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Response surface methodology applied to the study of the microwave-assisted synthesis of quaternized chitosan

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ABSTRACT

A quaternized derivative of chitosan, namely *N*-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride (QCh), was synthesized by reacting glycidyltrimethylammonium chloride (GTMAC) and chitosan (Ch) in acid medium under microwave irradiation. Full-factorial 2^3 central composite design and response surface methodology (RSM) were applied to evaluate the effects of molar ratio GTMAC/Ch, reaction time and temperature on the reaction yield, average degree of quaternization ($\overline{DQ}$) and intrinsic viscosity ($[\eta]$) of QCh. The molar ratio GTMAC/Ch was the most important factor affecting the response variables and RSM results showed that highly substituted QCh ($\overline{DQ}$ = 71.1%) was produced at high yield (164%) when the reaction was carried out for 30 min. at 85 °C by using molar ratio GTMAC/Ch 6/1. Results showed that microwave-assisted synthesis is much faster ($\leq$30 min.) as compared to conventional reaction procedures (>4 h) carried out in similar conditions except for the use of microwave irradiation.

1. Introduction

Chitosan is a β(1 → 4)-linked copolymer of 2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) found as a component of the cell wall of some fungi, however it is generally prepared through the deacetylation of chitin, an abundant polysaccharide present in the exoskeletons of crustaceans, mollusks and insects (Peniche, Argüelles-Monal, & Goycoolea, 2008; Rinaudo, 2006). Due its nontoxic nature and for being biocompatible and biodegradable, a range of applications has been reported for chitosan, including in wound dressing (Mogoşanu & Grumezescu, 2014), tissue engineering (Muzzarelli, 2009) and drug delivery (Sanyakamdhorn, Agudelo, & Tajmir-Riahi, 2013). Nevertheless, the application of chitosan is often limited by its poor solubility in water at neutral and alkaline pH. Thus, several strategies have been adopted for carrying out controlled chemical modifications on chitosan aiming to improve its water solubility and to expand its range of applications.

In this sense, *N*-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride (QCh), a polycationic derivative of chitosan, is a very interesting alternative as it is soluble in a wider range of pH and it displays improved properties, including antimicrobial activity (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003), mucoadhesivity (Sonia & Sharma, 2011), higroscopicity and moisture retention (Prado & Matulewicz, 2014), as compared to chitosan.

The synthesis of QCh is usually carried out by reacting chitosan with glycidyltrimethylammonium chloride (GTMAC) in alkaline, neutral or acid medium at relatively high temperature (>70 °C) for long reaction time (4–18 h) (Cho, Grant, Piquette-Miller, & Allen, 2006; Ruihua, Bingchao, Zheng, & Wang, 2012; Wu, Su, & Ma, 2006; Xiao et al., 2012). However, when the synthesis is carried out in neutral or alkaline medium, *O*-substitution occurs to an appreciable extent (Prado & Matulewicz, 2014; Ruihua et al., 2012). Additionally, the hydrolysis of GTMAC to 2,3-dihydroxypropyltrimethylammonium chloride is favored in such reaction media, negatively affecting the reaction yield. In contrast, when such a synthesis is carried out in acid medium, highly substituted QCh samples are produced and *N*-substitution predominates, preventing the formation of undesirable products as compared to procedures carried out in neutral and alkaline media (Cho et al., 2006; Prado & Matulewicz, 2014; Ruihua et al., 2012).

Numerous reports have shown that microwave heating has a high potential to accelerate chemical reactions, to increase reaction yield and to enhance product's purity and material's properties as compared to conventional experiments in which heating by convection or conduction is used (Caddick & Fitzmaurice, 2009; Gawande, Shelke, Zboril, & Varma, 2014; Moseley & Kappe, 2011; Nuchter, Ondruschka, Bonrath, & Gum, 2004; Zhu & Chen, 2014). The microwave heating involves two main mechanisms, namely dipolar polarization and ionic conduction, and it presents

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advantages such as rapid heat transfer, volumetric and selective heating. Thus, microwave heating has been used in chemical functionalization of polysaccharides, including chitosan (Ge & Luo, 2005; Liu, Wang, Yang, & Sun, 2012; Petit, Reynaud, & Desbrieres, 2015; Singh, Tiwari, Tripathi, & Sanghi, 2006) and cellulose (Biswas, Kim, Selling, & Cheng, 2014; dos Santos, Bukzem, Ascheri, Signini, & de Aquino, 2015). Petit et al. (2015) investigated the preparation of amphiphilic derivatives of chitosan by using microwave irradiation and they found that it is possible to obtain modified chitosan at lower reaction time as compared to conventional procedures and without any degradation of the polymer chain. Singh et al. (2006) described the synthesis of chitosan-*g*-polyacrylamide by using microwave irradiation and they reported that higher reaction yield was achieved in rather shorter time as compared to the reaction carried out under conventional heating. The recent literature also reports on the use of microwave heating in polycondensation reactions (Komorowska-Durka, Dimitrakis, Bogdał, Stankiewicz, & Stefanidis, 2015), ring-opening polymerizations (Hoogenboom & Schubert, 2006) as well as in radical polymerizations (Sugihara, Semsarilar, Perrier, & Zetterlund, 2012).

