A DRUG DELIVERY SYSTEM BASED ON STIMULI-RESPONSIVE ALGINATE/N-ISOPROPYLACRYL AMIDE HYDROGEL

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The release kinetics of paracetamol and theophylline from pH- and temperature-responsive hydrogel matrices prepared from sodium alginate and N-isopropylacryl amide crosslinked with N,N'-methylenebisacrylamide was investigated. The drug-loaded hydrogel matrices were studied by FT-IR spectroscopy. As the release kinetics of drugs is related to the kinetics of solvent diffusion into the hydrogels, studies on the swelling behavior become of great importance for appreciating the drug release from hydrogel matrices.

Swelling and release kinetic studies were performed at 37 °C in acid medium, at pH = 2.2, for simulating the pH of the gastric fluid in the stomach. The drug release kinetic data were analyzed and the release parameters were estimated by fitting the experimental data to three different theoretical models. The results obtained showed nearly similar release profiles for theophylline and paracetamol over a 9-day interval. The values obtained indicated an anomalous transport mechanism for both model drugs.

Keywords: alginate, N-isopropylacryl amide, stimuli-responsive, drug carrier, kinetic parameters

INTRODUCTION

Crosslinked hydrogel networks are widely investigated as therapeutic devices, scaffolds for tissue engineering and controlled/sustained drug release carriers, taking advantage of their special properties, such as high sorption capacity, hydrophilic surface and low interfacial tension in contact with body fluids, minimal irritation of the surrounding tissue,[1-4] good biocompatibility and carrier properties, and high permeability for nutrients and metabolites.[5-7]

Hydrogel-based drug delivery devices belong to the group of swelling-controlled drug delivery systems.[8] In contact with an aqueous medium, the polymer network swells and, as water penetrates inside the glassy network, the drug trapped inside the hydrogel gets dissolved and starts diffusing out of the network. Three driving forces contribute to this phenomenon, and namely, a penetration concentration gradient, a polymer stress gradient and the osmotic forces. In the case of non-swelling-controlled delivery systems, the relaxation rate of the polymer is very slow in comparison with the water transport inside the hydrogel, which follows Fickian diffusion. However, in many swelling-controlled delivery systems, an anomalous transport mechanism, characterized by an intermediate Fickian diffusion and Case II transport, has been observed.[9]

The release rate of drugs from a hydrogel polymer network is affected by certain factors – the swelling behavior of the hydrogel, pore size (*i.e.,* mesh size) of the polymer network, the affinity between drugs and the hydrogel constituting polymers, concentration within the gel, crosslinking density, hydrated/dehydrated state of the gel and *in vivo* degradation of the hydrogel.[10,11] The drug release from the

hydrophilic matrices is generally controlled by both diffusion and erosion phenomena, the latter dominating the release rate of low aqueous solubility drugs.[12] The physically entangled polymer systems slowly dissolve and release the drug simultaneously with polymer dissolution, while chemically crosslinked polymer gels degrade and the drug release rate depends on the rate of polymer degradation.[13] The solubility and permeability of the model drug molecules/ions selected for the release studies also represent important factors influencing the release behavior, because, once dissolved, the drug diffuses through the gel/film. This mass transport step can be more or less hindered due to the different sizes of the drug molecules and to their interactions with the macromolecules.[14]

The drug release behavior can be modulated by a careful selection of the loaded gel properties, which may be suitable for pharmaceutical as well as for cosmetic and food applications. To deliver drugs to the patient in a controlled rate, enabling an appropriate drug concentration and prolonged effectiveness, new types of matrices with high performance properties were prepared.

Hydrogels with *stimuli-responsive properties* are frequently applied as drug release carriers. Because of their intelligent design, these gels can sense changes in the surrounding environment, therefore these features are exploited to modify the drug release rates. Since swelling/shrinking of these hydrogels is controlled by external stimuli, it is important to adjust the dynamic swelling response for predicting the drug release.

Recent researches have been focused on developing new polymeric matrices as drug carriers, exhibiting both a responsive behavior to external stimuli (*e.g.*, temperature and pH) and biodegradability. These systems are obtained mainly by coupling a biopolymer with a thermo-sensitive macromolecule.[15-17]

Among thermo-sensitive polymers, *poly(N-isopropylacrylamide)* (PNIPAAm), one of the most widely studied, exhibits phase transition at lower critical solution temperature (LCST) – of around 32 °C –, in water,[18] close to the physiological temperature. Due to this property, PNIPAAm-based hydrogels swell below the LCST and collapse in an aqueous environment above this value of temperature, being thus suitable for controlled drug delivery. The LCST of PNIPAAm-based hydrogels can be modulated to increase by adding a hydrophilic component, or to decrease with a hydrophobic one.[19]

Nowadays, due to their biocompatibility, biodegradability and non-toxicity, polysaccharides, such as cellulose derivatives or those obtained from marine algae, are frequently used to prepare hydrogels, membranes, films or microcapsules carriers for biologically active agents.

Alginate (Alg), a natural polysaccharide derived from brown seaweeds, composed of D-mannuronic acid and D-guluronic acid residues, is a biodegradable polymer widely used in biomedical applications. Alginate hydrogels are pH-sensitive and biocompatible, with a relatively low cost and wide applicability as biomaterials.[15,20] These hydrogels can be obtained mainly by ionic or covalent crosslinking. Most of the recent studies were focused on the development of alginate beads prepared on the basis of sodium alginate ability to form gels with Ca^{2+} ions, which can be used for encapsulation of bioactive agents like cells, enzymes, proteins, vaccines, etc. Calcium alginate beads loaded with drugs like imipramine, propranolol, vitamin C, sodium diclofenac were investigated and some disadvantages, such as low drug entrapment efficiency and rapid release of the loaded drugs, were evidenced. It was also observed that the loading efficiency is lower in the case of water-soluble than of water-insoluble drugs, due to drugs leakage from alginate beads with high porosity, so that the matrix based only on calcium alginate was considered as less suitable as a controlled release system for oral delivery, for example.[21]

A few studies are dedicated to the different approaches made to obtain alginate microcapsules or macroscopic hydrogels by covalently crosslinking alginate, rather than by the above-discussed ionic crosslinking. The formation of an acetal-linked network through an acid-catalyzed acetalization reaction of alginate hydroxyls with bifunctional glutaraldehyde,[22] as well as the covalent

crosslinking of alginate with PEG-diamines having different molecular masses,[23] were reported.

