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Biocompatible and Biodegradable Alginate/Poly(*N*-isopropylacrylamide) Hydrogels for Sustained Theophylline Release

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ABSTRACT: Mixed-interpenetrated polymeric networks based on sodium alginate (ALG) and poly(*N*-isopropylacryl amide) (PNIPAAm) covalently cross-linked with *N,N*'-methylenebisacrylamide are studied for their biocompatibility, nontoxicity, and biodegradability aiming their application in drug delivery. The presence of drug-polymeric matrix interactions and the distribution of the drug in the polymeric network for theophylline-loaded ALG/PNIPAAm hydrogels are also investigated by spectroscopic and microscopic methods. The quantitative evaluation of theophylline loaded hydrogels performed by NIR-CI technique shows a better drug entrapment and a higher homogeneity of the samples with increased alginate content. The thermal behavior of the hydrogels is significantly modified by theophylline presence. The application of the ALG/PNIPAAm hydrogels as carriers for sustained drug release formulations was assessed by the theophylline release tests performed both by *in vitro* and *in vivo* studies. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40733.

KEYWORDS: drug delivery systems; polysaccharides; stimuli-sensitive polymers

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INTRODUCTION

Designing new polymeric materials with tailored properties for controlled drug release applications is a promising strategy to develop new pharmaceutical formulations with reduced administration frequency, noninvasive for the patients. In recent years, the development of new, intelligent drug delivery systems (DDS) which are able to detect and treat a damaged tissue, to capture and isolate undesirable pathogenic agents and trigger an action that will release drugs, proteins or therapeutic agents[1] at a desired rate or at a target site in the body has become the main goal of many scientists. Systems of this nature are able to maximize the efficacy of therapeutic agents, thereby improving patients quality of life.

Various crosslinked polymeric hydrogel as responsive systems were recently developed and investigated in particular as controlled DDS, able to deliver drugs at controlled rate for predefined periods, enabling an appropriate drug concentration for prolonged effectiveness, possessing a good mechanical strength under physiological conditions.[1–3]

Among the factors influencing the release rate of a drug from a polymeric hydrogel such as swelling behavior, mesh size, crosslinking density, hydration, drug concentration within the gel, the degradation behavior is also important.[4,5] For specific application of polymeric materials in drug delivery it is required that the polymer to degrade under physiological conditions and slowly release the encapsulated drug by a certain degradation mechanism. The biodegradable hydrogels can be degraded by bulk degradation or surface erosion mechanisms.[6,7] Bulk degradation is characterized by hydrolysis of chemical bonds of the polymer chain in bulk material, while degradation by surface erosion mechanism is characterized by loss of the material from the surface only, the later one being beneficial for delivery of molecules at a constant rate and for the maintaining of the mechanical and structural integrity of the material during a significant period of degradation.[8] Degradable hydrogel networks offer the same advantages as normal hydrogels, but also they contain bonds that can be cleaved hydrolytically or enzymatically, either in the polymer backbone or in the crosslinks formed by gel preparation.[9] Because the gels obtained from

natural polymers exhibit some limitations, recent efforts are focused on developing hybrid hydrogel systems, derived from both natural and synthetic materials, which display the most advantageous properties.[10]

One promising approach for drug delivery application is to combine the merits of both bioresponsive and biodegradable hydrogels.

Stimuli-responsive hydrogels have been comprehensively investigated in the literature for applications in the biomedical field. Of particular interest are the polymeric systems that mimic biological ones obtained by combining biopolymers such as polysaccharides with synthetic polymers to create dual stimuli-responsive materials, which exhibit thermo- and pH-sensitive behavior.[11–14]

Obtaining and characterization of dual temperature and pH-responsive hydrogels prepared from sodium alginate (ALG) and poly(*N*-isopropylacryl amide) (PNIPAAm) covalently crosslinked with *N*,*N*′-methylenebisacrylamide have been previously described.[15,16] In continuation of our previous research on these hydrogels,[15–18] in this work their biocompatibility, toxicity, and biodegradability was evaluated, completed by a thorough investigation on their ability to release drugs in a controlled manner which was tested by *in vivo* and *in vitro* studies using theophylline as model drug. These investigations demonstrated the ability of ALG/PNIPAAm hydrogels as carriers for sustained drug release formulations.

Theophylline is a dimethylated xanthine drug with similar action to caffeine and theobromine, being a potent bronchodilator widely used in the treatment of acute asthma.[19] The main actions of theophylline involve direct relaxation of bronchioles and the muscles of pulmonary sanguine vessels having a relaxing and bronchodilator effect upon smooth muscles.[20,21] Previous studies on theophylline actions showed that it may have anti-inflammatory and immunomodulatory effect when given in low doses, which makes the drug easier to use, with less side effects and interaction problems. Also, theophylline may increase responsiveness to corticosteroids.[22] Free theophylline concentration in plasma is normally 10 to 20 μM and rarely exceeds 50 μM during therapy.[19] Theophylline is characterized by a narrow therapeutic index within the therapeutic serum concentration ranges of 5–20 $\mu g\ mL^{-1}$,[23] it is metabolized extensively by the liver mainly by the microsomal system, while the metabolites are eliminated with the urine.[24]

Up to now, after our knowledge, previous studies performed using theophylline as model drug[25–28] emphasized mainly on the *in vitro* and/or *in vivo* release experiments, the specific properties of the drug-loaded matrices being overlooked. Therefore the physicochemical characteristics of the theophylline-loaded samples including structure, morphology, drug-polymeric matrix interactions and thermal behavior were investigated. Another objective of this work is to correlate the drug release with hydrogels' biodegradation behavior, as this area received a limited attention. The investigations on the biodegradability of the PNIPAAm/ALG polymeric hydrogels revealed their degradation by surface erosion and the theophylline release studies demonstrated their sustained drug release ability.

EXPERIMENTAL

Materials

Alginic acid from brown algae (*Macrocystis pyrifera* kelp) with a weight-average molecular weight M_w of 117,000 g mol^{-1}, (Fluka) and N-isopropylacryl amide (NIPAAm) (Aldrich) were used as feed materials to prepare the hydrogels used as polymeric matrices for drug entrapment by crosslinking with *N*,*N*′-methylene *bis*-acrylamide (Fluka) (BIS) following a methodology previously described, involving simultaneously radical polymerization and covalent crosslinking.[15] Three hydrogel samples with distinct NIPAAm-to-alginate percentage weight ratios (99/1, 80/20, and 75/25 NIPAAm/ALG) were tested. It is known that *N*-isopropylacrylamide monomer is toxic for human body, thus it is compulsory its careful removal. Instead poly(*N*-isopropylacrylamide) is biocompatible with many polymers and also with living organisms.[29–31] The prepared PNIPAAm/ALG hydrogels were intensively purified by dialysis (molecular weight cut-off 14,000 Da) against twice distilled water for 3 days at room temperature, until the measured pH in the washing water was ~7, before tested for their toxicity and biocompatibility. Afterwards the gel samples were lyophilized using a freeze-drying system (LABCONCO FreeZone) before investigation. The enzyme used for the enzymatic degradation study was alginate-lyase (Sigma, 29,000 units g^{-1} solid) from *Flavobacterium* sp. (E.C. 4.2.2.3.). Anhydrous crystalline theophylline supplied by BASF was chosen as model drug for the release experiments, purity 97 to 100% w/w. Twice-distilled water was used and all other reagents were of analytical grade.

Ethics Statement for Experiments with Animals

The experiments with animals were performed according to the institutional guidelines for care and use of laboratory animals and the experimental research protocol was approved by the Animal Research Ethics Committee from "Gr. T. Popa" University of Medicine and Pharmacy of Iasi, Romania (official paper no. 15559/21.09.2010) in rigorous accordance with international ethical regulations on laboratory animal work.[32,33]

The laboratory animals were euthanized by exposure to ether vapors overdose in closed containers, as ether is one of the inhalant anesthetics considered acceptable conditionally. The experiments were performed in compliance with safety regulations.

The selection of the administration path for the *in vivo* studies was performed based on the characteristics related to each medium.

Toxicity and Biocompatibility Studies of PNIPAAm/ALG Hydrogels

For the toxicity and biocompatibility tests, the drug unloaded hydrogel samples have been intraperitoneally delivered to mice with the aim to achieve a direct contact of the administered hydrogels with the internal tissues, avoiding interaction with gastric fluids which allowed in this manner a fast response and change in various parameters and therefore the most reliable results can be obtained.

