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Hydrogels based on chitosan–xanthan for controlled release of theophylline

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Abstract The aim of this paper is theophylline (THP) inclusion into xanthan–chitosan polyionic complex (Xa–CS) and the study of its in vitro and in vivo kinetic release. Xa–CS hydrogel was obtained by ionic complexation between two oppositely charged polysaccharides. THP was loaded into the Xa–CS matrix by diffusion of the drug solution. The obtained samples were characterized by FTIR spectroscopy, SEM microscopy and study of the swelling behavior. THP in vitro release experiments were carried out in conditions mimicking the gastrointestinal environment. The chosen drug dose for in vivo study was 15 mg THP/Kg body weight of THP powder or an equivalent dose in complex form. THP serum concentrations were determined by an HPLC assay. The THP peak serum concentration (C_{max}) was 7.18 μg/ml for free THP and AUC_{0-48} was 25.76 μg h/ml, while in the case of Xa–CS–THP, C_{max} was of 5.72 μg/ml and $AUC_{0-48} = 45.72$ μg h/ml. The in vivo study regarding the behaviour of the obtained formulation, showed an increase bioavailability of THP compared to the raw drug, suggesting the possible application of the complex Xa–CS as an oral controlled drug delivery system in the management of chronic pulmonary obstructive disease.

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1 Introduction

Theophylline (THP), an alkaloid found in *Camellia sinensis* leaves, is one of the most used bronchodilator drugs in chronic obstructive pulmonary disease [1, 2]. THP directly relaxes bronchioles and the muscles of pulmonary sanguine vessels, so that it has a relaxing and bronchodilator effect upon the smooth muscles [3]. Conventional dosage forms of THP should be administrated 3–4 times a day in order to avoid large fluctuations of plasma concentration [4]. The THP therapeutic range is narrow (10–20 μg/ml), although therapeutic effects may sometimes be achieved at plasma concentration of 5–10 μg/ml, with undesirable side effects occurring over this range [2, 5, 6]. THP pharmacokinetics obeys a non linear profile [7], with serum concentration sometimes increasing higher than predicted after administration, due to some mechanisms that are currently not fully understood. The average half life ($t_{1/2}$) is about 8 h, usually in the range of 6–12 h, while decreasing in smoking patients ($t_{1/2} = 5$ h) [8]. Due to its numerous side-effects, this drug is currently seldom administered in raw form.

By contrast, controlled release systems for THP provide constant serum concentrations for longer time periods, without imposing frequent dosage, therefore preventing various patient complications [9]. Several formulations were proposed for achieving THP controlled release. Systems such as micro- and nanoparticles based on natural and synthetic polymers with physically entrapped drug were obtained [9–17]. The conjugates obtained by THP esterification with poly(ethylene glycol) and methoxy(ethylene glycol) conferred therapeutic levels for prolonged time intervals, compared to the raw drug [18].

For better understanding of the drug release profile in biological systems THP in vitro–in vivo correlations were required due to its nonlinear pharmacokinetic profile

[19, 20]. The in vitro and in vivo drug delivery process from controlled release formulations depend upon the extent of cross-linking, morphology, size and density of the particulate system, its physicochemical properties, the pH of the dissolution media and the percentage of solid drug leftover in the delivery system [21, 22].

In this study a polyionic complex based on xanthan–chitosan (Xa–CS) was obtained and used for THP loading and in vitro and in vivo release studies were performed.

2 Materials and methods

2.1 Materials

CS (M_w = 94.8 kDa, with polydispersity index of 3.26 and deacetylation degree of 79.7%), was purchased from Vanson, Inc. (Redmond, WA, USA). Xa was obtained from BioChemika (viscosity, 1% (w/v) solution in water 1160 cPs), degree of substitution per side chain of 0.73 and 0.75 for acetate and pyruvate groups, respectively, as determined by proton NMR ^{1}H). Anhydrous THP was purchased from Sigma–Aldrich. All the other materials and reagents used in this study were of analytical grade of purity.