For improving the release properties of alginate-based matrices, systems with various compositions were tested. Polyelectrolyte complex membranes based on poly(ethylene imine) (PEI) treated alginate beads were tested[21] for prolonged release of propranolol hydrochloride as a model drug in a simulated intestinal fluid (SIF, phosphate buffer, pH 6.8). Hydrogels consisting of alginic acid and recombinant human serum albumin were evaluated as sustained drug release carriers for salicylic acid, benzoic acid, o-anisic acid and dibucaine chloride.[10] Bovine serum albumin (BSA) release from pH-sensitive hydrogel matrices obtained from methylcellulose and alginate blended with distinct salts ($CaCl_2$, Na_2HPO_4, NaCl) was studied in simulated gastric and intestinal media, showing a high loading efficiency and preserving the protein drug bioactivity, while the hydrogel blended with NaCl demonstrated potential application as targeted drug delivery in the intestine.[24]

Theophylline is frequently selected as a model drug in drug release experiments, because, according to the Biopharmaceutical Classification System, it belongs to Class I (high solubility-high permeability) drug, being well absorbed in the whole GI tract.[25] However, some authors noticed a moderate solubility of theophylline in water (8.3 g/L at 25 °C), while recent studies demonstrated[11] that co-solvents such as water/ethanol may enhance drug solubility, theophylline solubility in ethanol being of 12.5 g/L at 25 °C.

Paracetamol, as well as theophylline, were reported[14] to exhibit quite similar solubilities in various media at 37 °C, and namely: theophylline – 14 mg/mL in 0.1 N HCl and 11 mg/mL in phosphate buffer, pH 7.4; paracetamol – 19 mg/mL in 0.1 N HCl and 18 mg/mL in phosphate buffer, pH 7.4, respectively.

The *in vitro* release of theophylline from physically crosslinked *beta*-lactoglobulin (BLG) protein gels showed that the drug release depended mainly on the *solubility* of the drug in the medium (especially at low pH), on the physical state of the gel (either hydrated or dry) and the nature of the external solvent.[11,26] The drug release data obtained in the study of the *in vitro* and *in vivo* characteristics of chitosan/Kollicoat SR30D film-coated tablets of theophylline for targeted colonic delivery fitted well into the Korsmeyer–Peppas equation, indicating that the drug release was controlled by polymer relaxation.[27] The theophylline transport mechanism from pH-sensitive poly(acrylic acid-g-ethylene glycol) (P(AA-g-EG)) polymer networks was studied and the results obtained showed that, in most release cases, the drug release mechanism was anomalous (non-Fickian), indicating that, under certain conditions, such systems may provide release characteristics approaching a zero-order release. The pH of the dissolution medium appeared to have a strong effect on the drug transport mechanism.[9]

Polymeric systems exhibiting a stimuli-responsive behavior (thermo- and/or pH-sensitive) and their potential application in biomedical fields were obtained as semi-IPN hydrogels by associating[28] the PNIPAAm gels with calcium alginate (alginate-Ca^{2+}) or as comb-type macroporous hydrogels by grafting PNIPAAm on the surface or bulk of the pH-responsive alginate, using an aqueous $CaCl_2$ solution to crosslink the alginate.[29]

The present paper deals with the *in vitro* evaluation of a dually responsive (temperature and pH) hydrogel prepared from sodium alginate and poly(N-isopropylacrylamide) covalently crosslinked with N,N`-methylene-bis-(acrylamide), as a drug carrier for paracetamol and theophylline.

EXPERIMENTAL

Materials

The *alginic acid* (Alg) used in the experiments, extracted from brown algae (M_w = 48 000-186 000, $\eta_{red}^{25^oC}$ = 2.41 ml·g^{-1}, c = 0.2 g/dL), was purchased from Fluka. The monomer N-isopropylacryl amide (NIPAAm) was employed as received from Aldrich (97%). The crosslinking agent N,N'-methylene bis-acrylamide (BIS, Fluka), the initiator, potassium persulphate (KPS, Merck) and the accelerator, N,N,N',N'-tetramethylethylenediamine (TEMED, Fluka) were all used as received from the indicated suppliers. Analytical-grade reagents and twice distilled water were used for all experiments.

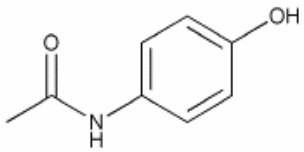
The *preparation* of the PNIPAAm/Alg stimuli-responsive IPN hydrogels, described elsewhere,[30]

started from the synthesis of N-isopropyl-acrylamide–itaconic acid copolymer hydrogels, described by Krušić and Filipović[31] by radical crosslinking copolymerization at 25 °C, considering also the synthesis of carboxymethylcellulose (CMC)–sodium alginate (Na-Alg) hydrogels by crosslinking with methylenebisacrylamide,[32] which supports the assumption that alginate might be crosslinked with NIPAAm by a different approach from that using Ca^{2+} ions, as well-known. Therefore, by combining the above-described methods, new NIPAAm-alginate gels were obtained, as a result of two simultaneously coupled reactions of radical copolymerization and crosslinking.

