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RESEARCH PAPER

Effect of the Type of Base and Number of Reaction Steps on the Degree of Quaternization and Molecular Weight of *N*-Trimethyl Chitosan Chloride

J. H. Hamman and A. F. Kotzé*

Department of Pharmaceutics, School of Pharmacy, Faculty of Health Sciences, Potchefstroom University for Christian Higher Education, Potchefstroom 2520, South Africa

ABSTRACT

N-*Trimethyl chitosan chloride (TMC), a chemically modified derivative of chitosan, is the first chitosan derivative shown to be an effective absorption enhancer for peptide and protein drugs across mucosal epithelia. TMC is synthesized by reductive methylation with methyl iodide in the presence of a strong base such as sodium hydroxide. In this reaction, the primary amino group on the C-2 position of chitosan is changed to a quaternary amino group. The charge density, as determined by the degree of quaternization, and probably also the molecular weight of TMC are important factors that influence the absorption enhancement effect and toxicity of this polymer. The molecular weight of the starting polymer decreases during the synthesis procedure due to factors such as the strong alkaline environment and elevated experimental temperatures. This study investigated the effects of two different bases, sodium hydroxide and dimethyl amino pyridine, together with a varying number and duration of reaction steps, on the degradation and the degree of quaternization of TMC polymers.* ^{1}*H-NMR (nuclear magnetic resonance) spectra showed a major increase in the degree of quaternization (21%–59%) of TMC with an increase in the number of reaction steps when sodium hydroxide was used as the base. Intrinsic viscosity values indicated that the use of dimethyl amino pyridine did not cause polymer degradation to the same extent as sodium hydroxide, but that the*

* Corresponding author. Fax: +27 18 2992251; E-mail: fmsafk@puknet.puk.ac.za

degree of quaternization of TMC stayed low (7.3%–9.6%) even when the number of reaction steps was increased. A combination of the two bases did not reduce polymer degradation, while the degree of quaternization was limited to relatively low values (12.5%–34.4%).

Key Words: *Chitosan; Degree of quaternization; Dimethyl amino pyridine; Polymer degradation; Sodium hydroxide;* N-*Trimethyl chitosan chloride.*

INTRODUCTION

Chitosan is a natural cationic polysaccharide derived from *N*-deacetylation of chitin (1). Chitosan has been investigated extensively in the pharmaceutical industry as an excipient (2–5); in recent years, it has also been shown that chitosan enhances the absorption of peptide and protein drugs across nasal and intestinal epithelia (1,6). However, chitosan is only soluble in acidic solutions, and this interferes with the biomedical applications of this polymer mentioned above (7). Water-soluble derivatives, with similar or increased absorption-enhancing effects, will be beneficial for promoting the absorption of macromolecular and hydrophilic drugs in the neutral environment of the small and large intestines.

For reasons other than pharmaceutical purposes, partial quaternization has been proposed in the past to increase the solubility of chitosan, and several methods have already been investigated (8). The conditions for the preparation of *N*-methylene chitosan using formaldehyde were reported by Hirano et al. (9), while Muzzarelli and Tanfani (10) used formaldehyde and sodium borohydride to synthesize *N*-trimethyl chitosan iodide. *N*-Trimethyl chitosan chloride (TMC) was also synthesized by reductive methylation of chitosan with methyl iodide in the presence of a strong base (7,8). In this procedure, the primary amino groups on the C-2 position of chitosan are changed to quaternary amino groups by substitution of the hydrogen atoms with methyl groups. The alkylation of the primary amine to the quaternary stage is simplified using a base to bind the acid generated during the reaction and to avoid protonation of the unreacted primary amino groups (8).

TMC shows good water solubility at a wide range of pH values; Kotzé et al. (11) were first to demonstrate the noble absorption enhancement effects of this polymer across intestinal epithelial cells (Caco-2). The same authors demonstrated the importance of TMC as an absorption enhancer for large hydrophilic compounds, especially at neutral and alkaline pH values, at which normal chitosan salts are ineffective as absorption enhancers (12). In several recent studies, it was shown that the charge density of TMC, as determined by the degree of quaternization, plays an important role in the absorption-enhancing properties of this polymer (13–15).

Theoretically, it could now be argued that the possible toxicity of TMC, if any, will probably depend on the molecular weight of the polymer, as was shown to be the case with chitosan (16). The mucoadhesive properties of TMC polymers may probably also depend on its molecular weight, but this has not yet been investigated.