2.2 Xa–CS complex preparation

The CS solution was prepared by dissolving 6.5 g CS powder in 300 ml 0.1 N HCl, neutralized with 0.1 N NaOH, followed by addition of distilled water until a total volume of 1 l and pH = 6.0 were reached. Xa solution was prepared under stirring by dissolving 6.5 g of dried powder into 1 l distilled water. The Xa–CS hydrogels were prepared as capsules in the following way: 880 ml of the previously degassed Xa solution were added dropwise through a syringe needle (1.1 mm diameter) using a peristalic pump (flow rate 3 ml/min) to 400 ml of CS solution. The obtained hydrogel capsules were kept for maturation under mild magnetic agitation for 30 min at room temperature. After filtration, the hydrogel capsules were washed with distilled water until neutral pH and freeze-dried (Alpha 1-4 LSC, Christ, Germany). Figure 1 shows an optical picture of the hydrogel capsules, with showing an average diameter of 3 mm. The shape of the formed capsules was not spherical but rather elongated, probably due to increased viscosity of Xa drops and to the complexation process under stirring. This does not pose a disadvantage regarding their behaviour during THP release.

2.3 Preparation of THP loaded capsules

The method for loading the obtained Xa–CS complex with THP consists in the diffusion of a drug solution into the hydrogel network. 0.1 g Xa–CS powder was suspended into 6 ml 4% THP solution (1:1 v/v, ethyl alcohol/water) under mild stirring. Total diffusion of the drug was reached after 30 min. This time period corresponds to the moment in which the hydrogel reaches it swelling equilibrium state, as determined from the swelling studies. The obtained Xa–CS–THP complex was then freeze-dried.

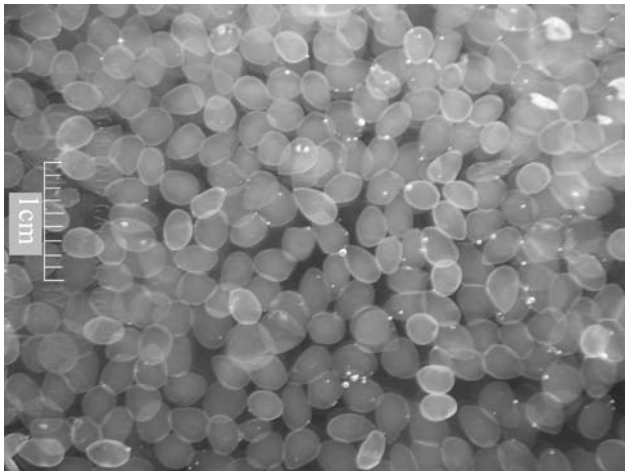

Fig. 1 Image of Xa–CS hydrogel capsules

2.4 Hydrogel capsules characterization

2.4.1 Determination of the swelling degree

The swelling degree (SD) of the freeze-dried capsules was determined in ethyl alcohol/water (1:1 v/v), phosphate buffer solution (pH 7.4) and hydrochloric acid solution (pH 1.5). The samples were weighed and suspended in this media, and the capsules were filtered at determined time intervals, blotted to remove excess solution and weighed again. The SD value was calculated using the following formula:

$$SD\% = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

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

2.4.2 Infrared spectroscopy–FTIR

The FTIR spectra were recorded using a FTIR Bomem MB 104 spectrometer. In order to collect the spectra, a small amount of freeze-dried capsules was mixed with potassium bromide (Merck IR spectroscopy grade) and compressed under vacuum to obtain tablets. The IR spectra, in

transmittance mode, were obtained in the spectral region of 450–4000 cm^{-1} using a resolution of 2 cm^{-1} and 120 co-added scans.

2.4.3 Morphology characterization

A scanning electron microscope (Vega II LSH with accelerating voltage of 30 kV—Tescan Company) was used to examine the morphology of the Xa–CS–THP complex capsules. Freeze-dried samples were attached to aluminium holders using a silver based adhesive. Samples conductivity was achieved by sputter coating with a 15 nm layer of gold.

