CELLULOSE/CHONDROITIN SULFATE HYDROGELS: SYNTHESIS, DRUG LOADING/RELEASE PROPERTIES AND BIOCOMPATIBILITY

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The present study investigates some hydrogels, based on natural, biodegradable and biocompatible polysaccharides, such as cellulose (C) and chondroitin sulfate (CS), as sustained release carriers, and evaluates their biocompatibility. Investigations were performed on cellulose/chondroitin sulfate (C/CS) hydrogels, in different mixing ratios. Swelling and drug delivery studies were conducted in a phosphate buffer solution (pH = 7.4) that simulates the pH of the intestinal fluid, at 37 °C.
Natural-based hydrogels were evaluated for the release of paracetamol and theophylline. The release profiles of the drugs from C/CS hydrogels depend on the CS content: for paracetamol, a decrease of the percent released and, for theophylline, a percentual increase with the increase of the CS content. The results showed good biocompatibility with the tested formulations.

Keywords: natural hydrogels, swelling, drug release, biocompatibility

INTRODUCTION

Hydrogels containing polysaccharides have been widely used in biomedical applications due to their biocompatibility with the human body. Furthermore, hydrogels resemble natural living tissue more than any other class of synthetic biomaterials, as due to their high water content and soft consistency, which makes them similar to natural tissues.[1,2]

Among the numerous polymers proposed for the preparation of hydrogels, polysaccharides have a number of advantages over the synthetic polymers initially employed in the field of pharmaceutics.[3]

Cellulose, the world's most abundant natural, renewable and biodegradable polymer, is a linear polysaccharide of D-glucose units linked by β(1→4) glycosidic bonds,[4,5] where every other glucose residue is rotated approximately 180° (Fig. 1a). As a result, cellobiose is a structural repeating unit of the glucan chains in cellulose.[5] The glucan chains in cellulose are parallel to each other and are packed side by side to form microfibrils, which stabilizes the structure, minimizing its flexibility.[6]

Cellulose is degradable by enzymes[7] and its solubility in water depends on its chain length, n (ranging from 500 to 5000).[8] Also, it is easily processable and thus available in a wide range of forms and shapes, *e.g.* as membrane sponges, microspheres and non-woven, woven or knitted textiles. The good match of the mechanical properties of cellulose derivatives with those of hard and soft tissues has been demonstrated.[9-13] It has been employed in the form of membranes (*e.g.* dialyse, biosensors),[14] bioadhesive cellulose gels, as vasco-surgical devices,[15] for investigations in bone tissue engineering,[16,17] cartilage tissue engineering,[18] tissue engineering in post-injury brain,[19] for connective tissue formation,[20] as drug delivery system[21] and scaffold for growing functional cardiac cell constructs *in vitro*.[22]

Chang *et al.*[23] prepared superabsorbent hydrogels from sodium carboxymethyl-cellulose and cellulose in a NaOH/urea

aqueous system, with epichlorohydrin as crosslinker, and evaluated the prolonged protein release behaviour of the hydrogels for application in biomedical fields. The hydrogels presented a release capacity of bovine serum albumine, and the release time was controlled by the CMC content.

Chondroitin sulfate is an important structural component in connective tissues and cartilages. It provides compressive strength to connective tissues by adjusting their water content, and possesses characteristic features, such as high water absorption, multifunctionality and biodegradability suitable for several bioapplications.[24,25] In addition, the presence of active functional groups in chondroitin sulfate, such as $–COO^-$ and $–SO_3^-$, provides access to biological functionalities, recently exploited in *in vivo* cartilage repair applications.[24]

It is a copolymer of the D-glucuronic acid and sulfated N-acetyl-D-galactosamine in C_4 or C_6, belonging to glycosaminoglycans (GAGs), which are primarily located on the surface of cells or in the extracellular matrix (Fig. 1b).

