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In vitro and *in vivo* theophylline release from cellulose/chondroitin sulfate hydrogels

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ABSTRACT

In vitro and *in vivo* release of the theophylline, loaded in mixed polysaccharidic cellulose/chondroitin sulfate (C/CS) hydrogels has been evaluated. The C/CS hydrogels in various mixing ratios obtained by a crosslinking technique were supplementary characterized by swelling studies in a pH 2.2 acidic solution at 37 °C, simulating the gastrointestinal medium, as *in vivo* theophylline delivery was done by oral administration. The hydrogels loading degree with theophylline was evaluated by near infrared chemical imaging (NIR-CI) technique and confirmed also by FT-IR spectroscopy. Based on PLS-DA (partial least squares-discriminate analysis) prediction, the drug loading was found up to 92.5%. The *in vitro* release profiles of theophylline from C/CS hydrogels showed that an increase of chondroitin sulfate leads to a decreased theophylline percent released, increased half release time and time to reach maximum percent released. During *in vivo* test, the raw theophylline was rapidly, absorbed, distributed, and eliminated. Comparatively with raw drug administration, the $t_{1/2}$ and AUC_{0-72} value were 4 times higher for theophylline loaded into 50/50 C/CS hydrogel. A good *in vitro–in vivo* correlation was found. A retarded release, controlled by CS content can be achieved by using mixed hydrogels as carriers.

1. Introduction

In recent years there is a continuous interest in the development of controlled drug release systems, to achieve the optimal therapeutic effect of drugs. The typical controlled release systems show a pattern of drug release, in which the drug concentration is maintained in the therapeutic window for an enough long period of time, thereby ensuring sustained therapeutic action.

Theophylline, an alkaloid found in *Camellia sinensis* leaves was discovered by Kossel in 1855 (Hennan, Aynesworth, & Martin, 1937; Lee, 1980; Vastag, Matthys, & Witt, 1984). It is a dimethylated xanthine that is similar in structure to caffeine and theobromine found in coffee, tea, cola beverages and chocolate (Greening, 1984). Theophylline is an effective drug used in the treatment of asthma and pulmonary disease (Rebuch & Contreras, 1982; Yu, Schwartz, & Sugita, 1996) and has been widely used as a model drug in various controlled release studies. Several formulations, were proposed to achieve *in vitro* and *in vivo* controlled release of theophylline, such as: polyisobutylcyanoacrylate (PICA) nanoparticles prepared by emulsifier-free polymerization in aqueous media at ambient conditions with promising performance as parenteral controlled drug delivery system (Mahasen, Iman, & Zinat, 1999); sustained-release preparations, administered once a day, namely tablet A (a swelling/disintegration-type matrix tablet consisting of hydrophilic hydroxypropylmethylcellulose granules and hydrophobic wax granules (cluster tablets) and tablet B (a matrix tablet consisting of hydrophobic ethylcellulose granules and hydrophilic cellulose granules), the obtained results indicating that the cluster tablets A showed significantly less inter-subject variation of theophylline plasma levels than the conventional matrix tablets B (Hayashi et al., 2007); spherical drug pellets coated with a rate-controlling membrane (Yuen, Desmukh, & Newton, 1993); floating controlled-release of theophylline loaded tablets having a density of 0.67, with a slower *in vitro* release rate and *in vivo* increased gastric retention time due to the presence of food (Desai & Bolton, 1993); locust bean gum and chitosan-based mucoadhesive tablets with high mucoadhesive strength and good swelling and *in vitro* drug release behavior (Senthil et al., 2010); chitosan–xantan hydrogels (Popa, Novac, Profire, Lupusoru, & Popa, 2010), *etc.*

In our previous paper the good biocompatibility and retarded *in vitro* release of a novel xanthine compound from the mixed polysaccharidic hydrogels of cellulose (C) and chondroitin sulfate (CS) have been demonstrated (Oprea et al., 2012).

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The aim of this study was to explore in depth the application of the C/CS hydrogels as carriers for theophylline administration, by their detailed characterization and *in vitro* and *in vivo* evaluation.

2. Experimental

2.1. Materials

Microcrystalline cellulose – Avicel® PH-101 with the polymerization degree of 183 was supplied by Sigma–Aldrich. Chondroitin sulfate (CS) was purchased from Roth, Germany. It was obtained from bovine tracheal cartilage. Epichlorohydrin used as crosslinking agent of analytical purity was purchased from Sigma–Aldrich.