2.4.4 THP loading assay

THP loading was studied by suspending 50 mg of dried Xa–CS–THP complex into 200 ml 0.1 N HCl, followed by incubation at 37°C for 48 h, under stirring. The obtained solution was then centrifuged to remove the insoluble polymeric fragments. The clear supernatant solution was analyzed by UV spectroscopy (NanoDrop, ND-1000, Wilmington, USA) at the wavelength of 270 nm. The THP concentration was determined using a calibration curve obtained with THP solutions of known concentration in the range of 0.125–2 μg/ml. All experiments were carried out in triplicate.

Three measurements were averaged for each data point and the equation of the calibration curve obtained by the least-square method was:

$$A = 0.0575 \times c \tag{2}$$

where A is the absorbance and c is the solution THP concentration, expressed in μg/ml.Encapsulation efficiency was determined using the following equation:

$$\%\ encapsulation\ \text{efficiency} = \frac{THP\ \text{loading}}{\text{theoretical THP loading}} \times 100 \tag{3}$$

2.4.5 THP in vitro release

For the in vitro kinetic study, flat-faced Xa–CS–THP complex 100 mg tablets, 12 mm in diameter were prepared by compression using a Carver laboratory press (Model 3912, SUA) at 2 tonnes force for 1 min. The release of THP from matrix tablets was performed at 37 ± 0.1°C using a standard dissolution tester DT 700 (Erweka, Germany) connected to a UV spectrophotometer (UV-300 Spectronic Unicam) using paddle method at 100 rpm and a dissolution medium volume of 1000 ml. The pH values of the release media were 1.5 (HCl solution) and 7.4 (phosphate buffer solution). Aliquots were drawn out automatically at specific time intervals and analyzed by spectrometric method.

The percentage of released drug was determined using Eq. 4 (Khare and Peppas 1995):

$$\text{Drug released}\ (\%) = \frac{R_t}{L} \times 100\% \tag{4}$$

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

2.4.6 In vivo drug release

Healthy Wistar rats with weights between 345 and 420 g were used for the in vivo study. Each rat was given a dose of 15 mg THP powder/kg body weight or an equivalent dose in complex form with 2 ml water through a catheter. Blood samples were drawn out at established time intervals up to 48 h. They were allowed to stand for 1 h, centrifuged to separate the serum which was kept frozen (−20°C) until further analysis. THP serum extraction was performed mainly as described in the literature [23], with a few minor changes. After thawing, 1 ml serum was mixed with 250 μl 10% $(NH_4)_2SO_4$ solution and homogenized for 1 min. THP was extracted for 15 min in 5 ml 2-propanol:dichloromethane (1:9 v/v) mixture. After 5 min centrifugation at 4000 rpm, the organic layer was drawn out and transferred into a glass tube and evaporated to dryness at 40°C. The dry extract was dissolved in 1 ml acetonitrile-10 mM aqueous sodium acetate (7:93 v/v), passed through a 0.45 μm syringe filter and used for further analysis. Plasma THP concentration was determined by high performance liquid chromatography (Shimadzu Model-CTO-20A HPLC system). The separation was performed on a 5 μm ZORBAX SB-C18 column (150 × 4.6 mm i.d.) under isocratic conditions with a mobile phase composed of acetonitrile-10 mM aqueous sodium acetate (7:93 v/v). Analyses were performed at room temperature under a flow rate of 1 ml/min using an injection volume of 100 μl. THP was detected by UV detector at 270 nm. THP concentration was determined with a calibration curve obtained with standard solutions of known THP concentrations in ethyl alcohol–water (1:1 v/v) in the range of 1–20 μg/ml, using HPLC software LC Solution Version 1.22 SP1 for integration and automatic determination of drug concentration in blood samples.