The readily water-soluble nature of chondroitin sulfate restricts its application as a solid-state drug delivery vehicle. Therefore, commonly, a crosslinking treatment is carried out to tailor the properties of chondroitin sulphate, as reported in several works,[26,27] or it is combined with other polymers, such as chitosan,[28,29] gelatin and hyaluronan,[30,31] collagen,[32,33] poly(vinyl alcohol)[34] or poly-(lactic-co-glycolic acid),[31] for producing more stable materials.

Studies on chondroitin sulfate-based hydrogels were reported by Wang *et al*.,[27] who used two methods to prepare chondroitin sulfate hydrogels: by crosslinking it directly with poly(ethylene glycol) diglycidyl ether, or by forming an interpenetrating polymer network. They characterized the release of a model drug, sodium diclofenac, and of a model protein, bovine serum albumin, from the synthesised hydrogels. Kuijpers *et al.*[35] evaluated chemically cross-linked gelatine-chondroitin sulfate hydrogels, impregnated in Dacron, as drug delivery systems for antibacterial proteins. Piai *et al*.[36] synthesized a hydrogel constituted of chitosan and chondroitin sulfate, the hydrogel with an excess of chondroitin sulfate (40% chitosan and 60% chondroitin sulfate) being used either as a chondroitin sulfate carrier, or to deliver the excess of chondroitin sulfate as active principles.

Moreover, since chondroitin sulfate is negatively charged, an interaction with positively charged molecules, such as polymers or growth factors, is anticipated, this being viewed as a key issue to facilitate the design of delivery systems. For instance, this characteristic is used to produce chondroitin sulfate–chitosan sponges as delivery systems for platelet-derived growth factor-BB (PDGF-BB) for bone regeneration, as reported by Park *et al*.,[29] who showed that such an interaction induces a more prolonged release of the growth factor.

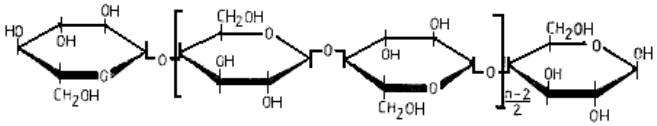
Chondroitin sulfate can be degraded by anaerobic bacteria, namely *Bacteroides thetaiotaomicron* and *B. ovatus*, which are resident in the large intestine.[37] This characteristic suggests that chondroitin sulfate is a potentially good candidate for the use as a colon-targeted drug carrier. Moreover, chondroitin sulfate is a good S/DMOAD (structure/disease modifying anti-osteroarthritis) drug. Administered orally, chondroitin sulfate reduces the pain in osteroarthritis patients,[38] especially over long periods, in comparison with diclofenac sodium.[27, 39-41]

Paracetamol (acetaminophen)-(Merck) (Fig. 2a) is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer) drug. The antipyretic effect is directly proportional with temperature: paracetamol has no efficiency with the decrease of normal temperature. It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) or opioid analgesics, paracetamol is also used in the management of more severe pain (such as cancer pain). Routes of administration: per os (oral), rectal, intravenously, for amelioration of surgical pain.[42]

As a broncho-dilatator drug, theophylline (1,3-dimethyl-7H-purine-2,6-dione)-(Merck) (Fig. 2b) relaxes directly the smooth muscles of the bronchial and pulmonary blood vessels. Also, it has been shown that aminofiline (theophylline-ethylene diamine) has a strong effect on contractility skirt at

healthy persons, and may reduce tiredness, thereby improving contractility in patients with chronic obstructive diseases.[43] Theophylline has significant effects on the circulatory system, especially when serum concentrations exceed 15 (20) mg/mL. Theophylline produces a moderate decrease in peripherical vascular resistance, sometimes important cardiac stimulation, raises the perfusion of most organs, increases diuresis and may have additional beneficial effects in the treatment of heart failure.[43]

The aim of the present study is to combine the properties of cellulose and chondroitin sulfate in mixed hydrogels to obtain new materials for medical and pharmaceutical applications. To this end, there has also been evaluated the biocompatibility and citotoxicity of cellulose/chondroitin sulfate hydrogels and their applicability in drug delivery systems using paracetamol and theophylline for achieving a controlled release profile, suitable for oral administration.