Reaction conditions, such as a strong alkaline environment and elevated reaction temperatures, decrease the molecular weight of the starting polymer during the synthesis of TMC (8). In this study, two different bases (separately as well as in combination) and a different number and duration of reaction steps were used to synthesize TMC to investigate the effect on the molecular weight and degree of quaternization of the product.

EXPERIMENTAL

Materials

Seacure 244 (93% deacetylated chitosan) was a gift from Pronova Biopolymer (Drammen, Norway). This material was milled in a Retsch mill (Retsch KG, Haan, Germany) to obtain a powder with relatively small particles to ease the solubility of the polymer. All the other chemicals used were commercially available and were used as received.

Method

We prepared 14 different TMC polymers by varying the number of the reaction steps and the type of base. Sodium hydroxide and dimethyl amino pyridine were used separately and in combination as the base. The method described by Sieval et al. (7) was used; by repeating the reaction step for the reductive methylation of chitosan several times under the same conditions, with the polymer obtained from each reaction step, TMCs with different degrees of quaternization were synthesized. The reaction route used to synthesize the TMC polymers is

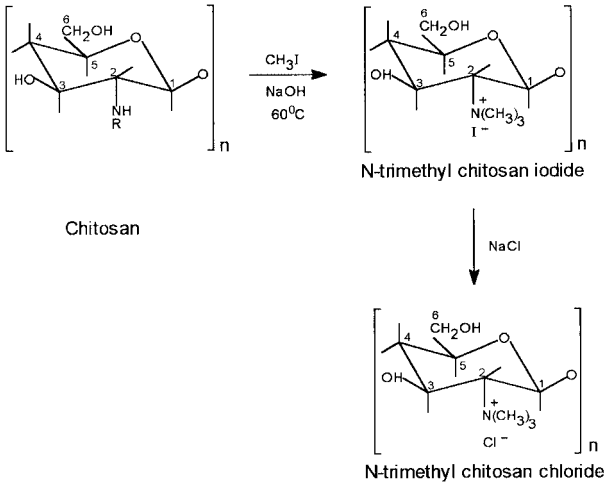

Figure 1. Route of synthesis for TMC using 93% deacetylated chitosan as the starting polymer; chemical structure of chitosan with R = H (93%), R = acetyl (7%).

schematically presented in Fig. 1. The reaction conditions for each step in the synthesis of the 14 TMC polymers with different degrees of quaternization are described below and are summarized in Table 1.

First Reaction Step

A mixture of 2 g chitosan, 4.8 g of sodium iodide, 11 ml of a 15% w/v aqueous sodium hydroxide solution or 10 ml of a 0.02% w/v dimethyl amino pyridine solution (depending on which type of base was used), and 11.5 ml of methyl iodide in 80 ml of *N*-methylpyrrolidone was stirred on a water bath at a temperature of 60°C for 45 min. The methyl iodide was kept in the reaction by a Liebig's condenser.

The product was precipitated with ethanol and isolated by centrifugation. After washing with ethanol and diethylether, the product was dissolved in 40 ml of a 5% w/v aqueous sodium chloride solution to exchange the iodide ion with a chloride ion. The polymer was precipitated with ethanol and isolated by centrifugation. This product was dissolved in 40 ml water and precipitated with ethanol to remove the remaining sodium chloride from the material.

Second Reaction Step

The product (obtained from step 1) was dissolved in 80 ml *N*-methylpyrrolidone and 4.8 g of sodium iodide, 11 ml of a 15% w/v aqueous sodium hydroxide solution or 10 ml of a 0.02% w/v dimethyl amino pyridine solution (depending on which type of base was used) and 7 ml of methyl iodide were added. The mixture was stirred on a water bath at a temperature of 60°C for 15 min. The product was precipitated with ethanol and isolated by centrifugation.

The iodide ion was exchanged with a chloride ion, and the remaining sodium chloride was removed as described in reaction step 1.

Third Reaction Step

The product (obtained from step 2) was dissolved in 80 ml *N*-methylpyrrolidone and 4.8 g of sodium iodide, 11 ml of a 15% w/v aqueous sodium hydroxide solution or 10 ml of a 0.02% w/v dimethyl amino pyridine solution (depending on which type of base was used) and 11.5 ml of methyl iodide were added. The mixture was stirred on a water bath at a temperature of 60°C for 30 min. The product was precipitated with ethanol and isolated by centrifugation.

