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Inclusion and release of theophylline from chitosan based microparticles

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Chitosan (CS) based microparticles for controlled release of theophylline (THP) were obtained by ionic gelation. The microparticles were characterized by laser diffraction analysis (particle size), scanning electron microscopy (SEM), FTIR spectroscopy, and thermogravimetric analysis. The average diameter of the CS-THP microparticles was 182 μm. The FTIR spectra confirmed that new hydrogen bonds had formed between the carbonylic group belonging to THP and the amino group of CS, while the thermogravimetric analysis showed a modified stability in the obtained particles and confirmed the presence of an interaction between the drug and CS. The THP release process was monitored for up to 24 h in simulated gastric fluid (pH 1.5) and in simulated intestinal fluid (pH 7.4). The release process of THP from microparticles was not influenced by the pH of dissolution media. In the first 30 min the drug was released in a rapid manner, and after this stage by a non-Fickian profile.

Key Words: Chitosan, theophylline, microparticles, controlled drug release.

Introduction

Chitosan (CS) (1,4)-[2amino-2-deoxy-β-D-glucan] (Figure 1a) is a polyaminosaccharide obtained from naturally occurring chitin by partial deacetylation. CS has amine groups that confer a polycationic character and allows the possibility of forming complex compounds with acids and polycarboxylic acids. CS is a biocompatible

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and biodegradable polymer that has antimicrobial activity, wound healing properties, and numerous medical applications, especially in the preparation of microparticles and microspheres with entrapped drugs for controlled delivery.[1–4]

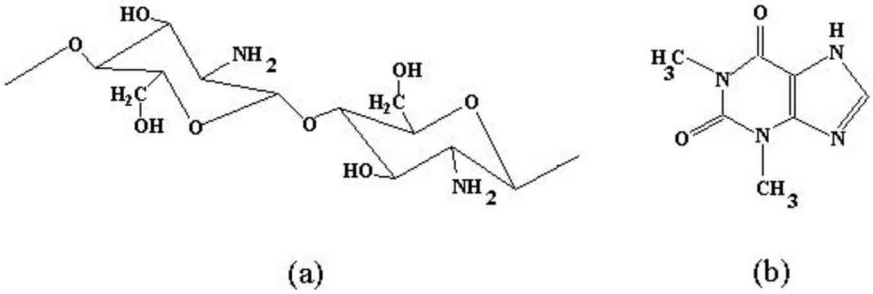


Figure 1. Chemical structures of (a) CS; (b) THP.

In acidic medium (pH values lower than 6.5), CS presents a polycationic character and it is soluble due to the transformation of neutral -NH_2 groups into positively charged –NH_3^+ groups. The amino groups can easily be used for attaching biologically active ligands.

Theophyline (THP) (1,3-dimethylxanthine), an alkaloid found in the leaves of *Cameliai sinesis*, is used clinically as a bronchodilator in the management of chronic obstructive pulmonary diseases.[5] The chemical structure of THP is depicted in Figure 1b.

THP has a short half-life (6 h), and so conventional dosage forms must be administered 3-4 times a day in order to avoid large fluctuations in plasma concentration. The therapeutic effects of THP appear for plasma concentration between 5 and 10 μg/mL, and toxic effects occur frequently for concentrations above 20 μg/mL.[6] Due to its numerous side-effects, this drug is now rarely administered as it is. Sustained release dosage forms for THP provide desirable serum concentrations for prolonged periods without frequent dosing, thereby preventing patient complications.[7] THP is soluble in acidic and alkaline medium as well.[8]

Experimental

CS sample provided by Kemestry Inc. (Sherbrooke, Canada) with M_w = 213,800 g mol^{-1}, deacetylation degree = 79.7%) and was used without further purification. Theophylline was provided by Sigma-Aldrich. All other chemicals and solvents were of analytical reagent grade. De-ionized double-distilled water was used throughout the study.

In 100 mL 1% acetic acid solution were solved 0.2 g THP and 1.0 g CS under magnetic stirring. A phosphate buffer solution (pH 8.0) was added dropwise with a peristaltic pump (flow rate = 3 mL/min) to obtain a final pH value of 7.0, to ensure a complete precipitation of the CS-THP complex. After centrifugation at 4500 rpm for 20 min, the precipitate was separated and freeze-dried (Alpha 1-4 LSC, Christ, Germany).

Size distribution and mean diameter measurements of CS-THP microparticles were recorded using a SALD-7001 type Laser Diffraction Particle Size Analyzer (Shimadzu, Japan). This system is an optic measurement device and uses laser radiation. In the laser diffraction particle size analyzer, the particle size distribution is calculated based on the light intensity distribution data of the diffracted light detected by sensor elements.

