric constant values (Albright & Gosting, 1946), the ionization degree is calculated using a mean field Poisson–Boltzmann model as described previously (Filion, Lavertu, & Buschmann, 2007; Lavertu, Filion, & Buschmann, 2008). The detailed model and all relevant equations are available in the Supplementary material section.

2. Materials and methods

2.1. Materials

Acetic anhydride (cat # 242845), sodium hydroxide standard solution – 1.0 N in H_2O (cat # 319511), sodium azide (cat #S2002), hydrochloric acid standard solution – 1.0 N in H_2O (cat #318949), deuterium oxide (cat # 151882), deuterium chloride solution – 35 wt.% in D_2O (cat # 543047) and D-(+)-glucosamine hydrochloride (cat # G1514) were purchased from Sigma–Aldrich. Glacial acetic acid (cat # 00598-320) and methanol HPLC grade (cat # 9093-03) were obtained from Anachemia Science and from J.T. Baker, respectively. Chitosan (Ultrasan) with a degree of deacety-lation (DDA) of 98.6% was provided by Piramal Healthcare Canada. Dextran 500 (cat # 17-0320-01) was purchased from Amersham Biosciences.

2.2. Hydrolysis kinetics of acetic anhydride

Acetic anhydride concentrations of 5, 15, 45, 200 mM were tested in 0, 50, 80 and 90% methanol-water mixtures (v/v), respectively. The extent of dissociation of acetic acid decreases as the proportion of methanol increases (i.e. its pK_a increases) and progressively higher concentrations of acetic anhydride are required to accurately measure conductivity as the proportion of methanol increases. Acetic anhydride was added to methanol-water mixtures while stirring and conductivity was monitored as a function of time using an Accumet[®] conductivity probe (Fisher Scientific, cat # 13-620-156) connected to an Accumet[®] Model 20 pH/conductivity meter from Fisher Scientific. The conductivity probe was calibrated using a 10 µS/cm standard solution (Fisher Scientific, cat # 09-328-1). Solutions were tested in a jacketed beaker coupled to a circulating bath (Neslab, model RT-111) to control temperature. Conductivity measurements were performed at 22.5 °C. The hydrolysis reaction rates were calculated from these conductivity measurements as described in Asprey, Wojciechowski, Rice, & Dorcas, 1996. Hydrolysis kinetics measurements were also performed on an aqueous solution of dextran 500 (nominal M_w = 500 kDa) 10% (w/w). According to the literature, such a solution has a dynamic viscosity (~20 cP (Xu, Li, Zhang, & Qi, 2009)) close to that of the chitosan solutions (Martinez, Chornet, & Rodrigue, 2004) tested in this study and was thus used to determine if hydrolysis rate was affected by solution viscosity.

2.3. pK_a measurements in different solvent mixtures

A 500 mM acetic acid solution was prepared by diluting glacial acetic in water. Sodium hydroxide was added to adjust the degree of ionization to 0.5 so that $pH \sim pK_a$. 1 ml of this solution was added to 9 ml water-methanol mixtures such that final compositions were 50 mM acetic acid in 0, 25, 50, 80 and 90% methanol (v/v). The pH of these solutions was measured using a glass body pH electrode (PerpHecT[®] ROSS, model #8220BNWP) calibrated with aqueous standard buffers. Under these conditions, the changes of pH from one solvent composition to another equal pK_a changes (Filion et al., 2007). pK_a values for various solvent compositions were calculated from these pH changes using a reference pK_a value of 4.76 for acetic acid in water (Bacarella, Grunwald, Marshall, & Purlee, 1955) and by correcting for the solvent effect using Eq. (15). Similarly, a 500 mM aqueous solution of glucosamine hydrochloride with a degree of ionization adjusted to 0.5 using sodium hydroxide was prepared. 1 ml of this solution was added to 9 ml water-methanol mixtures and pH was measured, as described above for acetic acid solutions. pK_a values were determined using a reference pK_a of 7.8 for glucosamine in water (Park, Choi, & Park, 1983). These glucosamine pK_a changes were then used to estimate the values of the intrinsic pK_a of chitosan (pK_0) for various water-methanol mixtures using a reference pK_0 value of 6.7 for chitosan in water (Filion et al., 2007). All pH measurements were done in the intersolvental pH^s_w scale (Inczédy, Lengyel, Ure, and International Union of Pure and Applied Chemistry (1998) and March (1992)) and were converted to the pH_s^s scale as described in Canals, Oumada, Roses, & Bosch, 2001. Note that all pK_a values in this study are reported on the ^s_spH scale.