The iodide ion was exchanged with a chloride ion, and the remaining sodium chloride was removed as described in reaction step 1.

Later Addition Step

Before the product was precipitated at the end of reaction step 1, 2, or 3, an additional 2 ml methyl iodide and 0.6 g sodium hydroxide pellets or 5 ml of a 0.02% w/v dimethyl amino pyridine solution (depending on which type of base was used) were added, while stirring was continued for another 45 min at a temperature of 60°C. The final products were dried under vacuum at 40°C.

The degree of quaternization of the different products was calculated from ^{1}H-NMR (nuclear magnetic resonance) spectra, obtained in D_2O at 80°C using a 600-MHz spectrometer (Bruker, Karlsruhe, Germany), as reported previously by Sieval et al. (7). The following equation was used to calculate the degree of quaternization of the TMC polymers from the ^{1}H-NMR spectra:

$$DQ(\%) = [(\int TM/\int H) \times 1/9] \times 100$$

where DQ(%) is the degree of quaternization as a percentage, $\int$ TM is the integral of the trimethyl amino group (quaternary amino) peak at 3.3 ppm on the ^{1}H-NMR spectrum, and $\int$ H is the integral of the ^{1}H peaks between 4.7 and 5.7 ppm on the ^{1}H-NMR spectrum.

Intrinsic viscosities of the synthesized TMC polymers were measured according to the method described in the British Pharmacopoeia for dextran; a size D glass U-tube viscometer was used at 25°C. Solutions of the TMC poly-

Table 1

Type of Base and Number of Reaction Steps Used to Synthesize Diferent N-*Trimethyl Chitosan Chloride Polymers*

Polymer	Type of Base	Number and Sequence of Reaction Steps
TMC-1	Solium hydroxide	One reaction step
TMC-2	Sodium hydroxide	Two reaction steps
TMC-3	Sodium hydroxide	Two reaction steps with a later addition step (30) min after the second step
TMC-4	Sodium hydroxide	Two reaction steps with a later addition step (45 min) after the second step
TMC-5	Sodium hydroxide	Three reaction steps with a later addition step after the third step
TMC-6	Dimethyl amino pyridine	One reaction step
TMC-7	Dimethyl amino pyridine	Two reaction steps
TMC-8	Dimethyl amino pyridine	Two reaction steps with a later addition step after the second step
TMC-9	Dimethyl amino pyridine	Three reaction steps with a later addition step after the second step
TMC-10	Dimethyl amino pyridine	Three reaction steps with two later addition steps after the second and third steps
TMC-11	Combination of sodium hydroxide and dimethyl amino pyridine	Two reactions steps, one with NaOH and one with dimethyl amino pyridine
TMC-12	Combination of sodium hydroxide and dimethyl amino pyridine	Two reaction steps, one with NaOH and one with dimethyl amino pyridine; later addition step with NaOH after the second step.
TMC-13	Combination of sodium hydroxide and dimethyl amino pyridine	Three reaction steps, one with dimethyl amino pyridine and two with NaOH; later addition step with NaOH after the second step
TMC-14	Combination of sodium hydroxide and dimethyl amino pyridine	Three reaction steps, two with dimethyl amino pyridine and one with NaOH; later addition step with dimethyl amino pyridine after the second step

mers were prepared in 0.2% v/v acetic acid in concentrations of 0.1%, 0.15%, 0.2%, and 0.25% w/v. The temperature of the solutions was kept at 25°C with a water bath. The flow-through times (time for the meniscus to fall from E to F in the glass U tube as described in appendix VH of the British Pharmacopoeia, 1988, A101) of the solutions and the solvent were measured in the U tube. The viscosity ratio for each solution was calculated with the following equation:

$$\text{Viscosity ratio} = \text{Flow-through time for the solution} / \text{Flow-through time for the solvent}$$

The α values (where α = [Viscosity ratio − 1]/Concentration) were calculated for each solution, and these α values were plotted on a graph as a function of concentration of the solutions for each polymer. The intercept on the *y* axis (α values) of a straight line through the points represents the intrinsic viscosity values of the TMC solutions.