The device has a stirring kit to maintain the particles in suspension during the measurements. The obtained CS-THP particles were measured immediately after synthesis.

A scanning electron microscope (Vega II LSH – Tescan Company) was used to observe the CS-THP particles morphology.

FTIR spectra were recorded using a FT-IR BOMEM MB 104 spectrometer. The experiments were done using a resolution of 2 cm^{-1} and were averaged over 120 scans, in the range of 4000-500 cm^{-1}. The samples were mixed with potassium bromide (Merck IR spectroscopy grade), dried and compressed under vacuum.

Thermogravimetric analysis was performed 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 using 4-6 mg sample weight. Operating parameters were kept constant for all the samples in order to obtain comparable data.

Loading efficiency was studied by dissolving microparticles (0.05 g) in 100 mL HCl 0.1 N and maintained for 24 h at 37 °C. The amount of loaded THP was determined by UV spectroscopy (NanoDrop, ND-1000, Wilmington, DE, USA) at 270 nm wavelength. The concentration of THP in solution was determined using a calibration curve obtained with known THP samples with concentration in the range of 0.125 to 2μg/mL at pH 1.5 and pH 7.4. All experiments were carried out in triplicate.

Three measurements were averaged for each data point and the equation of the calibration curve was obtained by the least-square method. The obtained equation (1) holds for both pH values:

$$A = 0.0051 \times c \tag{1}$$

where A is the absorbance and c is the THP concentration in solution, expressed in μg/mL.

THP loading efficiency was 60.2% and was calculated by equation (2).

$$\text{Loading efficiency } (\%) = m_a/m_t \times 100 \tag{2}$$

where m_a is the actual THP content and m_t is theoretical THP content.

The in vitro THP release profiles were determined as follows: 0.1 g of dried CS-THP microparticles was suspended in 50 mL of release medium and incubated at 37 °C under shaking (150 rpm) using a GFL Water-Bath Shaker. The release media were pH 1.5 (0.1 N HCl solution) and pH 7.4 (phosphate buffer solution) simulating the gastrointestinal tract media. Samples (1 μL) were periodically drawn out from the release media with microsyringes and the amount of released THP was determined by directly measuring the absorbance by spectrophotometric method. The in vitro release studies were performed in triplicate for each of the samples.

The percentage of released drug was determined using Equation (3)[9]:

$$\text{Drug released } (\%) = \frac{R_t}{L} \times 100\% \tag{3}$$

where L and $R_t$represent the initial amount of THP loaded and the cumulative amount of THP released at time t.

Results and discussion

The result of the laser diffraction measurement for the average diameter of the dispersed particles is shown in Figure 2. As can be seen from this figure, the average of randomly dispersed particles diameter was around 182

μm with a Gaussian distribution and standard deviation (S.D.) = 0.150.

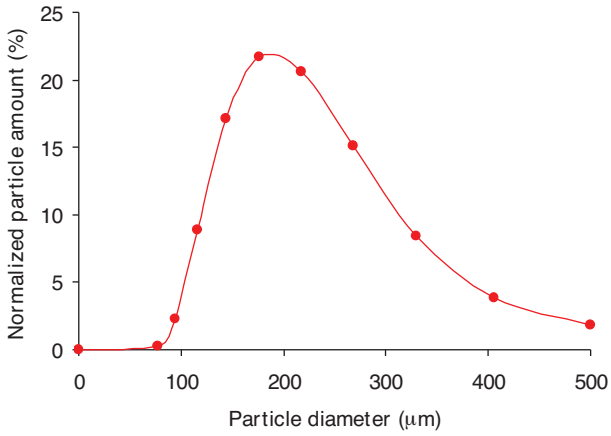
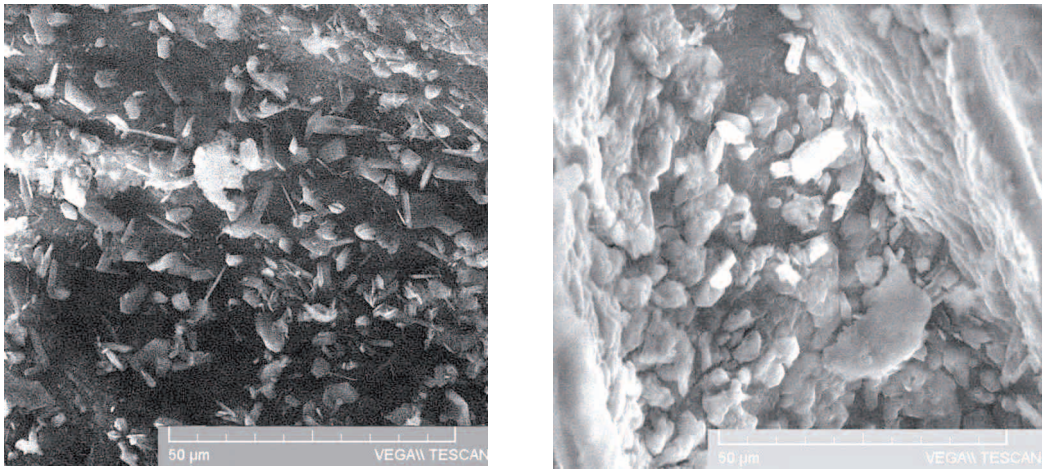


