CHARACTERISATION OF CHITOSAN AFTER CROSS-LINKING BY TANNIC ACID

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Abstract
Chitosan is widely applied in biomedical and cosmetic fields. For this reason, there is a need to modify this biopolymer to achieve new properties of chitosan-based materials. The properties of chitosan can be modified by several cross-linking methods. In this work, we used the chemical cross-linking of chitosan with tannic acid, after which cross-linked chitosan was shaped into a 3D structure by the lyophilisation process. For the material obtained, FTIR-ATR analysis was performed to characterise the structure of cross-linked chitosan. Moreover, mechanical properties and swelling properties were measured for chitosan before and after cross-linking. The results show that several properties of chitosan can be changed after cross-linking by tannic acid. The positions of bands in the FTIR spectra of chitosan were shifted after cross-linking. Mechanical properties were altered for cross-linked chitosan. However, the highest compressive modulus was observed for pure chitosan. The lowest compressive modulus was observed for chitosan cross-linked with 20 wt% of tannic acid. Swelling behaviour also depends on the cross-linking of chitosan. It decreases for chitosan after cross-linking by tannic acid. Overall, the properties of cross-linked chitosan depend on the amount of tannic acid used for modification. This method of cross-linking can be useful for obtaining materials with specific properties.

Key words: *chitosan, tannic acid, cross-linking, mechanical properties.*

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1. Introduction

Chitin is one of the most abundant natural polymers. Its derivative obtained in deacetylation process is called chitosan [1]. Chitosan is biocompatible, biodegradable and non-toxic for living organisms. It is commonly used as a component of biomaterials [2]. The properties of polymers can be modified by using several cross-linking methods. We can distinguish between physical and chemical cross-linking. As physical cross-linking methods, one can use UV radiation, temperature, and gamma radiation. All of the physical methods are easy to perform and cheap. The disadvantage of these methods, however, is the difficulty in controlling the cross-linking process, which means that it is very hard to obtain desirable degrees of cross-linking [3, 4]. Chemical cross-linking methods are based on the chemical reactions which take place after adding the chemical compound as a cross-linking agent to the polymer. Several chemical cross-linking agents can be used, such as glutaraldehyde or formaldehyde. Nevertheless, they enhance the toxicity of the material, which makes it impossible to use them as biomaterials for contact with living bodies [5]. Tannic acid is a polyphenolic compound containing several OH groups. It is used as a natural cross-linking agent, for example in leather production from hide. Tannic acid can react with the amino groups of chitosan and/or proteins and, as such, the reaction can modify the properties of chitosan and/or proteins [6].

The aim of this work was to study the properties of chitosan after treatment with tannic acid.

2. Materials and methods

Chitosan and tannic acid were purchased from Sigma-Aldrich. Chitosan was prepared as a 1% (weight) concentration solution in 0.5 M acetic acid. Tannic acid was added to the solution in weight ratios of 2, 5, 10 and 20% based on chitosan amount. Then, the mixture was stirred for 1 h. Composites were obtained as 3D structures after solution lyophilisation.

FTIR spectra were obtained using a Genesis II FTIR spectrophotometer (Mattson, USA) equipped in an ATR device with zinc selenide crystal.

Mechanical properties were measured for lyophilised structures by using a mechanical testing machine (Z.05, Zwick/Roell, Germany). Compressive modulus and maximum force of compression were measured using the point method.

Swelling behaviour was measured by immersion of the composite fragments in PBS solution. After 1, 2, 3, 4, 5, 6, 7 and 24 h, materials were dried and weighed. Mass changes were then calculated using the equation:

$$swelling = [(m_t - m_0)/m_0] \cdot 100\% \quad (1)$$

Such analyses were performed for chitosan samples with the addition of tannic acid in different ratios as well as for pure chitosan as a control.

3. Results and discussion

3.1. Infrared spectroscopy (ATR-IR)

Characteristic motions in FTIR spectra and groups of different chitosan specimens containing tannic acid are listed in ***Table 1***.

3.2. Mechanical properties

Mechanical properties were measured using the compression method. Compressive modulus and the maximum force of compression for chitosan composites with 2, 5, 10 and 20 wt% of tannic acid are listed in ***Table 2***.

The highest compressive modulus was observed for pure chitosan, while the lowest was observed for chitosan cross-linked with 20 wt% tannic acid. The maximum force of compression is highest for pure chitosan and lowest for chitosan cross-linked with 5 wt% of tannic acid.