2.4. Chitosan characterization

Chitosan DDA was determined by ¹H NMR as previously described (Lavertu et al., 2003). ¹H NMR measurements were performed using a Varian Inova 500 MHz spectrometer operated with VNMRJ 2.3A software and equipped with a 5 mm SW PFG ¹H/¹⁹F/X[¹⁵N-³¹P] probe. Molecular weight of starting chitosan was determined by size-exclusion chromatography (SEC) as previously described (Nguyen, Winnik, & Buschmann, 2009). Measurements were performed on a Gel Permeation Chromatography (GPC) system consisting of an LC-20AD isocratic pump (Shimadzu), an autosampler SIL-20AC HT (Shimadzu), an oven CTO-20AC (Shimadzu) coupled with a Dawn HELEOS II multiangle laser light scattering detector (Wyatt Technology Co.), a Viscostar II (Wyatt Technology Co.), an Optilab rEX interferometric refractometer (Wyatt Technology Co.), and two Shodex OHpak columns (SB-806M HQ and SB-805 HQ) connected in series. A chitosan dn/dc value of 0.214 was used (laser's wavelength of 658 nm) and the number and weight average molecular weights $(M_n \text{ and } M_w)$ were found to be equal to 132 kDa and 187 kDa, respectively. The water content of chitosan was determined using a heated centrifugal vacuum concentrator (Savant Speedvac, model SS11) by measuring the loss on drying of 100 mg of chitosan after 48 h at 60 °C. The percentage of water in chitosan was found to be 9.7% and the concentrations of chitosan solutions prepared from non-dried powder throughout this study were adjusted accordingly.

2.5. Chitosan reacetylation kinetics and efficiency

Chitosan was first dissolved in water using glacial acetic acid. The chitosan powder was dispersed in water using a magnetic stirrer prior to glacial acetic acid addition. In order to obtain a final concentration of 4.5 mg/ml after methanol addition (i.e. final concentration of 27.5 mM of glucosamine units of chitosan for all solutions in this study), the chitosan concentration in water prior to methanol addition was adjusted to 4.5, 9.0 or 22.5 mg/ml for 0,

Та	ble	1

Hydrolysis and reacetylation reaction rate constants in water-methanol mixtures.

% MeOH (v/v)	$k'_{H} ({ m min}^{-1})^{ m a} (imes 10^{3})$	$k_{\rm H}~({ m min^{-1}~M^{-1}})^{\rm a}~(imes 10^3)$	$k_{ m R}~({ m min^{-1}~M^{-1}})^{ m b}$
0	145 ± 1	2.63 ± 0.01	187 ± 17
50	68 ± 1	2.40 ± 0.01	108 ± 6
80	23.2 ± 0.1	2.04 ± 0.01	46 ± 2
90	8.6 ± 0.1	1.53 ± 0.01	-

^a n = 2, mean $\pm (max - min)/2$.

^b n = 3, mean \pm SD.

50 or 80% MeOH (v/v), respectively. Since acetic anhydride reacts with the neutral form of glucosamine and in order to maximize the reacetylation efficiency, the amount of glacial acetic acid was adjusted to a minimum that ensured polymer solubility for a given proportion of methanol. We found that for mixtures with 0 and 50% (v/v) of methanol, the final acetic acid concentration needed is 25 mM while 80 mM is required for 80% of methanol (v/v). Thus, acetic acid concentration prior to methanol addition was adjusted to 25, 50 or 400 mM for 0, 50 or 80% MeOH (v/v), respectively. Once chitosan was dissolved, an appropriate volume of methanol was added in order to reach a final composition of 0, 50 or 80% MeOH (v/v) and solutions were stirred for 2 h in order to ensure complete homogenisation and thermal equilibrium. 30 ml chitosan solutions were used to study reacetylation kinetics. Kinetics experiments were performed at room temperature (\sim 22.5 °C) in a 50 ml round bottom flask using 4.5 mg/ml chitosan water-methanol solutions prepared as described above. Acetic anhydride was added to stirred chitosan water-methanol mixtures at a molar ratio of acetic anhydride to glucosamine (*R*) of 0.45. At various time points (30 s, 1, 2, 4, 8, 15 and 30 min), 2 ml aliquots of the reaction mixture were pipetted in 3 ml of ice-cold 1 M sodium hydroxide solution in order to precipitate the reacetylated chitosan and stop the reaction. All samples were then repeatedly centrifuged at about $4000 \times g$ and washed with deionized water at least five times in order to remove acetic acid and sodium hydroxide. Once washed, samples were frozen and freeze-dried prior to analysis by ¹H NMR. Efficiency of the chitosan reacetylation as a function of the molar ratio of acetic anhydride to glucosamine (R) was determined using 5 ml chitosan solutions prepared as described above. Experiments were performed at room temperature (~22.5 °C) in 20 ml scintillation vials under magnetic stirring. Reaction times of 180 min and R values of 0.075, 0.145, 0.29 and 0.45 were used. In cases where a very small amount of acetic anhydride was required, the reagent was first diluted in methanol just prior to addition to the chitosan solution. The reacetylated samples were washed by centrifugation and degree of acetylation DA was measured by ¹H NMR as described above. Note that in order to facilitate analysis of reacetylated material, we limited the range of *R* so that the final product would be soluble.