Figure 2. Numeric size distribution for CS-THP microparticles.

SEM images of CS-THP freeze-dried microparticles are shown in Figure 3. These images show that THP microcrystals were distributed both on the microparticles' surfaces and also included in their fibrillar structures.



(a) (b)

Figure 3. SEM micrographs for CS-THP microparticles on magnification $\times 2000$ of surfaces (a) and cross-section (b).

Figure 4 depicts the FTIR spectra of CS, THP, and CS-THP microparticles. The spectrum of CS powder had 2 peaks around 897 and 1156 cm^{-1} corresponding to saccharine structure[10]; absorption bands at 1654 cm^{-1} and 1599 cm^{-1} were detected and attributed to amide I (C=O) and amide II (N-H), respectively,[11] and the band at 1381 cm^{-1} was attributed to the distorting vibration of C-CH_3.[12] At 1321 cm^{-1} was recorded the C-N stretch and finally at 1078 cm^{-1} appears the peak of the C-O stretch group. In the case of THP, the broad band at 3456 cm^{-1} was attributed to N-H stretching vibrations. Bands at 3059, 2986, 2918, and 2824 cm^{-1} were due to both aromatic and aliphatic C-H stretching vibrations. The band at 1717 cm^{-1} represented the imide group stretching of the heterocyclic ring. The sharp band at 1668 cm^{-1} was due to tertiary amide

group stretching vibration. N-H bending vibration was represented by a band at 1566 cm^{-1}. The band at 1242 cm^{-1} shows C-N stretching vibrations.[13] In the complex THP-CS the bands characteristic for CS and THP are found. The band at 1599 cm^{-1} was not seen in the spectrum of THP-CS microparticles. From this result, it was concluded that a hydrogen bond had formed between the carbonylic group of THP and the amino group of CS.