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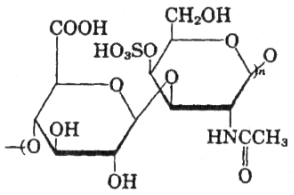
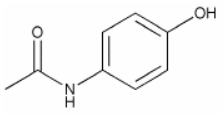


b)

Figure 1: Chemical structure of hydrogel components: a) structure of cellulose; b) structure of chondroitin sulfate



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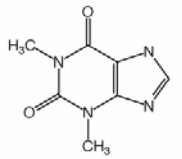

b)

Figure 2: Chemical structure of the drugs used: a) structure of paracetamol; b) structure of theophylline

EXPERIMENTAL

Materials

Microcrystalline cellulose (C) – Avicel HP-101 – (Sigma Aldrich) and chondroitin sulfate (CS) powder, received from the National Institute of Research and Development for Biological Science, Bucharest, were obtained by digestion of bovine trachea with papaine.

Preparation of cellulose/chondroitin sulfate hydrogels

The hydrogel samples were prepared in various mixing ratios (%), as follows: 90/10, 80/20, 70/30, 60/40, 50/50 C/CS.

Cellulose/chondroitin sulfate hydrogels were produced through crosslinking.

Cellulose was initially dissolved[44] in an alkaline solution of 9% NaOH. Freshly distilled epichlorohydrin was added to the cellulose/chondroitin sulfate mixtures, under vigorous, continuous stirring. The crosslinking reaction was performed for 8 h at 80 °C, the gel-like samples being placed on glass plates. The hydrogels were washed with warm water to remove the unreacted compounds. The samples were dried for 10 h, on a LABCONCO FreeZone device.

Investigation methods

FT-IR spectroscopy

The hydrogels loaded with paracetamol and 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}.

Swelling studies

Swelling studies were carried out for all formulations by direct immersion into a phosphate buffer solution simulating the intestinal fluid (pH = 7.4). The hydrogel samples were maintained for 24 h at 37 °C, periodically removed from the solution, gently wiped with a soft tissue to remove the surface water, weighed and then placed back into the vessel as quickly as

possible. The swelling degree at equilibrium was calculated according to equation:

$$Q_{max} = (W_t - W_d)/W_d \times 100(\%) \quad (1)$$

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

To determine the kinetics of solvent diffusion into the matrices (swelling), the following equation was used:[45]

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

where W_t and W_{eq} represent the amounts of phosphate buffer solution (pH 7.4) absorbed by the matrices at time *t* and at equilibrium, respectively, k_{sw} is the swelling constant characteristic of the system and n_{sw} is the power law diffusion exponent which considers the type of solvent transport. Equation (2) applies to the initial states of swelling (swelling degree below 60%), and linearity is observed when log Ft is represented as a function of log t.

Matrix characterisation

Hemolysis test

Blood was obtained from healthy patients, by routine venipuncture from the antecubital vein in tubes containing EDTA. Each hydrogel preparation was tested with the blood of a single patient. The blood was stored refrigerated for no more than 2 days until use. Prior to the hemolysis test, all hydrogel samples were sterilized by ultraviolet light trans-illumination for 2 min. Distilled water was used as a positive control and the plasma separated from the same blood – as a negative control. From each tube, 1.5 mL of blood were drawn and put into contact with hydrogels, in Eppendorf centrifuge tubes (2 mL). The blood samples in contact with the biomaterials were incubated at 37 °C for 2 h. After incubation, the samples were centrifuged at 5000 rpm for 6 min. The separated plasmas were diluted 11-fold with Tris (hydroxymethyl) amynomethane – Sigma-Aldrich), (62.5 mmol/L, pH 8.0 adjusted with HCl) prior to spectrophotometrical measurements. The remaining 0.5 mL of blood in each tube were centrifuged, again, at 5000 rpm and separated plasmas were diluted 11-fold with Tris, the resulting solutions being used as negative controls. The positive control was prepared by hemolysing blood with distilled water (1:11 dilution). The hemolysed solution was also incubated at 37 °C for 2 h. Finally, the positive control solution was diluted 100-fold for spectrophotometric analysis.[46]

