POLYELECTROLYTE COMPLEXES OF CHITOSAN WITH DEXTRAN SULPHESIS. SYNTHESIS AND CHARACTERIZATION

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Chitosan (CS), the unique cationic groups-containing polysaccharide, is a natural, renewable, nontoxic and biodegradable material, considered as an ecologically-friendly product. Several studies on the obtaining of intermolecular complexes (IMC) between CS and natural or synthetic anionic polyelectrolytes have been reported. CS and dextran sulphate (sodium salt form) (DS), among other applications, may be also used to obtain various complexes with special applications in protein, cell and enzyme encapsulation. The present investigation discussed some new results on CS interaction with DS, for obtaining suitable systems for controlled release of drugs and of other bioactive agents. The interaction between these two partners was investigated in solution by conductometric, potentiometric and turbidimetric titration, and in solid phase, by FTIR spectroscopy and thermogravimetric analysis. All titration methods confirmed the formation of complexes in a stoichiometric ratio between partners. Solid phase analysis of the obtained complex by FTIR spectroscopy evidenced the presence of new bands characteristic of electrostatic interaction.

Keywords: polysaccharides, chitosan, dextran sulphate, intermacromolecular complexes

INTRODUCTION

CS (2-amino-2-desoxy-b-D-glucan)[1-4] is a pseudonatural cationic polymer, easily prepared from the chitin of crabs and lobsters, by N-deacetylation with alkali.[1] Unlike its parent substance, it dissolves in diluted acids to produce a linear polyelectrolyte with high positive charge density, and forms salts with inorganic and organic acids – such as glutamic, hydrochloric, lactic and acetic acid.[2] Due to its biocompatibilty, biodegradability and low toxicity, CS represents an attractive biopolymer for a variety of biomedical and pharmaceutical applications.[2-4] Many of such applications involve a CS cationic character. For example, CS has been shown to possess cell binding activity,[5] a property which seems directly related to the polymer cationic polyelectrolyte structure and to the negative charge of the cell surface. Several researchers investigated the formation of interpolymer complexes between chitosan and anionic polymers, for subsequent use in controlled drug delivery.[6-8]

As a unique natural cationic polymer, CS has been studied as a partner of several intermacromolecular complexes (IMC) with natural or synthetic anionic polyelectrolytes, such as chondroitin sulfate,[9] poly(acrylic acid),[10-12] poly(galacturonic acid),[12,13] sodium alginate, K-caragenan.[12] Chitosan complexation with carboxymethylated polysaccharides, such as carboxymethylcellulose[14] or carboxymethyldextran, has been also studied.[12,15-17]

DS is a biodegradable and biocompatible polyanion similar to heparin, bearing a branched carbohydrate backbone and negatively charged sulfate groups. DS has been widely used in pharmaceutical formulations and was reported as an effective matrix material for controlled release formulations based on ionic interactions with basic drugs, such as insulin[13,15] and anti-angiogenesis peptide.[16]

This paper presents new results on CS-DS interaction, performed in solution, by conductometric, potentiometric and turbidimetric titration, as well as by solid phase analysis methods.

EXPERIMENTAL
Materials

CS purchased from Vanson Inc. (Redmond, WA, USA) had a DDA = 79.7%, PDI = 3.26, M_W = 94.8 kDa. CS was dissolved in 0.1N HCl to form chitosan chlorohydrate with a concentration of 5×10^{-4} eq/L.

DS with a M_w = 100.000 g/mol was purchased from Sigma-Aldrich, Sweden, and was used without preliminary purification. The substitution degree of DS, determined by a volumetric method,[17] was found to be of 2.8 sulphate groups per glucosyl residue. The concentration of DS solution was of 5×10^{-3} eq/L.

Figure 1 presents the structures of CS and DS in sodium salt form.

Methods

Potentiometric titration was carried out with a TitraLab 854 Titration Workstation. Titration was performed by the classical technique of titrating agent addition in equal increments – (Monotonic End-point Titration-MET).