Preparation of cellulose/chondroitin sulfate hydrogels and their characterization were described in detail in a previous paper (Oprea et al., 2012).

2.2. Investigation methods for hydrogels

2.2.1. Swelling tests

It is well-known that the water permeation into the matrix can be a prerequisite for the drug release (*e.g.* swelling of the matrix to enable drug diffusion or dissolving the drug in the matrix prior to diffusion).

Swelling studies were carried out by direct immersion in acidic solution pH 2.2 at 37 °C which simulate the gastric fluid medium conditions as *in vivo* experiments were done by oral administration. The hydrogels samples were periodically removed from the solution, gently wiped with a soft tissue to remove surface solution, weighed and than carefully placed back into the vessel as quickly as possible. The swelling degree (S) was calculated according to the equation:

$$S\ (\%) = \frac{W_t - W_d}{W_d} \times 100 \quad (1)$$

where W_t is the weight of the swollen samples at time t and W_d is the weight of the dry sample.

To determine the kinetics of solvent diffusion into the matrices (swelling) the following equation was used (Berens & Hopfenberg, 1978):

$$\frac{W_t}{W_{eq}} = k_{sw} t^{n_{sw}} \quad (2)$$

where W_t and W_{eq} represent the amount of pH 2.2 solution absorbed by the matrices at time t and at equilibrium, respectively; k_{sw} is the swelling rate constant or specific rate characteristic of the system and n_{sw} is the power diffusion law exponent which takes into account the type of solvent transport. Eq. (2) applies to initial states of swelling (swelling degree less than 60%) where linearity of ln Ft as a function of ln t is obtained.

2.2.2. FT-IR spectroscopy

The hydrogels loaded with theophylline were analyzed by FT-IR spectroscopy, using the KBr pellet technique. The spectra were scanned on a Bruker VERTEX 70 (USA) device, over the 4000–500 cm^{-1} range, at a resolution of 4 cm^{-1}.

2.2.3. Drug loading

The model drug used for *in vitro* and *in vivo* release measurements was theophylline. The loading was performed by a diffusion filling method which allows the partition of drug solution into the hydrogel network. Thus, 0.1 g C/CS hydrogel in powder form was suspended in a 4% theophylline solution (1:1, v/v, ethyl alcohol/water) under mild stirring and left to swell 24 h, period corresponding with the time in which the compositions reached their equilibrium swelling degree, as determined from the swelling studies (Popa et al., 2010). The loaded samples were then freeze-dried using a Labconco FreeZone device.

2.2.4. Near infrared chemical imaging technique (NIR-CI technique)

NIR spectra of theophylline-loaded hydrogels were recorded on a Specim's Ltd. SisuCHEMA controlled with Evince software package for processing the original image data. The system includes a Chemical Imaging Workstation for 1000–2500 nm NIR domains. The original image for each sample was taken with a NIR model spectral camera, respectively an imaging spectrograph type ImSpector N17E with 320 and 640 pixel spatial resolution at a rate of 60–350 Hz.

2.2.5. In vitro theophylline release

The dissolution medium was the acidic medium which simulates the pH of gastric fluid (pH 2.2). During dissolution testing, the media was maintained at 37 ± 0.5 °C. Aliquots of the medium of 1 ml were withdrawn periodically at predetermined time intervals and analyzed using a HP 8450A UV-visible spectrophotometer at λ = 271 nm, the wavelength characteristic to theophylline. In order to maintain the solution concentration the sample was carefully reintroduced in the circuit after analyzing. The concentrations of the drug were calculated based on calibration curves determined at the same wavelength.