3. Results and discussion

3.1. Hydrolysis of acetic anhydride

Hydrolysis kinetics of acetic anhydride in water–methanol mixtures are shown in Fig. 1. The reaction rate constants obtained from these fits using Eq. (4) are shown in Table 1. As expected, the hydrolysis rate decreases as the proportion of methanol increases. The value found for the pseudo-first order reaction in water ($k'_H =$ 0.145 min⁻¹) is in good agreement with the reported value in the literature of 0.16 min⁻¹ at 25 °C (Cleland & Wilhelm, 1956). Interestingly, the true second-order reaction rate (k_H) decreases as the proportion of methanol increases in the mixture. Measurements on a dextran 500 aqueous solution (10%, w/w) revealed that k'_H



Fig. 1. Hydrolysis kinetics of acetic anhydride for methanol–water mixtures monitored by conductivity measurements. (A) Conductivity as a function of time normalized to the fit value of the conductivity extrapolated to infinite time (c_{inf}). The experimental data (symbols) are fit to the model (dashed lines) using Eq. (4). Calculated k'_{H} values were obtained with $n = 2(\text{mean } k'_{H} \pm (\text{max} - \text{min})/2)$, and values are reported in Table 1. (B) Representation of acetic anhydride hydrolysis kinetics using Eq. (5). The theoretical curves (dashed lines) were obtained using the k'_{H} fit values found in (A).

in these conditions decreases by about 10% as compared to the aqueous solution. The density of this 10% (w/w) dextran solution was found to be 1.034 g/ml so that about 60% of this k'_H reduction can be explained by the reduced concentration of water in these conditions. Therefore, it appears that solution viscosity does not significantly affect hydrolysis kinetics, and values obtained in water-methanol mixtures (values in Table 1) can be used to model kinetics in presence of chitosan.

3.2. Variation of pK_a in water-methanol mixtures

The changes in pK_a of acetic acid and glucosamine as a function of methanol volume fraction in water–methanol mixtures are shown in Fig. 2. Acetic acid pK_a increases significantly as the proportion of methanol increases. The dissociation of a neutral acid such as acetic acid involves electrostatic work associated with the separation of two opposite charges. As methanol proportion increases in the system, the dielectric constant of the solvent decreases (the dielectric constant of methanol is ~33 at 25 °C versus 78.5 for water) and the work required to separate the two opposites charges increases compared to water alone, thereby reducing the



Fig. 2. Variation of the pK_a of acetic acid and glucosamine as a function of methanol volume fraction (v/v). The pK_a changes were determined by measuring pH changes in methanol–water mixtures with 50 mM of either acetic acid or glucosamine at a degree of dissociation of ~0.5. pK_a were calculated from the pH changes added to the known pK_a values in water of 4.76 (Bacarella et al., 1955) and 7.8 (Park et al., 1983) for acetic acid and glucosamine, respectively (n=2, mean \pm (max – min)/2). Our results for acetic acid are compared to the results of Shedlovsky and Kay (1956) (dotted line is a polynomial fit of these previous literature results).