The method used for measuring plasma hemoglobin concentration in all specimens was the polychromatic method of Noe *et al*.[47] Absorbance was measured at 380, 415 and 470 nm and the formula used for evaluation was (3):

$C\ (mg/L) = 1.65\ mA_{415} - 0.93\ mA_{380} - 0.73\ mA_{470}$

where C is hemoglobin concentration in mg/L, mA_{380}, mA_{415} and mA_{470} are the absorbances at 380, 415 and 470 nm, expressed in milliabsorbance units. The results were expressed as(4):

$$\text{hemolysis percent } (\%) = (C - C_n)/(C_p - C_n) \times 100$$

where C is the concentration of hemoglobin in the sample, C_n the concentration of hemoglobin in the negative control and C_p the concentration of hemoglobin in the positive control.

Drug loading and* in vitro *release studies

Drug loading of the hydrogel matrices was carried out by mixing paracetamol or theophylline with dried matrices in powdered form, followed by the addition of a certain quantity of an appropriate solvent (maximum amount of liquid uptaken during swelling). The mixture was left to swell at room temperature for at least one hour, while the drug penetrates and/or is attached to the matrices. The concentration of the drug solution = 16 mg/mL. In the end, the drug-loaded samples were freeze-dried using a Labconco FreeZone device.

In vitro release studies have been conducted by a standard dissolution set-up.[48]

The dissolution medium was a phosphate buffer solution mimicking the pH of intestinal fluid (pH = 7.4). During dissolution testing, the medium was maintained at 37 ± 0.5 °C. Aliquots of the medium of 1 mL were withdrawn periodically at predetermined time intervals and analyzed at λ_{max} values of 242, for paracetamol, and 271 nm for teophylline respectively, using a HP 8450A UV-visible spectrophotometer. To maintain the solution concentration, the sample was reintroduced in the circuit after analysis.

Drug concentrations were calculated based on the calibration curves determined for each drug, at their specific maximum absorption wavelengths.

A simple, semi-empirical equation using the Korsmeyer and Peppas model was used to kinetically analyze the data on the drug release from the studied matrix system applied in the initial stages (approximately 60% fractional release):[49-55]

$$M_t / M_\infty = k_r t^{n_r} \quad (5)$$

where M_t/M_α represents the fraction of the drug released at time *t*, Mt and M_∞ are the absolute cumulative amounts of drug released at time *t* and at infinite time (in this case, the maximum amount released under the experimental conditions used, at the plateau of the release curves), respectively, k_r is a constant incorporating the characteristics of the macromolecular matrix and of the drug, and n_r is the diffusion exponent, indicating the release mechanism. In the equation above, a value of n_r = 0.5 indicates a Fickian diffusion mechanism of the drug from the matrix, while a value $0.5 < n_r <$

1 indicates an anomalous or non-Fickian behaviour. When n_r = 1, a case II transport mechanism is involved, while n_r > 1 indicates a special case II transport mechanism.[56-59]

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

RESULTS AND DISCUSSION

FT-IR analysis

The FT-IR spectra of C/CS hydrogels loaded with paracetamol are shown in Figure 3, and band assignment – in Table 1. In the case of C/CS based hydrogels loaded with paracetamol, the broad absorption peak at 3325 cm^{-1} indicates the hydrogen-bonded OH groups. The peak at 3112 cm^{-1} is attributed to the aromatic C-H stretching vibrations, whereas those appearing at 1611 cm^{-1} and 1564 cm^{-1} are due to primary amide bond stretching and aromatic C=C stretching vibrations, respectively. N-H bending vibrations are seen at 1508 cm^{-1}. Absorption peaks are seen, also, at 1256, 1242 cm^{-1}, due to the aromatic C-N and aliphatic C-N stretching vibrations, respectively.

