

Synthesis and Characterization of *N*- Diethyl Methyl Chitosan

M. Reza Avadi^{1,2}, Gholamreza Mahdavinia³,
Asal Mirmohammad Sadeghi², Mohammad Erfan¹, Mohsen Amini⁴,
Morteza Rafiee Tehrani⁴ and Abbas Shafiee^{4,*}

(1) Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran

(2) Hakim Pharmaceutical Co, P.O. Box: 11365-5465, Tehran, I.R. Iran

(3) Polymer Research Laboratory, Department of Chemistry, Sharif University of Technology, Tehran, I.R. Iran

(4) Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, I.R. Iran

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ABSTRACT

Biodegradable polymers such as chitosan have been used extensively in biomedical fields in the form of sutures; wound dressing and as artificial skin. Colonic drug delivery for either local or systemic effects has been the subject of much research over the last decade. Chitosan exhibits poor solubility at pH values above 6 that prevent enhancing effects at sites of absorption of drugs. In the present work, *N*-diethyl methyl chitosan (DEMC) was prepared based on a modified two-step process via a 2² factorial design to optimize the preparative conditions. DEMC Polymer with different degrees of quaternization for pharmacological and pharmaceutical experiments was achieved. The reaction was optimized using different amounts of reactants. pH-metric titration and infrared methods predetermined the degree of deacetylation of the starting chitosan. DEMC Chloride was characterized using FTIR and ¹H NMR spectroscopies. Based on NMR calculation, high degree of quaternization was achieved through the optimized two-step process. Under our optimized conditions, (i.e. the second step, chitosan 1.5%, ethyl iodide 30%, NaOH 3.1%, 60°C, 6h), the prepared DEMC comprised a degree of quaternization of 79%. The prepared *N*-diethyl methyl chitosan chlorides were rapidly and completely dissolved in water at room temperature.

Key Words:

chitosan;
diethyl methyl chitosan;
synthesis;
NMR;
colon drug delivery.

INTRODUCTION

Colonic drug delivery is intended either for local or systemic therapies. The colon has been determined as an ideal site for protein and peptide absorption [1]. Two major problems in the oral administration of protein and peptide drugs are acidic and

enzymatic degradations, but by targeting delivery to colon it is determined that proteolytic degradation can be minimized [2].

Chitosan with excellent biocompatible and biodegradable properties have been used extensively in the

(*)To whom correspondence should be addressed.
E-mail: ashafiee@ams.ac.ir

pharmaceutical industry as drug delivery systems [3]. Deacetylation of chitin (Figure 1a), the second abundant biopolymer isolated from insects, crustacea such as crab and shrimps as well as fungi, leads to poly (β -(1-4)-D-glucosamine) or so called chitosan (Figure 1b) [4]. Chitosan is insoluble at neutral and alkaline pH values but forms salt with inorganic and organic acids such as hydrochloric acid, lactic acid and acetic acid. The amine groups of the polymer are protonated in acidic medium and a soluble polysaccharide with positive charge is attained. The physical properties of chitosan depend on the degree of *N*-acetylation and the distribution of *N*-acetyl groups [5]. Due to the primary amino group at the C-2 position of each polymer sub-unit, further chemical modifications are easy and feasible. Because of their permeation enhancing effect, enzyme inhibitory capabilities, and mucoadhesive properties, chitosan and its derivatives are able to reduce both absorption and enzymatic barriers, which makes these polymers important excipients for peroral peptide delivery systems [6]. With biotechnology progress peptide analogues that are resistant to enzymatic degradation, hydrophilicity and molecular size of these agents remain serious problem in absorption through intestinal epithelium. Toxicity and non-specific mechanisms of action of these agents may limit their application, so biocompatible and biodegradable polymers were selected as safe permeation enhancers [7]. It has also been shown that chitosan enhances the penetration of macromolecules across the intestinal barrier [8]. However, chitosan has poor solubility at pH values above 6 that prevent the enhancing effect at the sites of absorption. At those pH values, chitosan loses its positive charge density, so it aggregates and precipitates [9].

The mucus layer adjacent to the colonic mucosa acts as a diffusion barrier [10]. Changes in mucus layer by bacteria flora degradation or disease may affect the mucus layer [11]. There is unstirred layer on the mucosal surface that molecules should pass through this site by diffusion, so factors such as molecular size and polarity are important in movement of a drug molecule towards the mucosa. It is determined that some dietary fibres such as chitosan and pectin have chitosan-exchange properties [12]. Several alkylated chitosans (Figure 1c) have been synthesized [13,14], however, to the best of our knowledge, optimized preparation of diethyl methyl chitosan (DEMC) (Figure 1d), has not been yet reported for drug delivery purposes. This study deals with synthesis

of DEMC. A factorial design approach was followed to achieve the optimum experimental conditions.

