# HYDROGELS FOR THE CONTROLLED DELIVERY OF THEOPHYLLINE DERIVATES: PREPARATION AND CHARACTERIZATION

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**Abstract:** This paper presents the obtaining of xanthan-chitosan hydrogels with two new entrapped theophylline derivates. The synthesized products were physical-chemical characterized by FTIR spectroscopy and by scanning electron microscopy (SEM). The in vitro release studies of theophylline derivates from the obtained complexes were carried out in similar pH conditions mimicking the gastrointestinal media. The kinetic data were finally analyzed based on literature reported specific polymer-drug systems mathematical models.

Keywords: chitosan, xanthan, theophylline derivates, drug delivery.

#### 1. Introduction

Chitosan (CS) and Xanthan (Xa) involved in the complex forming, are by far the most studied polysaccharides [1-3]. CS is the N-deacetylated form of chitin,  $(1\rightarrow 4)$ -linked 2-acetamide-2-deoxy- $\beta$ -D-glucan and presents various substitution degrees for N-acetyl groups. CS also presents amino and hydroxyl groups which are capable of interaction with other partners [4]. Xa, a polysaccharide derived from Xanthomonas Campestris, is very used in food processing and oil industry as a thickener agent and emulsifier. Its backbone consists in  $(1\rightarrow 4)$ - $\beta$ -D-glucose units with a  $\beta$ -D-mannose terminal chain,  $\beta$ -D-glucuronic acid and  $\beta$ -D-mannose which present  $\beta$ -D- $(1\rightarrow 2)$  and  $\beta$ -D- $(1\rightarrow 4)$  linkages [5]. Xa has polyanionic properties, being capable to interact with polycations and to form insoluble

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polyelectrolyte hydrogels [6-7]. Controlled release systems containing theophylline derivates may provide constant plasmatic concentrations for prolonged time intervals, so frequent dosage will be no longer needed thus minimizing various patient complications [8].

## 2. Materials and methods

### 2.1 Reagents

Chitosan (CS), VANSON ( $M_n = 94.8$  kDa and deacetylation degree = 79.7 %) was kindly provided by Sherbrooke University, Canada, and used without further purification. Xanthan (Xa) was provided by BioChemika (viscosity, 1 % (w/v) solution in water 1160 cPs), with degree of substitution per side chain of 0.73 and 0.75 for acetate and pyruvate groups, respectively, as determined by NMR <sup>1</sup>H. Theophylline derivates were synthesized and kindly offered for further studies by the Faculty of Pharmacy from the University of Medicine and Pharmacy "Gr. T. Popa", Iasi, and their chemical structures are depicted in Figure 1.



Fig. 1. Chemical structures of theophylline derivates A and B

Derivates A and B were found to be soluble in hot alcohol/water (1:1, v/v), in DMSO, DMF and almost insoluble in water and HCl and alkaline aqueous solutions.

#### 2.2 Characterization

FTIR spectra were recorded using a FT-IR BOMEM MB 104 spectrophotometer. The experiments were carried out with a resolution of 2 cm<sup>-1</sup> and 120 co-added scans, in the domain of 4000 - 500 cm<sup>-1</sup>. The samples were mixed with potassium bromide (Merk IR spectroscopy grade), dried and compressed under vacuum at 10 000 psi.

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The microstructural and morphological studies were performed using TESLA BS-300 electronic microscope with accelerating voltage of 20 kV. Samples conductivity was achieved by sputter coating with silver.

The swelling degree (SD) of the freeze-dried Xa-CS capsules was determined in ethyl alcohol/water (1:1, v/v), phosphate buffer solution (pH 7.4) and hydrochloric acid solution (pH 2.4). The dried samples were weighed and suspended in this media, and at determined time intervals the capsules were drawn out, filtered, blotted to remove excess solution and weighed again. The SD value of Xa-CS capsules was calculated using the following formula

$$SD\% = \frac{W_s - W_d}{W_d} \times 100 \tag{1}$$

where  $W_s$  and  $W_d$  represent respectively the weight of swollen and of dry capsules. Each experiment was performed in triplicate, and the reported results were calculated as an average value.

