CHEMICAL MODIFICATION OF CHITOSAN WITH FATTY ACIDS

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Abstract
This paper presents the results of chitosan modification with fatty acids, namely linoleic and oleic acid. We used carbodiimide as the initiator for the reaction of carboxyl and aminoglycoside functional groups. The analysis of infrared spectra confirmed the formation of chitosan derivatives. Their hydrophilic-hydrophobic properties were determined by O/W emulsification and micelles formation in aqueous systems.

Key words: *chitosan, fatty acid, amphiphilic properties, micelles.*

1. Introduction

Chitosan, a natural polysaccharide, is produced by the complete or partial N-deacetylation of chitin. It consists of randomly distributed ß(1-›4)-2-amino-2-deoxy-D-glucopyranose and ß(1-›4)-2-acetamido-2-deoxy-D-glucopyranose (with the ratio depending on the deacetylation degree). Chitosan shows high biocompatibility and antibacterial properties, it is biodegradable and non-toxic polymer, thus it can be widely used in medical applications [1]. Moreover, the strong functionality of chitosan - two hydroxyl groups and one primary amine group, which can donate a free pair of electrons, make chitosan soluble in diluted aqueous acetic solvents and allows the formations of the coordination bonds, what offers a considerable opportunity of a chemical modification [2].

The modification of chitosan chains (which are hydrophilic) with hydrophobic compounds (such as carboxylic acids, including fatty acids) can result in products with an amphiphilic behavior (a simplistic scheme of preparation of chitosan-based amphiphilic materials is presented in ***Figure 1***). The amphiphilic chitosan derivatives are able to self-assemble and form nanoparticles (micelles) under appropriate conditions [3].

The driving force for micelles formation in O/W emulsion is to reduce a free energy of phase boundary and adsorb at the interfaces to separate hydrophobic groups from contact with aqueous solution. In a micelle, the chitosan derivative hydrophobic groups are directed towards the interior of the cluster and the polar head group is directed towards the solvent [4].

The aim of this paper was to investigate the chemical modification of chitosan with fatty acids, namely linoleic and oleic acids in order to obtain amphiphilic derivatives capable to self-assemble in W/O environment.

2. Materials and methods

2.1. Materials

Chitosan (Sigma-Aldrich) of viscosity 20 - 300 cP (1% in 1% acetic acid) and deacetylation degree 75 - 85% was dissolved in 1% acetic acid. Next, the solution of linoleic (LA) or oleic (OA) acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (1:1 mol/mol) in methanol solution was added drop wise

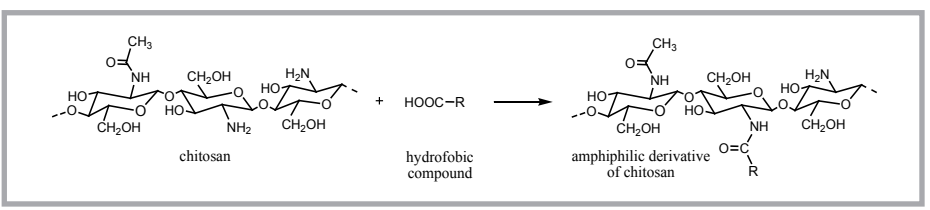

Figure 1. *Chemical modification of chitosan at primary amino group to obtain amphiphilic derivative.*

to chitosan solution, initiating the reaction of carboxyl groups of fatty acids and chitosan amino groups (0.34/1 mol/mol). The reactions were carried out for 24 hours at room temperature [5]. The products were precipitated in ammonia solution, centrifuged, washed with distilled water (until the pH was approximately neutral) and methanol. The precipitates were then dried for 24 hours under the vacuum at 30 °C.

2.2 Methods

The assessment of chemical structure of chitosan and its derivatives was performed by analysis of the Fourier transform infrared spectra on Nexus spectrometer in 400 - 4000 cm^{-1} range at 32 scans. All samples were dried under vacuum at 30 °C before the measurement.

Deacetylation degree (DD, %) of chitosan was determined by the conductometric titration method, taking the first derivative of the conductivity of the chitosan solution (dissolved in 0.1 mol HCl) and the volume of titration solution (0.1 mol NaOH), and using following equation:

$$DD = \frac{203.2 \cdot 100}{24.0 + \frac{1000m}{0.1(V_2 - V_1)}}$$

where m is the amount of chitosan in g, V_2 - V_1 is the volume of sodium hydroxide solution consumed in the titration of amino groups of chitosan [6]. The conductivity of the solution during the whole titration process was measures by a lab pH meter (SCHOTT Instruments handylab LF 11/LF 12).

2.3 Micelle preparation

The obtained derivatives of chitosan were dissolved in 1% acetic acid solution (0.001, 0.003 and 0.005 g/ml). The varying amounts of methylene chloride (served as an oil phase at concentration of 1, 3 and 5%, v/v) were added to the dissolved chitosan-fatty acid solution while shaking and homogenizing with IKA Basic Orbital Shaker. The solutions were held under the vacuum for 30 min at room temperature to remove the methylene chloride. To evaluate the efficiency of the process leading to chitosan self-assemblies, size of the formed particles was determined by Dynamic Light Scattering with the Zetasizer Nano-ZS (Malvern) apparatus.