Conductometric titration was performed with a Consort C831 conductivity meter.

Turbidimetric titration involves measurements of the intensity decrease of a light flow passing through a solution containing solid IMC particles. Turbidity measurements were carried out on a Brinkmann PC 900 turbidity meter (USA) equipped with a 20-23-634-5 type optical fiber cell. The device indicates the amount of transmitted light (transmittance), while system

turbidity, expressed in arbitrary units, is obtained as a difference (t% = 100 – T%).

The FTIR spectra were recorded using a FTIR BOMEM MB 104 spectrometer.

Thermogravimetic analysis was carried out using a Mettler Toledo TGA-SDTA851e system, in nitrogen atmosphere, with a flow of 20 mL/min, at a heating rate of 10 °C/min, from 25 to 900 °C.

RESULTS AND DISCUSSION

The electrostatic interaction between CS and DS is graphically described in Figure 2.

CS titration with DS was carried out in a concentration range in which the polymer interaction can be analyzed by all the three methods applied for the study in solution. In all cases, to reduce the dilution effects, the concentration of the titrating partner's solution was an order of magnitude higher than that of the titrated partner. In the following, the results of the concentrations at which the complex is separated from solution (C_{CS} = 5×10^{-4} eq/L and C_{DS} = 5×10^{-3} eq/L) are presented. In all cases, the value of the measured parameter was represented as a function of the molar (equivalent) ratio between the reacting partners, as determined from the working volumes and concentrations.

The electrostatic interaction between CS and DS in an aqueous solution was characterized by potentiometric and conductometric titration (electrochemical methods), while the global behavior of the system was followed by turbidimetric titration.
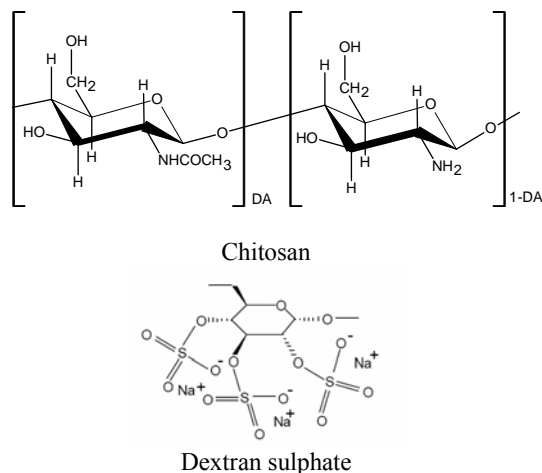

Chitosan

Dextran sulphate

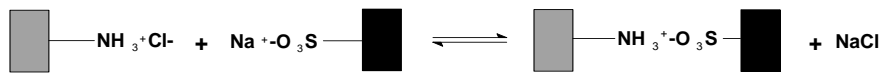Figure 1: Chemical structure of CS and DS sulphate

Figure 2: Interaction of CS with DS

The potentiometric titration curves of CS with 0.01N NaOH (1) and CS with DS (2) are presented in Figure 3. From the obtained potentiometric titration curve, it may be remarked that the interaction between the two partners occurred at a stoichiometric ratio of 1:1, as shown in Scheme 1.

In Figure 4, the conductometric titration curves of S with 0.01N NaOH (a) and with DS (b) are presented. On the titration curve of the CS with NaOH, a slight inflection point was observed, attributable to a low HCl excess during solution preparation, while a marked inflection point corresponds to the titration of $-NH_3^+Cl^-$ groups. In the case of conductometric titration of CS with DS (Fig. 4 b), the conductivity of the system decreases before the equivalence point, possibly because DS has a dissociation constant higher than that of the CS, releasing Na^+ ions. Both potentiometric and conductometric titrations indicate an electrostatic interaction of the partners, in an approximate stoichiometric ratio of 1:1.