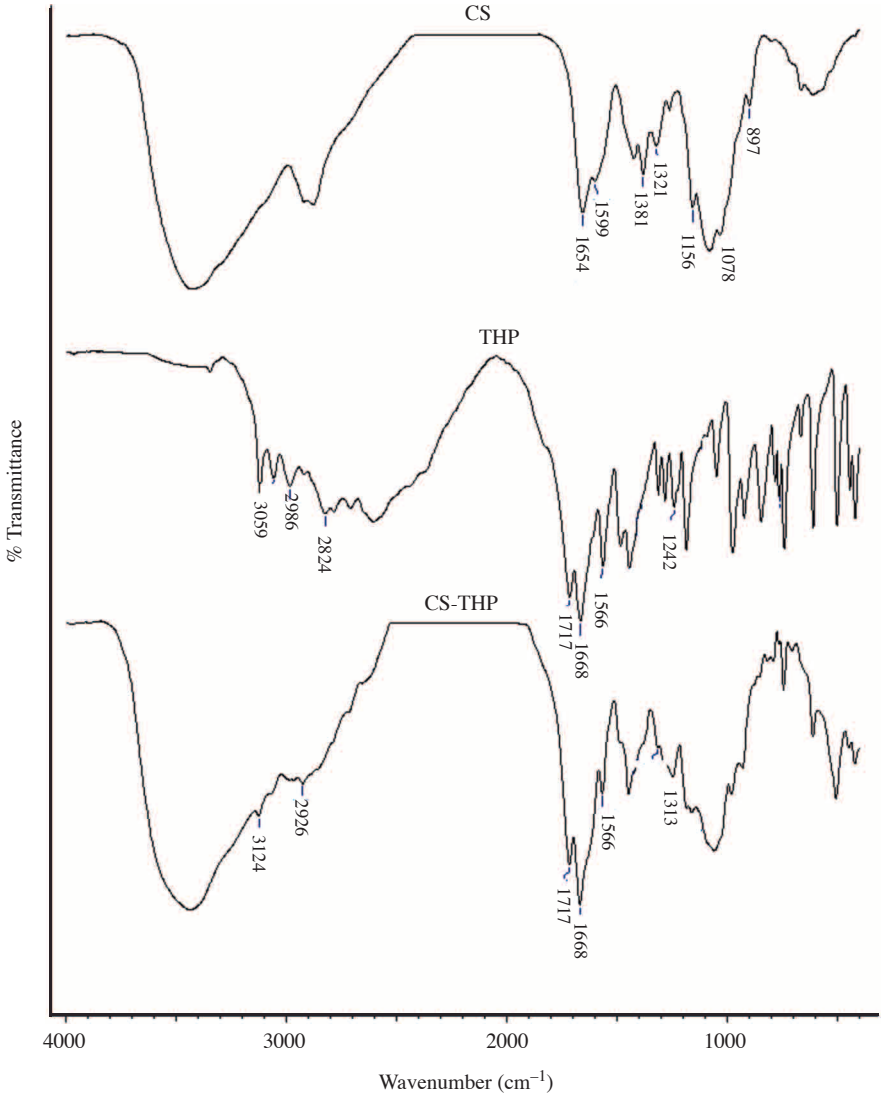


Figure 4. FTIR spectra of raw CS, THP, and CS-THP microparticles.

Thermal properties are interesting in view of the structure-property relationship and for practical applications.[14,15]. Thermogravimetric (TG) curves and differential thermogravimetric (DTG) curves, recorded in conditions described above for the precursors and CS-THP microparticles, are shown in Figures 5 and 6. The TG characteristics are presented in the Table, where $\mathbf{T}_{onset}$ is temperature at which the degradation begins in each step, $\mathbf{T}_{endset}$ - the temperature at which degradation is finished in each given step, $\mathbf{T}_{peak}$ - the temperature at which the degradation rate is maximum, **W%** - the percentage mass loss, and **Residue** - represents

the ash that is left after heating the sample to 900 °C.

Table. Thermogravimetric characteristics for CS, THP, and CS-THP microparticles.

Sample	Step	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	W (%)	Residue
CS	I	51	78	108	7.50	27.31
	II	275	300	502	65.19	
THP	I	269	349	366	73.63	9.78
	II	366	450	491	14.33	
	III	491	668	711	2.26	
CS-THP	I	54	79	114	7.66	42.32
	II	242	269	425	50.02	

Examining these curves and data from the Table, we can conclude that the thermal degradation runs in 2 or 3 stages. The first step corresponds to dehydration processes and mass loss in this stage yields information about the sample water content. The degradation peak for CS-THP complex is unique and was shifted compared to THP and CS degradation peaks, proving that a solid dispersion was obtained.

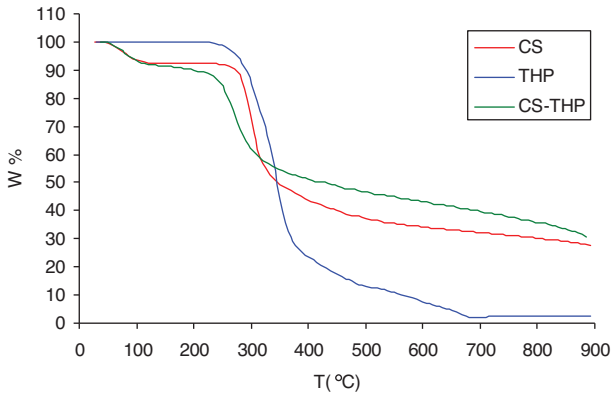


Figure 5. Thermogravimetric curves (TG) for the precursors and CS-THP.

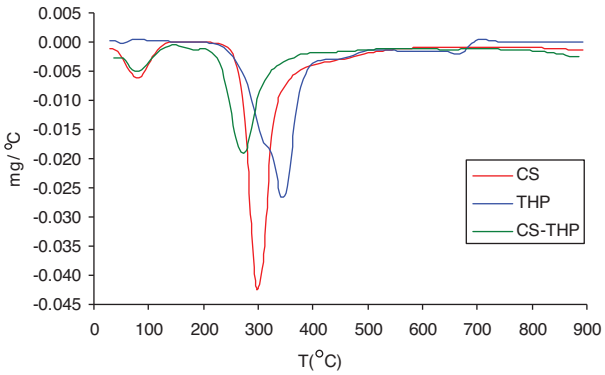


Figure 6. Differential thermogravimetric curves (DTG) for the precursors and CS-THP.

