XANTHAN/CHONDROITIN SULFATE HYDROGELS AS CARRIER FOR DRUG DELIVERY APPLICATIONS

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Abstract: Preparation, characterization and *in vitro* release studies of codeine from xanthan/chondroitin sulfate (X/CS) hydrogels prepared through a crosslinking technique are reported.

Swelling and drug delivery studies were conducted in phosphate buffer solution (pH=7.4) which simulates the pH of the intestinal fluid, at 37 °C.

The *in vitro* release test revealed that the percentage of codeine released in phosphate buffer solution increases with increasing the amount of chondroitin sulfate in the composition of hydrogels. The drug release behaviour of the hydrogels loaded with codeine fitted well with case II transport mechanism for all formulations.

The biocompatibility testing was made by hemolisys (plasma hemoglobin) technique.

INTRODUCTION

Hydrogels are insoluble, crosslinked polymer networks composed of hydrophylic homo- or hetero-co-polymers, which have the ability to absorb significant amounts of water. Due to their water content, hydrogels also possess a degree of flexibility very similar to natural tissue, which minimizes potential irritation to surrounding membranes and tissues.

Hydrogels have been used as prime carriers for pharmaceutical applications, predominantly as carriers for delivery of drugs, peptides or proteins. They have been used to regulate drug release in reservoir-based, controlled release systems or as carriers in swellable and swelling-controlled release devices (Peppas, 1987, 1997; Narasimhan, 1997).

Xanthan gum (Figure 1) is a high molecular weight extracellular polysaccharide, produced on commercial scale by the fermentation of gram negative bacterium *Xanthomonas campesteris.*

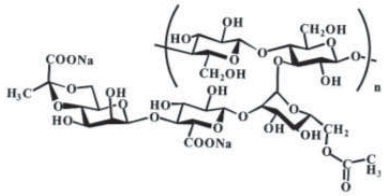

Figure 1. Xanthan structure

The molecule consists of a backbone identical to that of cellulose, with side chains attached to alternate glucose residues. It is a hydrophilic polymer, which until recently had been limited for use in thickening, suspending and emulsifying water based systems (Gwen, 1996). It appears to be gaining appreciation for fabrication of matrices, as it not only retards drug release, but also provides time-independent release kinetics with added advantages of biocompatibility and inertness, in ophthalmology (Ceulemans, 2002; Ludwig, 2005), implantology (Kumar, 2007) and tissue engineering (Silava, 2007), can also work effectively *in vivo* establishing constant drug plasma levels (Lu, 1991). It is also recommended for use in both acidic and alkaline systems. Xanthan gum has been evaluated as a hydrophilic matrix for controlled release preparation, using different model drugs including theophylline (Lu, 1991), cephalexin (Dhopeshwarkar, 1994), prednisolone (Watanabe, 1992), and indomethacin (Watanabe, 1993).

Chondroitin sulfate is an important structural component in connective tissues and cartilages. It provides compressive strength to connective tissues by regulating their water content, and possesses characteristic features, such as a high water absorption, multifunctionality and biodegradability suitable for bioapplications (Wang, 2007; Comper, 1990). In addition, the presence of active functional groups in chondroithin sulphate, such as –COO−, and $–SO_3–$, provides access to biological functionalities, which have been recently exploited in *in vivo* cartilage repair applications (Wang, 2007).

It is a copolymer of D-glucuronic acid and sulfated N-acetyl-D-galactosamine in C_4 or C_6 and belongs to the glycosaminoglycans (GAGs), which are primarily located on the surface of cells or in the extracellular matrix (Figure 2).

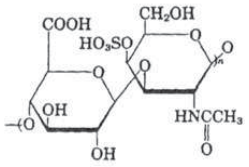

Figure 2. Chondroitin sulfate structure

Studies on chondroitin sulfate-based hydrogels were reported by Kuijpers et al. (Kuijpers, 2000) who evaluated chemically cross-linked gelatine-chondroitin sulfate hydrogels, impregnated in Dacron, as drug delivery systems for antibacterial proteins.

Varghesea et al. (Varghesea, 2008) synthesised a fast thermoresponsive hydrogel composed of poly(N-isopropylacrylamide) (PNIPAm) and chondroitin sulphate (CS) using precipitation polymerization suitable for controlled delivery applications of cationic drugs

The aim of this study is to combine the properties of X and CS in mixed hydrogels in order to obtain new materials for medical and pharmaceutical applications. With this aim have been also evaluated the biocompatibility of X/CS hydrogels and their applicability in codeine delivery systems, for achieving a controlled release profile suitable for oral and subcutaneous administration so the investigation were made in phosphate buffer solution (pH=7.4).