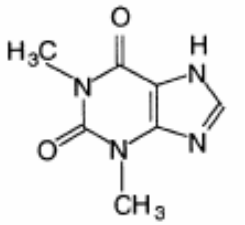
PNIPAAm – alginate sIPN hydrogels with distinct NIPAAm-to-alginate percentage mass ratios were prepared, namely: 100/0, 99/1, 99/5, 90/10, 85/15, 80/20 and 75/25 NIPAAm/Alg. Further increase of the Alg amount hindered the hydrogel formation reaction. The NIPAAm aqueous solution was mixed with a solution of sodium alginate obtained by dissolving the required amount of alginic acid in NaOH 0.5 M. To this mixture, potassium persulphate (KPS) and N,N'-methylenebisacrylamide (BIS) were added, up to a final concentration of BIS (w/v) of 2.0 wt% (2 BIS), 3.0 wt% (3 BIS), 4.0 wt% (4 BIS), 5.0 wt% (5 BIS), 6.0 wt% (6 BIS) and 7.0 wt% (7 BIS), followed by N,N,N',N'-tetramethylethylenediamine (TEMED) addition. The mixture obtained was vigorously stirred and then the final solution was kept undisturbed for a while, to remove the trapped air bubbles. The crosslinking reaction takes place at 25 °C, gel formation being observed within about 2 h. The gel samples obtained are stored for about 4 days to stabilize and reach equilibrium, then repeatedly washed with twice distilled water to remove the residual monomer or the non-crosslinked compound. The samples are then lyophilized using a LABCONCO FreeZone freeze-drying system, dehydrated porous aerogels being thus obtained. The obtained reaction yield was of about 65%. The presence of the alginate in the hydrogel structure has been proved[30] by elemental analysis, FT-IR and RAMAN spectroscopy, thermogravimetry (TG/DTG), scanning electron microscopy (SEM), swelling behavior, etc. The results are similar to those of other studies on radical crosslinking of systems containing alginate.[33,34]

The NIPAAm/Alg hydrogel samples with a composition of 75/25 NIPAAm/Alg, crosslinked with 7.0 wt% BIS, were selected for the drug release experiments, because the results obtained in previous studies[35-37] showed a slower drug release compared to that of other compositions tested.

The drug model was anhydrous crystalline theophylline, soluble in water to some extent (1 g/120 mL), supplied by BASF, while paracetamol was purchased from Rhodia Europe (France).



Paracetamol structure



Theophylline structure

Paracetamol (acetaminophen) (P), a derivative of para-aminophenol (N-acetyl-p-aminophenol), showing analgesic and antipyretic action, is one of the most widely used drugs, recently considered for the therapy of neurodegenerative diseases (such as Alzheimer's disease) characterized by oxidant and inflammatory stress.[38] Unlike analgesics, such as aspirin or NSAIDs, acetaminophen is not associated with gastrointestinal (GI) tract irritation and has no vaso-constrictive effects, which recommends it as an efficient alternative in the treatment of acute, mild or moderate migraine attacks.[39] Paracetamol is an important drug for the control of pain in dentistry and several studies have confirmed its effectiveness. According to the Biopharmaceutical Classification System (BCS), it is a class I (highly soluble and highly permeable) drug. Paracetamol is a non-steroidal anti-inflammatory drug with potent antipyretic and analgesic actions, but very weak anti-inflammatory activity. When administered to humans, it reduces the levels of prostaglandin metabolites in urine, yet without reducing the synthesis of prostaglandins by blood platelets or stomach mucosa. Paracetamol is also a weak *in vitro* inhibitor of both cyclooxygenase (COX)–1 and COX-2, rapidly absorbed from the gastrointestinal tract and distributed throughout most body tissues. The plasma half-life is of 1 to 4 h,[40] but it may be increased by liver damage and following overdosage. Paracetamol is

eliminated mainly by liver metabolism (conjugation) and subsequent renal excretion of metabolites. Approximately 85% of an oral dose appears in the urine within 24 h of administration, mostly as a glucuronide conjugate, with small amounts of other conjugates and unchanged drug.

Theophylline is a methylxanthine drug useful in the treatment of respiratory diseases, such as nocturnal asthma, the main actions of theophylline involving relaxation of bronchial smooth muscle, increase of blood pressure and renal blood flow, and some anti-inflammatory effects.

Investigation methods

Studies on the *swelling* of NIPAAm/Alg gels were performed by mass measurements in a pH 2.2 buffer solution, simulating the gastric fluid in the stomach, at 37 °C. The equilibrium swelling ratio (Q_{eq}) was calculated according to equation (1):

$$Q_{eq}(\%) = \frac{(W_{eq} - W_d)}{W_d} \times 100 \quad (1)$$

where W_{eq} and W_d are the mass of the equilibrium swollen sample and the initially dried hydrogel, respectively.

The morphology of the 75/25 NIPAAm/Alg hydrogels crosslinked with 2.0 and 7.0 wt% BIS was studied by *scanning electron microscopy (SEM),* using a VEGA II TESCAN scanning electron microscope. Prior to analysis, the samples were fractured in liquid nitrogen and were coated with a silver/carbon thin layer inside a cathodic spraying device. *Drug loading* of the hydrogel matrices was carried out by mixing the paracetamol and theophylline drugs with the dried hydrogel in powder form, after which a certain amount of solvent – buffer solution of pH 2.2 – was added and left to swell at room temperature for 1 h, while the drugs penetrated into and/or were attached to the matrices.

The drug-loaded samples, freeze-dried using a Labconco FreeZone device, were studied by *FT-IR spectroscopy* to evidence the potential interactions between the drugs and the hydrogel network, using a FT-IR spectrophotometer Digilab, Scimitar Series, USA. The dried formulations were ground to powder, mixed with KBr, compressed into a tablet and then recorded in the 500-4000 cm^{-1} range, at 4 cm^{-1} resolution.

The *release profiles* of paracetamol and theophylline from the 75/25 NIPAAm/Alg hydrogel sample crosslinked with 7.0 wt% BIS were determined based on the standard dilution test and on UV-VIS spectroscopy measurements performed with a Hewlett Packard 8540A type UV-VIS spectrophotometer. A calibration curve was first drawn for each drug, using a particular absorption band, λ = 242 nm, for paracetamol, and λ = 271 nm for theophylline, respectively, as well as standard solutions of active principles in acidic solution of pH = 2.2, with concentrations varying from 10^{-5} to 10^{-2} g/L. During the drug release experiments, a 2 mL sample was taken from the release medium at predetermined time intervals, and the paracetamol and theophylline concentration values in the release medium were determined, after which each amount was carefully reintroduced in the release medium.