3 Results and discussion

The complexation reaction between oppositely charged macromolecules leads to structure changes in both Xa and

CS polymers. An aqueous polyelectrolyte complexed hydrogel was formed by the interaction of the cationic amino groups of CS and the anionic groups of Xa and by hydrogen bonds established between macromolecular chains. These effects created an "ionic" reticular network of immobilized polymers [24]. It was found that the molecular weight of CS, the pH of CS solutions and the initial Xa concentration were the most critical parameters in Xa–CS network formation and had a strong influence upon the hydrogel properties, particularly on water retention capacity and on solubility under various pH conditions [24, 25].

Based on these considerations we took into account various Xa–CS complexation conditions. Preliminary tests were initially carried out using three Xa–CS mass ratios: 1.7, 2.2 and 2.7. The results showed that in the first case the obtained matrix was unstable and partially soluble in acidic conditions, while the third set of conditions produced a densely crosslinked network with limited swelling capacity that does not allow efficient drug entrapment. The matrix prepared when the 2.2 mass ratio was used showed both stability under acidic conditions and sufficient swelling degree and it was chosen for further study.

3.1 Hydrogel swelling studies and THP entrapment

In order to determine the optimal concentration and volume of the THP solution for drug loading of Xa–CS matrix with mass ratio of 2.2, the swelling studies were performed in media where THP has a good solubility or in which the release studies were performed. Maximum SD of freeze-dried Xa–CS capsules was determined for ethyl alcohol/water (1:1, v/v), phosphate buffer solution (pH = 7.4) and for HCl solution (pH 1.5) media and it is depicted in Fig. 2.

SD for all three media reaches the equilibrium after approximately 30 min. In acidic and alkaline media similar values for SD were obtained (4900%). The obtained maximum value for SD in ethyl alcohol/water (1:1, v/v) was 6400%. The high SD value observed in ethyl alcohol/water provides enhanced diffusion of a large volume of

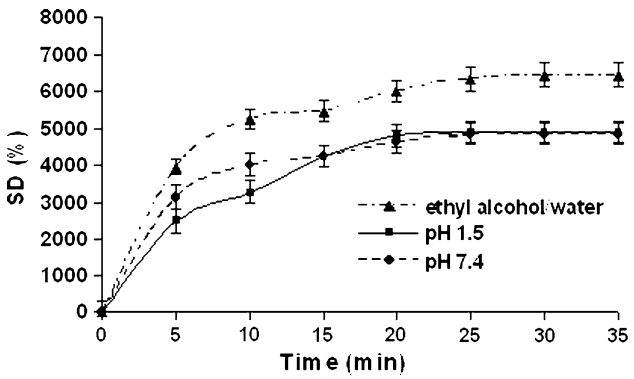

Fig. 2 Variation of SD of Xa–CS complex versus time

solution and therefore the inclusion of a large amount of THP into Xa–CS capsules. Based on these considerations the drug solution volume for entrapment into Xa–CS capsules was determined. The amount of THP is directly correlated with its solubility in ethyl alcohol/water (1:1, v/v) solution. The recipe was thoroughly described in the experimental section. Theoretical THP loading was 70% w/w, as determined from the optimum formulation. The entrapped THP content in Xa–CS matrix was calculated using Eq. 2 and it was found to be 62%. The encapsulation efficiency of 88.6% was established using Eq. 3.

3.2 Morphology study

SEM micrographs of Xa–CS and Xa–CS–THP systems are shown in Fig. 3. Analyzing hydrogels surfaces we can assert that as a consequence of how this system has been dehydrated and the polymer material has collapsed through the freeze-drying process, the capsules presented no formal pores on their surface but only folds. Furthermore, the cross-section presents a fibrillar structure resulted from Xa and CS chains complexation. General morphology of the THP loaded Xa–CS capsules evidenced the presence of acicular drug microcrystals both on their surfaces and into the matrix folds. Similar appearance was reported for solid dispersions of THP and CS prepared by spray-drying method and also for polyionic hydrogels obtained by complexation between Xa and CS containing various encapsulated enzymes [12, 24].

