

Synthesis and Physicochemical Characterization of Chemically Modified Chitosan by Succinic Anhydride

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ABSTRACT

The N-succinil-chitosan is a chemically modified derivative of the biopolymer chitosan. The succinic anhydride attached to the free amino groups presented along the chitosan's polymer chain imparts to the molecule different physicochemical properties not exhibited before the modification. These chemical modifications enhance chitosan's solubility in slightly acid, neutral and alkaline media. These properties are related to the long alkyl chains attached to hydrophilic parts. In this case the hydrophilic part of D-glucosamine promotes stronger interactions with the water molecules, and consequently, enhances the solubility of the chitosan polymer. Non-modified free chitosan is soluble only in acidic medium ($\text{pH} \leq 5.5$). These modifications made possible new applications of chitosan in biotechnological area since the solubility in neutral or slightly alkaline solutions is very important in a biological field.

Key words: N-succinil-chitosan, succinic anhydride, biopolymer, solubility

INTRODUCTION

Chitin is a biopolymer similar to cellulose and quite abundant in nature. It is extracted mainly from crustaceous skin or shells. (Singla, 2001; Khan, 2002; Tangpasuthadol, 2003). Due to the natural origin of chitin, different variants are found in environment. In addition, when chitin is submitted to different chemical processes, a series of polymers varying in the degree of deacetylation (DD), molecular weight (MW), viscosity, pKa, etc, may be generated (Chatelet, 2000; Canella, 2001; Singla, 2001). Chitosan is a natural polysaccharide composed of $\beta(1-4)$ -D-glucosamine units originated from the total or partial deacetylation of chitin in alkaline solutions (Singla, 2001; Dee,

2004). It is soluble in acid medium ($\text{pH} < 5.5$) due to the presence of free amino groups along the polymer chain. The presence of these amino groups allows the synthesis of different chitosan derivatives (Khan, 2002; Franco, 2004). The purpose of this study was to synthesize a N-succinil-chitosan derivative based on a methodology proposed by Yamagushi et al, 1981 with some modifications (Yamagushi, 1981).

MATERIAL AND METHODS

Chitosan samples were obtained from shrimp's shells and purchased from Farma Service

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Bioextract (São Paulo, Brasil). All chemicals were of analytical grade.

Sample preparation

Two samples of N-succinil-chitosan were prepared by reaction of chitosan (Chit) with succinic anhydride at 1:1 w/w (0.06:0.01, mol/mol) and 1:3 w/w (0.06:0.03 mol/mol) proportions named as CS1:1 and CS1:3, respectively. These molar quantities of chitosan/anhydride were smaller than what it was suggested in the original work of Yamagushi et al. (1981), who used an excess of anhydride (16,4 moles/amino group). The sample was precipitated with ethanol, not methanol as the authors proposed, without yield losses. Moreover, the previous solubilization of succinic anhydride in acetone was substituted by direct addition of the succinic anhydride to the reactional medium.

Sample characterization

The solubility of chitosan and its synthesized derivative was evaluated at different pH: acid (pH=4.0; 3% CH₃COOH solution), neutral (pH=7.0, water) and alkaline medium (pH=10.0; 0.1 mol L⁻¹ NaOH solution) at room temperature (25°C). Qualitative observations of the samples submitted to the solubilization tests were registered after 1, 5 and 10 hours.

The deacetylation degree (DD) of chitosan and its derivative was determined by infrared (IR) spectroscopy and ninhydrin titration. The substitution degree (SD) was determined using the same techniques. (Curotto, 1999; Canella, 2001; Khan, 2002). The spectra of chitosan sample (in the forms of KBr disks) were obtained using an IR

Instrument (Bomem MB-100 FTIR, Germany) with a frequency range of 4000-400 cm⁻¹. The degree of deacetylation of the chitosan sample by IR spectroscopy was calculated using the equation proposed by Domszy and Roberts that is given below:

$$DD=100-[(A_{1665}/A_{3450}) \times 100/1.33]$$

where A₁₆₆₅ and A₃₄₅₀ were the absorbance at 1655cm⁻¹ of the amide-I band as a measure of the N-acetyl group content and 3450cm⁻¹ of the hydroxyl band as an internal standard to correct for film thickness or for differences in chitosan concentration powder form. The factor '1.33' denoted the value of the ratio of A₁₆₆₅/A₃₄₅₀ for fully N-acetylated chitosan. It was assumed that the value of this ratio was zero for fully deacetylated chitosan.

The hygroscopic degree was measured under vacuum in 90.70% humidity dessicators containing BaCl₂ salt. Samples were previously freeze-dried during 24 h, accurately weighted and placed in the dessicator. The measurements were done in 1h intervals for 5h, until constant weight for chitosan and its derivative was reached.

RESULTS AND DISCUSSION

The solubility measurements are shown in Table 1 for three different pHs. Chit was perfectly soluble in acetic acid solution but precipitated at neutral and alkaline solutions.

Table 1 - Solubility tests of Chit and modified chitosans CS1:1 and CS1:3 in different solutions and pHs.