The FT-IR spectra of C/CS hydrogels loaded with theophylline are shown in Figure 4, and band assignment – in Table 1. The band appearing at 3121 cm^{-1} is due to aromatic C-H stretching vibrations, whereas that appearing at 2922 cm^{-1} is due to aliphatic C-H stretching vibrations. The peaks appearing in the 2824-2712 cm^{-1} region are attributed to a $N{=}CH_3$ bond, due to the unreacted electrons from H. Absorption peaks also appeared at 1716 and 1668 cm^{-1}, as due to C=O stretching vibrations. The C-N stretching vibrations are seen at 1049 cm^{-1}, while the one appearing at 1243 cm^{-1} is due to aromatic C=O stretching vibrations.

Table 1
Characteristic bands for C/CS-based hydrogels loaded with paracetamol and theophylline

Characteristic bands of C/CS-based hydrogels loaded with paracetamol (cm^{-1})	Characteristic bands of C/CS-based hydrogels loaded with theophylline (cm^{-1})
3325	-
3112	3121
-	2922
-	2824-2712
-	1716
1611	1668
1564	-
1508	-
1256	-
1242	1243
-	1049

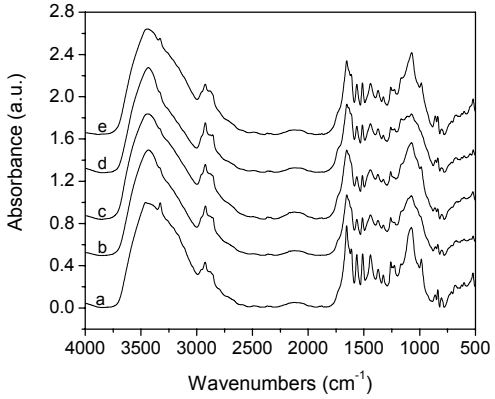

Figure 3: FT-IR spectra of (a) 90/10 C/CS, (b) 80/20 C/CS, (c) 70/30 C/CS, (d) 60/40 C/CS, (e) 50/50 C/CS hydrogels loaded with paracetamol

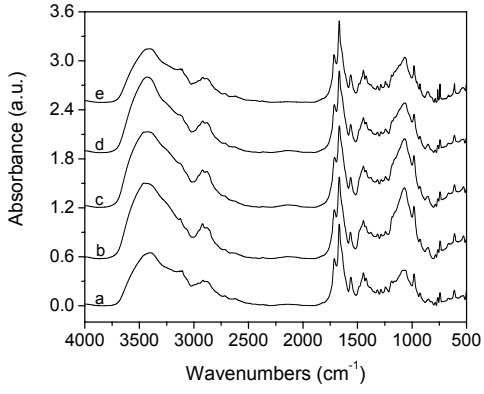

Figure 4: FT-IR spectra of (a) 90/10 C/CS, (b) 80/20 C/CS, (c) 70/30 C/CS, (d) 60/40 C/CS, (e) 50/50 C/CS hydrogels loaded with theophylline

FT-IR spectra (Figs. 3 and 4) evidenced the presence of hydrogen bonds and additional bands characteristic of paracetamol and theophylline, showing the interactions between active principles and hydrogels.

Swelling studies

Swelling of the polymeric matrices was analyzed to compare their water uptake capacities. Swelling of the matrix is an important parameter that allows to determine the release characteristics of matrix systems. A correlation of polymer swelling with drug release would help to predict the drug release mechanism for different types of polymer matrices.

Swelling studies were performed in a pH 7.4 buffer solution that simulates the pH value of the intestinal fluid at 37 °C.

The mass changes characteristic of water uptake and swelling started from the beginning and continued along the 6 h of experiment (Fig. 5). The matrices showed a high ability to swell, a swollen state being detected from the very first seconds by visual observations.

The swelling profiles (Fig. 5) show that the 50/50 C/CS hydrogel, with 50% cellulose and 50% CS, has the highest degree of swelling – 366.66 wt%. The maximum swelling degree for bicomponent C/CS hydrogels gradually decreases with increasing the percentage of cellulose, reaching – in the case of cellulose-based hydrogels – a value of 183.17 wt%.