3. Results and discussion

The starting point for chitosan modification with fatty acids was the determination of deacetylation degree of chitosan by the conductometric titration which is simple and low cost technique [6]. The first measurement point (V_1) was the volume of sodium hydroxide solution needed to equilibrate free H^+ ions from the excess of hydrochloric acid, while the second measurement point (V_2) was the volume of sodium hydroxide solution needed to protonate the amino groups. Calculated deacetylation degree (DD) of chitosan was ~ 78%.

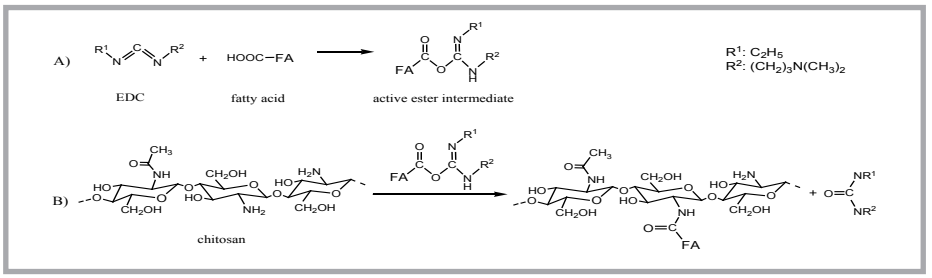

Figure 2. *Schematic representation of fatty acid activation (A), and its reaction with chitosan (B).*

The reaction of chitosan with fatty acids was catalyzed with water-soluble carbodiimide EDC·HCl, which reacts with carboxyl groups of fatty acids to form an active ester intermediates (***Figure 2.A***). Consequently, the intermediates can react with primary amine groups of chitosan to form an amide bond [7] (***Figure 2.B***).

Additionally, the advantage of using EDC as a catalyst is its easy removal from the reaction medium (rinsing with water) and more importantly, the reaction can be carried out in mild conditions (no need for high temperature or pressure) [7].

Chemical structure of the neat chitosan and modification products - chitosan grafted with linoleic or oleic acid chains was verified based on IR spectra showed in ***Figure 3***.

The comparison of infrared spectra of chitosan and modification product, CH-LA and CH-OA showed the characteristic absorption at 2924, 2854, 1464, 1182 cm^{-1} assigned to vibrational bands corresponding to CH_2 group, as well as the 2 vibrational bands at 1739 and 1590 cm^{-1}, corresponding to amide groups which became stronger for modification products. A decrease of absorption band was found at 3000 - 3600 cm^{-1} what can be related to diminished concentration of amino groups of chitosan after reaction with acids.

Self-assemble of chitosan derivatives towards micelles/aggregates was assessed in O/W emulsions. Continuous phase was formed by chitosan derivatives diluted in 1% acetic acid and dispersed phase was methylene chloride (which was evaporated after micelles formation). The hydrodynamic diameter of micelles was determined using Dynamic Light Scattering method (DLS). All samples showed different micelles formation ability which depended from CH/LA concentration and volume fraction of oil phase. Samples with the same volume fraction of oil phase (methylene chloride) and different CH/LA concentration showed strong correlation with these parameters. For the highest CH/LA concentration (0.005 g/ml), a large diameter size distribution was observed (from 400 to 4500 nm). Reducing the concentration to 0.003 g/ml led to more homogeneous micelles in terms of diameter size (in comparison to 0.005 g/ml), however the product was a binary mixture of small (400-800 nm) and large (3500-