3.3 FTIR spectra

Figure 4 depicts the FTIR spectra of raw Xa, CS, THP, Xa–CS hydrogel and Xa–CS–THP complex. The spectrum of CS powder (Fig. 4a), has shown characteristic absorption bands at 1654 cm^{-1} (amide I), 1599 cm^{-1} (NH_2), also the band at 1381 cm^{-1} that was attributed to the distorting vibration of C–CH_3 (amide II) [26]. Xa (Fig. 4b), presents the peaks at 1057 cm^{-1} (C–O linkage from alcohol group), 1719 cm^{-1} (characteristic to acetate and pyruvate groups), 2922 cm^{-1} (C–H streching) and 3427 cm^{-1} (–OH streching) [27, 28]. The FTIR spectrum for Xa–CS hydrogel is depicted in (Fig. 4c). It was found that the complexation between the two oppositely charged polymers did not affect the behaviour of the ester group which showed, as expected, a less intense absorption around 1730 cm^{-1}. All the other C–O absorption peaks caused by carboxylic groups mentioned above, including the peak at 1057 cm^{-1}, collapsed after complexation to form a single large band which appeared at 1663 cm^{-1}. The amide II (1381 cm^{-1}) is seen as a shoulder due to this effect. In the case of raw THP (Fig. 4d), the peak at 3456 cm^{-1} is due to N–H groups vibrations. The bands at 3059, 2986, 2918 and