To evaluate the drug release kinetics, the data were further analyzed with equation (2), proposed by Korsmeyer and Peppas:[41]

$$M_t / M_\infty = k \cdot t^n \quad (2)$$

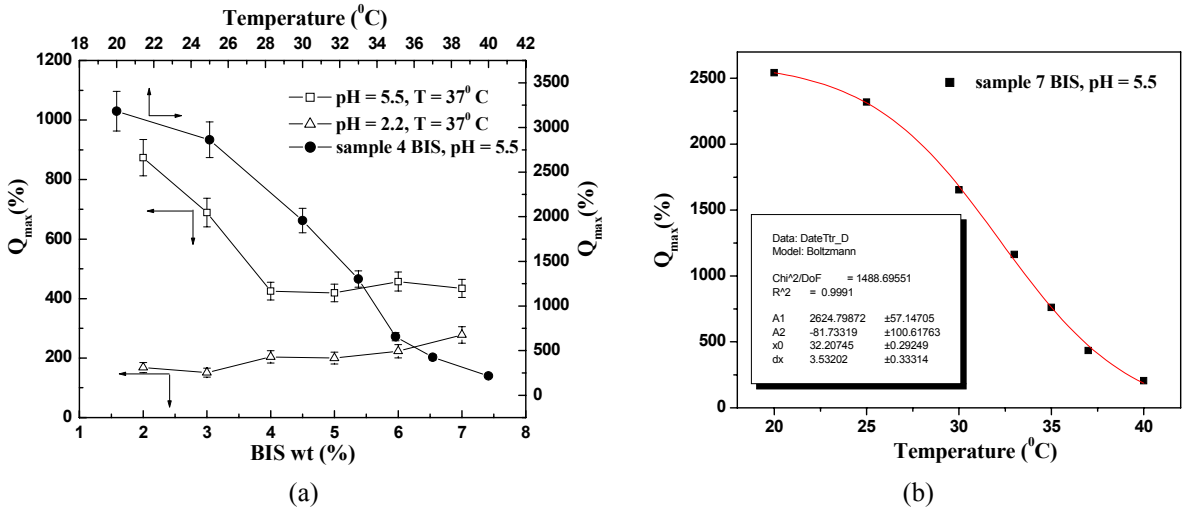
where M_t/M_α represents the fraction of the drug released at time t, k is a constant incorporating the characteristics of the macromolecular network system and n is the diffusion exponent, which is indicative of the release mechanism. In the kinetic equations, a value of n = 0.5 indicates a Fickian diffusion mechanism of the drug in the hydrogels, while a value $0.5 < n < 1$ indicates an anomalous or non-Fickian behavior. When n = 1, a case II transport mechanism is involved, while $n > 1$ indicates a special case II transport mechanism.[5,41]

RESULTS AND DISCUSSION

The *swelling measurements* performed on NIPAAm/Alg hydrogels under various conditions demonstrated their dual temperature and pH-responsive behavior, with an anomalous diffusion mechanism, as observed in some previous studies of ours.[30,35-37,42] The crosslinking degree influenced the behavior of the samples in a different manner, as depending on the solution pH and temperature (Fig. 1).

The *thermo-responsive behavior* of the samples was evidenced by measurements performed at temperatures below and also above the LCST of PNIPAAm (32 °C), when the hydrophobic interaction and collapse of the PNIPAAm chains determines a fast shrinking of the gels. The maximum swelling ratio dependence on temperature for the 75/25 NIPAAm/Alg hydrogel samples crosslinked with 4.0 wt% BIS (Fig. 1a) and 7.0 wt% BIS (Fig. 1b) was examined at pH = 5.5. It can be noticed that the swelling ratio decreased rapidly as the temperature increased toward LCST. Traditionally, in terms of swelling ratio changes, the phase separation temperature or LCST is regarded

as the temperature at which the phase separation degree, swelling ratio change *vs*. temperature change (dQ/d*T*), is the highest, or the temperature at which the swelling ratio of the hydrogel decreases most dramatically. The results obtained allowed the calculation (using an ORIGIN 6.1 program) of the volume phase transition temperature by the Boltzmann function (Fig. 1b), which had similar values of 32.2 °C and 32.6 °C, for both samples, obtained with different crosslinking amounts. Therefore, the amount of crosslinking agent in the samples does not significantly influence the transition temperature.



(a) (b)

Figure 1: Variation of the maximum swelling degree with pH and temperature for NIPAAm/Alg samples with different crosslinking agent concentrations: 2-7 wt% BIS (a) and temperature dependence for sample 7 BIS at pH = 5.5 (b)

Measurements performed at different pH values showed a *pH-sensitive behavior* of the samples. All NIPAAm/Alg hybrid hydrogels exhibit a lower swelling ratio in an acidic medium, which increases with an increase in pH. In an acidic medium (*i.e*., pH = 2.2), the swelling ratios of hydrogels are of 150-280%, while in water (pH = 5.5), the swelling ratio rises to 450-870% at 37 °C (further increase was hindered by the collapse of the PNIPAAm chains at this temperature) and, at 20 °C, the values reach up to 2500-3500%, which shows the versatility of the samples behavior according to changes in both pH and temperature. This increase in the swelling capacity at an increased pH can be attributed to the free carboxylic acid groups in the ionized Alg, which will break the hydrogen bonding and generate electrostatic repulsion among the Alg macromolecules. This repulsive force will push the network chain segments apart and attract more water into the hydrogel, which will result in a higher swelling ratio.

With the progressive decrease of the pH value from pH = 5, electrostatic repellence is suppressed, enhanced intermolecular hydrogen bonds and possible entanglements emerge and association structures are formed,[43] which explains the decrease in the swelling degree at a low pH. At a pH value of 6.6, the carboxylic acid groups are slightly dissociated. Two types of interactions play an important role, namely charge repulsion between the dissociated carboxylic groups and the hydrogen bonding formed between the carboxylic acid and the ionized carboxylate groups.[44] With the decrease of pH, the number of dissociated carboxylic groups in alginate chains decreases, which makes alginate lose its hydrophilicity to some extent. When some dissociated carboxylic groups in the alginate chains are gradually protonated, hydrophobic segments appear in the alginate chains. Cao *et al*.[33] showed that, depending on the pH of the medium, the alginate molecules can form hydrophilic–hydrophobic aggregates in an

aqueous solution. They postulated this dependence of "aggregation" on pH as a sign of the self-assembly caused by the partial protonation of the dissociated carboxyl groups in the alginate main chain. As the pH value decreases from 5.0 to 3.0, the hydrophobic segments in the alginate chains increase, and the hydrophilic segments decrease. Meanwhile, the weakening of the mutual repulsion of the ionized carboxyl groups promotes the development of intermolecular hydrogen bonds and possible entanglements.

