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New approach to the quaternization of chitosan and its amphiphilic derivatives

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Abstract

Regioselective quaternization of chitosan and its amphiphilic derivatives has been carried out by means of reaction with betaine in the presence of the coupling reagent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in aqueous media at pH 5.5 ± 0.5. This reaction results in preparation of *N*-/(trimethylammonio)acetyl/chitosan chloride and its amphiphilic derivatives. The degree of quaternization increases with increasing EEDQ/chitosan ratio and is partly accompanied by N-ethoxycarbonylation. That side-product formation can be minimized by increasing betaine/EEDQ ratio.

Keywords: Chitosan; Chitosan derivatives; Quaternization; Betaine; EEDQ

1. Introduction

Chitosan represents a partially or fully *N*-deacetylated natural polysaccharide chitin and is considered to be a polycationic copolymer of D-glucosamine and *N*-acetyl-D-glucosamine. Chitosan attracts the considerable interest since it is a biodegradable, nontoxic polymer. Chitosan exhibits antibacterial and antiviral activities and has found numerous applications in medicine, cosmetics and food technology [1,2]. Due to its polycationic nature, chitosan is considered to be a suitable polymer for polysoaps (polymer surfactants) preparation and construction of the non-viral gene delivery systems [3,4]. It has been also shown that chitosan enhances the absorption of drugs across nasal and intestinal epithelia [5–7]. Unfortunately, in spite of these advantages chitosan has an apparent $pK_a \sim 6.5$ and is only soluble in acidic aqueous solutions with pH values lower than 6.0. At higher pH values amino groups of chitosan macromolecules become unprotonated, and at aqueous basic media

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chitosan forms insoluble, gel-like particles. The introducing of aromatic or alkyl substituents in chitosan polymer chain results in decreased solubility of the chitosan derivative. This restricts the medical application of chitosan and its derivatives at the physiological pH values. One of the strategies to increase both the solubility and positive charge density of chitosan macromolecules is based on the introducing of quaternary ammonium groups in chitosan [8–24]. This modification has got the commonly accepted term ''quaternization of chitosan''. In this paper we use the term ''quaternization'' of chitosan in spite of this term belonging to a reaction between an amine and alkyl halide resulting in a tetralkylammonium salt formation from chemical point of view. The quaternization of chitosan makes it soluble at basic conditions and improves its properties as a drug carrier across epithelia [25–27] and increases also its antimicrobial activity [2,6,27]. Numerous studies of quaternization of chitosan have been carried out by means of reactions of chitosan with alkyl iodide [8–11,13–18,20,21,24] or glycidyltrimethylammonium chloride [12,19,22,28]. Unfortunately, these reactions are carried out mainly at basic conditions, where chitosan is used in form of heterogeneously dispersed particles, and under elevated temperature (70–90 °C). These conditions lead to the non-random quaternary group distribution and the formation of highly 3-O and 6-O alkylated chitosan side-products of decreased solubility [18,20] as well as two other byproducts: *N*-methyl- and *N*,*N*-dimethylchitosans [18]. The reduction in chitosan molecular weight also occurs due to elevated temperature and alkaline synthesis conditions [11,17,18].

Recently Holappa and coworkers published the method of preparation of chitosan betainates [23]. Betaine, a nutrient that plays an important biological role in the health of the cardiovascular system, was used as *N*-chlorobetaine chloride for N-acylation of O-protected chitosan derivative dissolved in an organic solvent. Unfortunately, this method included five synthetic protection–deprotection procedures and resulted in high degrading of chitosan polymer chains [29].

In this paper, we present a new, smooth and one-step method of preparation of quaternizated chitosans (chitosans-Q) of varying molecular weight and amphiphilic *N*-/2(3)-(dodec-2-enyl)succinoyl/chitosans (DDC-chitosans-Q), a new class of potential nonviral vectors for gene delivery [30], which can not be prepared by the methods previously described.

2. Experimental part

2.1. Materials

Chitosan (Mw 90 ± 5 kDa, degree of acetylation 20%), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and deuterium oxide (99.96%) were purchased from ALDRICH.

Betaine hydrochloride was a product of SIGMA.

Low molecular weight chitosan having a degree of deacetylation of 95% and molecular weight Mw 4.6 ± 0.1 kDa (Oligochitosan), *N*-/2(3)-dodec-2-enylsuccinoyl/Oligochitosan 4.6 ± 0.1 kDa (DDC-Oligochitosan) and *N*-/2(3)-dodec-2-enylsuccinoyl/Chitosan 90 ± 5 kDa (DDC-Chitosan) were prepared by the method described in [31].

2.2. Equipment and measurements

2.2.1. Fourier transform infrared (FTIR) spectra of the freeze-dried samples were recorded in KBr discs with Perkin–Elmer 1420 Spectrometer.

2.2.2. ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded with an NMR spectrometer Bruker AVANCE and AVANCE 600 by dissolving the samples of the chitosans in H_2O/D_2O (4:1 v/v) or D_2O/DCl (1%) at concentration of 1–10 mg/ml at 293 K with suppression of the water peak. The chemical shifts were referenced from the shift of H_2O protons used as the internal standard (4.7 ppm).

Molecular weight determination: molecular weights (M_w) of the samples were determined by high performance gel permeation chromatography (HPGPC) using Ultrahydrogel 500 column ''Waters'' (size: 4.8 × 300 mm) and 0.15 M ammonium acetate/0.05 M acetic acid (pH 4.2) solution as the eluent at 30 °C and flow rate 0.5 ml/min. The weight-average molecular weight M_w was calculated by MultiChrom software (''Ampersand'', Russia). The column was preliminary calibrated using dextran standards (M_w: 1080, 4440, 9890, 43500, 66700, 123600, 196300 kDa) produced by SIGMA.

Calculations: the degrees of quaternization (DQ, mol.%) and N-ethoxycarbonylation (DE, mol.%) of Chitosan-Q and Oligochitosan-Q were calculated from a ^{1}H-NMR spectrum using the Eqs. (1)–(4):