Generally speaking, drug release from CS-based particulate systems depends upon the extent of cross-linking, morphology, size and density of the particulate system, physicochemical properties of the drug, and the pH of the dissolution media.[16]

The in vitro release profiles of THP from particles at pH 1.5 (HCl solution) and at pH 7.4 (phosphate buffer) are depicted in Figure 7. We observe that THP was released to completion within 8 h, with release behaviors similar under both pH conditions.

During the first 30 min, about 65%-70% of the loaded THP was released from the microparticles. This behavior may be attributed to the rapid dissolution of THP adsorbed on the microparticles' surface.

Similar THP release profiles were obtained in the case of CS-THP solid dispersions obtained by spray-drying method and in the case of glutaraldehyde cross-linked protein based microcapsules loaded with THP. In the first case almost 70% of the loaded drug was released in the first 30 min. At pH 1.2, the dissolution profiles

of the drug from physical mixture and solid dispersions were almost the same. In the alkaline medium (pH 6.8), the release rate of the spray-dried pharmaceutical was sustained more than the original and the physical mixture. In the second case, the complete core release from the protein based microcapsules was observed after 20 min in simulated gastric fluid and after 30-50 min in simulated intestinal fluid, at 37 °C.[17,18]

After the 30 min rapid dissolution, the drug continued to be released from the obtained CS-THP microparticles until completed between 0.5 and 8 h. For a brief evaluation of the drug release profile in this time interval a semi-empiric mathematical model was applied, developed by Korsmeyer and Peppas, with the following equation[19]:

$$M_t/M_\infty = kt^n \tag{4}$$

where M_t / M_∞ represents the released drug fraction plot of time t, k – kinetic constant cumulating both polymer and drug properties, and n – the exponent used to characterize the transport mechanism. For n = 0.4 the drug is released by Fickian diffusion, for 0.45 < n < 0.89 the non-Fickian case is applied (anomalous diffusion), and for n = 0.89 – a zero order release profile.[20,21]

As shown in Figure 8, it was found that between 0.5 and 8 h (n = 0.8254 for pH 1.5 and n = 0.7788 for pH 7.4) the drug was released from CS-THP microparticles by a non-Fickian mechanism.

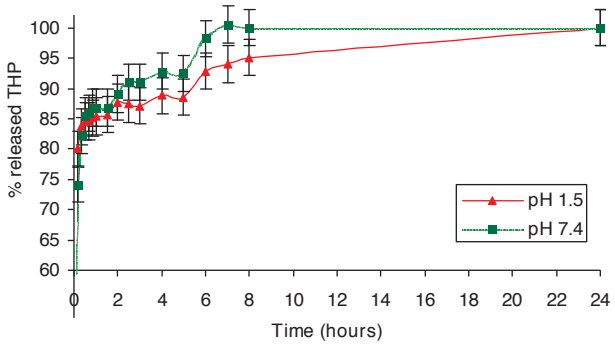


Figure 7. THP in vitro release profiles from CS-THP microparticles. Each result show the mean ± SD (n = 3).

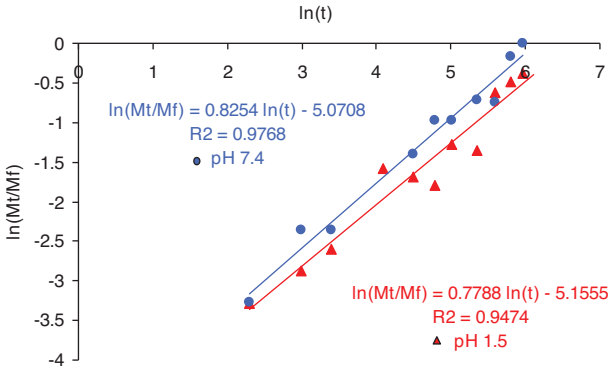


Figure 8. Ln(M_t / M_f) versus ln(t) plot for CS-THP microparticles.

Drug release was not affected by the medium pH, because the release process was controlled by the drug diffusion. This in vitro release behavior suggests that the interaction between THP and positively charged CS polymer is weak, allowing the THP to be released from microparticles by a dissociation mechanism.

Conclusions

Chitosan based microparticles for the controlled theophylline (THP) release were obtained by ionic gelation. THP release profiles at pH=1.5 and pH=7.4 do not exhibit obvious differences, due to the presence of weak interactions, namely hydrogen bonds between the carbonylic group of THP and the amino group of CS. The maximum amount of released THP was achieved after 8 h in both pH conditions.

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