#### 2.2. Theophylline derivates loading

CS solution (0.65 %, w/v) was obtained by dissolving 6.5 g CS powder in 300 mL 0.1 N HCl, neutralized with 0.1 N NaOH (approximately 100 mL) until a pH = 6.0 was reached and completed with distilled water until a total volume of 1 L was reached. Xa solution was prepared under stirring by dissolving 6.5 g of dried powder into 1 L distilled water. Xa-CS hydrogels were prepared as capsules at 2.2 weight ratio as follows: 132 mL Xa solution (0.65 %, w/v) were added dropwise using a peristaltic pump (flow rate 3 mL/min) into 60 mL CS solution (0.65 %, w/v) under mild magnetic stirring and kept for maturation for 1 h at room temperature. The obtained hydrogel capsules were then filtered, washed with distilled water to remove the unreacted polymers, freeze-dried and used for theophylline derivates loading.

Xa-CS hydrogel loading was achieved by mixing 0.1 g of dried Xa-CS capsules with 6 mL drug solution (theophylline derivates A and B) (1.67 % w/v, ethyl alcohol/water) under mild stirring. After 2 h, the entrapped hydrogels were freezedried and submitted for further analysis. The determined loading efficiency was found to be 75.0 % in case of compound A and 66.3 % in case of compound B.

#### 2.3. In vitro release of theophylline derivates

The in vitro release studies of theophylline derivates A and B from Xa-CS hydrogel were performed in similar pH conditions mimicking the physiological gastrointestinal media (gastric acidic medium, pH = 2.4, and in intestinal weak

alkaline medium, pH = 7.4) as follows: 50 mg tablets were submersed in 200 mL 0.1 HCl respectively in 200 mL phosphate buffer solution and maintained under stirring (150 rpm) into a thermostated bath at 37° C. Aliquots were drawn out from the release media at specific time intervals and analyzed by spectrophotometric method at a wavelength of 270 nm.

# 3. Results and discussion

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# 3.1. Xa-CS hydrogel swelling studies

Xa-CS hydrogel swelling studies were performed in two characteristic gastrointestinal media and in ethyl alcohol/water solution, which also was used for solving theophylline derivates and for loading into Xa-CS complex. The swelling curves of Xa-CS capsules are depicted in Figure 2. The maximum SD value obtained for Xa-CS hydrogel capsules was reached in ethyl alcohol/water (1:1, v/v) with a value of 6400 %, swelling equilibrium being achieved after 30 minutes, while for pH = 2.4 and pH = 7.4 the maximum equilibrium SD value was 4900 % after 25 minutes from the swelling process debut.



Fig. 2. SD variation vs. time for Xa-CS hydrogel

# 3.2. FTIR spectroscopy

Figure 3 depicts the FTIR spectra of raw Xa, CS, theophylline derivates (A and B), Xa-CS hydrogel and for the obtained complex with loaded theophylline derivates (Xa-CS-A and Xa-CS-B). The spectrum of CS powder showed characteristic absorption bands at: 1654 cm<sup>-1</sup> (amide I), 1599 cm<sup>-1</sup> (NH<sub>2</sub>) and also

the band at 1381 cm<sup>-1</sup> attributed to the vibration of C-CH<sub>3</sub> (amide II) [9]. Xa presents the peaks at 1057 cm<sup>-1</sup> (C-O linkage from alcohol group), 1719 cm<sup>-1</sup> (C=O characteristic to acetate and piruvate groups), 2922 cm<sup>-1</sup> (C-H stretching) and 3427 cm<sup>-1</sup> (-O-H streatching) [10,11]. The FTIR spectrum for Xa-CS complex evidenced that the reaction which took place between the two oppositely charged polymers did not influenced ester groups, as expected, and the characteristic band at 1730 cm<sup>-1</sup> was a little bit intensified. The absorption peaks characteristic for C-O linkages from carboxylic groups collapsed after the complexation forming a single large band at 1663 cm<sup>-1</sup> and the band at 1381 cm<sup>-1</sup> (amide II) was shifted due to this effect. In case of theophylline derivates A and B the characteristic bands appear at 3456 cm<sup>-1</sup> (N-H vibrations) and at 3059, 2986, 2918 and 2824 cm<sup>-1</sup> (attributed to aliphatic and aromatic groups vibrations) [12]. The absorption peak at 1717 cm<sup>-1</sup> represents the heterocyclic imides ring and the band at 1668 cm<sup>-1</sup>



Fig. 3. FTIR spectra

appears due to tertiary amidic group vibration. N-H bending vibration appears at 1566 cm<sup>-1</sup> and a band at 1242 cm<sup>-1</sup> shows the C-N stretching vibrations [13].

