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The relationship between the absolute molecular weight and the degree of quaternisation of *N*-trimethyl chitosan chloride

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Abstract

N-Trimethyl chitosan chloride (TMC) is a partially quaternised derivative of chitosan with enhanced water solubility and superior drug absorption enhancing properties, especially in neutral and basic environments where chitosan is insoluble. The degree of quaternisation of TMC plays an important role on the solubility, the absorption enhancing ability and mucoadhesive properties of this polymer. A range of TMC polymers with different degrees of quaternisation were synthesised by varying the number and duration of reaction steps. Size exclusion chromatography (SEC) and multi-angle laser light scattering (MALLS) were used to determine the absolute molecular weight, radius and polydispersity of these TMC polymers. The viscosities of TMC polymer solutions were measured at different concentrations and the calculated intrinsic viscosity values were used as a further indication of the molecular weight of each polymer. The decrease in absolute molecular weight correlated well with the intrinsic viscosities of the TMC polymers and was related to the increase in their degrees of quaternisation. This polymer degradation was explained by increased exposure time of the polymer molecules to reaction conditions, such as the strong alkaline environment and elevated temperatures, that is necessary to produce higher degrees of quaternisation during the synthesis of TMC polymers. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *N*-Trimethyl chitosan chloride; Chitosan; Molecular weight; Size exclusion chromatography/multi-angle laser light scattering; Intrinsic viscosity

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1. Introduction

Chitosan [α (1 → 4) 2-amino-2-deoxy β-D-glucan], a polysaccharide derived from the deacetylation of chitin, is a glucosaminoglycan with a chemical formula of $(C_6H_{11}O_4N)_n$ (Paul & Sharma, 2000). It has been shown that chitosan enhances the absorption of peptide and protein drugs across nasal (Illum, Farraj, & Davis, 1994) and intestinal epithelia (Artursson, Lindmark, Davis, & Illum, 1994; Borchard et al., 1996) in acidic environments. Chitosan has an apparent pK_a value of 5.60 (as measured by potentiometric titration) and is only soluble in acidic solutions with pH values lower than 6.0 (Sieval et al., 1998). This interferes with the biomedical application of chitosan, especially at the physiological pH value (7.40) where chitosan is insoluble and ineffective as an absorption enhancer (Kotzé, Luessen, De Boer, Verhoef, & Junginger, 1999a).

N-Trimethyl chitosan chloride (TMC) is a partially quaternised derivative of chitosan with improved solubility and easy preparation (Le Dung, Milas, Rinaudo, & Desbrieres, 1994). This derivative of chitosan has excellent absorption enhancing effects across mucosal epithelia, even in neutral environments and it has also been shown that the degree of quaternisation of TMC has an important effect on its absorption enhancing properties (Kotzé et al., 1999b; Thanou et al., 2000). During the synthesis of TMC, the number of positive charges on the polymer chain is increased, causing the molecule to expand in solution due to repelling forces between the functional groups. Furthermore, degradation of the polymer chain occurs during synthesis due to the strong alkaline environment and elevated temperatures (Domard, Rinaudo, & Terassin, 1986; Hamman & Kotzé, 2001). According to Gurny, Meyer, and Peppas (1984), the molecular weight of mucoadhesive polymers has an effect on their mucoadhesive properties up to a value of 100,000 g/mol, where after any further increase had no noticeable effect. Current formulation studies use the mucoadhesive properties of chitosan and TMC polymers to increase the contact time of the active ingredients with the epithelium and its absorption via the paracellular transport pathway through the tight

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Table 1
Number of reaction steps for the synthesis of TMC polymers

Polymer	Number and sequence of reaction steps
TMC 1	Step 1
TMC 2	Steps 1 and 2
TMC 3	Steps 1–3
TMC 4	Steps 1–4

junctions. It is therefore important to investigate the influence of the synthesis procedure on the molecular weight of the resulting polymers, due to chain degradation, to optimise TMC production processing parameters for future dosage form development studies. The aim of this study was to synthesise TMC polymers with different numbers of fixed positively charged amino groups and to investigate the relationship between the degree of quaternisation and the absolute molecular weight of these TMC polymers.