A simple, semi-empirical equation using Korsmeyer and Peppas model was used to kinetically analyze the data regarding the drug release from studied matrices system which is applied at the initial stages (approximately 60% fractional release) (Korsmeyer, Lustig, & Peppas, 1986):

$$\frac{M_t}{M_\infty} = k_r t^{n_r} \quad (3)$$

where M_t/M_∞ represents the fraction of the released drug, M_t and M_∞ are the absolute cumulative amount of drug released at time t and at infinite time (in this case maximum release amount in the experimental conditions used, at the plateau of the release curves), respectively, k_r is a constant incorporating characteristics of the macromolecular matrix and the drug, n_r is the diffusion exponent, which suggests the release mechanism. In the equation above a value of n_r = 0.5 indicates a Fickian diffusion mechanism of the drug from matrix, while a value $0.5 < n_r < 1$ indicates an anomalous or non-Fickian behavior. When n_r = 1 a case II transport mechanism is involved while $n_r > 1$ indicates a special case II transport mechanism (Korsmeyer & Peppas, 1984; Serra, Domenechc, & Peppas, 2006).

The corresponding drug-release profiles were represented through plots of the cumulative percentage of drug release *versus* time.

2.2.6. In vivo theophylline release

Healthy Wistar rats weighing between 350 and 420 g purchased from Cantacuzino Institute, Bucharest, Romania were used for the study. The animals were maintained in identical laboratory conditions for one week before starting the experiments having access at food and water *ad libitum*. *In vivo* tests were performed in accordance with the ethical rules stated in the paper "*Ethical guidelines for investigations of experimental pain in conscious animals*" (Zimmermann, 1983).

The tested theophylline-loaded hydrogels have been orally delivered as suspensions in sodium carboxymethylcellulose of 0.5 wt%. A dose of 15 mg THP powder/kg body weight was administrated for each rat. Blood samples were drawn out at established time intervals up to 72 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

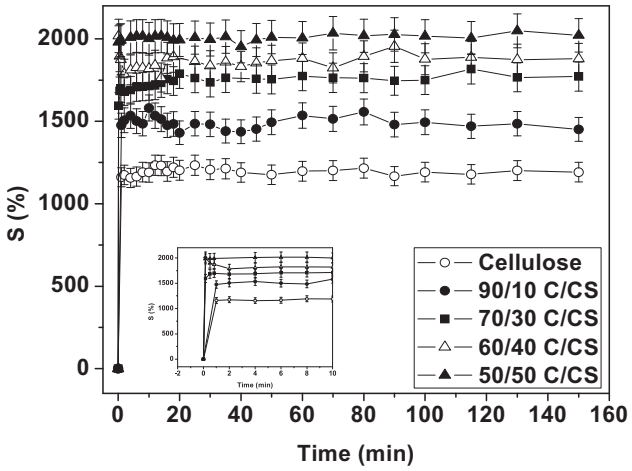

Fig. 1. Swelling profiles of C/CS hydrogels with different compositions in pH 2.2 medium.

described in the literature (Labačevski et al., 2003) with a few minor changes. After thawing, 1 ml serum was mixed with 250 μl 10 wt% $(NH_4)_2SO_4$ 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 mm × 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 (Popa et al., 2010). Each experiment was repeated four times and the mean value with standard deviation was reported. Statistical data analysis was performed using the Student *t* test and ANOVA with $p < 0.05$ as the minimal level of significance.

3. Results and discussion

3.1. Matrix characterization

3.1.1. Swelling studies in acidic medium

Swelling studies were performed in acidic medium (pH 2.2) at 37 °C. The mass changes characteristic of water uptake and swelling started from the beginning and continued until 150 min of experiment (Fig. 1). These matrices showed a high ability to swell, visual observations denoting that the matrices appeared swollen even from first seconds. Time to reach maximum degree of swelling is about 16 min for cellulose-based hydrogel and less than 4 min for C/CS hydrogels, then a plateau (equilibrium) is reached. The rate to reach this equilibrium also increases with CS content (see insert and Table 1). The swelling behavior is similar with that found in 90:10 water:ethanol solution, at 37 °C (Oprea et al., 2012).

The equilibrium swelling degree of the C/CS hydrogels increases with the amount of CS from 1329% for cellulose hydrogel to 1991% for 60/40 C/CS and of 2116% for 50/50 C/CS (Table 1).

Table 1
Swelling kinetic parameters values of the C/CS-based hydrogels.

C/CS hydrogels (%)	S (%)	n_{sw}	k_{sw} (min^{-n})
Cellulose	1329	0.01	0.08
90/10	1612	0.02	0.1
70/30	1928	0.03	0.12
60/40	1991	0.018	0.11
50/50	2116	0.004	0.14

3.1.2. Kinetic of swelling in acidic medium

The kinetic parameters of swelling in acidic medium of the C/CS hydrogels with various compositions are given in Table 1.