Toxicity Study. White Swiss strain male mice weighing between 20 and 25 g (Cantacuzino Institute, Bucharest, Romania) were maintained under standard laboratory conditions (12/12 h

Table I. Hematological and Immune Systems Parameters (Mean ± Standard Deviation STD) at Mice i.p. Injected with 99/1, 80/20 and 75/25 of PNIPAAm/ALG Hydrogel Suspensions

		Mice group intraperitoneally injected with PNIPAAm/ALG hydrogels with various composition		
Hematological parameter	Control mice group	99/1	80/20	75/25
White blood cells (WBC) ($\times 10^9$ L^{-1})	5.64 ± 0.13	5.62 ± 0.11	5.62 ± 0.11	5.63 ± 0.14
Polymorphonuclear cells (PMN) ($\times 10^9$ L^{-1})	1.51± 0.06	1.52 ± 0.05	1.50 ± 0.06	1.48 ± 0.07
Lymphocytes ($\times 10^9$ L^{-1})	3.68 ± 0.11	3.63 ± 0.07	3.65 ± 0.07	3.71 ± 0.09
Monocytes ($\times 10^9$ L^{-1})	0.35 ± 0.05	0.36 ± 0.02	0.35 ± 0.03	0.35 ± 0.04
Eosinophils ($\times 10^9$ L^{-1})	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.04 ± 0.02
Basophils ($\times 10^9$ L^{-1})	0.05 ± 0.03	0.052 ± 0.02	0.052 ± 0.02	0.045 ± 0.03
Polymorphonuclear cells (PMN) (%)	26.82 ± 0.97	27.08 ± 0.46	26.8 ± 0.84	26.34 ± 0.85
Lymphocytes (%)	65.26 ± 1.05	65.05 ± 1.28	65.52 ± 1.43	65.93 ± 1.05
Monocytes (%)	6.23 ± 0.71	6.44 ± 0.34	6.29 ± 0.54	6.23 ± 0.7
Eosinophils (%)	0.78 ± 0.36	1 ± 0.28	0.98 ± 0.28	0.69 ± 0.34
Basophils (%)	0.93 ± 0.47	0.93 ± 0.45	0.92 ± 0.45	0.81 ± 0.51
Red blood cells (RBC) ($\times 10^9$ L^{-1})	9.39 ± 0.06	9.39 ± 0.08	9.38 ± 0.06	9.36 ± 0.02
Hemoglobin level (HGB) (g dL^{-1})	11.47 ± 0.05	11.38 ± 0.19	11.47 ± 0.05	11.48 ±0.04
Hematocrit level (%)	41.03 ± 0.04	40.98 ± 0.04	41 ± 0.06	41.07 ± 0.21
NBT test (%)	13.83 ± 0.75	13.83 ± 0.41	13.83 ± 0.75	14 ± 0.63
Platelets (PLT) ($\times 10^9$ L^{-1})	253 ± 38.82	252.9 ± 5.43	252.9 ± 10.35	252.9 ± 12.06
Immune system parameter				
Serum opsonic capacity (*S. aureus* × 1000 mL^{-1})	771.67 ± 58.45	766.67 ± 55.74	768.33 ± 56.72	770 ± 59.33
Phagocytic capacity of peritoneal macrophages (*S. aureus* × 1000 mL^{-1})	716.67 ± 51.64	711.67 ± 51.93	715 ± 50.5	716.67 ± 51.64
Bactericidal capacity of peritoneal macrophages (*S. aureus* × 1000 mL^{-1})	696.67 ± 8.16	693.33 ± 8.16	695 ±8.37	696.67 ± 8.16
Splenic T lymphocytes (%)	12.5 ± 0.55	12.67 ± 0.52	12.33 ± 0.52	12.5 ± 0.55

light/darkness, 23 ± 2°C) for one week before and throughout the experiment having access at food and water *ad libitum*. Each hydrogel composition (99/1, 80/20 and 75/25 NIPAAm/ALG) was tested on five mice. The tested hydrogels have been delivered intraperitoneally as suspensions. The animals were carefully observed for any behavioral changes and mortality immediately after dosing and during the subsequent 14 days. At the end of the observational period, the animals were euthanized with an overdose of ethyl ether.

Biocompatibility Study. Hydrogel samples were administered to white Swiss strain male mice weighing between 25 and 35 g treated as before, which were randomly assigned to four groups of seven animals each, denoted as groups I to IV: group I (control) received physiological serum, while group II received 99/1 of NIPAAm/ALG, group III received 80/20 of NIPAAm/ALG, and group IV received 75/25 of NIPAAm/ALG. The tested PNIPAAm/ALG samples were intraperitoneally delivered as suspensions, in a single daily dose of 160 mg/kg body for 14 consecutive days, following the European Community guidelines. The control group received a daily dose of physiological serum (0.2 ml /10 g body). Each injection was performed through a syringe equipped with a gauge 21G needle.

The biological samples, such as blood, intraperitoneal liquid and spleens were harvested in order to determine the hematology and biochemistry parameters.

Blood samples were collected in EDTA tubes from all the animals in each group by cardiac puncture after general anesthesia of animals with ethyl ether for estimation of hematological parameters such as white blood cells (WBC), polymorphonuclear cells (PMN), red blood cells (RBC), hemoglobin level (HGB), hematocrit level, platelets (PLT) using an HEMAVET 950 FS device from Drew Scientific Group (England).

To determine the phagocytosis capacity of peripheral blood neutrophils the blood was collected into heparinized test tubes and evaluated by Nitroblue tetrazolium (NBT) test, revealing the metabolic changes that occur during the process of phagocytosis correlated quantitatively.[34] Briefly, the cells were incubated for

Table II. Serum Biochemical Parameters (Mean ± Standard Deviation STD) at Mice Treated with 99/1, 80/20, and 75/25 of PNIPAAm/ALG Hydrogel Suspensions for 14 Days

		Mice group intraperitoneally injected with PNIPAAm/ALG hydrogels with various composition		
Serum biochemical parameter	Control mice group	99/1	80/20	75/25
AST (U L^{-1}) mean ± STD	23.17 ± 1.17	3 ± 2	33.5 ± 1.87	33.67 ± 1.97
ALT (U L^{-1}) mean ± STD	73.33 ± 1.75	101.67 ± 1.86	102 ± 1.79	102.17 ± 1.94
LDH (U L^{-1}) mean ± STD	497.5 ± 3.33	502.83 ± 2.32	503.17 ± 2.32	503.33 ± 2.34

30 min at 37°C and then aliquots of NBT solution were added into the cells and incubated for 1 h at 37°C. Blood smears were observed through a light microscope in immerse magnification to determine the percentage of positively stained neutrophils and showed active involvement in phagocytosis.

Serum opsonic capacity and peritoneal macrophages function (phagocytosis and bactericidal activity) were assessed after 14 days of treatment using the Shortmann and Palmer technique.[35] Bacteria used were *Staphylococcus aureus*, grown in broth overnight, centrifuged and washed twice in phosphate-buffered saline. The final suspensions were adjusted with a nephelometer to a standard concentration of approximately 1×10^6 colony forming units/mL. Spleenic lymphocytes with rosetting capacity were also assessed after 14 days of treatment.[36]

The collected serum was used to estimate biochemical parameters such as aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and lactate dehydrogenase (LD) using a CORMAY ACCENT 200 device (Poland).

Statistical analysis of the data was performed using the GraphPad Prism software (version 5.0). Statistical significance of differences between data was evaluated by one-way ANOVA using the Tukey test. A value of $P < 0.05$ was considered significant. All data are presented as mean ± standard deviation.

Biodegradability of PNIPAAm/ALG Hydrogels

The biodegradability of PNIPAAm/ALG gels was studied *in vitro* by chemical degradation performed in PBS (pH 7.4) and by enzymatic degradation in the presence of alginate-lyase from *Flavobacterium* sp. (E.C. 4.2.2.3.) 0.4 mg mL^{-1}.

The alginate-lyase was chosen for enzymatic degradation study due to its specificity for alginate component of the hydrogels, allowing us to observe comparatively the effect of degradation in PBS and in the enzyme presence.

The biodegradability was evaluated by residual mass measurements, ATR-FTIR spectroscopy and SEM images observation before and after degradation.

Dried pre-weighed gel samples were incubated in phosphate-buffered saline (PBS pH 7.4) and in alginate-lyase solution (0.4 mg mL^{-1} in PBS) respectively. The biodegradation studies were carried out at 25°C for 24 h. Samples were removed at specified time intervals from solutions, washed with twice distilled water thoroughly and dried by freeze-drying followed by vacuum drying for 48 h. The degradation level was determined based on the residual mass (RM %), calculated with the following eq. (1):

$$\mathrm{RM}(\%) = \frac{W_r}{W_i} \times 100 \quad (1)$$

where W_i and W_r are the initial mass and the residual mass of the dried gel samples, at a moment "*t*."

Infrared spectra were collected on wet gel samples freshly removed from the degradation medium by attenuated total reflectance (ATR-FTIR) method using a Bruker Vertex 70 Spectrometer equipped with a 45° angle diamond. Three recordings were performed for each sample. Spectra were measured at a resolution of 4 cm^{-1} in the range 4000 to 800 cm^{-1} for a total of 64 scans.

The morphology of the hydrogels after degradation was examined using a VEGA II TESCAN SEM microscope. The gel samples dried in the fully-hydrated state were frozen in liquid nitrogen, freeze-fractured and subsequently coated with gold.

Preparation of Theophylline-Loaded Hydrogel Samples

The PNIPAAm/ALG hydrogel samples loaded with theophylline for *in vitro* release study were obtained by mixing the drug with the dried matrices in powder form (5 wt % drug reported to dry hydrogel) followed by swelling at room temperature for 1 h in a certain quantity of solvent for each composition studied which corresponds to the maximum quantity of liquid uptake measured during swelling experiments—while the drug penetrates and/or attached into matrices. The solvents were the buffer solutions used for release study. Finally the theophylline-loaded samples were freeze-dried using a Labconco FreeZone device.