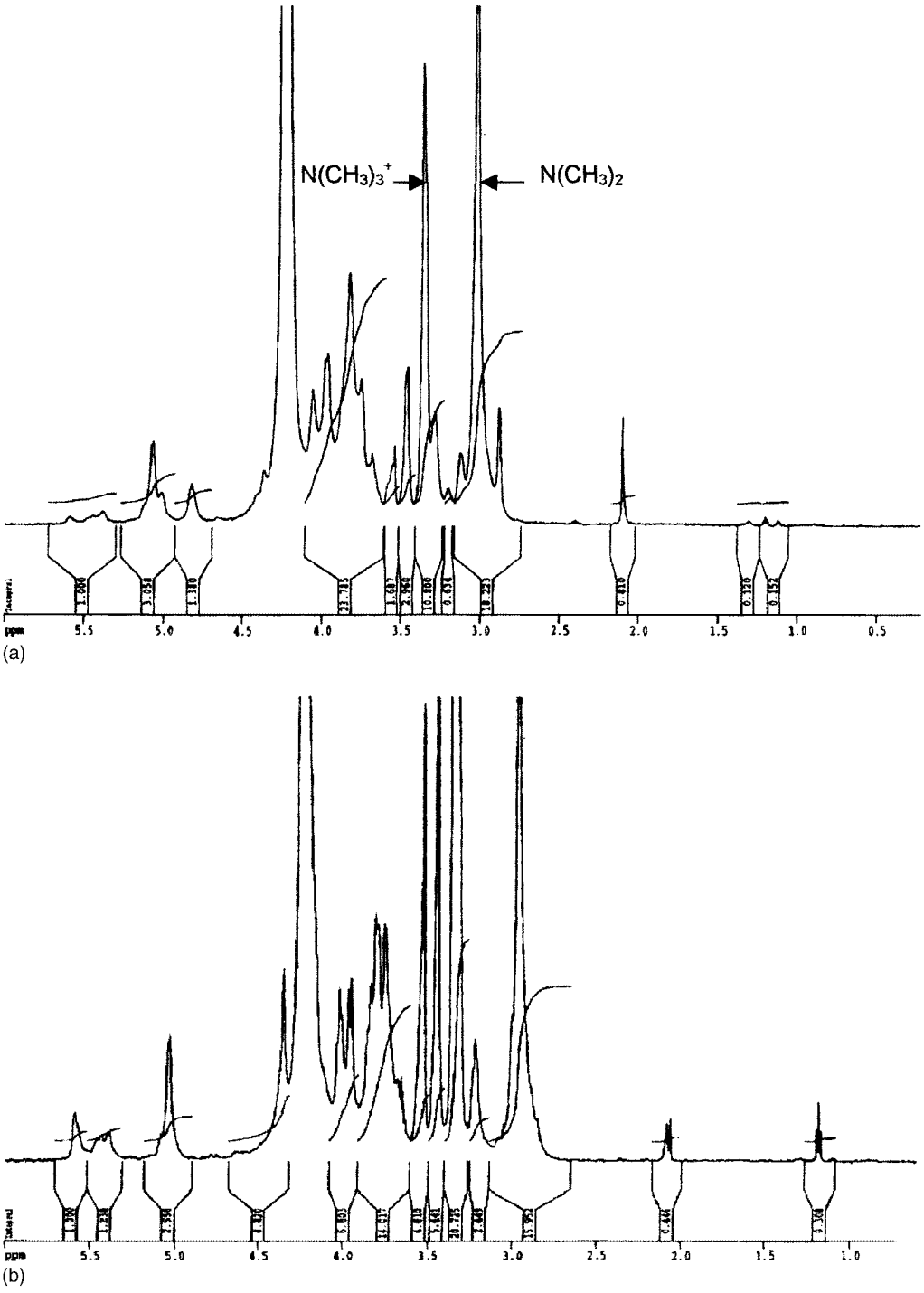

Figure 2. (a) ^{1}H-NMR spectrum of TMC prepared in a one-step reaction with sodium hydroxide as the base; (b) ^{1}H-NMR spectrum of TMC prepared in a three-step reaction with sodium hydroxide as the base.