The values obtained for swelling parameter, n_{sw} in acidic medium of the C/CS hydrogels with different mixing ratios varies in range between 0.004 and 0.03 indicating an anomalous transport mechanism. The kinetic rate constant values, k_{sw} increase from 0.08 $min^{-0.01}$ to 0.14 $min^{-0.004}$ for cellulose-based and 50/50 C/CS hydrogel formulation, respectively.

3.2. Theophylline-loaded hydrogels characterization

3.2.1. FT-IR analysis

FT-IR spectra of C/CS hydrogels loaded with theophylline are shown in Fig. 2.

The spectra of the loaded hydrogels contain theophylline bands. The bands at 3430–3450 cm^{-1} assigned to —OH groups are wider and shifted to lower wavenumbers increasing CS content and also band at 2882 cm^{-1} assigned to CH_2 symmetric stretching because of the possible interactions between components by hydrogen bonding. The band appearing at 3121 cm^{-1} is assigned to the aromatic C—H stretching vibrations, whereas that appearing at 2922 cm^{-1} corresponding to aliphatic C—H stretching vibrations. The peaks appearing in region 2824–2712 cm^{-1} are attributed to N=CH_3 bond. The C—N stretching vibrations are seen at 1049 cm^{-1}, while the one that appeared at 1243 cm^{-1} is assigned to aromatic C=O stretching vibrations. A slight shift of bands position from 1717 cm^{-1} to 1712 cm^{-1} and from 1667 cm^{-1} from 1663 cm^{-1} attributed to —CO—N(R)—CO— theophylline characteristic group confirms presence of the theophylline in hydrogels and also the interactions between drug and hydrogels.

3.2.2. NIR-CI studies

Theophylline distribution into C/CS hydrogels and its homogeneity were evaluated by the near infrared chemical imaging (NIR-CI) technique. The prediction of loading degree was evaluated based the new near infrared chemical imaging maps. The chemical imaging provides a simple method for evaluating the spatial drug distribution. The non-destructive character and minimal sample preparation required, show the feasibility of this technique which is used more to investigate samples with pharmaceutical applications.

Both PLS-DA (partial least squares-discriminate analysis) and PCA (principal component analysis) models were used to determine the homogeneity and prediction of the two components, namely the drug and polymeric matrix. PLS-DA model based on multivariate inverse least squares discrimination method is used by Evince to classify the components. This could be achieved because of these two models based on mathematical processing. Assigning a color for each component, class of principal components, allows visual assessment of the degree of homogeneity of the components. As noted with a value between 0 and 1 of the same original component facilitated obtaining quantitative information. For the first component value 1 represents available percentage of 100% and the value tends to zero when there is an unknown structure. It is appreciated the correct information available upon the cube of information can