For the *in vivo* experiments the drug loading was performed by a diffusion filling method allowing the equilibrium partitioning of drug into the hydrogel[37] from a solution of 4 wt % drug concentration in ethanol/water 1/1 mixture which swells the polymer network. The drug-loaded hydrogels were prepared as suspensions, by swelling ~0.1 g of PNIPAAm/ALG hydrogel in powder form in the 4 wt % theophylline solution (1:1, v/v, ethyl alcohol/water) under mild stirring for 24 h, period corresponding to the time necessary for all compositions to reach their equilibrium swelling degree, as determined from the swelling studies.[15,28]

Characterization of Theophylline-Loaded PNIPAAm/ALG Hydrogels

Near Infrared Chemical Imaging Technique (NIR-CI Technique). NIR spectra were recorded on a SPECIM'S Ltd. SisuCHEMA controlled with Evince software package for processing

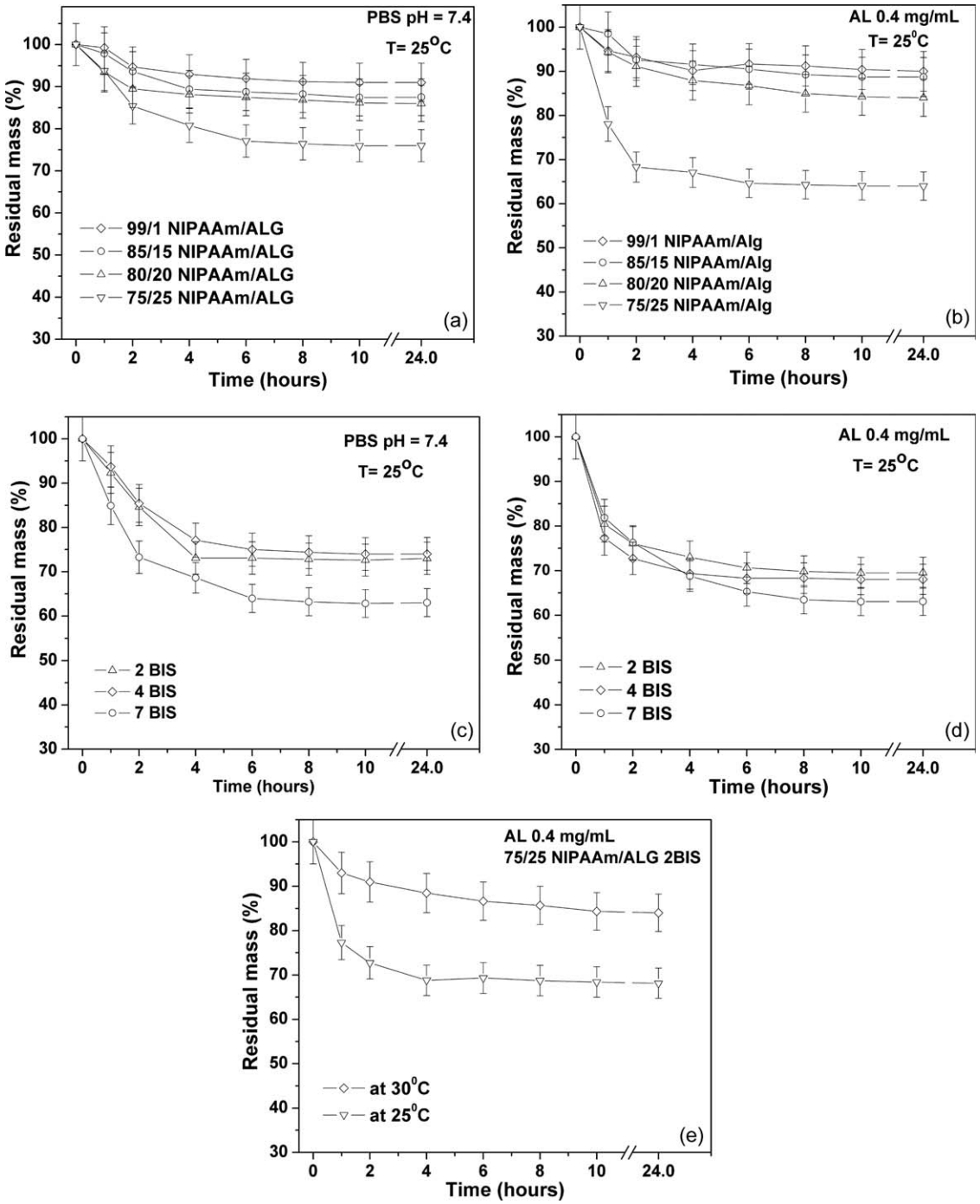

Figure 1. Degradation curves of PNIPAAm/ALG hydrogels with different composition at 25°C in PBS pH 7.4 (a) and in AL 0.4 mg/mL (b); of 75/25 NIPAAm/ALG hydrogels with various cross-linking degrees at 25°C in PBS pH 7.4 (c) and AL 0.4 mg/mL (d); enzymatic degradation of 75/25 NIPAAm/ALG hydrogel in AL 0.4 mg/mL, at different temperatures (e).

the original image data. The system includes a Chemical Imaging Workstation for 1000 to 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 to 350 Hz. Each sample was scanned between 1000 and 2500 nm.

Microscopy (SEM). The morphology of the drug-filled polymeric networks fractured after immersion in liquid nitrogen was observed after metallization with gold with a FEI Quanta 200 FEG Scanning Electron Microscope. Magnification is indicated on pictures.

Thermogravimetric Analysis. The thermal behavior of the PNIPAAm/ALG hydrogels loaded with theophylline was evaluated using a Q 500 (TA Instruments) thermal analyzer. The Thermal Gravimetric Analysis (TGA) was performed on samples of ~5

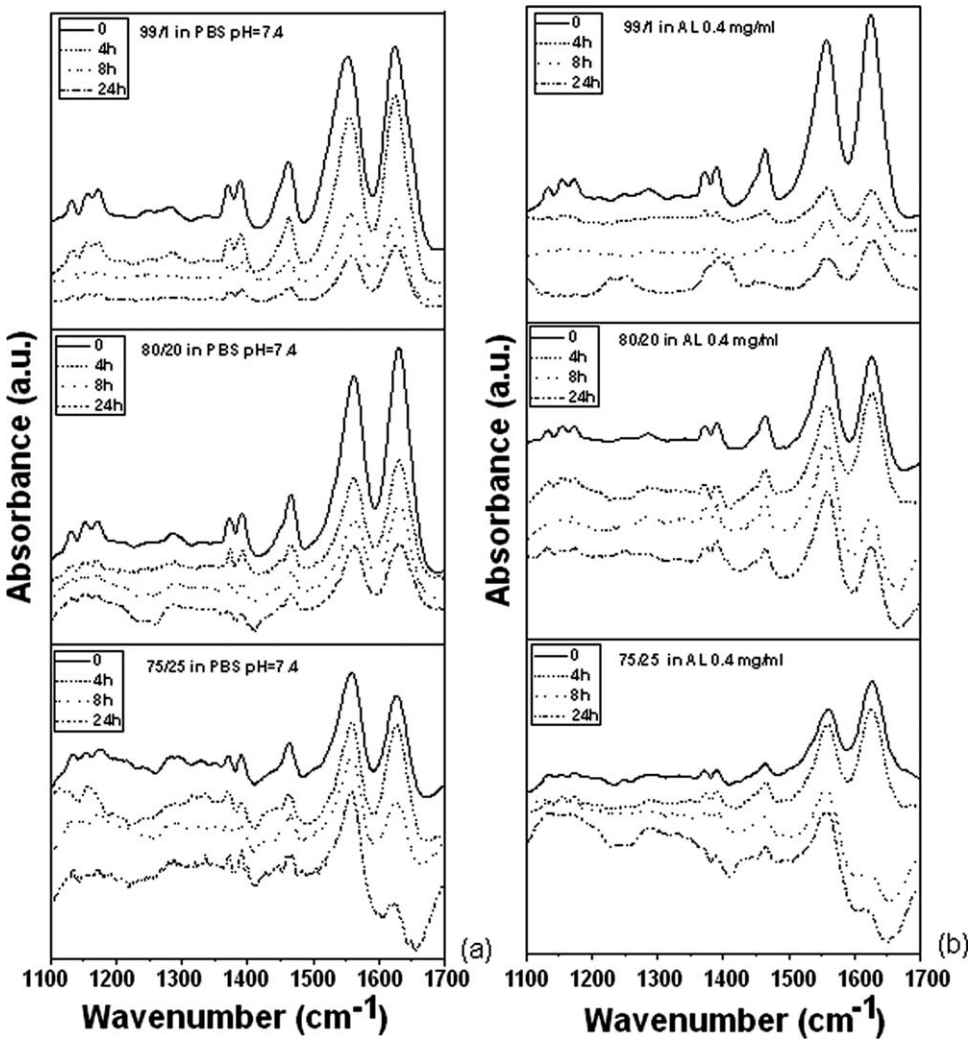

Figure 2. ATR-FT-IR spectra of hydrogel samples in PBS pH = 7.4 (a) and under alginate-lyase action (b) before degradation and at different time intervals.

mg weight, in air, from 15 to 700°C, at a heating rate of 20°C min^{-1}.