EXPERIMENTAL

Materials and Methods

Chitosan (98% deacetylated, viscosity of 1% w/v solution, 264 mPa.s) was a gift from Primex Iceland. Ethyl iodide, sodium borohydride, formaldehyde, sodium fluorescein and brilliant blue were obtained from Sigma. Sodium hydroxide, *N*-methyl pyrrolidone (NMP) and sodium iodide were purchased from Merck and the other materials were as pharmaceutical and analytical grade.

Determination of the Degree of Deacetylation

Two different routes were followed to determine the degree of deacetylation (DD). According to a modified acid-base titration method [15], chitosan (0.05g) dissolved in 20.0 mL of 0.10 N HCl was titrated pH-metrically with a standardized solution of 0.10 N NaOH

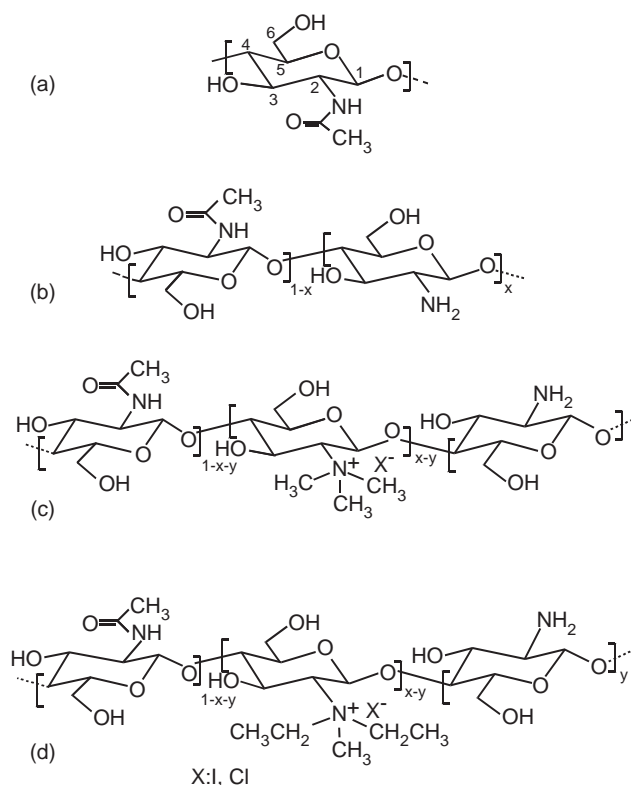


Figure 1. Structural repeating units of chitin (a), chitosan (b), TMC (c) and DEMC (d).

solution. The diagram has two equivalent points related to the excess of HCl and the protonated amino groups. The degree of deacetylation was calculated by the following equation [15].

$$DD = 16.1 (Y-X) f/w \quad (1)$$

Where, Y and X are the consumed NaOH volume of the equivalent points, f is molarity of the NaOH solution and w is the initial chitosan weight. We also used infrared spectroscopy for determining DD according to previously reported methods [16]. In infrared spectroscopy, examination of fully *N*-acetylated chitosan has shown that the value of the ratio was 1.33. On assumption of that value, this ratio is zero for fully deacetylated chitosan and there is a rectilinear relationship between the *N*-acetyl group content and the absorbance of the amide I band, so the percentage of acetylated amine group is given by:

$$N\text{-acetyl (\%)} = (A_{1655}/A_{3450}) \times (100/1.33) \quad (2)$$

Molecular Weight Determination

For determination of the chitosan average molecular weight (MW), five different concentrations of chitosan solution in acetic acid-sodium acetate buffers were prepared. The relative viscosity was obtained with a capillary viscometer at 30–0.05 C. Then intrinsic viscosity was determined and the molecular weight of chitosan was calculated based on the Mark-Houwink equation, $[\eta] = k.MW^a$ [17] where, $k = 1.64 \times 10^{-30} DD^{14}$ and $a = -1.02 \times 10^{-2} DD + 1.82$. Thus, the intrinsic viscosity and MW of chitosan were determined to be 1050 cm³/g and 1026000 g/mol, respectively.

Preparation of Diethyl methyl Chitosan

DEMC was prepared by a two-step method reported by Kim et al. [18]. In the first step 700 mg of chitosan was dissolved into 70 mL of 1% acetic acid solution and 1.5 mL of formaldehyde solution (37%) was added. After stirring for 1 h, the pH of solution was adjusted to 4.5 by adding 1 M NaOH solution and 2 mL of 10% NaBH₄ solution was added and stirring continued for 1.5 h. Methyl chitosan precipitant was obtained by adding 1 M NaOH solution and adjusting the pH of solution to 10. The precipitant was washed with distilled water and it was Soxhlet-extracted with ethyl

Table 1. Factorial design parameters and levels for optimizing the experimental conditions of the diethyl methyl chitosan preparation.