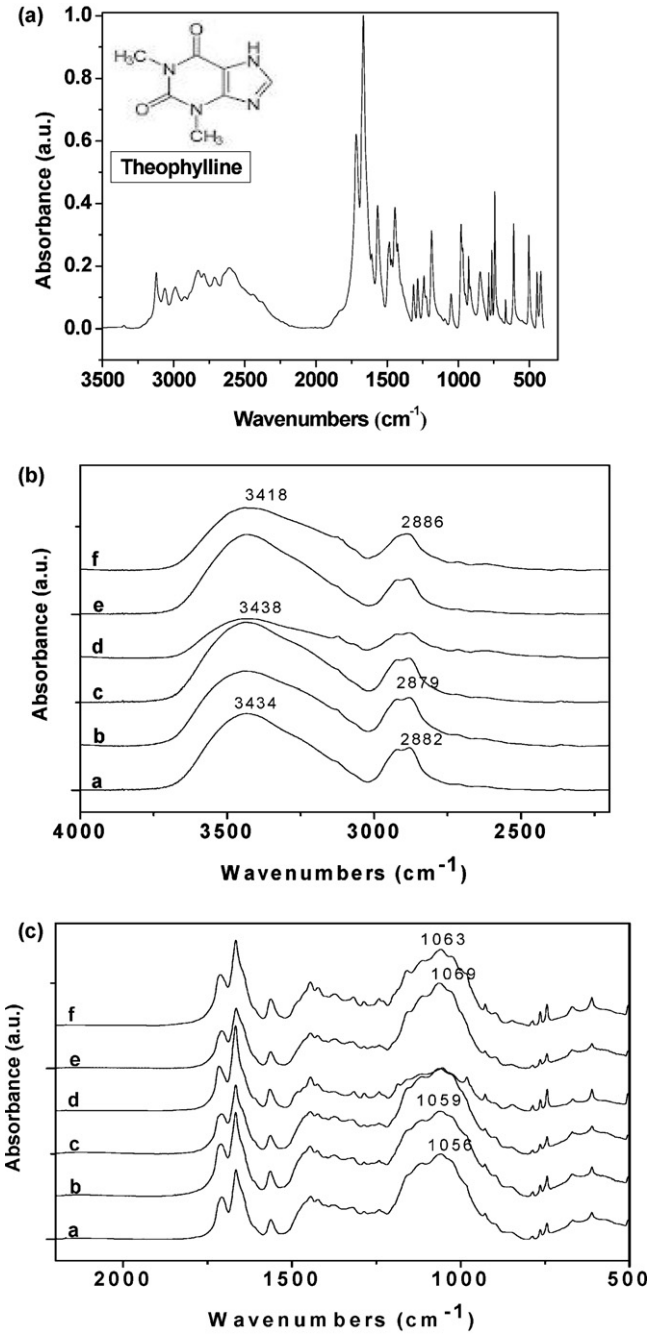

Fig. 2. FT-IR spectra of theophylline (a) and C/CS hydrogels loaded with theophylline: (a) cellulose-based hydrogel, (b) 90/10 C/CS, (c) 80/20 C/CS, (d) 70/30 C/CS, (e) 60/40 C/CS, (f) 50/50 C/CS in different spectral ranges: 2200–4000 cm^{-1} (b) and 500–2200 cm^{-1} (c).

be extracted. The final images have for every pixel a complete spectrum that includes contributions from all the chemical components present in system.

Fig. 3 corresponds to the PLS-DA model for 50/50 C/CS composition.

A visibly uniform distribution, corresponding to a high homogeneity degree of the drug in the cellulose/chondroitin sulfate hydrogel matrix was observed. Based on PLS-DA prediction, a drug loading up to 92.5% into the 50/50 C/CS composition was found from theophylline loaded amount.

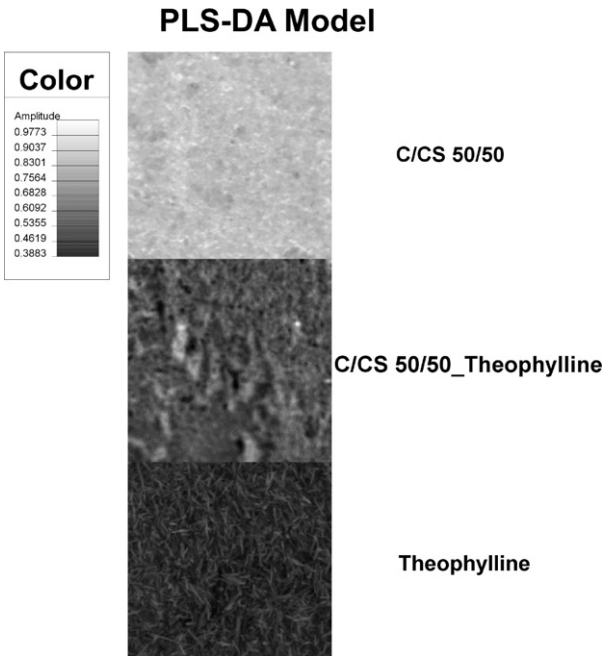

Fig. 3. PLS-DA model for 50/50 C/CS hydrogel.

Fig. 4 shows the NIR spectra of theophylline, 50/50 C/CS composition and theophylline/50/50 C/CS system in the full range of the near infrared region with an air background.

The obviously overlap between the polymers and theophylline bands in near infrared region (Table 2) empower for the use a multivariate regression for the simultaneous determination of drug content in samples.

The data of Table 2 confirm also FT-IR spectra results.

3.2.3. *Theophylline in vitro release studies*

The release profiles of theophylline from C/CS hydrogels are shown in Fig. 5.