In Vitro Theophylline Release Study from PNIPAAm/ALG Hydrogels

The *in vitro* release studies for theophylline have been performed by a standard dissolution test[38] carried out in conditions which mimic the gastrointestinal environment, using saline phosphate buffer solution of pH 7.4 and acidic buffer solution of pH 2.2 as dissolution medium. During the experiment, the temperature was maintained at 37 ± 0.5°C. The measured concentration of drug in solution was 18 mg/100 mL. Aliquots of the medium of 1 mL were withdrawn at predetermined time intervals and analyzed at λ_{max} value of 271 nm, the wavelength characteristic for theophylline, using a HP 8450A UV–visible spectrophotometer. In order to maintain constant the measured drug concentration, the sample was carefully reintroduced in the circuit after analysis. The theophylline concentrations were calculated based on calibration curves determined at the same wavelength.

The drug release kinetics was evaluated with a semi-empirical eq. (2) based on Korsmeyer-Peppas model, which is applied at the initial stages (approximately 60% fractional release).[39]

$$\frac{M_t}{M_\infty} = kt^n \tag{2}$$

where M_t/M_∞ represents the fraction of the drug released, M_t and M_∞ are the absolute cumulative amount of drug released at time t and at infinite time respectively (in this case the maximum amount released in the experimental conditions used, at the plateau of the release curves), k is a constant incorporating the characteristics of the macromolecular drug-loaded system and n is the diffusional exponent characteristic for the release mechanism. In the equation above, a value of $n \approx 0.5$ indicates a Fickian diffusion mechanism of the drug from the hydrogel network, while a value $0.5 < n < 1$ indicates an anomalous or non-Fickian behavior. When $n = 1$, a case II transport mechanism is involved with zero order kinetics, while $n > 1$ indicates a special case II transport mechanism.[40] The corresponding theophylline release profiles are represented

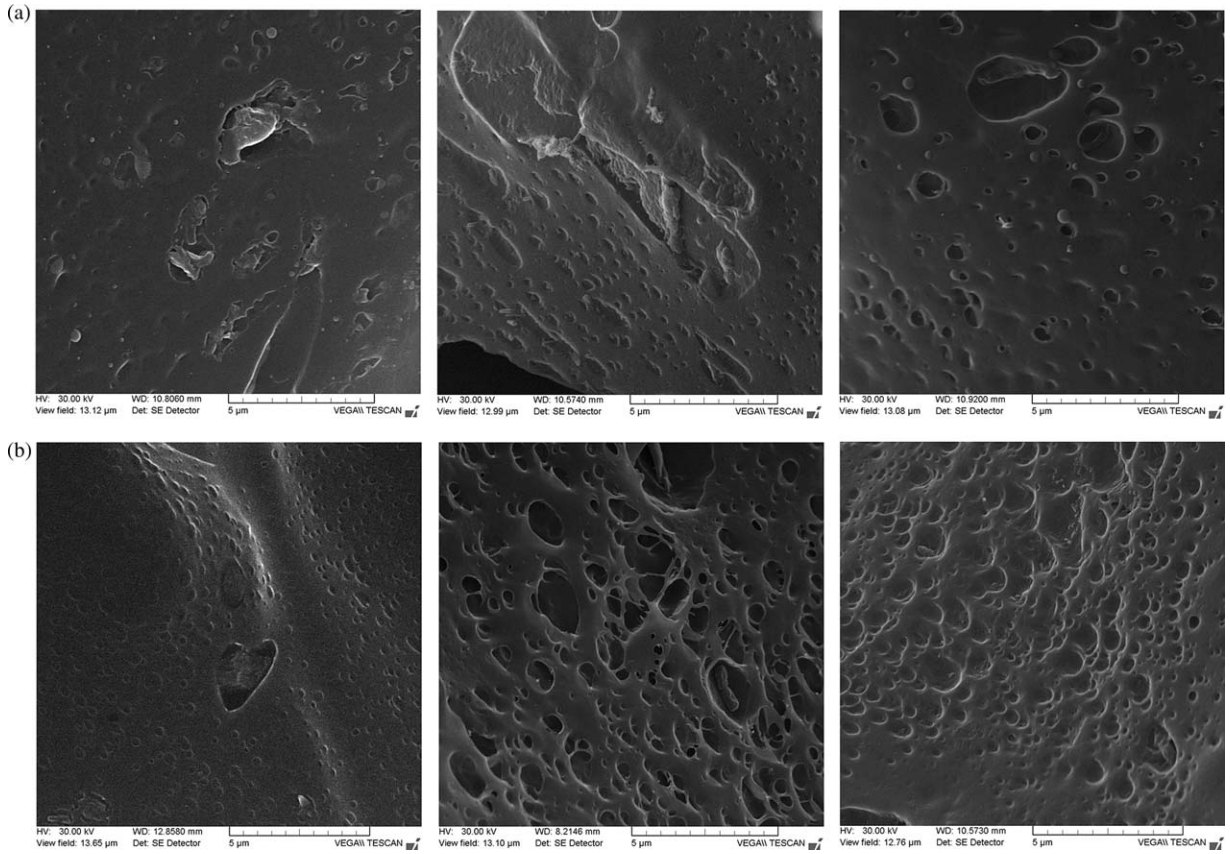

Figure 3. SEM micrographs of hydrogel samples 99/1 NIPAAm/ALG, 80/20 NIPAAm/ALG, and 75/25 NIPAAm/ALG degraded in PBS (a) and in alginate-lyase medium (b) for 24 h.

through plots of the cumulative percentage of drug released *versus* time.

***In Vivo* Theophylline Release from PNIPAAm/ALG Hydrogels Pharmacokinetic Analysis.** The theophylline release pharmacokinetic evaluation studies from PNIPAAm/ALG hydrogels were conducted on healthy male Wistar rats weighing between 330 and 450 g, purchased from Cantacuzino Institute, Bucharest, Romania, using the retro-orbital sinus bleeding technique.

For the pharmacokinetic and *in vivo* release studies the oral delivery was chosen, this being the usual way for drug administration. The animals were maintained in identical laboratory conditions as before, with free access to food and water. The raw theophylline and theophylline-loaded hydrogels were delivered by gastrogavage as suspensions, in a dose of 15 mg kg^{-1} body weight for each rat.

The blood samples withdrawn at predetermined time intervals up to 72 h were allowed to stand for 1 h, centrifuged at 4000 rpm for 10 min to separate the serum, which was kept frozen (−20°C) until further analysis. Theophylline serum extraction was performed mainly as described in the literature with a few minor changes.[27,41] 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 withdrawn and transferred into a glass tube and evaporated to dryness at 40°C. The dry extract was dissolved in 1 mL acetonitrile-10 m*M* aqueous sodium acetate (7:93 v/v), passed through a 0.45 μm syringe filter and used for further analysis. Plasma theophylline concentration was determined by high performance liquid chromatography HPLC (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 m*M* aqueous sodium acetate (7:93 v/v). Analyses were performed at room temperature under a flow rate of 1 mL min^{-1} using an injection volume of 100 μL. Theophylline was detected by UV detector at 270 nm. The concentration was determined with a calibration curve obtained with standard solutions of known theophylline concentrations in ethyl alcohol–water (1:1 v/v) in the range of 1 to 20 μg mL^{-1}, using HPLC software LC Solution Version 1.22 SP1 for integration and automatic determination of drug concentration in blood samples.[27]

The pharmacokinetic (PK) parameters such as maximum plasma concentration (C_{max}), time of maximum concentration (t_{max}) and plasma elimination half-life ($t_{1/2}$) were obtained directly from the plasma concentration–time plots. The area under the plasma concentration–time curve up to 72 h (AUC_{0-72}) was calculated using the linear trapezoidal rule. The relative bioavailability (test/reference ratios) of the PNIPAAm/ALG/T hydrogel formulations, compared to

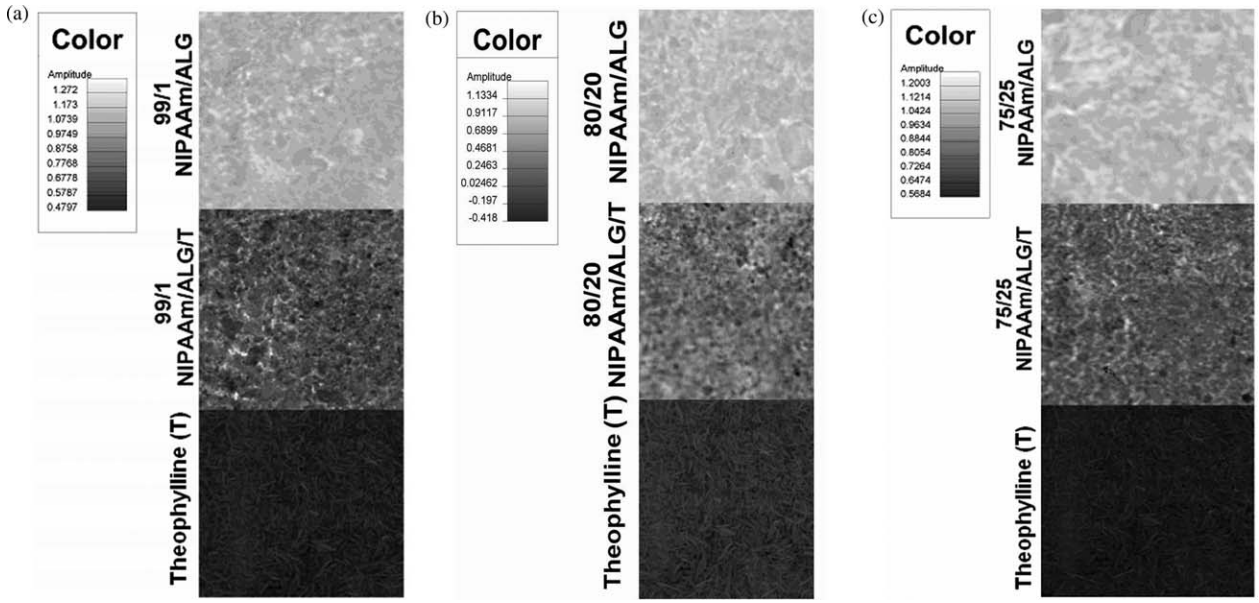

Figure 4. PLS-DA model for NIPAAm/ALG/T polymeric systems: (a) 99/1 NIPAAm/ALG/ T, (b) 80/20 NIPAAm/ALG/ T, (c) 75/25 NIPAAm/ALG/T.

raw theophylline, was calculated as the ratio (AUC_{sample}/$AUC_{theophylline}$) × 100.