	Solubility		
	pH=4.0	pH=7.0	pH=10.0
Chit	++++	-	-
SC1:1	++	++	+++
SC1:3	-	++	++++

This was expected due to the presence of amino groups along the polymer chain which were protonated under low pH. SC1:1 became partially solubilized in the entire pH range due to the increasing substitution of the amino groups by carboxylic groups, which became negatively charged above pH 6.0. The highly substituted SC1:3 appeared insoluble in pH 4.0 due to the predominance of carboxylic groups compared to

amino groups but totally solubilized at high pH, where the complete dissociation of carboxylic acid groups occurred.

Deacetylation degree (DD) of original chitosan sample was determined to verify how many amino groups were available to react with succinic anhydride. Substitution degree (SD) was determined for the modified samples. In Figure 1 both results of DD for Chit and SD for SC1:1 and

SC1:3 are shown. It was possible to verify that the initial Chit presented a DD of 45% while the substituted samples, SC1:1 and SC1:3, presented a SD of 10 and 20%, respectively. The increased substitution of amino groups by carboxylic groups was directly related to the increase in solubility related previously. The relatively low substitution

degree obtained for both modified samples could be explained in part due to the hydrolysis of the reactant..

In Fig. 2 the capacity of the samples to absorb humidity at room temperature are shown. It was observed that the modified samples absorbed more humidity than Chit.

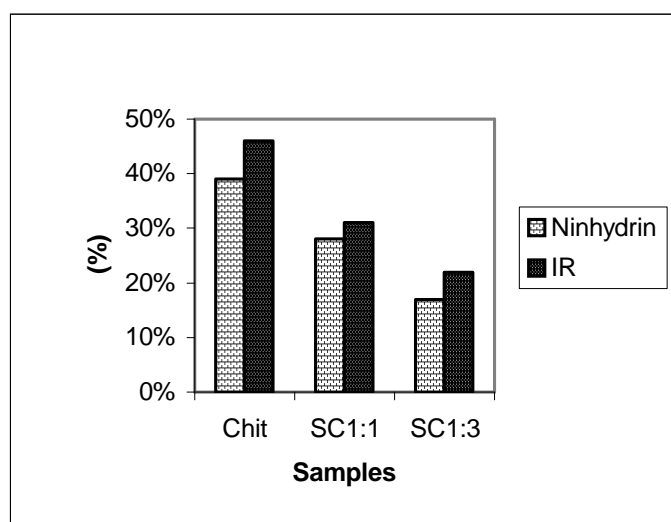


Figure 1 - Deacetylation of Chit and substitution degree of modified samples determined by ninhydrin titration and infrared spectroscopy

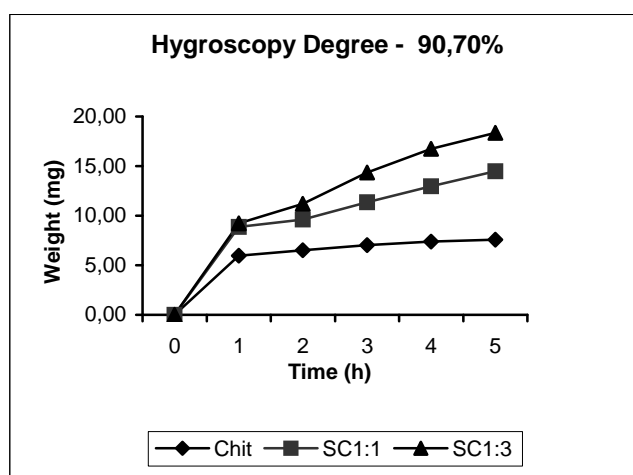


Figure 2 - Hygroscopy degree of the modified Chitosan samples in 90.70% humidity environment.

All samples absorbed more humidity in the first hour followed by shorter increase in the next 5h when the weight becomes constant. Among all

samples, SC1:3, which was the most substituted sample, presented a larger hygroscopic degree as a function of time and temperature.

CONCLUSION

The different physicochemical properties determined in this study indicated that N-succinyl-chitosan could be applied in many biotechnological fields. The molar quantities of chitosan/anhydride used in these study were quite lower than what it was done in the original Yamaguchi's 1981 work and, the modifications introduced in the sample preparation resulted in optimization of the process, economy of reactants without losses of physicochemical properties. The solubility of substituted chitosan samples in neutral and alkaline media increase the possibility of use in cosmetics and pharmaceutical. The hygroscopic capacity of the modified samples could be useful to film formation, to membranes formulated for wound healing and as additive in cosmetics and toiletries.

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RESUMO

A N-succinil-quitosana é um derivado quimicamente modificado do biopolímero quitosana. A inserção de substituintes de anidrido succínico nas aminas protonadas presentes ao longo da cadeia do polímero quitosana, conferem diferentes características físico-químicas a molécula de quitosana.

Esta modificação química, possibilitou à quitosana, solubilidade em pHs que variam do ácido (2.0 a 3.0) até alcalino (13.0 a 14.0). Estas propriedades são atribuídas ao alongamento da cadeia alquílica, que afasta a parte hidrofílica da cadeia fechada da D-glicosamina, facilitando o acesso da água, a qual irá estabelecer uma interação mais forte com a molécula de quitosana. Esta propriedade não está presente em amostras de quitosana pura, a qual sabe-se que solubiliza-se apenas em pH abaixo de 5.5. Estas modificações na quitosana possibilitam novas aplicações na área de biotecnologia, uma vez que a solubilidade em meio neutro e levemente alcalino é importante na área biológica.

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