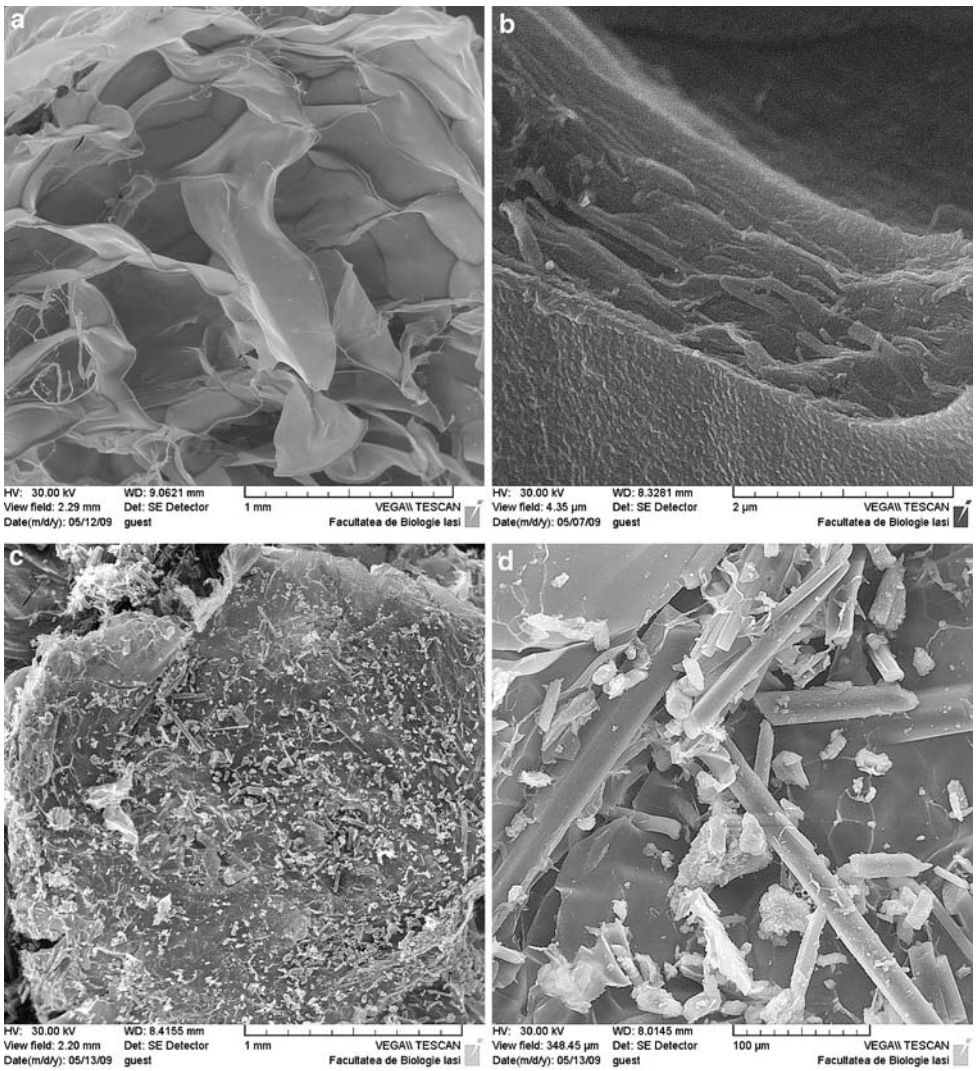

Fig. 3 Scanning electron micrographs of **a** surface of Xa–CS, **b** Xa–CS cross-section, **c** surface of Xa–CS–THP, **d** Xa–CS–THP cross-section

2824 cm^{-1} are attributed to the vibrations of aromatic and aliphatic groups [29]. The band at 1717 cm^{-1} represents the heterocyclic ring imide group stretching. N–H bending vibration is represented by a band at 1566 cm^{-1}. A band at 1242 cm^{-1} shows C–N stretching vibrations [17]. The FTIR spectrum of Xa–CS–THP complex shows the characteristic absorption bands found for the raw products. In the Xa–CS–THP spectrum, the drug characteristic bands overlapped the characteristic bands for Xa–CS complex, that show lower intensity due to lower overall concentration. This is due to the fact that the percentage of entrapped THP is high (62%). The band located in the 3200–3500 cm^{-1} region is attenuated due to formation of hydrogen bonds among the three partners.

3.4 In vitro release of THP from complex

The in vitro release of THP from Xa–CS complex was investigated under simulated conditions mimicking the gastrointestinal tract environment [9, 17, 30–32]. The release profiles of THP from Xa–CS–THP complex in HCl solution (pH 1.5) and phosphate buffer solution (pH 7.4) are shown in Fig. 5. We notice that THP was released to completion within a 12 h period, with release behaviours similar under both pH conditions.

When the polymeric matrix is relatively stable in the elution medium, it is expected that the drug diffusion step is rate determining for the release kinetics. As shown in previous studies, diffusion controlled drug release is a three staged process. The release medium first penetrates the matrix, causing it to swell; in the second stage the glassy polymeric material switches to rubbery state; the third and last stage involves the drug diffusion through the swollen matrix [21]. This mechanism is expected to occur in the present system under both pH conditions. Therefore the empirical equation developed by Ritger and Peppas [33] can be applied in order to obtain the diffusion parameters from the early stage release data:

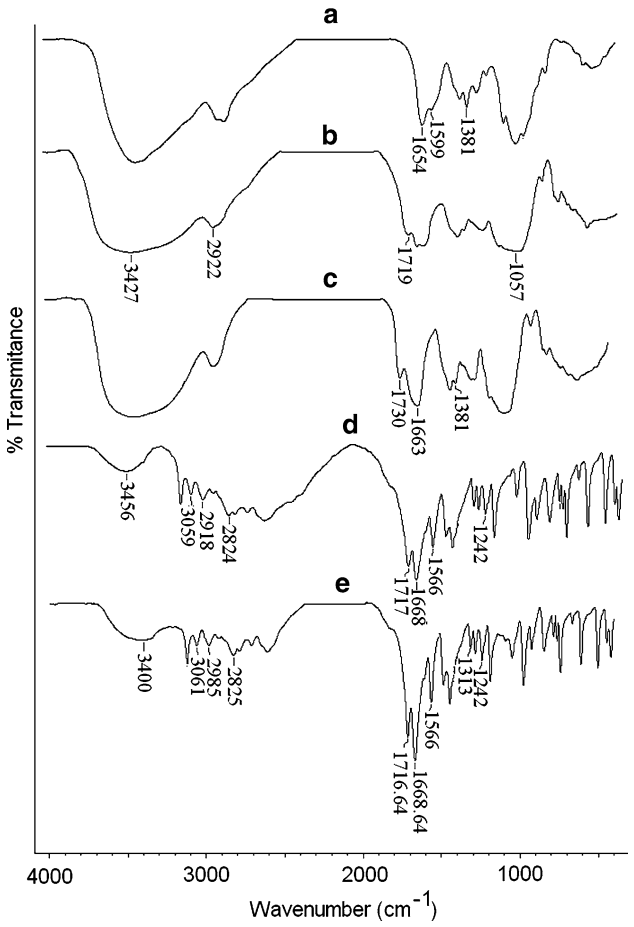

Fig. 4 FTIR spectra of *a* CS, *b* Xa, *c* THP, *d* Xa–CS, *e* Xa–CS–THP

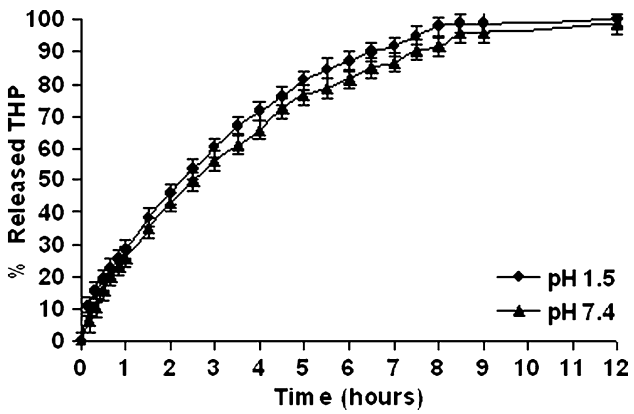

Fig. 5 In vitro THP release profile of Xa–CS–THP

$$\frac{M_t}{M_\infty} = kt^n \tag{5}$$

where M_t/M_∞ represents the fraction of released drug at time t, k is a constant characteristic to drug–polymer interaction and n is an empirical parameter characteristic to the release mechanism. When $\ln(M_t/M_\infty)$ is plotted versus ln (t), the value of the diffusional exponent is obtained.

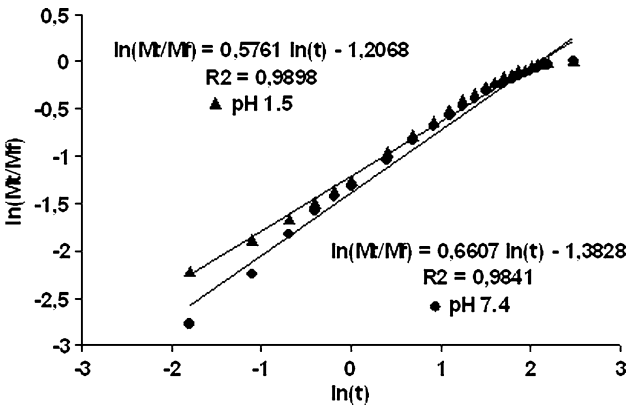

Fig. 6 $\ln(M_t/M_f)$ vs. $\ln(t)$ plot for Xa–CS–THP

According to Lee and Jou [34], the diffusion relative rate and polymer relaxation process cause three different classes of diffusion, characterized by distinct values of the diffusional exponent. When $n = 0.5$ the rate of diffusion is much smaller than the rate of relaxation and the process is called Fickian diffusion. Secondly, if $n = 1.0$, the diffusion process is much faster than the polymer relaxation. In this case the controlling step is the velocity of an advancing front, which forms the boundary between swollen gel and glassy core. Thirdly, in non-Fickian diffusion ($n = 0.5$–1.0) the diffusion and relaxation rates are comparable.