Codeine or metylmorphine (Figure 3) is an opiate used for its analgesic, antitussive and antidiarrheal properties. It is one of the most effective orally-administrated opioid analgesics and has a wide safety margin.

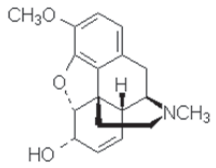

Figure 3. Chemical structure of codeine

It may be mentioned that these types of formulations based on xanthan and chondroitin sulfate have not been studied or proposed as hydrogels for biomedical applications.

MATERIALS AND METHODS

Xanthan gum (X) was purchased from Sigma Aldrich and chondroitin sulfate (CS) from Roth.

Xanthan/chondroitin sulphate (X/CS) hydrogels were produced by a crosslinking technique in various mixing ratios: 90/10, 80/20, 70/30, 60/40, 50/50 X/CS in presence of epichlorhydrin, as crosslinking agent, purified by washing with warm water and dried for 10 hours by using a LABCONCO FreeZone device.

In this study were used 80/20, 60/40 and 50/50 X/CS formulations.

Codeine was purchased from Centre for the Study and Therapy of Pain (CSTD), "Gr.T.Popa" Medicine and Pharmacy University, Iasi.

The *kinetics of the swelling* were carried out for all formulations by direct immersion in a phosphate buffer solution which simulate intestinal fluid (pH=7.4). The hydrogels samples were maintained for 24 hours at 37 ^{0}C, periodically removed from the solution, gently wiped with a soft tissue to remove surface water, weighed and than placed back into the vessel as quickly as possible. The swelling degree at equilibrium was calculated according to the equation (1).

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

where W_t is the weight of the samples after swelling in phosphate buffer solution 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: (Berens, 1978)

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

where W_t and W_{eq} represent the amount of phosphate buffer solution, 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 takes into account the type of solvent transport. Eq. (2) applies to initial states of swelling (swelling degree less than 60%) and linearity is observed when log Ft as a function of log t is represented.

The *drug loading* of the hydrogel matrices was carried out by mixing codeine with dried matrices in powdered form and then a certain quantity of the appropriate solvent (maximum amount of liquid uptaken during swelling) was added and left to swell at room temperature at least 25 ^{0}C, while the drug penetrates and/or attached into matrices. The drug-loaded samples were freeze-dried using a Labconco FreeZone device.

During the *in vitro drug release* studies, at predetermined time intervals, samples of 1 ml were withdrawn from the release medium and concentrations of codeine was determined at λ_{max} value 284 nm using a HP 8450A UV–visible spectrophotometer. In order to maintain the solution concentration the sample is reintroduced in the circuit after analyzing.

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) (Higuchi, 1961; Ritger, 1987; Chen, 2007; Peppas, 1985, 1986, 1989) (3):

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

where M_t/M_α represents the fraction of the drug released at time t, 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 is indicative of the release mechanism. In the equation above a value of $n_{sw}/n_r = 0.5$ indicates a Fickian diffusion mechanism of the drug from matrix, while a value $0.5 < n_{sw}/n_r < 1$ 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 (Katime, 2001; Korsmeyer, 1984; Serra, 2006; Berg, 2006).

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

Percent hemolysis test

Blood was obtained from healthy patients drawn by routine venipuncture from the antecubital vein in tubes containing EDTA. The blood was stored refrigerated for no more than 2 days until its use. Each hydrogel preparation was tested with blood from a single patient. Prior to hemolysis test all the hydrogel samples were sterilized by ultraviolet light trans-illumination for 2 min. Distilled water was used as positive control and plasma separated from the same blood as 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 hydroxymethyl amynomethane – Sigma-Aldrich, (Tris) (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 hydroxymethyl amynomethane (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 (ISO, 2002).

The method used for measuring plasma hemoglobin concentration in all the specimens was the polychromatic method of Noe et al. (Noe, 1984). Absorbance was measured at 380 nm, 415 nm and 470 nm and the formula used for evaluation was:

$$C(mg/L) = 1.65\ mA_{415} - 0.93\ mA_{380} - 0.73\ mA_{470} \quad (4)$$

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

$$\text{hemolysis percent } (\%) = (C - C_n)/(C_p - C_n) \times 100 \quad (5)$$

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.

RESULTS AND DISCUSSION

Swelling kinetic studies

Swelling studies were performed in phosphate buffer solution of pH = 7.4 which simulate the pH of intestinal fluids at 37 ^{0}C, the results being presented in table 1.