Factor	Low level	High level
CH ₃ CH ₂ I (mL)	1.5	3.0
NaOH solution (wt%)	15	30

Table 2. Water solubility of different types of DEMC according to factorial design system.

DEMC samples	Solubility (%)	Dissolution time (min)
A1	8.5	14.5
A2	5.5	19
A3	6.5	17.5
A4	9.5	15

alcohol and diethyl ether (1:1) for 3 days. In the second step, 200 mg of methyl chitosan was dispersed in 10 mL of *N*-methyl pyrrolidone and sodium hydroxide, ethyl iodide and sodium iodide were added as described in Table 1. Reaction was carried out with stirring for 5 h at 60 C. Finally acetone was added and the precipitant of chitosan derivative was collected. For exchanging I⁻ with Cl⁻, the polymer was dissolved in 4 mL of 10% sodium chloride solution. The polymer was precipitated with acetone, centrifuged and dried to obtain a white water-soluble powder. The ¹H NMR spectrum was obtained in D₂O using a 400 MHz spectrometer (Variant unity plus) and the degree of quaternization was calculated. Step II of synthesis was carried out based on 22 factorial design.

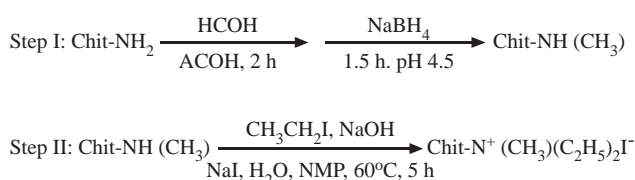
Determination of Water Solubility

Dried diethyl methyl chitosan (0.10 g), which were synthesized according to factorial design procedure, was dispersed in 1 mL of distilled water and stirred with enough water (Table 2) at room temperature until the sample was completely dissolved.

RESULTS AND DISCUSSION

A two-step synthesis of DEMC was reported by Kim et

al. [18]. In the first step *N*-alkyl chitosan was prepared by introducing a methyl group into the amine group of chitosan via Schiff's base followed by reducing the C=N bond, and in the next step ethyl iodide was used to produce diethyl methyl chitosan. The second stage of synthesis is based on direct alkylation of methyl amino group in the presence of sodium iodide and sodium hydroxide in a water/NMP medium. We followed a similar approach with some modifications to synthesize DEMC. The I⁻ was then exchanged to Cl⁻ by dissolving the quaternized polymer in a 10% NaCl solution to obtain DEMC chloride having higher solubility than the iodide counterpart.



Our preliminary studies on the second step showed that an inorganic base such as NaOH aqueous solution was better than organic bases (e.g., amines) due to larger pK_a of the latter than chitosan in order to neutralize hydroiodic acid produced during the reaction [13]. Sodium hydroxide is a strong base; able to fix the acid liberated and avoids the protonation of the NH₂ groups unreacted. Since we used chitosan with 94% DD, our experiments showed that higher NaOH concentration in a range of 15-30% resulted in higher degree of quaternization (Table 3).

The reaction temperature is reported to be 50 C for the second stage [18]. Actually, our observation was shown that the colour of reaction was changed from red to dark brown when it was heated at higher than 60 C. A

Table 3. Results for 22 factorial experiments (run in triplicate). The amount of ethyl iodide and NaOH solution (wt%) are as the independent variables and the degree of quaternization is the response factor (the dependent variable).

Formulation	Exp. 1	Exp. 2	Exp. 3	Mean±SD
A1	54.23	58.14	50.32	54.23±3.91
A2	42.56	48.69	41.32	44.19±3.95
A3	61.23	58.35	54.69	58.09±3.29
A4	81.25	78.65	77.10	79.00±2.10

similar behaviour was observed when the mixture was heated at 50 C for longer than 6 h, and the produced dried product exhibited low water solubility. The coloration and insolubility problem was critical especially at higher base concentration, higher temperature and longer reaction period. It seems that the chitosan is O-alkylated and/or degraded under harsher conditions [19].

Methyl iodide and sodium hydroxide were reported to be the two most effective reaction variables as mentioned in the case of trimethyl chitosan synthesis [20]. Therefore, after prevailing some practical problems, their concentrations were adjusted in two levels based on a concise experimental design (Table 1) and precisely conducted four reactions in triplicate and the degree of quaternization was selected as dependent variable (Table 3). The optimum amounts of reactants were found to be 3.0 mL ethyl iodide and 30.0% NaOH (formulation A4) because the highest degree of quaternization (79%) was achieved using this method. Finally, since sodium iodide is reported to be an agent for adjusting the overall concentration of reactants in the reaction medium [21], no appreciable changing was gained by increasing its amount in the range of 480 to 600 mg.