2. Experimental

2.1. Materials

Seacure 244 (93% deacetylated chitosan) was a gift from Pronova Biopolymer (Drammen, Norway). The chitosan was milled in a Retsch-mill (Retsch K G, Haan, Germany) to obtain a powder to improve the solubility rate of the polymer. Iodomethane, sodium hydroxide pellets, sodium iodide, sodium chloride, acetic acid, ammonium acetate, absolute ethanol and diethylether (Merck, South Africa) and *N*-methyl-2-pyrrolidone (Riedel-de Haën, South Africa) were used as received. All chemicals used in the synthesis process were of analytical grade.

2.2. Synthesis of TMC polymers

TMC polymers were synthesised by reductive methylation of chitosan that was accomplished by a chemical reaction between chitosan and iodomethane in the presence of sodium hydroxide based on the methods described by Sieval et al. (1998) and Hamman and Kotzé (2001). The reaction step was repeated several times with the product obtained from each step to increase the degree of quaternisation of the TMC polymers. The reaction steps used to synthesise different TMC polymers (TMC 1–4) are described below and the compilation of reaction steps for each of the polymers are presented in Table 1.

Reaction step 1. A mixture of 2.0 g chitosan, 4.8 g of sodium iodide, 11 ml of a 15% (w/v) aqueous sodium hydroxide (NaOH) solution and 11.5 ml of iodomethane in 80 ml of *N*-methylpyrrolidone was stirred on a water bath at a temperature of 60 °C for 45 min. The iodomethane was kept in the reaction by using a Liebig's condenser. The product was precipitated from solution with ethanol and isolated by centrifugation.

Reaction step 2. The product obtained from the previous reaction step was dissolved in 80 ml *N*-methylpyrrolidone and 4.8 g of sodium iodide after which 11 ml of a 15% (w/v) aqueous NaOH solution and 11 ml of iodomethane were added. This mixture was stirred on a water bath at a temperature of 60 °C for 30 min. The product was precipitated from solution with ethanol and isolated by centrifugation.

Reaction step 3. Before precipitation of the product in step 2, an additional 2 ml iodomethane and 0.6 g NaOH pellets were added to the mixture while stirring was continued for another 45 min at a temperature of 60 °C. The product was precipitated from solution with ethanol and isolated by centrifugation.

Reaction step 4. The product obtained from the previous reaction step was dissolved in 80 ml *N*-methylpyrrolidone and 4.8 g of sodium iodide after which 11 ml of a 15% (w/v) aqueous NaOH solution and 11.5 ml of iodomethane were added. The mixture was stirred on a water bath at a temperature of 60 °C for 30 min. The product was precipitated from solution with ethanol and isolated by centrifugation.

Ion exchange. After washing with ethanol and diethylether, the final products were dissolved in 40 ml of a 5% (w/v) aqueous NaCl solution to exchange the iodide-ion with a chloride-ion. The polymer was precipitated from solution using ethanol and was isolated by centrifugation. The product was finally dissolved in 40 ml water to remove the remaining NaCl from the material and precipitated from solution with ethanol. This product was dried in a vacuum oven at 40 °C for 12 h.

3. Characterisation of TMC polymers

3.1. Degree of quaternisation

^{1}H-NMR spectra of the TMC polymers were obtained with a 600 MHz BRUKER DMX-600 spectrometer (Karlsruhe, Germany) by dissolving samples of the polymers in D_2O at 80 °C with suppression of the water peak. The degree of quaternisation was calculated with data obtained from the ^{1}H-NMR spectra according to a previously described method (Sieval et al., 1998; Hamman and Kotzé, 2001) with Eq. (1)

$$\%DQ = \left[\frac{[(CH_3)_3]}{[H]} \times \frac{1}{9} \right] \times 100 \tag{1}$$

where %DQ is the degree of quaternisation as a percentage, $[(CH_3)_3]$ is the integral of the trimethyl amino group at 3.1 ppm and [H] is the integral of the ^{1}H peaks between 4.7 and 5.7 ppm.