The release profiles of theophylline from C/CS hydrogels depend on CS content. Thus, an increase of CS content leads to a gradually decrease of theophylline percent released from 95% in case of cellulose-based hydrogel to 78% for 80/20 C/CS, 73% for 70/30 C/CS reaching up to 58% for 50/50 C/CS composition. This behavior is caused probably because of drug-matrix interactions for hydrogels containing a higher amount of CS, through hydrogen and ionic interactions leading to slower release rate and smaller released amount as it was showed by spectroscopic results. The prolonged release of theophylline from these matrices, due to the presence of CS, is proved also by the time to reach maximum percent released and half release time values. Half release time values vary from 14 min for cellulose-based hydrogels to 60 min in case of 50/50 C/CS composition. Time to reach maximum percent released varies also, from 160 min for cellulose-based hydrogel to 310 min for 70/30 C/CS reaching in case of 50/50 C/CS to 370 min (Table 3).

3.2.4. *Kinetic study of theophylline release*

The kinetic parameters for theophylline released in acidic solution (pH 2.2) from C/CS-based hydrogels are showed in Table 3.

The release mechanism of theophylline from C/CS hydrogels is described as an anomalous transport for all tested formulations which appeared by coupling Fickian diffusion with the relaxation of the hydrogel network. The values of release rate constant, k_r showed a decrease from 0.05 $min^{0.76}$ to 0.01 $min^{-0.77}$ with CS content increasing in hydrogels composition.

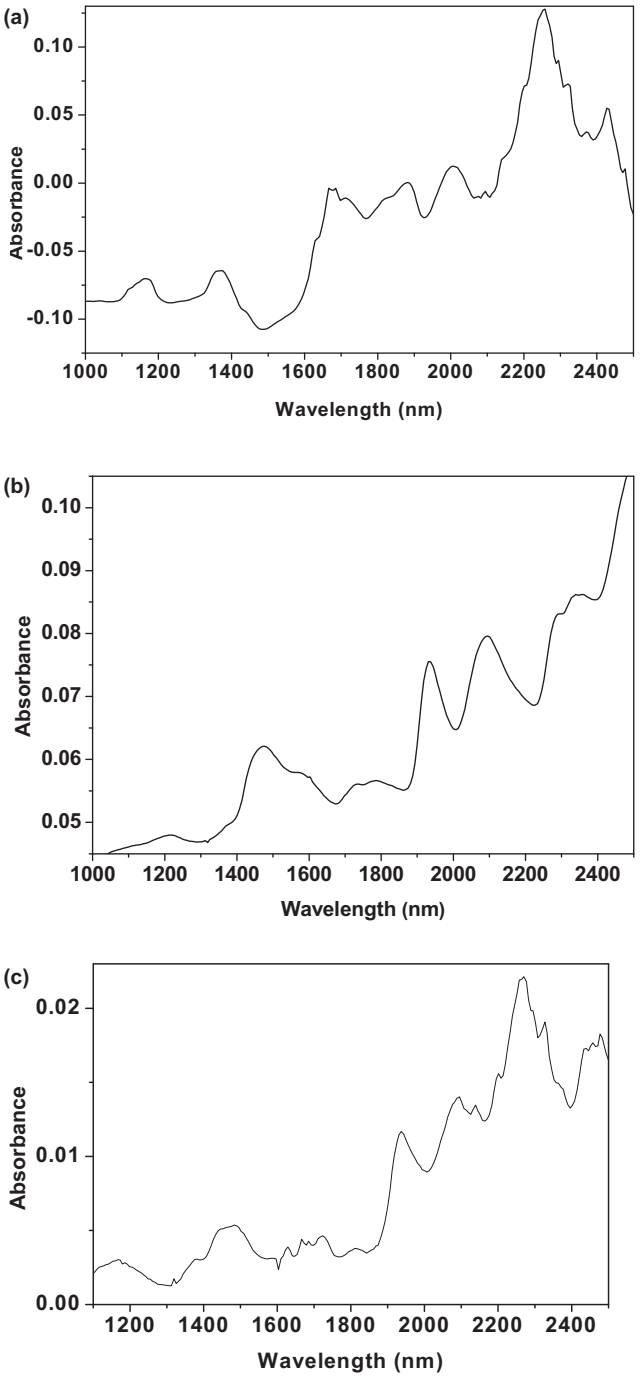

Fig. 4. Near-IR reflectance spectra of: theophylline (a), 50/50 C/CS hydrogel (b) and 50/50 C/CS/theophylline system (c).