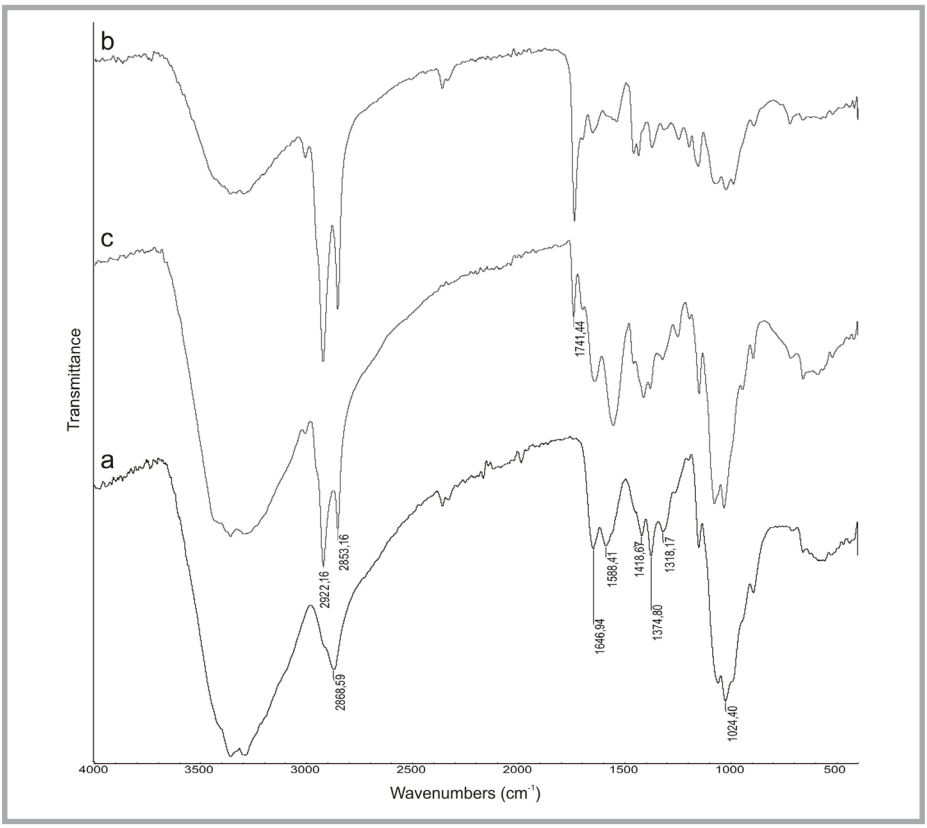

Figure 3. *IR spectra of the neat chitosan (a) and its derivatives- with linoleic (b) and oleic (c) acid.*

6500 nm) particles (which are probably the result of aggregation of smaller micelles). For the lowest concentration of CH/LA (0.001 g/ml), the hydrodynamic diameters were smaller, had the lowest volume size distribution and showed relatively narrow micelle size distribution (300-900 nm) (***Figure 4.A***). In further work we will determine a critical micelle concentration (CMS) – the concentration at which the micellization starts.

The volume fraction of oil phase in the emulsion also affects the size and distribution of the particles. As it is shown in ***Figure 4.B***, the higher methylene chloride concentration, the higher volume fraction of one type of particles with uniform size, and much reduced particles size distribution.

4. Conclusion

Chemical modification of chitosan with hydrophobic fatty acids, namely linoleic and oleic acid, has been successfully performed. We demonstrated that carbodiimide can be used as efficient catalyst for chemical modification of chitosan. The product of modifica-

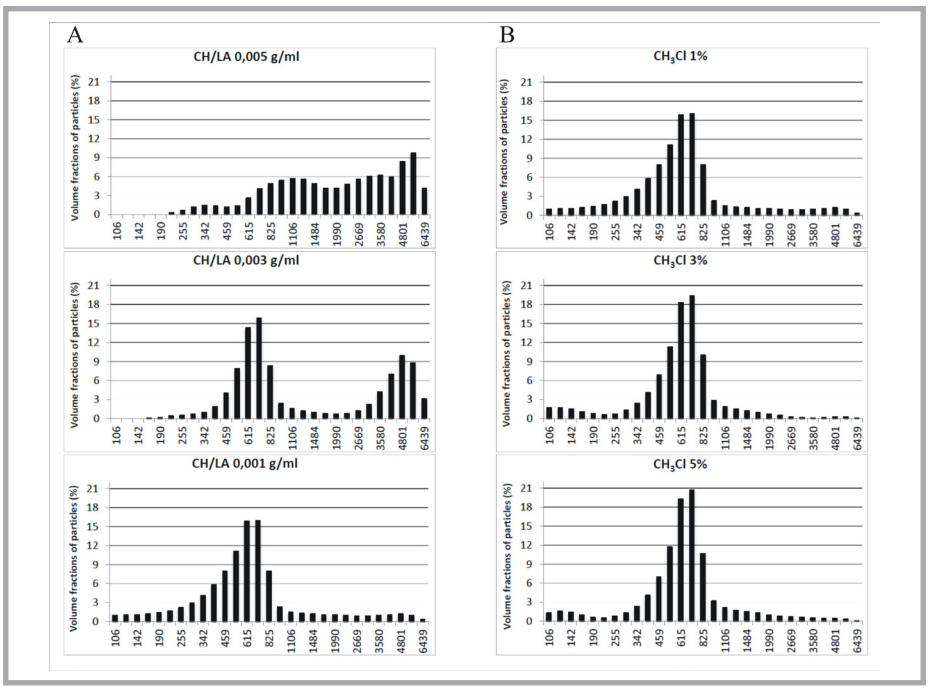

Figure 4. *DLS measurement for samples: A) with the same volume fraction of methylene chloride (1%) and different CH/LA concentration; B) with the same CH/LA concentration (0.001 g/ml) and different volume fraction of methylene chloride.*

tion: amphiphilic derivatives were capable to self-assemble into micelles with the diameter depended from the emulsification conditions. The average hydrodynamic diameter was 670 nm for the highest volume fraction of CH_3Cl and the lowest concentration of CH/LA.

5. *Acknowledgments*

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