Each experiment was repeated four times and the results were expressed as mean ± standard deviation. Statistical data analysis was performed using the Student′s *t*-test and analysis of variance (ANOVA) with $P < 0.05$ as the minimal level statistically significant.

RESULTS AND DISCUSSION

Toxicity Tests

The first test consisted on intraperitoneal (i.p.) administration of a single dose of 2000 mg kg^{-1} body hydrogel suspension at a mouse, for each composition. Because the mice survived up to 2 weeks after administration, four other mice were injected for each composition, and their survival was assessed. It was found that all mice survived 14 days after i.p. administration of hydrogel suspensions. No behavioral or physical changes such as abdominal swelling were observed in treated mice following injection or on subsequent days. Throughout the study period, animals showed no signs of peritonitis, lethargy, muscle loss, dehydration or anorexia, symptoms which are associated with animal toxicity.[42,43]

The acute toxicity of a 5000 mg kg^{-1} body dose was tested according to OECD guidelines.[44] Due to technical problems occurred at administration of the solutions with such a high concentration, one single dose of 3200 mg kg^{-1} body was tested. It was found that after a single dose of 3200 mg kg^{-1} body of hydrogel suspension intraperitoneally administered to mice, for each composition, they survived 14 days after administration. It has been concluded that the LD_{50} for PNIPAAm/ALG hydrogels, after i.p. administration as suspensions, is bigger than 3200 mg kg^{-1} body, showing their nontoxicity. The results are in good accordance with those found by using other type of hydrogels containing PNIPAAm.[45]

Biocompatibility of PNIPAAm/ALG Hydrogels

The *in vivo* biocompatibility of PNIPAAm/ALG hydrogels was examined during 14 consecutive days after i.p. injection of gel suspensions at mice, following the effects on hematological and immune system parameters comparatively with a control group of mice, which received physiological serum (Table I).

Clinical chemistry and hematology data are of great importance to determine the effects induced on the body by the tested hydrogel matrices.

The values of hematology parameters such as WBC, RBC, PLT, HGB concentration, hematocrit level showed no significant variations between mice groups treated with 99/1, 80/20 and 75/25 of PNIPAAm/ALG hydrogels compared with control mice group, being in the range of normal limits reported for healthy mice.[46] Statistical analysis revealed no significant influence of the studied compounds on the neutrophils phagocytic capacity, on the phagocytosis activity of immune cells, serum opsonic capacity, phagocytosis and bactericidal capacities of peritoneal macrophages, spleenic lymphocytes with rosetting capacity of tested mice compared to control group, after 14 days of testing.

As it concerns the clinical biochemistry parameters, a statistically significant increase of ALT and AST values was observed in the experimental groups compared with the control mice group after 14 days of testing (Table II), but the values are in the range of normal limits. There was no significant effect on LD levels in case of mice injected with PNIPAAm/ALG hydrogel suspensions compared with that obtained for control mice group.

Based on the toxicity and biocompatibility results it can be considered that the injected PNIPAAm/ALG hydrogels are

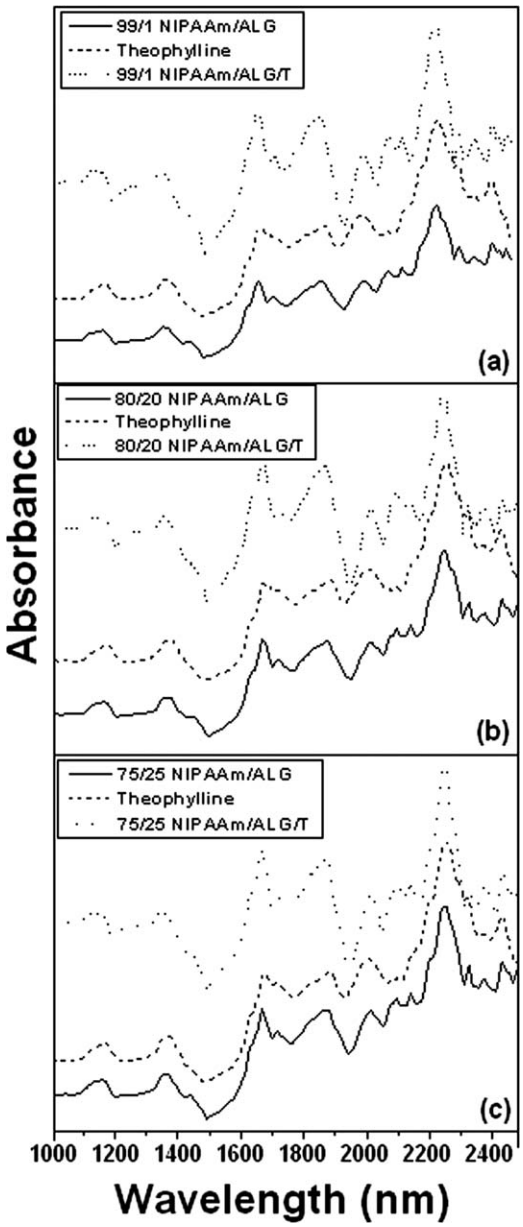

Figure 5. Near-IR reflectance spectra of the hydrogel samples, theophylline, and theophylline-loaded mixed hydrogels: (a) 99/1 NIPAAm/ALG/T, (b) 80/20 NIPAAm/ALG/T, (c) 75/25 NIPAAm/ALG/T.

nontoxic, they did not produce any significant changes in the hematology of tested rats and had no hepato-toxicity effects, which is in accordance with the biocompatibility results on PNIPAAm-based materials previously reported.[2,30,31]

All presented results indicate a good biocompatibility of PNIPAAm/ALG hydrogels with living tissues, so they could be considered as potential carriers for controlled drug delivery and because of their sensitivity to external pH and temperature stimuli, they show reversible on-off swelling characteristics[15–18] which are important for sensors, acuators and biomedical applications as it was also found for other hydrogels containing PNIPAAm[2,45,47]

Biodegradability of PNIPAAm/ALG Hydrogels

Weight Loss Measurements. The degradation curves obtained at 25°C both in PBS and in alginate-lyase presence, using as substrate PNIPAAm/ALG hydrogels with various compositions and crosslinking degrees are depicted in Figure 1(a–d). A gradual mass loss was observed, especially up to 6 h for all samples studied.

The residual mass decreased at 76% in PBS and respectively at 64% in AL for sample 75/25 NIPAAm/ALG in respect with 86 to 91% in PBS and 84 to 90% in AL for samples with higher ALG content. The residual mass reached a value with 12% lower in AL than in case of PBS degradation for the sample with 25 wt % ALG in composition, demonstrating the increased susceptibility to enzymatic degradation of the samples with higher alginate content, as it was expected due to the specificity of the enzyme action. The results obtained showed the increased mass loss under enzyme action as a function of composition, but also a significant mass loss in PBS (with a difference of 12 wt %) demonstrating the biodegradable character of these gels. Concerning the degradation of samples with different crosslinking degrees there are not significant differences, but the slightly increased mass loss of the samples having higher crosslinking degrees can be attributed to the susceptibility to hydrolysis of the amide bonds found there in higher number.

The PNIPAAm/ALG hydrogels are partially degradable, as residual mass measurements showed. Their degradability is associated not only with biodegradable alginate bonds, but also with amide hydrolysable bonds introduced by crosslinking while crosslinked NIPAAm part of the hydrogel network remains intact.

The thermo-sensitive behavior of these hydrogel samples influences their enzymatic degradation susceptibility [Figure 1(e)]. Due to shrinking of the gels at temperatures near LCST (32°C), enzyme diffusion is hindered and mass lost became insignificant compared with the one observed at room temperature, so the residual mass is higher 84% at 30°C in respect with 68% at room temperature. The degradation behavior of the 75/25 PNIPAAm/ALG hydrogel in AL 0.4 mg/mL at 37°C is similar with that at 30°C, where the hydrogel is very closed to PNIPAAm LCST and starts to shrink.