At pH = 5.5, a significant decrease in the Q_{max} values with the increase in the crrosslinking agent (BIS) content was observed from 2 BIS to 4 BIS while, at higher amounts of crosslinking agent, no significant changes could be observed. At an acidic pH value (pH = 2.2), the influence of the BIS content was quite insignificant (Fig. 1a). The slight increase in the swelling degree with increasing the BIS content being justified by the morphology observed for the gel samples, evidenced through SEM, the sample with a higher BIS content (7 BIS) has a slightly increased pore number than that with a low BIS content (2 BIS), so that water sorption is slightly increased (Fig. 2a, b).

The 75/25 NIPAAm/Alg sample crosslinked with 7.0 wt% BIS was selected for drug release experiments, as our previous studies[35-37] showed a slower sustained release than in other compositions tested, thus appearing as most suitable as a drug release carrier.

The *FT-IR spectra* obtained for the NIPA Am-Alg-Theophylline (T) and NIPAAm-Alg-Paracetamol (P) systems are presented in Figures 3 and 4, in the 2500-4000 and 900-1900 cm^{-1} spectral regions, respectively. Band assignment was done according to literature data.[45-50] (Tables 1 and 2).

Table 1
Assignments of FT-IR spectra vibration bands for components and NIPAAm-Alg-T system

Bands of NIPAAm-Alg hydrogel (cm^{-1})	Bands of theophylline (T) (cm^{-1})	Bands of NIPAAm-Alg-T (cm^{-1})	Band assignments
3430, s, broad band		3430, s, broad band	-OH groups
	1717, s	1707, sh	-C=O and -C=N – purine ring
	1667, s		C=C, -C=O (O=C-N) – purine ring
1645, s		1655, s	overlapped amide I + COO^-, asymmetric + C-N-H
	1563, m		C=C, -C=N, C-N-H – purine ring
1545, s		1545, s	amide II, N-H
1455, m		1453, m	COO^-, symmetric
1378, 1359, m		1378, 1359, m	isopropyl group
	1303, 1273, 1232, w	1272, 1227, w	N-H, -C=N – purine ring
	948, m; 905, w	965, 907, w	purine ring

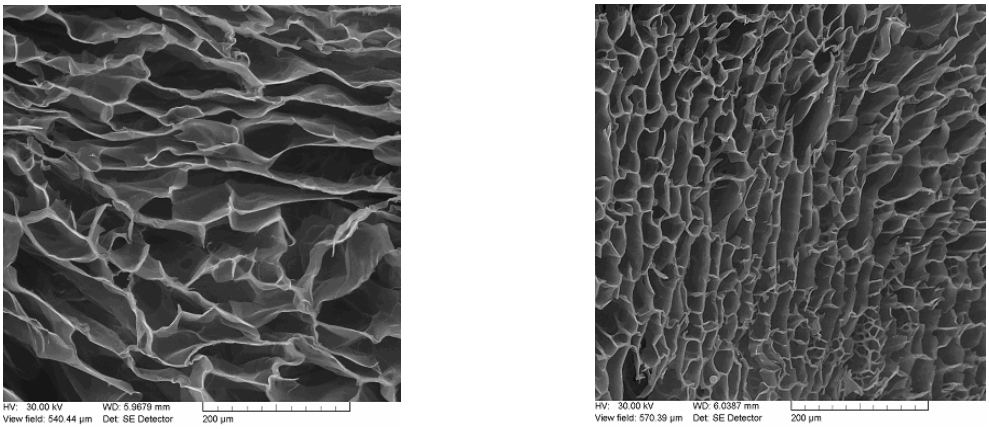
s – strong, m – medium, w – weak, sh – shoulder

Table 2
Assignments of FT-IR spectra vibration bands for components and NIPAAm-Alg-P system

Bands of NIPAAm-Alg hydrogel (cm^{-1})	Bands of paracetamol (P) (cm^{-1})	Bands of NIPAA m-Alg-P (cm^{-1})	Band assignments
3400, s, broad band		3404, s, broad band	-OH groups
	3284, s (sharp peak)		N-H and OH phenolic

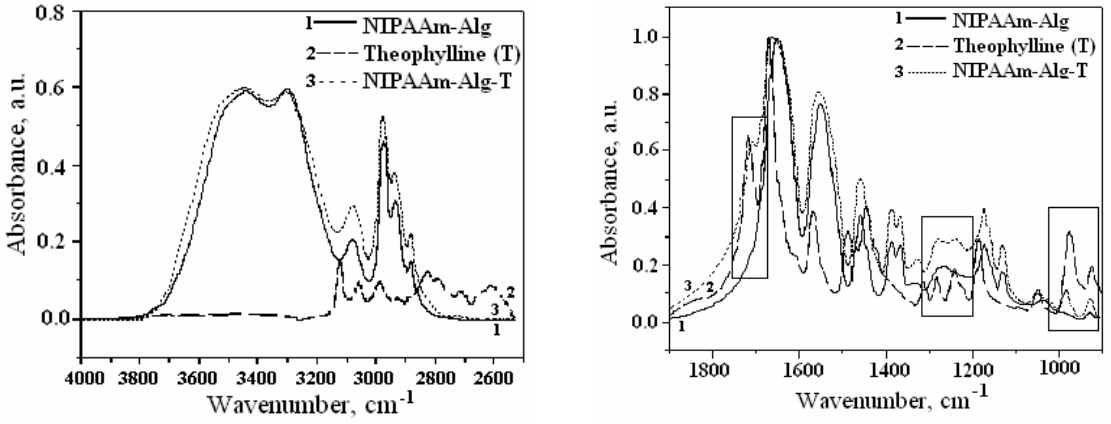
			groups
	3110, s (broad band)		-OH phenolic groups and phenolic ring
1656, s		1656, s	overlapped amide I + COO^-, asymmetric
	1662, s (sharp peak)		C=O (C-N-H)
	1618, m		C=C, aromatic ring
1556, s		1558, s	amide II, N-H
	1518, s	1526, sh	C-N-H, C=C – aromatic ring
1473, m		1473, m	COO^-, symmetric
	1452, s (sharp peak)		CH_3, C_6H_5, NH
1400, 1382, m		1400, 1382, m	isopropyl group
	1384, 1340, m		C-H
	1272, 1243, s (double peak)	1274, m (broad peak)	C=O (C-N-H), OH phenolic ring
	1035, w		C-N
	980, w	930, w	substituted aromatic ring