In this work the parameters k and n were calculated from the plot of ln (M_t/M_∞) versus ln (t) (Fig. 6) with the obtained experimental data. The data show that in acidic pH $n = 0.58$ and $k = 0.30$, while $n = 0.66$ and $k = 0.25$ in alkaline pH. Both diffusional exponents are characteristic for a non-Fickian diffusion process.

3.5 THP in vivo release profile

In order to compare the in vivo release behaviour of raw anhydrous THP with THP encapsulated in the Xa–CS complex, the samples were administered orally to laboratory rats. The time dependence of the plasma drug levels for up to 48 h after administration are plotted in Fig. 7.

The mean plasma level of raw THP gradually increased to a C_{max} value of 7.18 μg/ml at $t_{max} = 4$ h, while $t_{1/2} =$ 11.5 h. The serum concentration of THP released from the complex reached a maximum value of $C_{max} = 5.72$ μg/ml at $t_{max} = 4$ h with a $t_{1/2} = 13$ h, and remained constant in the range of 5–24 h with an average concentration value of 4.03 μg/ml, later decreasing with a relatively low rate in the time interval of 24–36 h. It was noticed that between 5 and 24 h the serum drug concentration remained constant and the in vivo release of THP from the complex obeyed a zero order release kinetic. The AUC_{0-48} was 25.76 μg h/ml for raw THP compared to 45.72 μg h/ml for THP entrapped in Xa–CS polyionic complex.

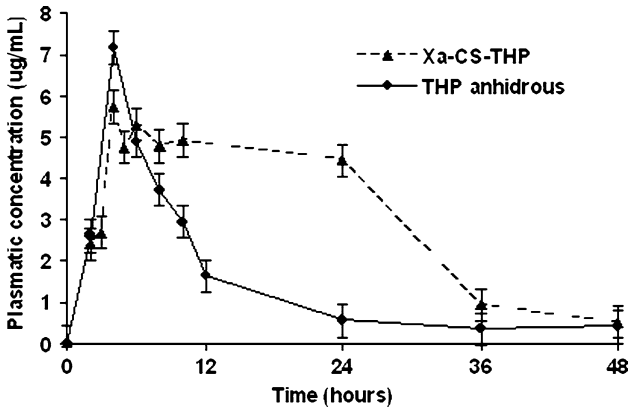

Fig. 7 Plasma THP concentrations vs. time after oral administration in rats. Each result shows the mean ± S.D. ($n = 4$)

The significant modification of pharmacokinetic parameters may be due to that CS has been found to enhance the drug absorption through gastrointestinal mucosa without producing damage to the biological system. Although there was no evidence of Xa–CS support decomplexing during swelling or drug release steps, the complex still contains free amino groups for CS as evidenced on FTIR spectra, which might contribute to improved mucoadhesivity. The mechanism of action of CS was suggested to be a combination of muchoadhesion and a transient widening of the tight junctions between epithelial cells [35]. Also previous studies show that the Xa–CS matrix was able to modify the dissolution rate of water insoluble drugs, consequently increasing their bioavailability [36].

4 Conclusions

Xa–CS hydrogel capsules intended for the controlled release of THP were obtained by complexation between two oppositely charged polysaccharides. The analysis results confirmed the formation of the Xa–CS hydrogel with THP entrapped into the matrix. THP in vitro release profiles at pH = 1.5 and pH = 7.4 did not exhibit significant differences due to the fact that the matrix is equally stable in both dissolution media. The equal stability in both dissolution media could be an advantage, the drug being released with constant rate regardless pH changes during its passage through gastro-intestinal tract.

The experimental values of the empiric diffusional exponent show that the release profile obeys a non-Fickian diffusion process for up to 12 hours of elution under both investigated pH conditions. Compared to the raw drug, the entrapped THP proved increased bioavailability.

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