The values presented in table 1 show that the 50/50 X/CS hydrogel, with 50% xanthan and 50% CS, has the highest degree of swelling – 1642 wt% comparing with 80/20 X/CS composition with a swelling degree of 1134.3 wt%. It can be observed that all tested formulations are superabsorbants and the increase of CS content significantly increases the swelling degree of X/CS hydrogels. The high water quantity uptake and faster swelling can be attributed to the existence of groups with negative charges in CS structure, such as $–COO^-$ and $–SO_3^-$, which helps the gels to swell highly, conferring a high concentration of negative charge in the regions that contain them (Comper, 1990).

Table 1. The influence of composition, swelling degree and the values n_{sw} and k_{sw} of the X/CS hydrogel

Hydrogels	Xanthan (wt%)	CS (wt%)	Q_{max} (%)	n_{sw}	k_{sw} (min^{-nsw})
80/20 X/CS	80	20	1134.354	0.14	0.70
60/40 X/CS	60	40	1495.874	0.12	0.73
50/50 X/CS	50	50	1642.025	0.06	0.83

Table 1 presents the values obtained for the kinetic parameters of swelling, k_{sw} and n_{sw}, of the X/CS hydrogels swollen in a pH 7.4 phosphate buffer solution, at 37 °C.

The values obtained for swelling parameter, n_{sw}, in case of X/CS hydrogels with different mixing ratios, varies between 0.06 – 0.14 indicating an anomalous transport mechanism and the values of swelling rate constant, k_{sw}, are increasing with increasing of CS content.

In vitro codeine release studies

The release profiles of codeine from X/CS hydrogels are shown in figure 4.

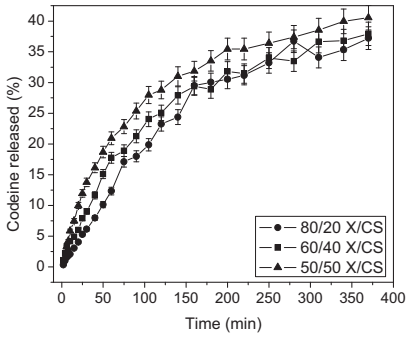

Figure 4. Release profiles of codeine from X/CS-based hydrogels with different compositions, in phosphate buffer solution (pH=7.4) at 37 ^{0}C

The release profiles showed that the percentage of codeine released increases with increasing the amount of chondroitin sulfate in the composition of hydrogels. Thus, the 50/50 X/CS hydrogel released approximately 43 % codeine comparative with 80/20 X/CS composition witch release about 38 % within 400 minutes.

The kinetic parameters for codeine released in from X/CS-based hydrogels with various compositions are presented in table 2.

Table 2. The kinetic parameters of codeine released from X/CS hydrogels

Hydrogels	First order kinetic model	
	k 10^{-3} (min^{-1})	R
80/20 X/CS	3	0.99
60/40 X/CS	1.9	0.99
50/50 X/CS	4.83	0.99

From the obtained release profiles the diffusion exponent n_r was calculated according to Eq. 3 (Table 2). The release of codeine in phosphate buffer solution is described as a case II transport mechanism (zero order kinetics) for all formulations.

Hemolysis test

The hemolysis test showed that the hemolysis percentages of all the blood samples in contact with the hydrogels based on X/CS were negative. All hemolysis percentages were less than 1% (table 3) as compared to the positive control, value which is below 5% limit admitted for this test (ISO, 2002).

Table 3. Hemolysis percentage of the X/CS hydrogel formulations tested

Hydrogels	Hemolysis percentage (%)
80/20 X/CS	-0.036
60/40 X/CS	0.0381
50/50 X/CS	-0.0611

The results obtained for hemolysis test, for both types of formulations, showed a good biocompatibility between hydrogels and blood.

CONCLUSIONS

Xanthan/chondroitin sulfate hydrogels were produced by a crosslinking technique in presence of epichlorhydrin, as crosslinking agent.

The swelling of xanthan/chondroitin sulfate hydrogels shows a relationship with CS concentration, so an increase of CS content in hydrogels composition leads to a higher swelling ratio.

The results of controlled release tests showed that an increase of CS content leads to an increase of codeine percent released.

The release of codeine from X/CS hydrogels was described as case II transport mechanism for all formulations.

The biocompatibility testing was made by hemolysis (plasma hemoglobin) the results obtained showed a good biocompatibility with tested formulations.

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