Table 2 presents the values obtained for the kinetic parameters of swelling, k_{sw} and n_{sw}, of the C/CS hydrogels swollen in a pH 7.4 phosphate buffer solution, at 37 °C.

The values obtained for the swelling parameter, n_{sw}, in the case of C/CS hydrogels with different mixing ratios varies between 0.12 and 0.2, indicating an anomalous transport mechanism during swelling.

Matrix characterisation

Hemolysis test

The hemolysis test showed that the hemolysis percentages of all blood samples in contact with the hydrogels based on C/CS were negative. All hemolysis percentages were below 1% (Table 3), as compared to the positive control showing a value below 5%, the limit accepted for this test.[46]

The results obtained for the hemolysis test showed good biocompatibility between hydrogels and blood.

In vitro *release studies*

C/CS-based hydrogels loaded with paracetamol

The release profiles of paracetamol from C/CS hydrogels are shown in Figure 6.

The results obtained showed that the release of paracetamol from C/CS hydrogels depends on the CS content. Thus, an increase in the CS content leads to a decrease of the paracetamol percent released from 98%, from the 90/10 C/CS hydrogel, to ~40% for the 60/40 C/CS hydrogel.

The release profiles (Fig. 6) showed that, in the case of 50/50 and 60/40 C/CS hydrogels, the active principle is released at a slower release rate, compared to other mixtures, this behaviour being attributed to an increase in the chondroitin sulfate content in hydrogel composition, which probably decreased the pore size, leading to more compact hydrogels.

C/CS-based hydrogels loaded with theophylline

The release profiles of theophylline from C/CS hydrogels are shown in Figure 7.

Table 2
Values of swelling kinetic parameters of C/CS-based hydrogels

C/CS hydrogels (%)	n_{sw}	k_{sw} (min^{-nsw})
Cellulose	0.21	0.61
90/10	0.17	0.52
80/20	0.12	0.59
70/30	0.15	0.59
60/40	0.2	0.53
50/50	0.16	0.58

Table 3
Hemolysis percentage of three tested C/CS-based hydrogel formulations

C/CS hydrogels (%)	Hemolysis percentage (%)
80/20	0.0512
60/40	-0.1136
50/50	0.1561

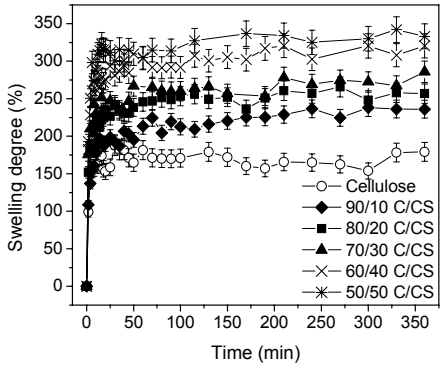

Figure 5: Swelling profiles of C/CS hydrogels with different compositions

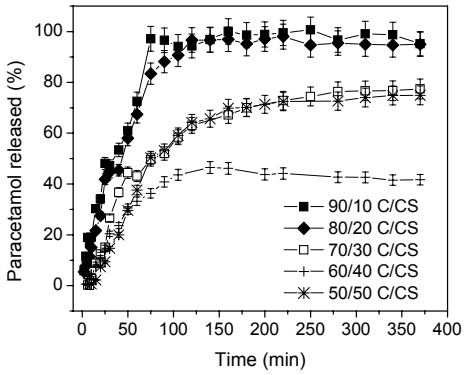

Figure 6: Release profiles of paracetamol from C/CS-based hydrogels in phosphate buffer solution (pH 7.4) at 37 °C

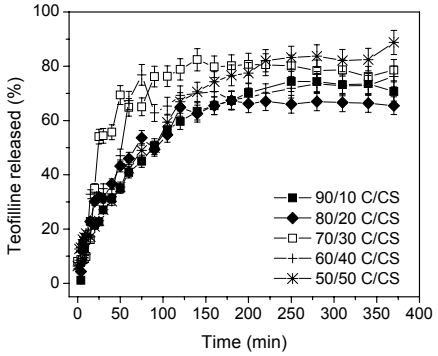

Figure 7: Release profiles of theophylline from C/CS hydrogels in phosphate buffer solution (pH 7.4) at 37 °C