s – strong, m – medium, w – weak, sh – shoulder



(a) (b)

Figure 2: SEM micrographs of 75/25 NIPAAm/Alg hydrogel sample crosslinked with 2.0 wt% BIS (a) and 7.0 wt% BIS (b)



(a) (b)

Figure 3: FT-IR spectra of NIPAAm-Alg/Theophylline (T) system: (a) 2500-4000 cm^{-1} and (b) 900-1900 cm^{-1}

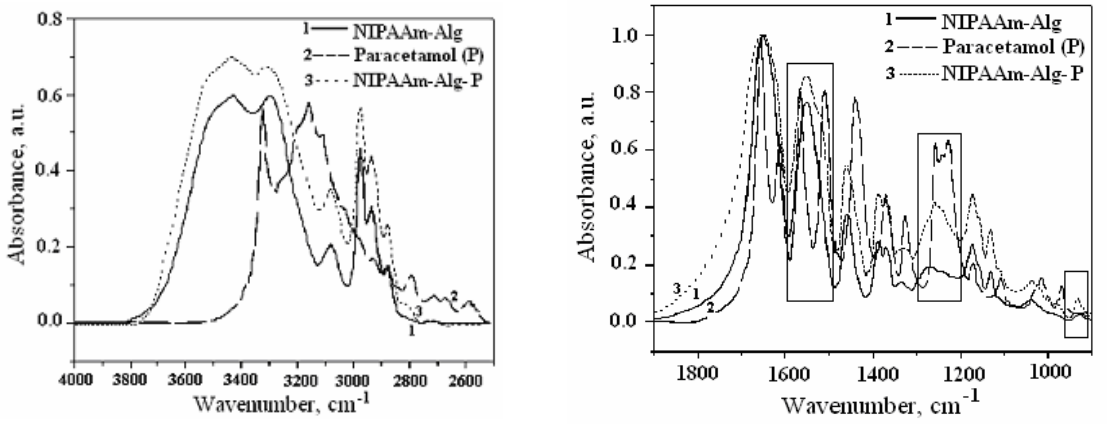


Figure 4: FT-IR spectra of NIPAAm-Alg/Paracetamol (P) system: (a) 2500-4000 cm^{-1} and (b) 900-1900 cm^{-1}

The presence of theophylline in the NIPAAm-Alg-T system is noticeable (Fig. 3a and b) only in the fingerprint region, namely through the shoulder at 1707 cm^{-1} attributed to the –C=O (positions 2 and 6) and -C=N bond in the purine ring assigned to the sharp peak at 1717 cm^{-1} from the theophylline spectra, the bands from the 1272-1227 cm^{-1} region, which can be attributed to N-H and -C=N bonds from the purine ring – corresponding to those of theophylline at 1273 and 1232 cm^{-1}, and the weak peaks at 965 and 907 cm^{-1}, which can be assigned to the substituted purine ring and are slightly shifted compared to those of pure theophylline, at 948 and 905 cm^{-1}. Therefore, some interactions through hydrogen bonding between the -C=O and N-H groups from the purine ring of theophylline and carboxyl or amide groups from the NIPAAm/Alg hydrogel matrices are possible.

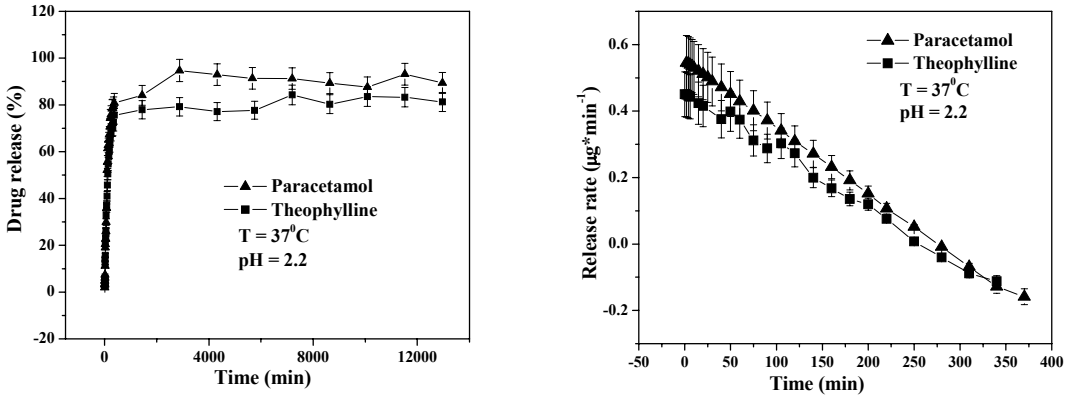
The FT-IR spectra of the NIPAAm-Alg-P system (Figs. 4a and b) are more complex. The paracetamol presence in the system can be identified only through the shoulder at 1526 cm^{-1}, assigned to the C-N-H and C=C bonds from the aromatic ring, which corresponds to the sharp peak at 1518 cm^{-1} from the paracetamol spectra, the broad peak at 1274 cm^{-1}, attributed to phenolic OH, which gave a double peak at 1272 and 1243 cm^{-1} for paracetamol, and the band at 930 cm^{-1} corresponding to the one at 980 cm^{-1} in the spectra of paracetamol, which can be assigned to the di-substituted aromatic cycle. Weak matrix-drug interactions, involving the OH and NH groups of the drug and complementary groups in the matrix, are also possible.