The titration curve using turbidimetric measurements (Fig. 5) evidences a sudden increase in turbidity at a [DS]/[CS] ratio of about 1:1, which agrees with the results evidenced by electrochemical methods; this suggests that the interaction between the partners involves mainly electrostatic forces. It can be also noticed that the titration curve of CS with NaOH evidenced a slight turbidity at [NaOH]/[CS] = 1:1, confirming that non-ionized CS is not water-soluble.[15]
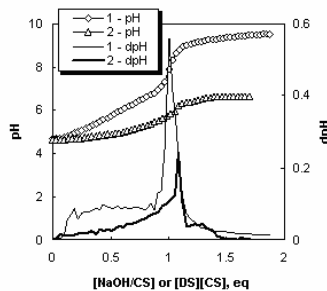

Figure 3: Potentiometric titration of CS with NaOH (1) and with DS (2) ($C_{CS} = 5×10^{-4}$ eq/L; $C_{DS} = 5×10^{-3}$ eq/L; $C_{NaOH} = 0.01N$)
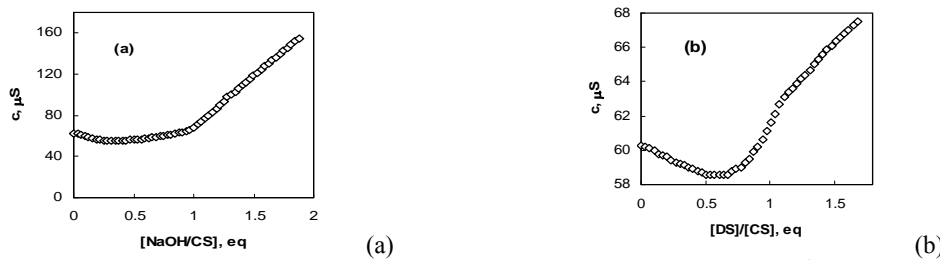

(a)　　　　　　　　　　　　　(b)

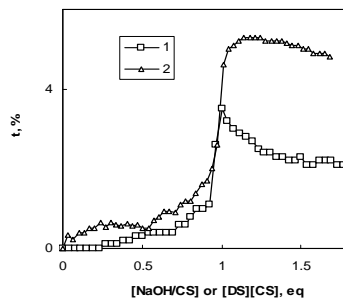Figure 4: Conductometric titration of CS with NaOH (a) and with DS (b) ($C_{CS} = 5×10^{-4}$ eq/L; $C_{DS} = 5×10^{-3}$ eq/L; $C_{NaOH} = 0.01N$)

Figure 5: Turbidimetric titration of CS with NaOH (1) and with DS (2) ($C_{CS} = 5×10^{-4}$ eq/L; $C_{DS} = 5×10^{-3}$ eq/L; $C_{NaOH} = 0.01N$)

The CS-DS complex prepared by the electrostatic interaction between partners was filtered, washed twice with deionized water, freeze-dried and characterized by FT-IR spectroscopy and thermogravimetric analysis.

The thermogravimetric curves for CS, DS and IMC are shown in Figure 6, while the main thermogravimetric parameters (T_{onset}, T_{peak}, T_{endset}, W%) are listed in Table 1. A complex process of thermal decomposition takes place in two to four stages, as depending on the chemical structure.

Thermogram analysis showed that the temperature at which weight loss begins is below 100 °C, when the traces of solvents/water used in sample preparation are removed.

DTG curves (Fig. 6b) evidenced a different behavior for the complex, compared to the individual partners, for which thermal degradation proceeds in two stages. The second one is accompanied by the most significant weight loss (approximately 64%), in a temperature range of 220-340 °C. The corresponding temperature at the maximum rate of degradation is different with more than 20 °C, compared to the raw materials.

Considering the temperature at which thermal degradation starts as a criterion for thermal stability, it is evident that thermal stability decreases in the following order:

CS > IMC > DS.