3.2. Molecular weight

The absolute molecular weights of the synthesised TMC

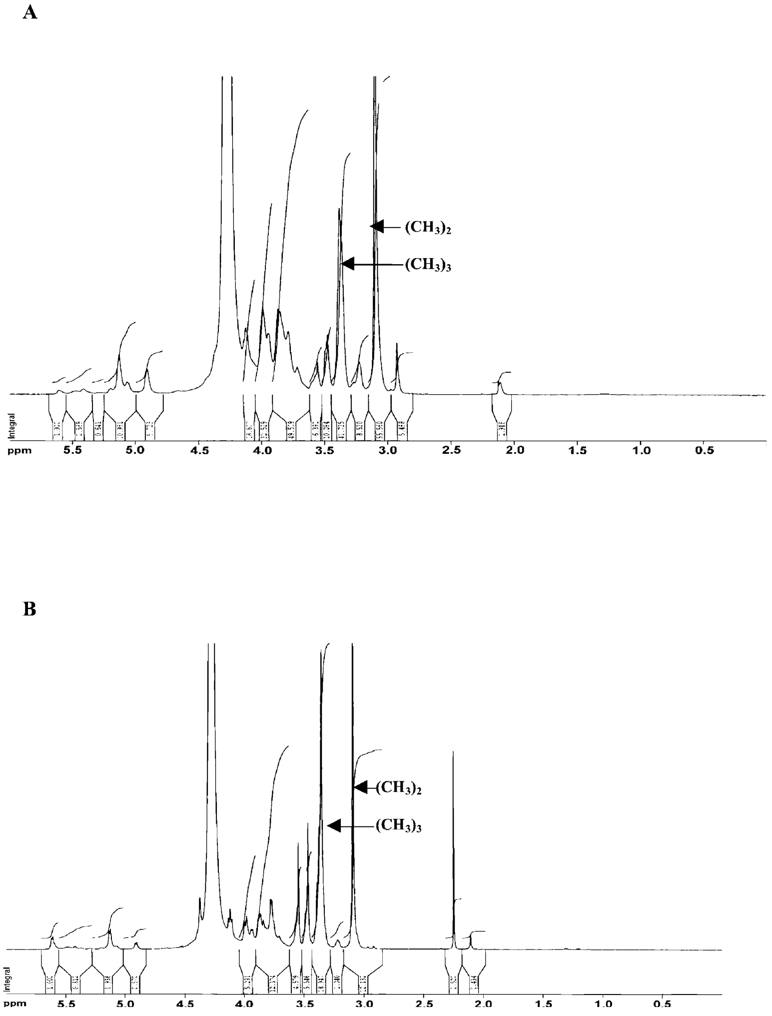

Fig. 1. ^{1}H-NMR spectra of (A) TMC 1 (22% quaternised) and (B) TMC 4 (59% quaternised) as the lowest and highest quaternised polymers, respectively.

polymers were measured with a size exclusion chromatography (SEC) (Hewlett Packard 1100, USA) connected to a multi-angle laser light scattering (MALLS) detector that consisted of a laser photometer (Dawn DSP, Wyatt Technology Corporation, USA) coupled to a refracting index detector (ERC 7515A, Japan).

The TMC polymers were dried in a vacuum oven (at 40 °C) for 24 h and prepared in solutions of 5 mg/ml from which 0.8 ml samples were filtered through 0.2 μm membrane filters and collected in chromatographic sample vials. The mobile phase consisted of a 0.2 M ammonium acetate solution and the pH was adjusted to 4.50 with acetic acid. The experimental set-up consisted of an HP 1100 vacuum degasser, isocratic pump and auto-sampler connected to a TSK-guard PWH (Toso Haas, Japan) inline column. The size exclusion columns included a TSK G6000 PW (Toso Haas, Japan; inside diameter = 7.5 mm, length = 30 cm, particle size > 17 μm, pore size > 1000 Å) column connected in series with a TSK G5000 PW (Toso Haas, Japan; inside diameter = 7.5 mm, length = 30 cm, particle size = 7.5 mm, pore size = 1000 Å) column. Samples of the TMC solutions (100 μl) were injected at a flow rate of 0.8 ml/min and were analysed with the laser photometer (He/Ne laser, $\lambda = 633$ nm) and the refracting index detector. The data from the detector was interpreted with a computer using Astra® for Windows (Wyatt Technology Corporation, USA) and the molecular weight of the sample was calculated with Eq. (2)

$$\frac{K^*c}{R(\Theta)} = \frac{1}{M_\mathrm{w}P(\Theta)} + 2A_2c \qquad (2)$$

where $R(\Theta)$ is the excess intensity of scattered light at dawn angle Θ, c is the sample concentration, M_w is the weight-average molecular weight, A_2 is the second viral coefficient and K^* is the optical parameter equal to $4\pi^2n^2(\mathrm{d}n/\mathrm{d}c)^2/(\lambda_0^4N_\mathrm{A})$ (n is the solvent refractive index, dn/dc is the refractive index increment, N_A is Avogadro's number and λ_0 is the wavelength of the scattered light in vacuum).