# 3.3. Morphology study

SEM micrographs of Xa-CS hydrogel and for Xa-CS complex with loaded theophylline derivates A and B are shown in Figure 4. The cross-section of Xa-CS capsules presents a fibrillar structure resulted from Xa and CS chains complexation. The general morphology of the loaded Xa-CS capsules evidenced the presence of acicular drug microcrystals into the matrix folds.



Fig. 4. Scanning electron micrographs of: (a) – surface of Xa-CS, (b) – cross-section of Xa-CS, (c) – Xa-CS-A complex, (d) – Xa-CS-B complex

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### 3.4. In vitro release of theophylline derivates

The in vitro release profiles of theophylline deivates A and B from Xa-CS hydrogel at pH 2.4 (HCl solution) and pH 7.4 (phosphate buffer solution) are depicted in Figures 5 and 6. In the first 8 hours of elution approximately 35 % of entrapped derivate A was released and respectively 45 % of entrapped derivate B and after 120 hours of elution in both pH conditions theophylline derivates A and B were almost totally released from Xa-CS tablets ( $\approx$  98 %). Even if theophylline derivates are slightly soluble in water, analyzing their in vitro release profiles we can assert that Xa-CS-A and Xa-CS-B complexes increased theophylline derivates solubility in both acidic and alkaline dissolution media. These results confirm the fact that Xa-CS hydrogel, in which A and B derivates were entrapped, has the capability to increase the solubility of slightly water soluble drugs.



Fig. 5. In vitro release profile of derivate A from Xa-CS complex at pH 2.4 and pH 7.4



Fig. 6. In vitro release profiles of derivate B from Xa-CS complex at pH 2.4 and pH 7.4

The most commonly used equation for diffusion-controlled matrix system is an empirical equation described by Ritger and Peppas [14] in which the early time release data can be fitted to obtain the diffusion parameters:

$$\frac{M_t}{M_{\infty}} = kt^n \tag{2}$$

where,  $M_t/M_{\infty}$  is the fractional drug release at time t, k is a constant characteristic of drug-polymer interaction and n is an empirical parameter characterizing the release mechanism. When  $\log M_t/M_{\infty}$  is plotted against log t, the value of the diffusional exponent was obtained. Based on the diffusional exponent Lee et al. distiguished three classes of diffusion according to the relative ratess of diffusion and polymer relaxation. The first is Fickian diffusion (n = 0.5), in which the diffusion rate is much smaller than the relaxation rate. In this case, the system is controlled by Fickian diffusion. The second, in Case II (n = 1.0), where the diffusion process is much faster than the relaxation process. The controlling step is the velocity of an advancing front, which forms the boundary between swollen gel and glassy core. The third class is non-Fickian diffusion (n = 0.5 - 1.0), which describes those cases where the diffusion and relaxation rates are comparable [15]. The parameters k and n were calculated from the plot of  $\ln(M_t/M_f)$  versus ln(t) (Figure 7 and 8) are presented in Table 1.



Fig. 7. ln(Mt/Mf) vs. ln(t) plot for Xa-CS-A

The obtained results indicate that the transport mechanism in acidic pH, where n = 0.47 - 0.53, and in alkaline pH, where n = 0.53 - 0.54, is controlled by non-Fickian diffusion.



Fig. 8.  $ln(M_t/M_f)$  vs. ln(t) for Xa-CS-B

<b>Table 1.</b> Release exponent n and constant k	obtained from appl	lied Peppas equati	on for Xa-CS-A
and Xa-CS-B			

Complex	pH = 2.4		<i>pH</i> = 7.4			
	п	k	$R^2$	п	k	$\mathbb{R}^2$
Xa-CS-A	0.527	0.094	0.987	0.527	0.078	0.906
Xa-CS-B	0.440	0.157	0.984	0.540	0.106	0.969

### Conclusions

Drug delivery systems based on natural polymers were prepared by the entrapment of two new synthesized theophylline derivates into Xa-CS hydrogel matrix, in order to obtain new therapeutic nitrogen oxide donors systems. FTIR analysis and SEM microscopy confirmed the presence of entrapped theophylline precursors into Xa-CS complex. The in vitro release experiments carried out in simulating conditions mimicking the gastrointestinal tract environment showed that theophylline derivates A and B were released in the first 8 hours of elution about 35-45 % and almost totally ( $\approx$  98 %) after 120 hours by non-Fickian diffusion.

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