The release profiles of theophylline from C/CS-based hydrogels with different compositions (Fig. 7) show that the increase of the chondroitin sulfate content in C/CS hydrogels leads to an increased percent of released theophylline, so that, in the case of 50/50 and 60/40 C/CS compositions, the percents of released drug are of approximately 83 and 75%, respectively, while, for 90/10 and 80/20 C/CS formulations, the percents of released drug are around 70 and 65%, respectively.

The differences among the release profiles of the drugs used in this study, from C/CS-based hydrogels, can be explained by the increased solubility of theophylline over paracetamol. Consequently, paracetamol solubility in water is of 0.1-0.5 g/100 mL, being much lower than in other polar solvents, such as alcohols,[60] while theophylline is classified as highly soluble, since its solubility at each pH exceeds the threshold value of 1.80 mg/mL (maximum dose divided by 250 mL).

Kinetic mechanism

C/CS-based hydrogels loaded with paracetamol

The kinetic parameters for paracetamol release in a phosphate buffer solution (pH = 7.4) from C/CS-based hydrogels with various compositions are presented in Table 4.

The CS content in C/CS hydrogels and the release rate constants are connected, so that an increase in the CS content leads to a decrease of the release rate constants.

The values of the diffusion exponent (n_r) obtained for paracetamol release from C/CS hydrogels indicates an anomalous transport mechanism for 90/10, 80/20 C/CS hydrogels, which appeared by coupling Fickian diffusion with a relaxation of the hydrogel network and a case II transport mechanism for 70/30, 60/40, and 50/50 C/CS hydrogels – in such a case, the mechanism being due to network relaxation, as the gels swell.

C/CS-based hydrogels loaded with theophylline

The kinetic parameters for theophylline released in a phosphate buffer solution (pH = 7.4) from C/CS-based hydrogels are listed in Table 5.

In this case, the values of the diffusion parameters (n_r) showed a diffusion-controlled mechanism for all compositions.

Table 4
The kinetic parameters of paracetamol released from C/CS-based hydrogels

C/CS hydrogels (%)	Korsmeyer-Peppas equation			First order kinetic model	
	n	R	k 10^4 (min^{-n})	k 10^4 (min^{-1})	R
90/10	0.73	0.99	387.6	193	0.98
80/20	0.8	0.99	260.6	118.6	0.98
70/30	1.42	0.98	13.5	73.9	0.97
60/40	1.17	0.99	32.4	58.1	0.99
50/50	1.47	0.99	8.9	54.3	0.98

Table 5
The kinetic parameters of theophylline released from C/CS-based hydrogels

C/CS hydrogels (%)	Korsmeyer-Peppas equation			Higuchi model	
	n	R	k 10^2 (min^{-n})	k 10^2 ($min^{-0.5}$)	R
90/10	0.59	0.99	3.56	5.25	0.99
80/20	0.58	0.98	4.44	5.95	0.98
70/30	0.14	0.96	6.64	3.23	0.96
60/40	0.5	0.98	2.64	2.64	0.99
50/50	0.41	0.96	5.93	4.42	0.97

CONCLUSIONS

Cellulose/chondroithin sulphate hydrogels were produced by a crosslinking technique.

The swelling degree of cellulose/chondroitin sulfate hydrogels is influenced by their compositions. Thus, an increase in the CS content in hydrogel composition leads to a higher swelling ratio.

The release profiles of the drugs from C/CS hydrogels depend on the CS content, so that an increase in the CS content leads to a decrease in the percent of released drug and to a slower release rate, in the case of paracetamol, and to an increased percent of released drug, in the case of theophylline.

The biocompatibility test was made by the hemolysis (plasma hemoglobin) technique, the results obtained showing good biocompatibility with the tested formulations.

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