The *drug release profiles and release rates* of paracetamol and theophylline from the 75/25 NIPAAm/Alg hydrogel network at 37 °C in pH 2.2 buffer solution are presented in Figure 5a and b. Almost similar release profiles and release rates of theophylline and paracetamol were obtained for 9 days, the release percent for theophylline being of ~80%, while that for paracetamol was of ~90% from the loaded drug quantity of $9 \cdot 10^{-4}$g. The similarity of the release profiles is not unexpected, taking into account their very close solubility. The release rate linearly decreases during the release period.

The drug release kinetic parameters (diffusion exponent *n* release and release rate constant *k*) for theophylline and paracetamol from the NIPAAm/Alg hydrogel matrices were calculated. The values obtained from the Korsmeyer-Peppas equation are presented in Table 3. The values of the release parameter *n* indicated an anomalous transport mechanism for both loaded drugs, which can be explained by coupling Fickian diffusion with the relaxation of the hydrogel network.

The kinetic data of the drug release were analyzed by fitting the experimental data to the theoretical models – Higuchi ($n = 0.5$) and to the first-order kinetic model ($n = 1$), on estimating the release rate constant (Table 4). The results obtained indicate a better correlation of the experimental values of the release parameters for both paracetamol and theophylline with the first-order kinetic

model, the correlation factor taking the highest values R ≈ 0.98. For both drugs under investigation, the release rate constant takes close values when applying the Korsmeyer-Peppas equation and the first order kinetic model while, for the Higuchi model, the values are quite different and R takes lower values (of 0.96), indicating that this model is not suitable for describing the release of these drugs from the NIPAAm/Alg gel matrix.



(a) (b)

Figure 5: Release profiles (a) and release rates (b) of paracetamol and theophylline from 75/25 NIPAAm/Alg hydrogel samples crosslinked with 7.0 wt% BIS at 37 °C in a pH 2.2 buffer solution

Table 3
Kinetic parameters of paracetamol and theophylline release from 75/25 NIPAAm/Alg hydrogel by the Korsmeyer-Peppas equation

Drugs	75/25 NIPAAm/Alg hydrogel			
	n	R	$k \cdot 10^{-3}$ (min $^{-n}$)	R
Paracetamol	0.94	0.98	6.93	0.99
Theophylline	0.88	0.98	9.03	0.99

Table 4
Kinetic parameters of paracetamol and theophylline release from 75/25 NIPAAm/Alg hydrogel, by the Higuchi model and first-order kinetic model

Drugs	75/25 NIPAAm/Alg hydrogel			
	Higuchi model		First-order kinetic model	
	$k \cdot 10^{-2}$ (min$^{-0.5}$)	R	k 10^{-3} (min^{-1})	R
Paracetamol	3.486	0.96	5.39	0.98
Theophylline	4.9	0.96	5.4	0.98

The NIPAAm/Alg gels may be assigned to the group of swelling-controlled delivery systems, exhibiting an anomalous transport mechanism, with k values of 5-9·10^{-3} min^{-1} for both studied drugs. The results obtained, agreeing with previous studies performed on theophylline or paracetamol release from various other matrices, also ascertain the fact that the investigated gels appear as suitable matrices for prolonged drug therapy, with a stimuli-responsive behavior.

CONCLUSIONS

Hydrogels prepared from sodium alginate and NIPAAm were evaluated as drug carriers for paracetamol and theophylline. Swelling measurements evidence the temperature- and pH-responsive behavior of the NIPAAm/Alg

hydrogels. The drug loading into the hydrogel matrices was proved by FT-IR spectroscopy, which evidenced some interactions between the components, probably through hydrogen bonding. The drug release profiles were very similar for paracetamol and theophylline, the values obtained for the kinetic parameters indicating an anomalous transport mechanism. The experimental data correlate well with the first-order kinetic model.

The relaxation of the hydrogel network at equilibrium swelling provides a better drug entrapment inside the matrices and its subsequent slower release, the system obtained being suitable for sustained drug release.