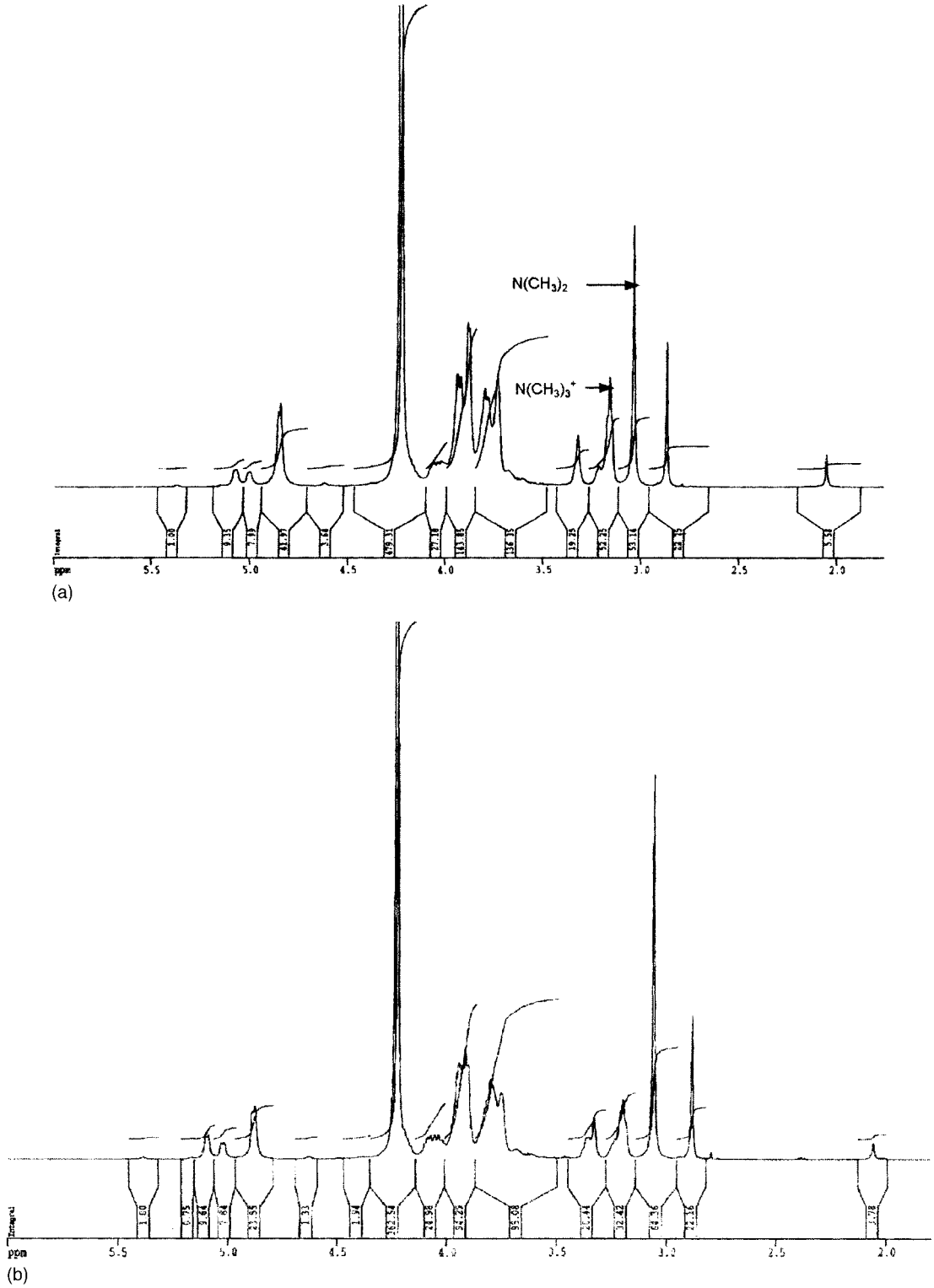

Figure 3. (a) ^{1}H-NMR spectrum for TMC prepared in a one-step reaction with dimethyl amino pyridine as the base; (b) ^{1}H-NMR spectrum for TMC prepared in a three-step reaction with dimethyl amino pyridine as the base.

RESULTS AND DISCUSSION

The ^{1}H-NMR spectra for TMC products obtained after one- and three-step syntheses with sodium hydroxide and after one- and three-step syntheses with dimethyl amino pyridine as the base are shown in Figs. 2 and 3, respectively.