3.3. Swelling behaviour

Chitosan cross-linked with tannic acid in a weight ratio of 20% and pure chitosan were immersed in PBS (pH 7.4) at room temperature. Specimens were then dried and weighed before and after 1, 2, 3, 4, 5, 6, 7 and 24 hours of swelling. Weight changes and percentage of swelling are shown in ***Table 3***. After one hour of immersion in PBS, the weight of the chitosan sponge increased by several times. However, after two hours of immersion in PBS, the samples dissolved and it was impossible to measure the weight change. Swelling was cal-

Table 1. *Characteristic motions in FTIR spectra and groups of different chitosan specimens: pure chitosan and chitosan with 5 and 20 wt% tannic acid.*

Specimen	Wavenumber [cm^{-1}]	Functional group	Motion
Chitosan	3249	O-H	stretch
	1549	N-H	bend
	1064	C-O-C	stretch
Chitosan with 5 wt% of tannic acid	3314	O-H	stretch
	1516	N-H	bend
	1066	C-O-C	stretch
Chitosan with 20 wt% of tannic acid	3311	O-H	stretch
	1640	C=O	bend
	1064	C-O-C	stretch

Table 2. *Compressive modulus and maximum force of compression for chitosan composites with 2, 5, 10 and 20 wt% of tannic acid (TA).*

Specimen	Compressivemodulus [kPa]	Maximum force of compression [N]
Chitosan	1.02±0.20	1.075 ± 0.132
Chitosan/2wt% TA	0.58±0.20	0.746 ± 0.044
Chitosan/5wt% TA	0.98±0.15	1.170 ± 0.003
Chitosan/10wt% TA	0.36±0.16	0.780 ± 0.014
Chitosan/20wt% TA	0.43±0.16	0.880 ± 0.059

Table 3. *Weight of chitosan and chitosan cross-linked with 20 wt% of tannic acid (TA) composites after 1, 2, 3, 4, 5, 6, 7 and 24 h of immersion in PBS.*

Specimen	Weight of immersion [mg] after in PBS time				
	0	1 h	Swelling %	2 h	4 h
Chitosan	0.0126	0.3351	2260	Sample dissolved	
Chitosan/20wt% TA	0.0154	0.3227	1995		

culated from equation number 1 for composites before and after 1 h of immersion. Swelling for chitosan was 2260% and for composite with the addition of 20% tannic acid was 1995%.

4. Conclusions

The properties of chitosan can be modified by chemical cross-linking using different amounts of tannic acid as a cross-linking agent. In particular, mechanical and swelling properties were altered after reaction with tannic acid. Differences in mechanical and swelling properties depend on the amount of tannic acid added to chitosan. Both the compressive modulus and the maximum force of compression were found to decrease after adding tannic acid as the cross-linking agent, which shows that chitosan cross-linked with tannic acid is stiffer than that before such a treatment. Swelling behaviour also depends on the cross-linking of chitosan and decreases with the addition of tannic acid. The cross-linking of chitosan with tannic acid can be considered a useful method for the preparation of chitosan materials with specific properties.

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6. References

1. Sionkowska A, Kaczmarek H, Wisniewski M, Skopinska J, Lazare S, Tokarev V; (2005) The influence of UV irradiation on the surface of chitosan films. Surf Sci 600, 3775-3779, DOI: 10.1016/j.susc.2006.01.090.
2. Sionkowska A; (2011) Current research on the blends of natural and synthetic polymers as new biomaterials: review. Prog Polym Sci 36, 1254-1276, DOI: 10.1016/j.progpolymsci.2011.05.003.
3. Kumbar SG, Kulkarni AR, Aminabhavi TM; (2002) Cross-linked chitosan microspheres for encapsulation of diclofenac sodium: effect of cross-linking agent. J Microencapsul 19, 173-180, DOI: 10.1080/02652040110065422.
4. Schiffman JD, Schauer CL; (2007) Cross-linking chitosan nanofibres. Biomacromol 8, 594-601, DOI: 10.1021/bm060804s.
5. Hennink WE, Nostrum CF; (2002) Novel cross-linking methods to design hydrogels. Adv Drug Deliver Rev 54, 13-36, DOI: 10.1016/S0169-409X(01)00240-X.
6. Rivero S, Garcia MA, Pinotti A; (2010) Cross-linking capacity of tannic acid in plasticised chitosan films. Carbohyd Polym 82, 270-276, DOI: 10.1016/j.carbpol.2010.04.048.