As it was mentioned, the thermo-responsive materials that exhibit sol–gel transition near body temperature such as those containing PNIPAAm are frequently employed for tissue engineering and drug delivery applications because their transitional properties make them easy to deliver and manipulate. Disadvantages to using PNIPAAm are related to its inability to degrade *in vivo* as well as its apparent structural similarity to toxic acrylamides.[48] The existence of a nondegradable polymer such as PNIPAAm which is not resorbable in the human body may cause a chronic inflammatory response and make multiple doses difficult, so bioerodable polymers are preferred for a controlled drug release system.[49] However, copolymerization of PNIPAAm with degradable polymers can create a material that is thermoresponsive, biodegradable, and lacking observed cytotoxicity.[50] Incorporation of alginate in hydrogels imparts

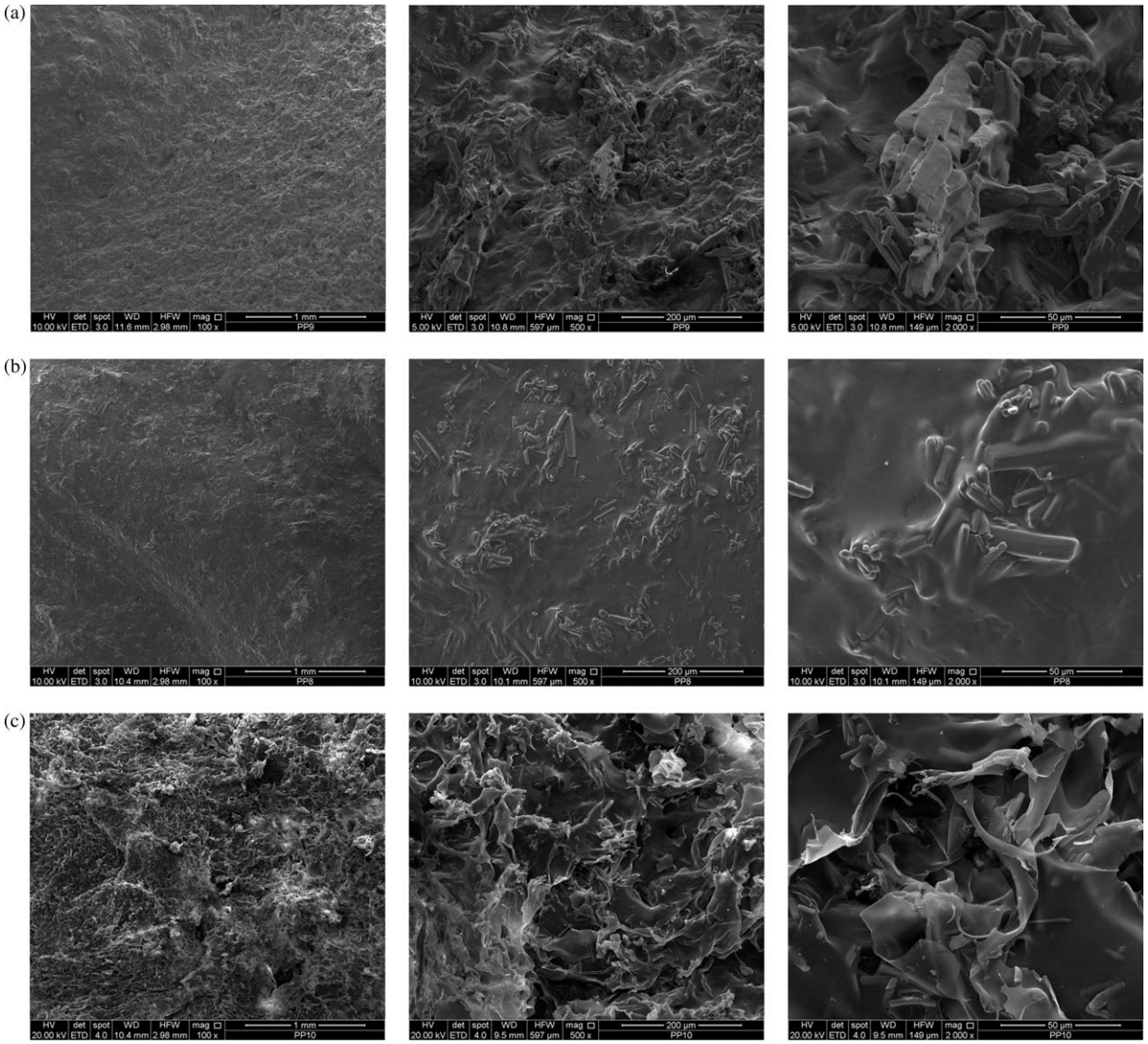

Figure 6. SEM micrographs of theophylline-loaded NIPAAm/ALG systems: (a) 99/1 NIPAAm/ALG/T, (b) 80/20 NIPAAm/ALG/T, (c) 75/25 NIPAAm/ALG/T.

biodegradability characteristics which are detectable even within 24 h. On the basis of the biocompatibility tests (performed during at least 2 weeks) it can be concluded that hydrogels degradation occurs during those tests and both hydrogels and degradation products did not show any detrimental effects to adjacent tissues. This was also demonstrated for other hydrogels containing PNIPAAm.[45,49,50]

ATR-FT-IR Spectra

The hydrogels degradation was studied also by the structural modifications observed up to 24 h exposure in PBS pH 7.4 and under alginate-lyase action, respectively by ATR-FTIR spectroscopy (Figure 2). There are no major structural modifications either in PBS or in AL, the mass loss being explained by the degradation of the hydrogels by surface erosion due to hydrolysis, which justifies the low mass lost (up to ~40%). From ATR-FT-IR spectra it can be noticed that amide I and amide II bands modified slightly during degradation, by decreasing in intensity both in PBS and AL, while the bands characteristic to isopropyl groups of PNIPAAm are hardly identified after 8 h of degradation in both cases.

Morphology of Degraded PNIPAAm/ALG Hydrogels. The morphological aspects of the hydrogels after 24 h of exposure to PBS and respectively under enzyme action, were examinated by SEM, both on the surface and in fracture of the samples. The images taken in fracture did not show a significant destruction of the hydrogels′ internal structure, instead a serious damage at the surface occurred (Figure 3). The detailed images of the pores walls mainly on the surface of the hydrogels evidenced the advanced degradation of samples 80/20 NIPAAm/ALG and 75/25 NIPAAm/ALG compared with 99/1 NIPAAm/ALG hydrogel, both in PBS and under alginate-lyase action. After 24 h of degradation, the porous surface of the hydrogels is affected, the

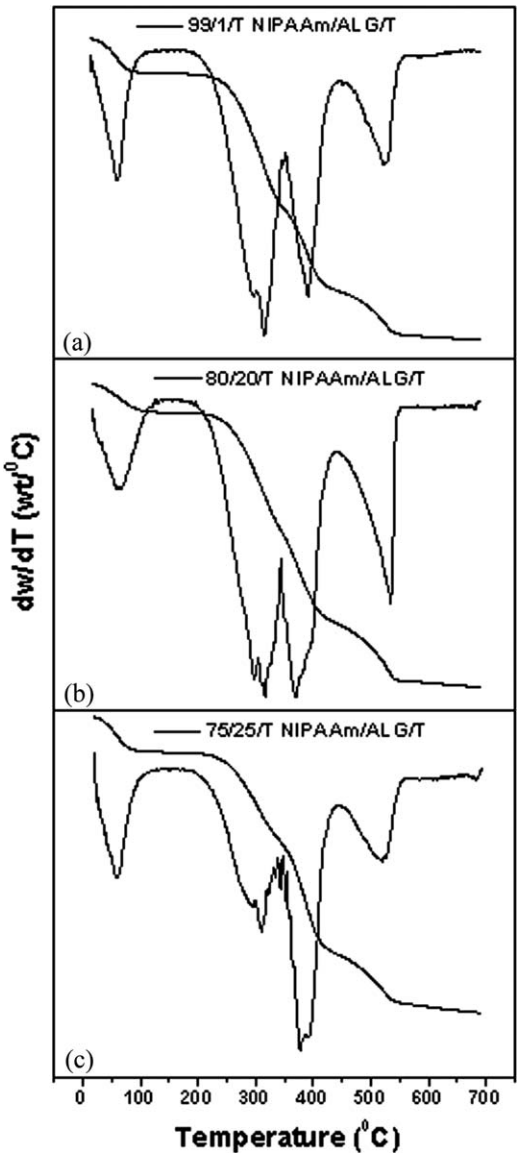

Figure 7. TG/DTG curves of NIPAAm/ALG/T systems with various compositions.

pores walls are thinner and with many cavities within, visible at higher magnification. The cavities number and dimensions are increasing for samples with 20 wt % and 25 wt % ALG in composition, especially when exposed under enzyme action. The average dimensions increased from 0.36 μm for 99/1 NIPAAm/ALG to 1.3/3 μm for 75/25 NIPAAm/ALG. The morphological aspect of the degraded samples confirms the degradation by surface erosion.

Characterization of Drug-Loaded Hydrogel Matrices

Near Infrared Chemical Imaging. The nondestructive character, minimal sample preparation required, and the possibility to predict chemical and physical sample parameters from one single spectrum are only some of the NIR-CI method advantages over other analytical techniques, showing its feasibility to investigate samples with pharmaceutical application.[51] The NIR-CI technique allowed the quantitative evaluation of theophylline loaded in the PNIPAAm/ALG polymeric matrices and to appreciate the homogeneity of the drug distribution within the polymeric networks.