dissociation constant. Solute-solvent interactions (solvation) also influence dissociation, but they are generally outweighed by electrostatic interactions (Bosch, Bou, Allemann, & Roses, 1996). Fig. 2 shows our results to be in agreement with acetic acid pK_a values reported in the literature (Shedlovsky & Kay, 1956). For example, the p K_a value measured at 80% MeOH (v/v) is within 0.02 pH units of the reported value in the literature. We found that glucosamine pK_a varies to a much lower extent than acetic acid pK_a , as expected since dissociation of cationic acids involves no charge separation (no electrostatic contributions) and thus depends only on solute-solvent interactions. As stated above in the Section 2, these glucosamine pK_a variations are used as an estimate for the variations of chitosan intrinsic pK_a (pK_0). The principal implications of this progressive decrease in the pK_a difference between acetic acid and chitosan (pK_a (glucosamine) – pK_a (acetic acid) = ~3.0 in water vs. pK_a (glucosamine) – pK_a (acetic acid) = ~1.5 in 80% MeOH) as the amount of methanol increases are (1) a larger amount of acetic acid is required to solubilise chitosan in mixtures with more methanol (25 mM vs. 80 mM acetic acid to dissolve 27.5 mM of chitosan (glucosamine units) in 50 and 80% MeOH mixtures, respectively) and (2) the production of acetic acid in the course of the reaction by either the reacetylation of chitosan or the hydrolysis of acetic anhydride ionizes chitosan (or acidifies the solution) to a lesser extent as the proportion of methanol increases and should result in higher reaction efficiency.

3.3. Kinetics of chitosan reacetylation

The kinetics of reacetylation of chitosan in water–methanol mixtures are shown in Fig. 3A. Most of the reacetylation occurs within a few minutes and the reaction is completed after 30 min for all conditions tested here. The reacetylation rate constant fit values from these experimental values are 183, 108 and 46 min⁻¹ M^{-1} for 0, 50, and 80% MeOH (v/v), respectively (see Table 1). The reason why the reacetylation rate decreases as the proportion of methanol increases is currently unknown, but it could be related to changes in the chitosan chain conformation or viscosity of the solution in presence of methanol.

The theoretical evolution of the fraction of neutral amine moieties as a function of time during reacetylation is shown in Fig. 3B. The degree of ionisation of chitosan increases significantly more in the course of the reaction in aqueous solutions versus 50 and 80% MeOH (v/v). This can be explained by the lower pK_a of acetic acid in pure water which results in a more important acidification of the solution as reacetylation and hydrolysis reactions progress. Calculated initial/final values of $1 - \alpha$ for these reactions are 0.23/0.03, 0.4/0.14 and 0.39/0.21 for 0, 50 and 80% MeOH (v/v), respectively. It is mostly the further ionization of the polymer in water in the course of the reaction that limits the efficiency of reacetylation and to a lesser extent the higher hydrolysis rate for these conditions.

3.4. Efficiency of the reacetylation reaction

Final DA values obtained using various amount of acetic anhydride are compared to theoretical values in Fig. 4. Theoretical curves were calculated using the reaction constants shown in Table 1 and obtained from Fig. 3 results. For the range of acetic anhydride to glucosamine ratios (R) tested, the reduction of the efficiency of the reaction as R increases can be seen clearly for 0% MeOH. As discussed above, this result is mostly due to the acidification of the solution by production of acetic acid by both reactions and not to acetic anhydride hydrolysis or glucosamine consumption (for this range of R). Since acetic acid pK_a is higher in 50 and 80% MeOH than in water, a reduction of the efficiency with increasing R is not clear in the range of R tested for 50 and 80% MeOH (v/v), but reacetylation in 80% MeOH appears to be slightly more efficient than 50% MeOH for the largest R values. The improved efficiency of the reaction as the proportion of methanol increases has been reported in the literature (Aiba, 1994) but without explanation. The theoretical reduction of the efficiency for larger R is shown in Fig. 4B. These calculations were performed by assuming that gelation or precipitation of the polymer occurring at high DA values would not affect significantly reaction kinetics. The results illustrate how increasing methanol proportion increases reacetylation efficiency for higher R values. The curves also reveal that even in a twofold excess of



Fig. 3. Kinetics of reacetylation of chitosan as a function of methanol volume fraction (v/v). The solution compositions are 27.5 mM glucosamine units of chitosan and 25 or 80 mM acetic acid in 0 and 50 or 80% MeOH. (A) Experimental degrees of acetylation DA (1 – DDA) values as a function of time and fit using the system of differential Eqs. (6), (7) and (10). The reaction rate constant of reacetylation (k_R) values obtained are given in Table 1 (n = 3, mean \pm SD). (B) Theoretical evolution of the proportion of the reactive neutral form of glucosamine in the course of the reaction, calculated from fit k_R values found in (A) for the same experimental conditions.