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REFERENCES

[1] H. Park and K. Park, *Pharm. Res.*, **13**, 1770 (1996).
[2] K. Smetana Jr., *Biomaterials*, **14**, 1046 (1993).
[3] J. M. Anderson, *Eur. J. Pharm. Biopharm.*, **40**, 1 (1994).
[4] J. M. Anderson and J. J. Langone, *J. Control. Release*, **57**, 107 (1999).
[5] I. Katime, R. Novoa and F. Zuluaga, *Eur. Polym. J.*, **37**, 1465 (2001).
[6] K. H. Bae, J. J. Yoon and T. G. Park, *Biotechnol. Progr.*, **22**, 297 (2006).
[7] R. P. Dumitriu, A. M. Oprea, I. E. Răschip and C. Vasile in "Environmentally Degradable Materials Based on Multicomponent Polymeric Systems", C. Vasile and G. E. Zaikov (Eds.), Brill Academic, Leiden, Netherlands, 2009, *in press*.
[8] P. Colombo, P. Santi, R. Bettini, C. S. Brazel and N. A. Peppas, in "Handbook of Pharmaceutical Controlled Release Technology", D. L. Wise, L. Brannon-Peppas, A. M. Klibanov, R. S. Langer, A. G. Mikos, N. A. Peppas, D. J. Trantolo, G. R. Wnek and M. J. Yaszemski (Eds.), New York, Dekker, 2000, pp.183-209.
[9] L. Serra, J. Doménech and N. A. Peppas, *Biomaterials*, **27**, 5440 (2006).
[10] D. Tada, T. Tanabe, A. Tachibana and K. Yamauchi, *Mat. Sci. Eng. - C*, **27**, 870 (2007).
[11] T. T. Reddy, L. Lavenant, J. Lefebvre and D. Renard, *Biomacromolecules*, **7**, 323 (2006).
[12] K. Tarara, K. Yamanoto and T. Nishihata, *Int. J. Pharm.*, **133**, 17 (1996).
[13] M. S. Latha and A. Jayakrishnan, *J. Pharm. Pharmacol.*, **46**, 8 (1994).
[14] S. Muschert, F. Siepmann, B. Leclercq, B. Carlin and J. Siepmann, *Eur. J. Pharm. Biopharm.*, **72**, 130 (2009).
[15] J. Shi, N. M. Alves and J. F. Mano, *Macromol. Biosci.*, **6**, 358 (2006).
[16] S. Y. Kim, S. M. Cho, Y. M. Lee and S. J. Kim, *J. Appl. Polym. Sci.*, **78**, 1381 (2000).
[17] E. Marsano, E. Bianchi and A. Viscardi, *Polymer*, **45**, 157 (2004).
[18] H. G. Schild, *Prog. Polym. Sci.*, **17**, 163 (1992).
[19] D. Schmaljohann, J. Oswald, B. Jorgensen, M. Nitschke, D. Beyerlein and C. Werner, *Biomacromolecules*, **4**, 1733 (2003).
[20] A. D. Augst, H. J. Kong and D. J. Mooney, *Macromol. Biosci.*, **6**, 623 (2006).
[21] A. Halder, S. Maiti and B. Sa, *Int. J. Pharm.*, **302**, 84 (2005).
[22] A. W. Chan, R. A. Whitney and R. J. Neufeld, *Biomacromolecules*, **9**, 2536 (2008).
[23] P. Eiselt, K. Y. Lee and D. J. Mooney, *Macromolecules*, **32**, 5561 (1999).
[24] H.-F. Liang, M.-H. Hong, R.-M. Ho, C.-K. Chung, Y.-H. Lin, C.-H. Chen and H.-W. Sung, *Biomacromolecules*, **5**, 1917 (2004).
[25] M. Han, Q. L. Fang, H. W. Zhan, T. Luo, W. Q. Liang and J. Q. Gao, *J. Pharm. Sci.*, **98**, 2626 (2009).
[26] E. Rytting, K. A. Lentz, X.-Q. Chen, F. Qian and S. Venkatesh, *AAPS J.*, **7**, E78 (2005).
[27] L.-F. Fan, W. He, Y.-Z. Chang, B. Xiang, Q. Du, F. Wang, M. Qin and D.-Y. Cao, *Int. J. Pharm.*, **375**, 8 (2009).
[28] M. R. de Moura, F. A. Aouada, M. R. Guilherme, E. Radovanovic, A. F. Rubira and E. C. Muniz, *Polym. Test.*, **25**, 961 (2006).
[29] J. H. Kim, S. B. Lee, S. J. Kim and Y. M. Lee, *Polymer*, **43**, 7549 (2002).
[30] R. P. Dumitriu, G. R. Mitchell and C. Vasile, *Polym. Int.*, submitted, 2009.
[31] M. Kalagasidis Krušić and J. Filipovic, *Polymer*, **47**, 148 (2006).
[32] A. Pourjavadi, S. Barzegar and G. R. Mahdavinia, *Carbohyd. Polym.*, **66**, 386 (2006).
[33] Y. Cao, X. Shen, Y. Chen, J. Guo, Q. Chen and X. Jiang, *Biomacromolecules*, **6**, 2189 (2005).
[34] J. Kadokawa, S. Saitou and S. Shoda, *Carbohyd. Polym.*, **60**, 253 (2005).
[35] R. Dumitriu, C. Vasile, G. Mitchell and A. M. Oprea, in "Monomers, Oligomers, Polymers, Composites and Nanocomposites Research: Synthesis, Properties and Applications", R. A. Pethrick, G. E. Zaikov and J. Pielichowski (Eds.), Nova Science Publishers, 2009, pp. 401-409.

[36] R. P. Dumitriu, A. M. Oprea and C. Vasile, *Solid State Phenomena*, **154**, 17 (2009).
[37] C. Vasile, R. P. Dumitriu, C. N. Cheaburu and A. M. Oprea, *Applied Surface Science*, **256**, 65 (2009).
[38] D. Tripathy and P. Grammas, *Microvasc*. *Res*., **77**, 289 (2009).
[39] H. C. Diener and V. Limmroth, *Curr*. *Med*. *Res*. *Opin*., **17**, 13 (2001).
[40] N. Qin, S. P. Zhang, T. L. Reitz, J. M. Mei and C. M. Flores, *J*. *Pharmacol*. *Exp*. *Ther*., **315**, 1298 (2005).
[41] R. W. Korsmeyer and N. A. Peppas, *J*. *Control*. *Release*, **1**, 89 (1984).
[42] R. P. Dumitriu and C. Vasile, in *Procs*. *Int*. *Conf*. *(Bio)Degradable Polymers from Renewable Resources*, Vienna, Austria, 2008, pp. 240-244.
[43] J. Yang, S. Chen and Y. Fang, *Carbohyd*. *Polym*., **75**, 333 (2009).
[44] H. Bu, A. L. Kjřniksen and B. Nyström, *Eur*. *Polym*. *J*., **41**, 1708 (2005).
[45] R. M. Silverstein, G. C. Bassler and T. C. Morrill, "Spectrometric Identification of Organic Compounds", 5th Ed., New York, John Wiley & Sons Inc., 1991.
[46] C. H. Willits, J. C. Decius, K. L. Dille and B. E. Christensen, *J*. *Am*. *Chem*. *Soc*., **77**, 2569 (1955).
[47] P. J. A. Ribeiro-Claro and A. M. Amado, *Spectrochim*. *Acta*, *Part A*, **61**, 2796 (2005).
[48] A. L. Abuhijleh, H. A. Ali and A.-H. Emwas, *J*. *Organomet*. *Chem*., doi: 10.1016/j.jorganchem. 2009.07.031 (2009).
[49] S. Talegaonkar, A. Y. Khan, R. K. Khar, F. J. Ahmad and Z. I. Khan, *Iran*. *J*. *Pharm*. *Res*., **6**, 95 (2007).
[50] M. L. Ramos, J. F. Tyson and D. J. Curran, *Anal. Chim. Acta*, **364**, 107 (1998).