Characterization of DEMC Chloride

The solubility for all formulations was tested. As we expected, DEMC with higher degree of quaternization exhibited higher solubility so that the formulation A4 led to a DEMC that was rapidly and completely dissolved in water at room temperature.

The FTIR spectrum of chitosan (Figure 2a) shows peaks assigned to the saccharide structure at 898 and 1154 cm⁻¹ and a strong amino characteristic peak at

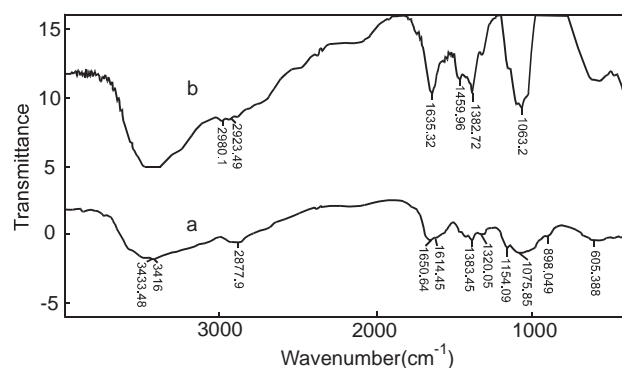


Figure 2. FTIR Spectra of chitosan (a) and *N*-diethyl methyl chitosan chloride (b).

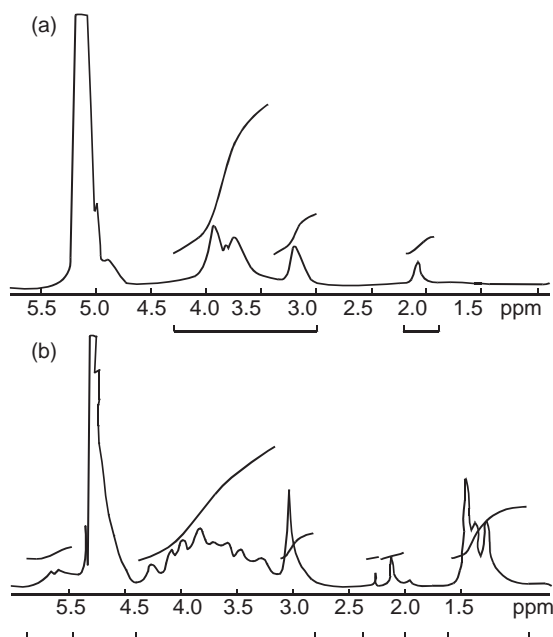


Figure 3. ^1H NMR Spectra of (a) chitosan in CF_3COOD and (b) *N*-diethyl methyl chitosan (DEMC) in D_2O .

around 1614 cm^{-1} . The absorption bands at 1650 and 1320 cm^{-1} are characteristic of *N*-acetylated chitin and have been reported to be the amide I and III bands, respectively [22]. The peak at 1614 cm^{-1} disappeared in Figure 2b due to conversion of NH_2 to *N*-diethyl methyl substituent.

The ^1H NMR spectrum of DEMC chloride is shown in Figure 3. The signal at 1.3 ppm is attributed to CH_3 groups of the ethyl substituted, while $\text{H}_2\text{-H}_6$ protons of the polysaccharide backbone superimpose the CH_2 groups. The intense band at 4.8 ppm is related to HDO (solvent). In this region, as observed more clearly from an extended spectrum, some different anomeric protons (H_1s) are appeared at 4.05 , 4.23 and 5.1 ppm . They can be attributed to mono *N*-acetyl glucosamine unit, mono *N*-substituted and disubstituted glucosamine units, respectively. The integral of CH_3 of ethyl groups versus the other protons was used to calculate the degree of quaternization [23]. The results are given in Table 3.

As a complementary experiment, we found that the peak at 1.3 ppm and 3 ppm assigned to alkyl of the quaternized amino group did not shift when a droplet of CF_3COOD was added to the solution. This is a good proof that the amount of unquaternization under our preparative conditions was negligible.

CONCLUSION

In this study, diethyl methyl chitosan, DEMC, a polyelectrolyte with different degrees of quaternization was attained to use for our later pharmacological and pharmaceutical experiments. A modified two-step procedure and a factorial design approach were employed to prepare *N*-diethyl methyl chitosan chloride with high degree of quaternization. Under our optimized conditions, (i.e., chitosan 1.5%, ethyl iodide 30%, NaOH 3.1%, 60 C , 6 h), the prepared DEMC comprised a degree of quaternization of 79%. The prepared *N*-diethyl methyl chitosan chlorides were rapidly and completely dissolved in water at room temperature.

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