Fig. 4. Experimental and theoretical final DA values (reaction time of 3 h), DA_{inf}, for various acetic anhydride to glucosamine ratios $R(n_{ACOAC}/n_{Gluc})$ in methanol–water mixtures (v/v). (A) Comparison of theory and experimental values for solution compositions of 27.5 mM chitosan (glucosamine units) and 25 or 80 mM acetic acid for 0 and 50 or 80% MeOH, respectively and *R* ranging from 0.075 to 0.45 (*n* = 3, mean \pm SD). (B) Theoretical values of DA_{inf} values for a larger range of *R* values. These curves illustrate the inhibition of the reacetylation reaction by the progressive ionization of chitosan as DA and *R* increase. For comparison, the final DA values for the same bimolecular reaction combined with the hydrolysis kinetics in 50% MeOH but without taking into account the changes in chitosan's ionization degree (i.e. α = constant) have been included (top curve in dark grey).

acetic anhydride vs. glucosamine (R=2), a complete reacetylation of chitosan cannot be attained. A theoretical curve of reacetylation (in grey) without taking ionization changes into account (i.e. assuming $1 - \alpha$ is constant in the course of the reaction) has been shown for 50% MeOH (v/v) in Fig. 4B to illustrate how progressive ionization reduces significantly the final DA obtained for higher Rvalues. In this case (i.e. assuming $1 - \alpha$ constant), the reacetylation efficiency at high R values is limited only by acetic anhydride hydrolysis and the reduction of the concentration of amino moieties by the reacetylation reaction (the ratio of reaction speed of the two bimolecular reactions $v_R/v_H = k_R[glucosamine]/k'_H$ decreases as glucosamine is consumed).



Fig. 5. Comparison of theoretical DA_{inf} values vs. *R* with the results of Vachoud et al. (1997). The values of Vachoud et al. were obtained in a different solvent, 1,2-propanediol at 50% (v/v) with water, using 30 mM chitosan (glucosamine units) and 42 mM acetic acid. The pK_a and reaction rates are affected by the nature of the solvent but the prediction of our model can be compared with these results to illustrate the behavior of the reaction as *R* increases (target DA increases) revealing the difficulty to reach a DA of 1 for these conditions. Compositions of solutions for theoretical calculations are chitosan (glucosamine units) 27.5 mM, acetic acid 39 and 80 mM, for 50 and 80% MeOH, respectively. Hydrolysis and reacetylation rate constants shown in Table 1 were used.

The saturation of the reaction at high *R* has been reported in the literature (Vachoud et al., 1997) and it has been recognized that ionization of the polymer was limiting the reaction and that steric hindrance may also play a role in this efficiency reduction. In their study, Vachoud et al. (1997) used conditions similar to those used here. They used a concentration of chitosan of 0.5% (w/v) with 42 mM acetic acid in 50% (v/v) water-1,2-propanediol solvent. 1,2-Propanediol is more viscous than methanol and has a dielectric constant similar to methanol's one. Despite this difference in the nature of the alcohol used in the reaction, we applied our model with similar conditions in the presence of 50 and 80% MeOH (v/v)to compare experimental values of Vachoud et al. (1997) determined by ¹H NMR and infrared IR with our theoretical predictions (results are shown in Fig. 5). It is worth noting that IR determined reacetylation levels were closer to modeled values at 50% MeOH (v/v) than for reacetylation determined by ¹H NMR which were closer to theoretical values obtained at 80% MeOH (v/v). Keeping in mind that these experimental results were obtained in a different solvent and despite the fact that Vachoud et al. (1997)¹H NMR and IR results are somewhat less consistent for high R values, comparison of theoretical and experimental values indicate the model is

Table 2

Theoretical efficiency of reacetylation in water-methanol mixtures.

Solvent composition (% MeOH (v/v))	Initial fraction of neutral glucosamine ^a $1 - \alpha (t=0)$	Initial ratio of reacetylation rate to hydrolysis rate $v_{R(t=0)}/v_{H(t=0)}$ $(= k_R[NH_2]_{t=0}/k'_H)$	Theoretical maximum efficiency ^b $(v_{R(t=0)}/v_{R(t=0)} + v_{H(t=0)})$	Predicted efficiency for $R = 1$ (without/with buffer) ^c
0	0.23	8.2	89%	47%/74%
50	0.40	17.8	95%	67%/83%
80	0.39	21.7	96%	78%/87%

^a Experimental conditions are 27.5 mM glucosamine and 25 or 80 mM acetic acid for 0 and 50% or 80% MeOH (v/v), respectively.