The release of theophylline in acidic medium is controlled by the presence of CS, its increasing content in hydrogels composition leading to the prolonged nature of releasing process.

After the *in vitro* release studies of theophylline, performed on C/CS matrices with different compositions, the abilities of releasing in a sustained and prolonged manner of theophylline from the 50/50 C/CS composition recommends it also for *in vivo* therapeutic evaluation.

3.2.5. *In vivo release profiles of theophylline*

The raw theophylline and theophylline-loaded hydrogel have been orally delivered in rats as suspensions in sodium carboxymethylcellulose of 0.5 wt%. A dose of 15 mg THP powder/kg body weight was administrated for each rat. The chosen hydrogel composition for *in vivo* release study of theophylline was 50/50 C/CS which showed a prolonged release during *in vitro* testing in acidic medium.

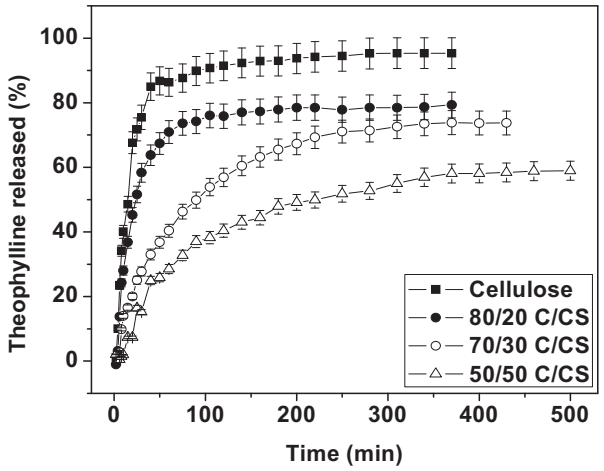

Fig. 5. The release profiles of theophylline from C/CS hydrogels in acidic solution at 37 °C.

Fig. 6 presents the pharmacokinetic release profiles of raw theophylline and theophylline-loaded in 50/50 C/CS formulation.

The *in vivo* release profiles showed the sustained release of theophylline loaded in 50/50 C/CS formulation compared with raw theophylline which is removed from the body in the first 17 h from administration. The prolonged release behavior of theophylline loaded in 50/50 C/CS formulation was proved by the presence of drug traces recorded up to 50 h from administration time. The evaluated pharmacokinetic parameters are given in Table 4.

Theophylline was detectable in plasma within first hour after its administration in rats. The mean plasma level of raw theophylline was recorded at a C_{max} value of 10.46 μg/ml at t_{max} of 4 h, while the $t_{1/2}$ is about 4.9 h which indicated a fast absorption of pure theophylline. The theophylline serum concentration, released from 50/50 C/CS composition, reached a maximum value of C_{max} of 10.75 μg/ml with a t_{max} of 4 h and a $t_{1/2}$ of 19 h, exhibiting a delayed absorption in blood. Serum theophylline concentration remained approximately constant in the range of 2–8 h with an average value of 10.47 μg/ml, later decreasing with a relatively low rate in 24–72 h interval.

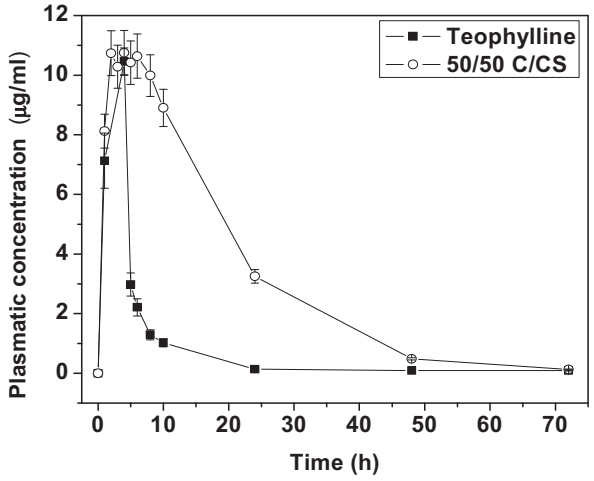

Fig. 6. Plasma theophylline concentration *versus* time. Each point is presented as mean ± S.D., $n = 4$.