The function $P(\Theta)$ describes the angular dependence of scattered light. The expansion of $1/P(\Theta)$ to first order gives:

$$1/P(\Theta) = 1 + (16\pi^2/3\lambda^2)\langle r_\mathrm{g}^2\rangle \sin^2(\Theta/2) + f_4\sin^4(\Theta/2) + \cdots \qquad (3)$$

Table 2
The degree of quaternisation (DQ), specific refractive index increment (d*n*/d*c*), weight-average radius moments (R_w), weight-average molecular weight (M_w) and intrinsic viscosities ($[\eta]$) of the synthesised TMC polymers

Polymer	DQ (%)	d*n*/d*c* (ml/g)	R_w (nm)	M_w (g/mol × 10^5)	$[\eta]$
Chitosan	–	0.17	37.4	1.48	22.20
TMC 1	22.1	0.14	35.8	2.02	7.70
TMC 2	36.3	0.13	41.2	1.95	4.49
TMC 3	48.0	0.12	36.1	1.66	3.36
TMC 4	59.2	0.12	19.6	1.43	2.80

At low angles, the angular dependence of light scattering depends only on the mean square radius $\langle r_g^2 \rangle$ (alternatively called radius of gyration) and is independent of molecular conformation or branching. A plot of $K^* c/R(\Theta)$ vs. $\sin^2(\Theta/2)$ (Zimm plot) yields a curve whose intercept gives M_w and whose slope at low angles gives $\langle r_g^2 \rangle$ (Wyatt Technology, 1999).

3.3. Intrinsic viscosity

Intrinsic viscosities of the synthesised TMC polymers were measured according to the method described in British Pharmacopoeia (2000) for dextran with a size D glass U-tube viscometer at 25.0 °C. Solutions of the TMC polymers 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 25.0 °C in a waterbath throughout the experiment. The flow-through time of each solution and the solvent were measured in the U-tube. The viscosity ratio for each solution is expressed by the ratio of the viscosity of the solution (η) to the viscosity of the pure solvent (η_0) at the same temperature (Florence & Attwood, 1988). This ratio is referred to as the relative viscosity (η_{rel}) as shown in Eq. (4):

$$\eta_{rel} = \eta/\eta_0 \tag{4}$$

The specific viscosity (η_{sp}) of the solution is defined by Eq. (5):

$$\eta_{sp} = \eta_{rel} - 1 \tag{5}$$

The extrapolated value (where $c = 0$) of the straight line when η_{sp}/c is plotted as a function of c (concentration) is the intrinsic viscosity ($[\eta]$) of the polymer, which gives an indication of the molecular weight of the polymer.

4. Results

4.1. Degree of quaternisation

Fig. 1 shows the ^{1}H-NMR spectra of the highest and lowest quaternised TMC polymers, respectively, while the calculated degrees of quaternisation, molecular weights and intrinsic viscosities of all the synthesised TMC polymers are presented in Table 2. Synthesis of TMC leads to methylation of the amino groups in the C-2 position of chitosan to form quaternary amino groups with fixed positive charges on the repeating units of the TMC polymer chain. There is also some degree of mono- and dimethylation of the chitosan polymer structure and methylation on the 3 and 6 hydroxyl groups of the chitosan polymer chain that increases with an increase in the number of reaction