The PLS-DA models corresponding to the PNIPAAm/ALG/T polymeric systems with different NIPAAm-to-ALG weight ratios are depicted in Figure 4. A visibly higher homogeneity degree can be noticed in the sample 75/25 NIPAAm/ALG/ T structure compared with the other two compositions, which corresponds to a more uniform drug distribution within the matrice.

Based on PLS-DA prediction, a drug loading of 73% was recorded for 75/25 NIPAAm/ALG/ T compared with 63% for 80/20 NIPAAm/ALG/ T and 55% for 99/1 NIPAAm/ALG/ T as a consequence of structural morphology. Thus, the highest drug loading degree and a better drug distribution was recorded for the 75/25 NIPAAm/ALG/ T system due to the presence of some drug-polymeric matrix interactions by hydrogen bonding, possible between C=O and NH groups, as it was previously evidenced by FT-IR.[17]

As it is known, the swelling degree and the mesh size of a hydrogel have a major influence on the drug loading capacity[52] thus it was expected the obtaining of a better drug entrapment (73%) within the sample with a higher hydrophylic alginate content, which was demonstrated to exhibit the highest swelling capacity and enhanced porosity compared with the other samples.[15] These results are in agreement with those obtained by NIR investigations performed on other theophylline-loaded hydrogels.[28]

The Near-IR compared spectra of the hydrogels, theophylline and drug-loaded hydrogels PNIPAAm/ALG with an air background are presented in Figure 5. Intense absorption bands corresponding to the functional groups of the components are observed in the full range of the spectra.[53] The obvious overlap between the polymers and theophylline bands in near infrared region empower for the use of multivariate regression for the simultaneous determination of drug content in samples.

The bands corresponding to the functional groups from hydrogels are mostly overlapped with theophylline bands, but in the spectra of the drug-loaded hydrogels they are shifted to lower wavelengths, which can be attributed to H-bond formation. The bands corresponding to saccharidic O-H maintained the same wavelength in the spectra of neat hydrogels compared with those of PNIPAm/ALG/T. Theophylline showed specific bands at 2008 nm attributed to N-H deformation, at 2430 nm corresponding to C-N-C bond and the shoulders at 2295 and 2322 nm for C-H bending. A strong, sharp band attributed to C=O bond appeared in all spectra, at: 2256 nm for theophylline, in the interval 2248–2250 nm for neat hydrogels and shifted at lower wavelengths 2242 to 2246 nm for drug-loaded hydrogels. Therefore some interactions through hydrogen bonding between -C=O and N-H groups from purine ring of theophylline and carboxyl or amide groups from PNIPAAm/ALG hydrogel are possible.

Microscopy Study. Theophylline was dispersed in the polymeric matrices as stick-shaped microparticles, their morphological

Table III. TGA Data

	Dehydration step				Main decomposition process					
NIPAAm/ALG/T	T_i (°C)	T_m (°C)	T_f (°C)	Δw (%)	T_i (°C)	T_m (°C)/mass loss step			T_f (°C)	Δw (%)
99/1	14	59.4	99	11	175	315	390	523	565	85
80/20	17	60	102	10	170	316	369	535	567	87.5
75/25	20	62	108	11.4	175	310	379	519	570	82

T_i is the onset decomposition temperature, T_m is temperature corresponding to the maximum mass loss rate; T_f is the final decomposition temperature and Δw (%) represents the weight loss.

aspect and distribution being identified and studied through SEM (Figure 6).

It can be noticed that the drug microparticles, with various dimensions in the range 8 to 46 μm were better dispersed within the polymeric networks with increased alginate content, while in the sample with the lowest alginate content (1 wt %) the theophylline seems to agglomerate in clusters. Thus, SEM images confirmed the NIR-CI results.

Thermogravimetric Analysis

The TG/DTG curves for NIPAAm/ALG/T hydrogel samples prepared in different compositions and loaded with theophylline, obtained at a heating rate of 20°C min^{-1} are shown in Figure 7. The analysis of the TG curves for theophylline-loaded samples showed mass loss in four consecutive steps up to 700°C, compared with PNIPAAm/ALG hydrogels without theophylline, which had only two steps of mass loss, as it was found presviously.[16] The first step, which can be associated with the process of dehydration up to 100°C (T_m ~60°C) and presents a reduced weight loss of 10 to 11 wt % is followed by three other mass loss steps on the range 170 to 570°C with a total mass loss of 82 to 88 wt %, corresponding to the main decomposition processes (Table III). DTG curves of the samples evidenced a complex decomposition process between 170 and 570°C, due to some drug-polymeric matrix interactions. The temperature range of decomposition summarized in Table III modified by variations in the composition, the drug influence on the thermal behavior of the mixed hydrogels being also evidenced.

In Vitro Theophylline Release

The *in vitro* release study of theophylline was performed in conditions similar with the gastrointestinal environment, at pHs 2.2 and 7.4. The theophylline release profiles at both pHs are depicted in Figure 8(a,b). The release rate and the amount of theophylline released into environments with different pHs (2.2 and 7.4) is influenced by the hydrogels composition, namely by the alginate content in the hydrogel samples.

At pH 2.2 the release curves for samples 99/1/T and 80/20/T of PNIPAAm/ALG hydrogels have similar aspect, with only smaller released percents for sample 80/20/T, while for sample 75/25/T the curve is visibly smoother compared with the other two samples, showing the lowest released percent during 600 min [Figure 8(a)]. The data obtained from the percentage theophylline released (%) *versus* time plots (Table IV) show that an increase of ALG content in the samples leads to the increase of the half release time from 47 min for 99/1/T to 150 min for 75/25/T, while the time to reach maximum released amount of theophylline increased from 320 min to 600 min for the same PNIPAAm/ALG samples. A decrease of the theophylline percent released was recorded: total release (100%) from the 99/1/T composition in only the first 320 min to 92% from 75/25/T at 600 min. PNIPAAm/ALG hydrogel samples with compositions 80/20/T and 75/25/T reached the 100% theophylline released after 24 h. This behavior can be explained by the effect of the enhanced swelling capacity of the highly absorbent hydrogels and of the presence of hydrogen bonding interactions between the loaded drug and the polymeric matrix on the drug release rate by leading to a slower release rate and

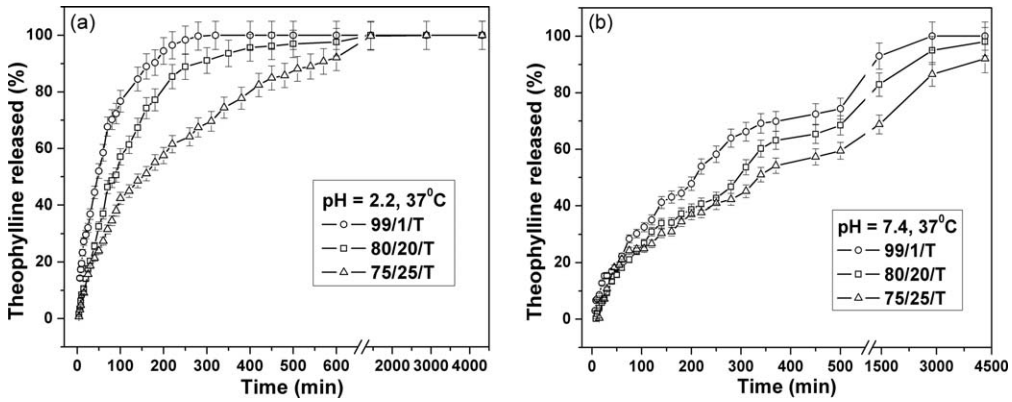

Figure 8. Theophylline release profiles from PNIPAAm/ALG hydrogels with various compositions in buffer solution of pH 2.2 (a), and pH 7.4 (b) at 37°C.

Table IV. Kinetic Parameters and *In Vitro* Release Characteristics of Theophylline from PNIPAAm/ALG Hydrogels

PNIPAAm/ALG/T (%)	Half release time (min)	Time to reach maximum amount released (min)	Maximum released amount (%)	n	k (min^n)	R
pH = 2.2						
99/1/T	47	320	100	0.96	17.88×10^{-3}	0.99
80/20/T	80	600	98	0.99	6.2×10^{-3}	0.99
75/25/T	150	600	92	1	4.55×10^{-3}	0.99
pH = 7.4						
99/1/T	127	500	74	0.64	1.6×10^{-2}	0.99
80/20/T	138	500	68	0.83	5.73×10^{-3}	0.99
75/25/T	146	500	59	0.7	4.38×10^{-3}	0.99

smaller released amount of drug and therefore on the ability to control drug release characteristics, as other studies have shown.[28,54] By increasing the amount of hydrophilic alginate in the matrice composition the theophylline release rate decreased, this behavior being well correlated with the swelling capacity of these samples that can be attributed to the increased diffusion pathway created by the electrostatic repulsion among free ionized carboxylic acid groups in ALG[15] and moreover because of some drug-matrix interactions through hydrogen bonding occurring in these hydrogels with higher amount of ALG in composition, leading to a slower release rate and a smaller released amount before 600 min, thus a retarded drug release.