Table 2
The wavelength corresponding to the functional groups on NIR spectra for 50/50 C/CS hydrogel, theophylline and theophylline/50/50 C/CS system.

Wavelength (nm)	50/50 C/CS	Theophylline	Theopylline/50/50 C/CS
1166	–	C—H second overtone	C—H second overtone
1370	–	C—H combination	–
1475	N—H stretch first overtone	–	N—H stretch first overtone
1660	–	C—H stretch first overtone	–
1720	–	–	C—H stretching mode, first overtone
1735	C—H stretch first overtone	–	C—H stretching mode, first overtone
1940	O—H bend second overtone	–	O—H bend second overtone
2005	–	O—H combination	–
2095	C—H combination	–	C—H combination
2256	–	H—O and C—H combination	H—O and C—H combination
2289	C—H stretch/CH_2 deformation	–	–
2327	–	C—H stretch/CH_2 deformation	C—H stretch/CH_2 deformation
2340	C—H stretch/C—H deformation	–	–
2428	–	C—H and C—C combination	–
2470	–	C—N—C stretch overtone	C—N—C stretch overtone

Table 3
The kinetic parameters and release characteristics of theophylline from C/CS hydrogels evaluated according to Korsmeyer–Peppas equation.

C/CS (%)	Half release time (min)	Time to reach maximum amount released (min)	Maximum released amount (%)	Korsmeyer–Peppas equation		
				n_r	R	k_r (min^{-nr})
Cellulose	14	160	95	0.76	0.96	0.05
80/20	16	200	78	0.66	0.99	0.05
70/30	49	310	73	0.6	0.98	0.03
50/50	60	370	58	0.77	0.98	0.01

Table 4
Pharmacokinetic parameters obtained for raw theophylline and theophylline-loaded 50/50 C/CS hydrogel.

Parameters	Raw theophylline	Theophylline-loaded 50/50 C/CS hydrogel
C_{max} (μg/ml)	10.46	10.75
t_{max} (h)	4	4
$t_{1/2}$ (h)	4.9	19
AUC_{0-72} (μg h/ml)	58.11	232.44
Relative bioavailability (%)	–	400

The value of area under the curve (AUC) or time dependence of plasma after administration of a single dose of drug was determined to be about 58.11 μg h/ml for raw theophylline compared to 232.44 μg h/ml for theophylline loaded in 50/50 C/CS formulation. These findings were in accordance with the results of Mahasen et al. (1999) who obtained an AUC_{0-25} value for drug-loaded nanospheres of about 1.39 times higher than that of theophylline solution.

The significant differences between pharmacokinetic parameters may be due to the presence of chondroitin sulfate which increases the bioavailability up to 400% for the tested formulation and drug absorption through gastrointestinal mucosa without producing damage to the biological system, compared with raw theophylline.

These *in vivo* absorption characteristics are in accordance with those obtained for *in vitro* evaluation of 50/50 C/CS composition, the prolonged release ability of this matrix characterizing both cases; the increased content of CS in hydrogels composition causes the controlled and sustained behavior of release process.

4. Conclusions

The cellulose/chondroitin sulfate-based hydrogels in various mixing ratios were *in vitro* and *in vivo* evaluated as carriers for theophylline release. The swelling profiles in acidic medium revealed dependence on CS content showing that an increase of CS percent in hydrogels composition leads to higher swelling ratios. A visibly uniform distribution, corresponding to a high homogeneity degree of the theophylline in the cellulose/chondroitin sulfate hydrogels structure was found by the NIR-CI studies. The *in vitro* release profiles in acidic medium showed that the increase of chondroitin sulfate content in C/CS hydrogels leads to a decrease of theophylline percent released while the half release time and time to reach maximum values. The kinetic evaluation of *in vitro* release is described by an anomalous mechanism for all formulations. The *in vivo* release profiles showed the sustained release behavior and the increased bioavailability of theophylline loaded in 50/50 C/CS formulation compared to the raw drug. A good *in vitro–in vivo* correlation of theophylline release from 50/50 C/CS formulation was found. The 50% percent of CS in mixed hydrogels with cellulose causes a controlled and prolonged nature of release process.

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