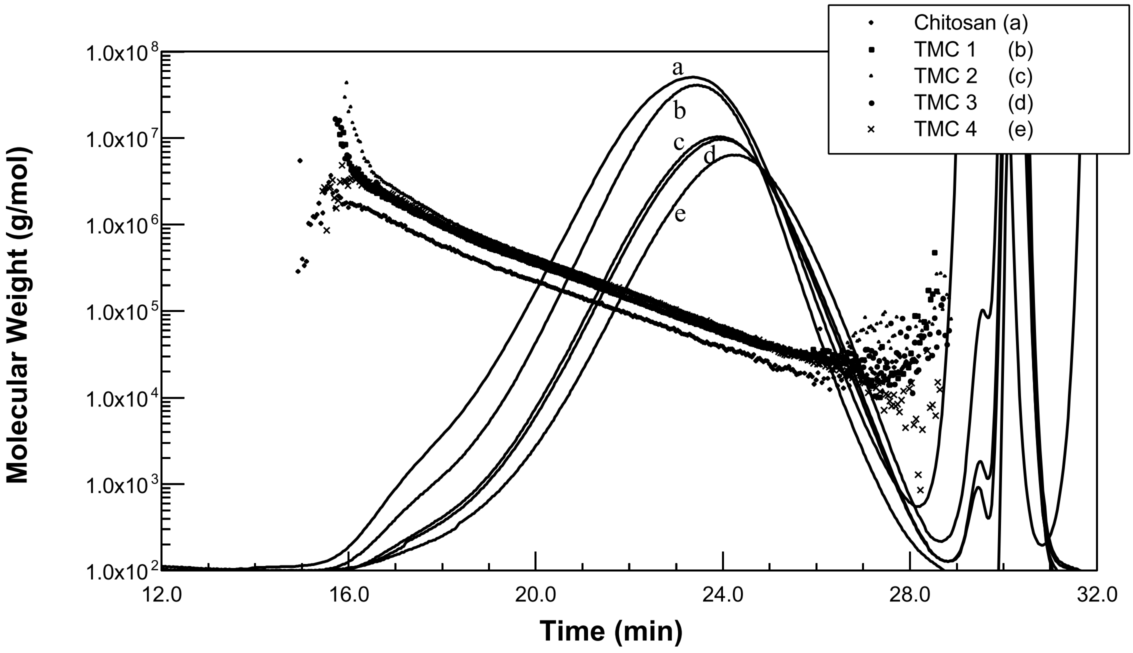

Fig. 2. Molecular weight plotted as a function of time (point values) and concentration as detected by the refractive index detector (solid lines) for the different TMC polymers.

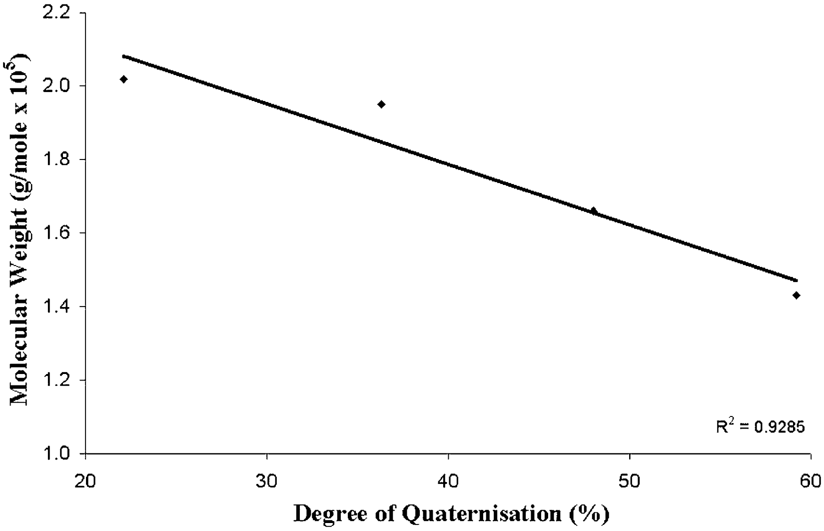

Fig. 3. The molecular weight of the TMC polymers determined by SEC/MALLS as a function of the degree of quaternisation.

steps (Hamman & Kotzé, 2001), which is probably of no importance to the absorption enhancing characteristics of these TMC polymers. According to the results of a study by Sieval et al. (1998), the peak at 3.3 ppm is assigned to the trimethyl amino group, the peak at 3.1 ppm is assigned to dimethyl amino groups and the peaks between 4.7 and 5.7 ppm are assigned to ^1H protons. As evident from the enlargement of the peak at 3.3 ppm, a longer reaction time resulted in an increase in the degree of quaternisation of the polymers. The synthesis procedure with a one step reaction resulted in a TMC polymer with a degree of quaternisation of 22.1%, while the degree of quaternisation increased to 59.2% for a reaction with four steps. Intermediate degrees of quaternisation were obtained with two and three step reactions.