^b This upper limit is reached only for small extent of reacetylation (i.e. when glucosamine amount and ionization degree are approximately constant in the course of the reaction, see Eq. (8)).

^c The calculations are done with a buffer concentration of 150 mM (acid and conjugated base present in equivalent amount of 150 mM, i.e. initial pH \sim =pK_a) with pK_a of 5.8, 6.1 and 6.3 for 0, 50 and 80% MeOH mixtures, respectively. These particular choices of pK_a ensure that the initial degrees of ionization is unchanged vs. the case without buffer. A further increase of 0.5 units of the pK_a's would result in predicted efficiencies being further improved to 83, 90, 91% (0, 50, 80% MeOH, respectively).



Fig. 6. . Influence of the presence of a buffer on the kinetics of reacetylation (theoretical) in water and 50% MeOH. Similar results are obtained at 80% MeOH but have not been included here for clarity. The calculations are done with a buffer concentration of 150 mM (acid and conjugated base present in equivalent amount of 150 mM, i.e. initial pH ~ =pK_a). (A) Final DA values as a function of *R* in methanol–water mixtures compared to the values in the presence of buffer. The curves predict a significant improvement on the efficiency of the reaction for large extent of reacetylation. (B) Evolution of the fraction of neutral glucosamine as a function of Chitosan in absence of buffer, as expected (calculations done with *R* = 4). (C) Comparison of final DA for different values of *pK*_a, showing that increasing buffer *pK*_a can further improve the efficiency of the reaction.

able to adequately predict the behavior of the reacetylation reaction without considering steric hindrance at high DA as a limiting factor of the reaction that was proposed previously (Qun et al., 2007; Vachoud et al., 1997).

3.5. Addition of a buffer could increase reaction efficiency

From the analysis presented above, the main factor limiting the efficiency of the reacetylation reaction is the acidification of the

solution in the course of the reaction that results in an increased degree of ionization of the polymer. The presence of methanol in the solution improves the reaction efficiency mostly by modulating the pK_a of acetic acid and to a lesser extent by reducing the effective hydrolysis rate of acetic anhydride. As pointed above, despite an improved efficiency in presence of methanol or other alcohols, a large excess of acetic anhydride is required to achieve high degree of acetylation. One way to circumvent this acidification problem and to better control the reacetylation process would be to buffer the solution to minimize pH changes in the course of the reaction. Theoretical results in the presence of a buffer (150 mM) in 0 and 50% MeOH (v/v) are shown in Fig. 6. In Fig. 6A, the buffer pK_a used are such that the initial ionization degree is the same as in the absence of any buffer. Results show that there is a significant improvement in the reaction efficiency, especially in water (improvement in 80% MeOH (v/v) is seen for higher DA than for 0 and 50% MeOH (v/v) and has been omitted in the figure for clarity purposes). From these results, one can see that the use of a buffer in 0% MeOH (v/v) could extend the linear region (i.e. \sim constant efficiency) of the curve DA vs. *R* from DA = 0–0.2 to \sim 0–0.6. The theoretical evolution of the fraction of neutral amino groups as a function of DA in presence of a buffer vs. unbuffered system is shown in Fig. 6B and illustrates how the buffer should limit polymer ionization as the reaction evolves. As expected, increasing the buffer's pK_a should result in further improvement of the reaction, especially for high extent of reacetylation (Fig. 6C). Theoretical efficiencies for low extent of reacetylation and for R = 1 (with and without buffer) are shown in Table 2.

4. Conclusion

We found in this study that reacetylation using acetic anhydride of chitosan dissolved in water-methanol mixtures using acetic acid is a fast reaction as compared to acetic anhydride hydrolysis. The reacetylation reaction rate decreases as the proportion of methanol increases in the solution and the improved efficiency of the reaction in presence of methanol is mostly due to an increase in the pK_a of acetic acid that limits acidification of the solution and ionization of the polymer in the course of the reaction. By using a theoretical model based on second-order kinetics combined with acid-base dissociation equations of chitosan, we have been able to predict the saturation of the reacetylation reaction reported in the literature at high R values without relying on any steric hindrance contribution, which appears to be unnecessary. Our findings suggest reacetylation be performed in 80% MeOH (v/v) where the efficiency of the reaction is predicted to be close to 100% for DA < ~0.6. Theory indicates that the use of a buffer to limit ionization of the polymer as the reaction evolves would be beneficial to increase efficiency when a high extent of reacetylation is targeted or when the reaction is performed in water. Taken together, our results permit a better understanding and control of chitosan reacetylation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.08.096.

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