Response surface methodology (RSM) is a set of statistical and mathematical techniques effective for developing, improving, and optimizing processes that involves a response of interest that is influenced by several independent variables (Myers, Montgomery, & Anderson-Cook, 2009). RSM is based on the fit of a polynomial equation to the experimental data that describes the relationship between a dependent variable, or response, and the independent variables as well as the interactions among these latter. Simultaneously, this technique allows to optimize the levels of the independent variables to attain the best possible response in a faster and more economical manner when compared to classic one-variable-at-a-time approach (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Myers et al., 2009). The synthesis of QCh involves reactional parameters such as molar ratio chitosan/GTMAC, reaction time and temperature that influence a series of responses related to the important properties of this chitosan derivative as its degree of quaternization and intrinsic viscosity as well as the reaction yield (Cho et al., 2006; Prado & Matulewicz, 2014; Ruihua et al., 2012). In this context, the RSM can be considered a useful tool to evaluate how the independent variables related to the reaction conditions used to synthesize QCh, as well as the interactions among them, affect the properties of this chitosan derivative.

Aiming to provide new insights for the preparation of N-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride (QCh), this study focus on the preparation of QCh samples in acid medium under microwave irradiation by using full-factorial 2^3 central composite design and response surface methodology (RSM) to evaluate the effects of molar ratio GTMAC/Ch, reaction time and temperature on the reaction yield, average degree of quaternization ($\overline{DQ}$) and intrinsic viscosity ($[\eta]$) of QCh. The parent chitosan as well as the resulting derivatives are characterized by Fourier transform infrared (FTIR) and ^{1}H NMR spectroscopy, capillary viscometry, thermogravimetric analysis (TGA), X-ray diffraction and with respect to water-solubility as a function of pH.

2. Materials and methods

2.1. Materials

Commercial chitosan (Cheng Yue Planting Co Ltd., China) was dissolved in 1% aqueous acetic acid solution to result in Cp = 3 g/L, the resulting solution was filtered through 0.45 μm membrane (Millipore®), and then it was neutralized by addition of 1 mol L^{-1} NaOH solution to provoke the precipitation of chitosan. The solid was thoroughly washed with distilled water and with ethanol/water mixtures of increasing ethanol content (70%, 80%, and 90%). The purified chitosan was dried at 30 °C and named as sample Ch. Glycidyltrimethylammonium chloride (GTMAC) was acquired from Sigma-Aldrich (Saint Louis, MO; USA) and its was used as received as well as other reactants and solvents employed in this study.

2.2. Synthesis of N-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride

Purified chitosan (0.5 g) was suspended in 30 mL of deionized water and 150 μL of glacial acetic acid were added to the suspension which was kept at constant stirring at room temperature for 10 min. An aqueous solution of GTMAC was added dropwise to the chitosan suspension which was then submitted to microwave irradiation at a power of 200 W in a monomode microwave reactor (Discover-LabMate, CEM, USA) under constant stirring at the desired temperature and during a given time. Following, excess acetone was added to the reaction medium to result in the precipitation of the product which was filtered, thoroughly washed with acetone and dried at 35 °C for 24 h. The reaction yield was calculated based on the weights of the parent chitosan and the resulting product.

2.3. Experimental design

A full-factorial 2^3 central composite design was used to analyze the main effects and interactions of the reaction variables, namely molar ratio GTMAC/Chitosan, reaction time and temperature, on the average quaternization degree ($\overline{DQ}$) and intrinsic viscosity of QCh and on the reaction yield of the microwave-assisted synthesis of QCh. The choice of the parameters and their levels was based on our own previous experimental studies. Thus, 11 independent runs of experiments were carried out in duplicate, including 2^3 orthogonal factorial and six replicate at the center point. The independent variables and their levels are shown in Table 1. All the experiments were carried out at random, in order to minimize the effect of unexplained variability in the observed responses due to systematic errors.

Also, to compare the microwave-assisted synthesis of QCh to reaction performed by using conventional heating, two additional runs were carried out using the same reaction conditions as used to produce samples QCh1 ($X_1 = 4/1$; $X_2 = 20$ min.; $X_3 = 75$ °C) and QCh8 ($X_1 = 6/1$; $X_2 = 30$ min. and $X_3 = 85$ °C) except for the use of microwave irradiation, resulting in samples QCh1-CH and QCh8-CH, respectively. Such reactions were carried out in a 100 mL one-necked round bottom flask immersed in a preheated oil bath at the given temperature for the desired time.

2.4. Characterization

2.4.1. ^{1}H NMR spectroscopy

The ^{1}H NMR spectra of the parent chitosan and its derivatives were acquired at 85 °C by using an spectrometer Agilent 400/54 Premium Shielded 9.4 T, operating at 399.8 MHz for ^{1}H. For these analyses, the samples were dissolved in HCl/D$_2$O 1% (v/v) at a

Table 1
Uncoded and coded levels of the independent variables related to the synthesis of *N*-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride.

Independent variables	Symbol	Levels		
		−1	0	1
GTMAC/Chitosan	X_1	4	5	6
Time (min)	X_2	20	25	30
Temperature (°C)	X_3	75	80	85