4.2. Molecular weight

A summary of the results obtained with the SEC/MALLS characterisation of the TMC polymers is presented in Fig. 2. The concentrations of the polymers, calculated by the refractive index detector are plotted as solid lines, while the point values represent the molecular weight as determined by the light scattering apparatus for each time period. The average molecular weight of each polymer sample was calculated from these plotted values with Eqs. (2) and (3). The weight-average molecular weight of the TMC polymers, as determined with SEC/MALLS, decreased with an increase in the degree in quaternisation (Table 2). The straight line shows the relationship between the degree of quaternisation and the molecular weights of the products

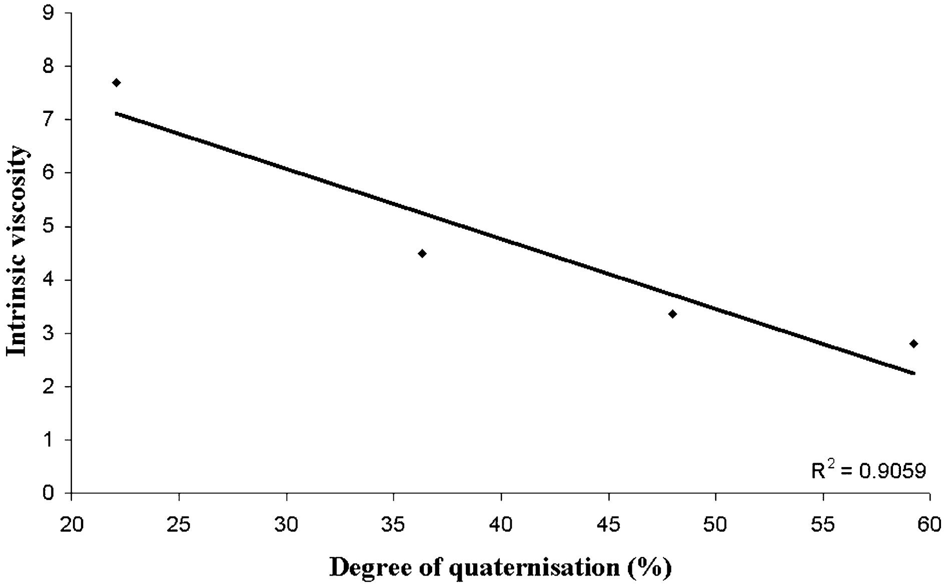

Fig. 4. Intrinsic viscosity of the TMC polymers plotted as a function of the degree of quaternisation.

when molecular weight is plotted as a function of the degree of quaternisation (Fig. 3). These results clearly show that an increase in the degree of quaternisation (determined by the specific reaction time) leads to a decrease in the absolute molecular weight of the TMC polymers. It should be noted that the molecular weight of the polymer chain increases during the reductive methylation process due to addition of methyl groups to the amino group of repeating monomers. However, a net decrease in the absolute molecular weight is observed due to degradation of the polymer chain caused by exposure to the specific reaction conditions.

4.3. Intrinsic viscosity

The intrinsic viscosities of the TMC polymers are also presented in Table 2. A higher number of reaction steps resulted in a decrease in the intrinsic viscosity, indicating that the molecular weight decreased with increased exposure time to the reaction conditions during the synthesis procedure. The intrinsic viscosities of the TMC polymers, plotted as a function of the degree of quaternisation, is presented in Fig. 4. These results clearly indicate that the intrinsic viscosity, as an indication of the molecular weight, decrease with an increase in the degree of quaternisation of the TMC polymers.

5. Conclusion

The decrease in molecular weight, as determined by the SEC/MALLS characterisation, with an increase in the degree of quaternisation of the TMC polymers correlated well with the decrease in the intrinsic viscosities of the TMC polymers. The decrease in intrinsic viscosities and the absolute molecular weight of the starting polymer during the synthesis process correlated well with the increase in the number of reaction steps, which is an indication of the total reaction time and exposure to the reaction conditions. This decrease in molecular weight can therefore be explained by the increased exposure time of the polymer molecules to reaction conditions such as the strong alkaline environment and elevated temperatures during the synthesis of the TMC polymers. The molecular weight of TMC may have an important effect on its absorption enhancing, toxicity and mucoadhesive properties. However, the degradation of the TMC polymer chain, under the experimental conditions used, is not high enough to influence the mucoadhesive properties as it was found to be above 100,000 g/mol for each of the polymers. This is an important factor when exploiting the mucoadhesive characteristics of TMC polymers in novel dosage form design, since the molecular weight of the TMC polymers can be disregarded as a variable given that the molecular weight of the starting chitosan polymer is well in excess of 100,000 g/mol. Whether this will be the same with the absorption enhancing properties is still unknown. Current and future investigations aim to synthesise TMC polymers to investigate the effect of molecular weight at a specific degree of quaternisation on the absorption enhancing properties of these polymers.

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