The calculated degrees of quaternization and intrinsic viscosity values for the different TMC polymers are listed in Table 2. In a previous study, Sieval et al. (7) assigned the peak at 3.1 ppm to dimethyl amino groups and the peak at 3.3 ppm to trimethyl amino groups. As evident from the enlargement of the peaks assigned to the quaternary amino groups (Figs. 2a and 2b), the degree of quaternization increased significantly with an increase in the number of reaction steps when sodium hydroxide was used as the base. However, the spectra also indicate some methylation on the 3 and 6 hydroxyl groups of chitosan, especially with increased reaction steps. In contrast to the results obtained with sodium hydroxide as the base, only weak quaternization of the amino groups was observed when dimethyl amino pyridine was used as the base, even with an increase in the number of reaction steps (Figs. 3a and 3b). This could probably be explained by methylation of dimethyl amino pyridine at a higher rate than chitosan. The base should also be strong enough to maintain a global pK_a value in the solution superior to that of chitosan throughout the whole reaction time (8), and this requirement was probably not met by dimethyl amino pyridine. When a combination of the two bases was used, a slight increase in the degree of quaternization was found, but it was still lower, as for the polymers prepared with sodium hydroxide alone (Table 2).

As evident from Table 2, the intrinsic viscosities of all the TMC polymers were markedly decreased in comparison with the starting polymer. Intrinsic viscosities were measured to indicate probable changes in molecular size of the starting polymer during the synthesis procedure. In general, increased reductions in the intrinsic viscosity were observed when more reaction steps were used compared to synthesis procedures with fewer reaction steps when sodium hydroxide and dimethyl amino pyridine or a combination of these were used as bases. This could be explained by the longer contact time of chitosan with the different bases at an elevated temperature when more reaction steps were used. It is also clear that the intrinsic viscosities of the TMC polymers prepared with dimethyl amino pyridine did not decrease to the same extent in comparison to the TMC polymers prepared with sodium hydroxide. This could be explained by the much weaker basic properties of dimethyl amino pyridine compared to sodium hydroxide. A combination of sodium hydroxide and dimethyl amino pyridine was also not effective in reducing the degradation of the starting polymer, and intrinsic viscosity values similar to those found when sodium hydroxide was used as the base were recorded.

Table 2

Degree of Quaternization (DQ) and Intrinsic Viscosity [η] Values of Different N-*Trimethyl Chitosan Chloride Polymers*

Polymer	Base	DQ	[η][a]
TMC-1	Sodium hyroxide	22.1	7.70
TMC-2	Sodium hydroxide	36.3	3.55
TMC-3	Sodium hydroxide	48.0	2.19
TMC-4	Sodium hyroxide	59.2	2.80
TMC-5	Sodium hydroxide	57.8	n.d.
TMC-6	Diemthyl amino pyridine	9.6	16.42
TMC-7	Dimethyl amino pyridine	7.3	16.78
TMC-8	Dimethyl amino pyridine	8.6	11.98
TMC-9	Dimethyl amino pyridine	8.2	9.28
TMC-10	Dimethyl amino pyridine	8.6	6.53
TMC-11	Combination of two bases	12.5	3.26
TMC-12	Combination of two bases	34.4	3.15
TMC-13	Combination of two bases	25.0	1.41
TMC-14	Combination of two bases	17.9	3.18

[a] [η] for the starting polymer (chitosan) = 22.20; n.d. = not determined.

CONCLUSION

The results of this study confirm that the degree of quaternization of TMC, and therefore its charge density, which is an important parameter for absorption enhancement, can be increased by an increase in the number of reaction steps in the synthesis procedure. This was especially effective when a strong base such as sodium hydroxide was used. Complete quaternization of chitosan is not possible due to the presence of some acetyl groups on chitosan and most likely also to possible steric effects of the attached methyl groups on adjacent quaternary amino groups. Although the decrease in molecular weight of the starting polymer, which might have an effect on the toxicity profile and mucoadhesive properties of this polymer, can be lowered with the use of dimethyl amino pyridine as the base, the degree of quaternization of TMC is limited to relatively low values. A combination of sodium hydroxide and dimethyl amino pyridine as the base in the synthesis process was not effective in producing polymers with high degrees of quaternization, and significantly lower values of quaternization, compared to polymers prepared with sodium hydroxide, were found. Current studies are directed at determination of the molecular weight of the different TMC polymers and determination of the effect of molecular weight on the toxicity and mucoadhesive properties of TMC polymers.

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