The theophylline release kinetic parameters were calculated. The values obtained for the diffusion exponent (n) at pH = 2.2 correspond to an anomalous behavior for the sample with 1 wt % ALG and to a case II transport mechanism for the samples containing higher alginate amount, namely 20 wt % and 25 wt %. The slope of the release curves at both pHs varies with samples composition. Testing many kinetic models, the most suitable one was found the zero order kinetic model ($n = 1$), according to the fitted results for theophylline released in pH 2.2 buffer solution from the three hydrogel samples, shown in Table IV. The decrease of the release rate constant values (k) from 17.88×10^{-3} min for 99/1/T to 4.55×10^{-3} min for 75/25/T indicates a significantly prolonged delivery by increasing ALG content of PNIPAAm/ALG hydrogels.

The theophylline release profiles in PBS at pH 7.4 [Figure 8(b)] show a step-way manner of release for all three compositions tested, with significantly smaller released percent values compared with those recorded at pH 2.2. The amount of theophylline released varies with hydrogel's composition in the same way, decreasing at higher alginate content: from 74% for 99/1/T to only 59% for 75/25/T at 500 min. After 24 h release time, the order is maintained: the hydrogel with 1 wt % ALG released 93% of theophylline, the one with 20 wt % ALG released 83%, while the sample containing 25 wt % ALG released only 67% of theophylline. The total release was recorded at 48 h for 99/1/T and it seems to take place after 72 h for 80/20/T and 75/25/T of PNIPAAm/ALG hydrogels. The values obtained for the diffusion exponent (n) at pH 7.4 indicate an anomalous transport for all samples, coupling Fickian diffusion with the relaxation of the hydrogel network. The decrease of the release rate constant values (k) from 1.6×10^{-2} min$^{0.64}$ for 99/1/T to $4.38 \cdot \times 10^{-3}$ min$^{0.7}$ for 75/25/T indicates once again the slower release rate at increased ALG content of the PNIPAAm/ALG samples (Table IV).

The slower release rate and the smaller theophylline released percents in medium with pH 7.4 compared with that with pH 2.2 can be attributed to the particular and complex temperature- and pH-sensitive swelling behavior[15] of these hydrogels correlated with the ability of the gel components to form hydrogen bonds with the entrapped theophylline.[54] A conjugated effect of temperature and pH should be considered when discussing the drug release correlated with swelling behavior of these gels, which have been demonstrated as dual-responsive.[15] Since the mixed IPN hydrogels have a higher content of PNIPAAm in all compositions tested, the temperature-response will be more drastic and faster than pH effect. The drug release studies have been performed at 37°C—physiological temperature, above the LCST of PNIPAAm, where the hydrogels present significantly lower swelling capacity than at 25°C, with a fast swelling in the first minutes, followed by the expulsion of some

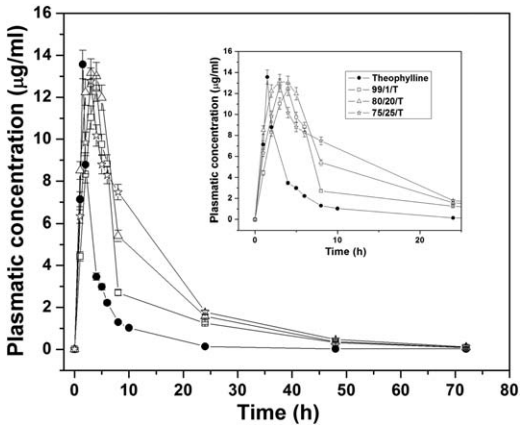

Figure 9. Plasma theophylline concentration *versus* time. Each point is presented as mean ± SD, $n = 4$.

Table V. Pharmacokinetic Parameters of Theophylline Release After Administration of Reference Theophylline and Three Theophylline-Loaded Hydrogel Compositions Tested on Rats

Sample	C_{max} (μg mL^{-1})	$t_{1/2}$ (h)	AUC_{0-72} (μg h mL^{-1})	Relative bioavailability (%)
T	13.56	2.7	48.7	-
99/1/T	12.42	6.9	117	240
80/20/T	13.16	7.5	163.4	335
75/25/T	12.76	11	176	361

of the absorbed water due to the collapse of PNIPAAm chains and then equilibrium values are established at both pHs.[15] The acidic pH (e.g. pH 2.2) doubled by temperature effect determines a fast initial swelling until equilibrium was reached; this is correlated with a rapid drug release, attributed to the anion-anion electrostatic repulsion between the carboxyl groups on the alginate chains, followed by their gradual protonation and formation of intermolecular hydrogen bonds leading to association structures,[55,56] which may hinder subsequent hydrogels swelling and formation of hydrogen bonds with loaded theophylline. This leads to an easier dissociation of the hydrogen bonds possibly formed between the drug and the polymeric matrix and it accelerates the complete drug release in the acidic media, as it was found for other hydrogels with dual (pH and temperature) responsive behavior containing PNIPAAm, such as (PNIPAAm)-*co*-acrylic acid hydrogel cages.[57] At pH 7.4, although the temperature-response was also prevalent, the samples with higher alginate content exhibited a gradual swelling[15] which is correlated with the slower drug release rate. Since carboxyl groups of alginate are almost completely ionized, strengthening of electrostatic repulsion among them may promote the development of hydrogen bonds between the hydrogel matrice and theophylline loaded, determining its subsequent slower release.

The results obtained for the *in vitro* release of theophylline at both pHs showed that increasing hydrophilic alginate content in the hydrogels composition modifies their properties, enhancing the ability to retain the drug within the hydrogel network through hydrogen bonding for longer time, being thus suitable for prolonged drug release. It can be concluded that PNIPAAm/ALG bicomponent hydrogels offer a satisfactory solution for sustained delivery of theophylline, which is mainly diffusion-controlled and depends on hydrogel composition.

In Vivo Theophylline Release—Pharmacokinetic Analysis

The *in vivo* release of raw theophylline and theophylline loaded in three hydrogel compositions, namely 99/1, 80/20, and 75/25 PNIPAAm/ALG was studied.

The mean plasma theophylline concentration *versus* time curves after a single oral dose are represented in Figure 9 and mean values of pharmacokinetic parameters (C_{max}, $t_{1/2}$, and AUC_{0-72}) are summarized in Table V.

The *in vivo* release profiles show the sustained release of theophylline loaded in PNIPAAm/ALG hydrogels, depending on their composition, compared with raw theophylline, which is removed from the body within the first 24 h from administration. The prolonged release of theophylline loaded in PNIPAAm/ALG formulations was proved by the presence of drug traces recorded up to 55 h after administration time.

Pure theophylline was detected in plasma within the first hour after its administration in rats. The mean plasma level of raw theophylline was recorded at a C_{max} value of 13.56 μg mL^{-1} achieved at a t_{max} of 1.5 h and the elimination half-life ($t_{1/2}$) of about 2.7 h, which indicated a fast absorption of pure theophylline, data which are consistent with previous *in vivo* theophylline release studies in rats.[27,28,58–61] The theophylline serum concentration released from 99/1/T composition, reached a C_{max} value of 12.42 μg mL^{-1} with a t_{max} of 4 h and a $t_{1/2}$ of 6.9 h, while in the case of 75/25/T composition C_{max} value is 12.76 μg mL^{-1} with a t_{max} of 3 h and a $t_{1/2}$ of 11 h, exhibiting thus a significantly delayed absorption in blood. After reaching the maximum value, serum theophylline concentration decreased with a relatively low rate up to 72 h for all samples studied, having the highest plasma level concentrations for 75/25/T sample.

Theophylline pharmacokinetics varies largely, obeying a nonlinear profile[62] and the average half life ($t_{1/2}$) was generally reported in the range of 4 to 9 h.[28,58,60,63]

The value of the area under the curve (AUC) or time dependence of plasma after administration of a single dose of drug was determined to be about 48.71 μg h mL^{-1} for raw theophylline compared with ~176 μg h mL^{-1} for theophylline loaded in 75/25/T PNIPAAm/ALG formulation. It can be noticed the increasing values obtained for AUC_{0-72} calculated for the three compositions studied with increasing alginate content. These findings are in accordance with previous results reporting on increased AUC values for theophylline-loaded polymeric samples.[27,28,59]

The alginate in higher amount increased also the bioavailability up to 361% for the tested formulations and thus the drug absorption through gastrointestinal mucosa without producing damage to the biological system, compared with raw theophylline.

CONCLUSIONS

Mixed-interpenetrated polymeric networks based on alginate and poly(*N*-isopropylacryl amide) (PNIPAAm) were investigated as carriers for sustained theophylline release.

The nontoxicity, biocompatibility and biodegradability of these hydrogels were evaluated. The quantitative evaluation of

theophylline loaded in these formulations showed a better drug entrapment and a higher homogeneity of the sample with higher alginate content compared with the other two samples tested, results confirmed also by NIR and microscopic examination. Loading capacity varies from 55% to 73%, increasing in the sample with higher alginate content. The theophylline presence clearly modified the thermal behavior of the hydrogels. The theophylline release studies performed both *in vitro* and *in vivo* demonstrated the sustained drug release ability of the PNIPAAm/ALG hydrogels as diffusion-erosion controlled drug delivery systems, dependent on their composition, with an enhanced bioavailability. Prolonged release of this drug in asthma and chronic obstructive pulmonary disease treatment could be beneficial because of low frequency of delivery.

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