The FT-IR spectra for CS, DS and IMC are drawn in Figure 7.

In the CS spectrum, the already mentioned characteristic bands[5] may be observed at 1655 cm^{-1} (amide I), 1598 cm^{-1} (NH$_2$) and 1381 cm^{-1} (amide II). The absorption bands characteristic of the polysaccharide skeleton are as follows: 1155 cm^{-1} for C-O-C, 1078 cm^{-1} for C-O and 1032 cm^{-1} for C-O.

The FT-IR spectrum for DS showed two characteristic absorption bands, one at 1260 cm^{-1}, related to an asymmetrical S=O vibration, and the other one at 817 cm^{-1}, indicating a symmetrical C-O-S vibration associated to the C-O-SO$_3$ group.[17] At 1024-980 cm^{-1}, the characteristic band for C-O-S from the pyranosic ring or α-(1-6) linkage was observed.

Absorption bands at 1598 cm^{-1} (for CS) and 1259 cm^{-1} (for DS) may be used to monitor the complex formation, on taking into account that they are characteristic of these polymers. The FT-IR spectrum of the complex evidenced that the band at 1598 cm^{-1} shifted to 1529 cm^{-1}, while the characteristic band for DS remained unchanged, which clearly indicates the presence of both polymers in the composition of the final IMC.[15]

Table 1
Thermogravimetric data on CS, DS and IMC decomposition

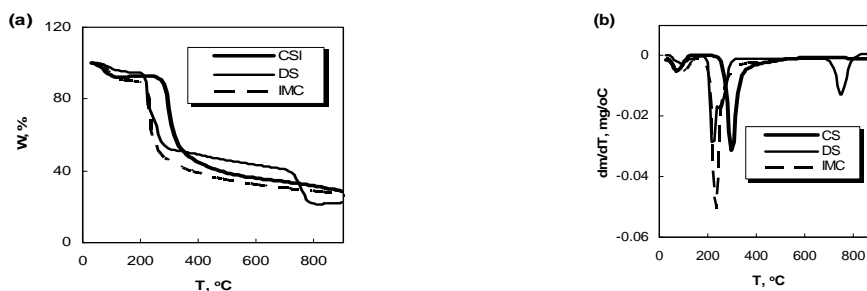| Sample	Degradation stage	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	W%	Residue
CS	I	48	71	105	7.54	28.35
	II	276	300	466	64.11	
DS	I	68	88	127	5.09	19.92
	II	218	223	220	18.46	
	III	220	256	276	30.46	
	IV	727	751	781	26.07	
IMC	I	59	85	128	10.37	25.97
	II	220	233	340	63.66	

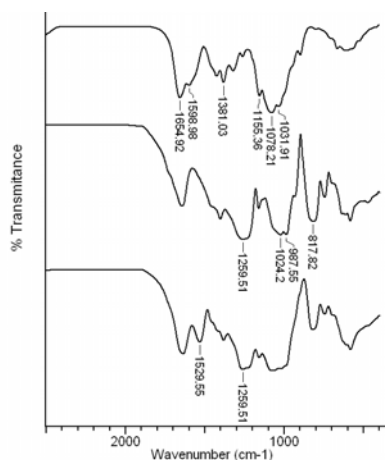Figure 6: TG (a) and DTG (b) curves of CS, DS and IMC

Figure 7: FTIR spectra of CS (a), DS (b) and IMC (c)

CONCLUSIONS

The interaction between ChCl and DS was studied in aqueous solution, by potentiometric, conductometric and turbidimetric titration, as well as in solid state, by FTIR spectroscopy and thermogravimetric analysis. All titration methods evidenced the formation of a complex in a stoichiometric ratio between partners, suggesting that the interaction proceeds through electrostatic forces. The FTIR spectrum of the solid complex evidenced some bands characteristic of electrostatic interaction. Thermal analysis confirmed the formation of a new complex and offered useful information for future biomedical applications.

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