Preparation, Characterization, and Swelling and Drug Release Properties of a Crosslinked Chitosan-Polycaprolactone Gel

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ABSTRACT: For applications in biotechnology to prepare biopolymers containing functional groups is essential. In addition, these materials have to be strong to provide physical support for practical applications. Recently, chitosan, polycaprolactone (PCL), and their various combinations were used for this purpose. In this work, we described the preparation and characterization of a new biodegradable polymeric gel containing chitosan and PCL. The gel preparation reactions were performed in suitable acetic acid solutions to obtain the products in high yields. A crosslinking agent was added to produce crosslinked gels. Swelling behavior of chitosan/PCL gels in different compositions was studied, and the results were compared. The chitosan/PCL gels show a rather large equilibrium swelling in water and in the phosphate buffered saline solution. Acrylic acid (AA) was added to these gels during preparation process to obtain a stable material for various applications. These polymeric gels were characterized by Fourier transform infrared. Their physical and morphological properties were investigated by using differential scanning calorimeter and scanning electron microscope techniques, respectively. Cell growth experiments indicate that chitosan, a positively charged polysaccharide, is not suitable for cell proliferation studies. On the other hand, the drug release studies were successful and, 59% of lidocaine, was released from a chitosan/PCL/AA hydrogel in buffer solution at pH = 7.4 at 37°C. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2885–2894, 2011

Key words: chitosan; polycaprolactone; lidocaine; gel preparations; drug release

INTRODUCTION

During the past two decades, significant advances have been made in the development of biodegradable polymeric materials for biomedical applications. Degradable polymeric biomaterials are preferred candidates for developing therapeutic devices such as temporary prostheses, three-dimensional porous structures as scaffolds for tissue engineering and as controlled/sustained release drug delivery vehicles.[1] Each of these applications demands material with specific physical, chemical, biological, biomechanical, and degradation properties to provide efficient therapy. In recent years, biodegradable polymers have attracted attention of researchers to be used carriers for drug delivery systems.[2–6]

Polylactides, polyglycolides, polycaprolactone (PCL) and their blends, and copolymers have been used for the preparation of biomaterials. Biodegradable polymers should be bioabsorbed at a predetermined date and the space initially occupied by them should be fully replaced by the regenerated tissue. Our laboratories have extensively studied the preparation, characterization, and various properties and applications of these biomaterials in drug release and cell growth potentials.[7–11]

Most classical polymeric materials lack functional groups to improve cell adhesion properties causes inability of modification by these polymers. To overcome this limitation, potentially degradable polyesters that have side chains with functional groups are synthesized and investigated.[7–19]

In a recent study, we introduced L-aspartate groups as a bioactive moity to modify PLLA/PEO/PLLA macromer to improve biocompatibility and thus to enhance cell affinity of this multiblock terpolymer.[10] We have also used a triblock copolymer of poly(ethylene glycol)/poly(D,L-lactide-*co*-glycolyde)/poly(ethylene glycol) and its modification

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with type-I collagen to form a surface, which is suitable for cell adhesion and proliferation.[11]

Intveld et al. have reported ring-opening polymerization of morpholine-2,5-dione derivatives that provides a method to prepare a wide range of degradable polyesteramides.[13] Langer and coworkers have synthesized a copolymer composed of L-lactic acid and lysine units, which was further modified to create comb-like graft copolymers.[14,15] Morita and co-workers reported the synthesis of poly(ε-caprolactone-*co*-glycolic acid-*co*-L-serine) copolymer.[16] Quchi et al. reported the preparation of poly(lactic acid-glycolic acid-lysine) terpolymer.[17] Lavic et al. have synthesized PLGA-polylysine block copolymer by compiling PLGA to poly(ε-carbobenzoxy-L-lysine).[18] Jo and co-workers have reported the preparation of poly(ethylene glycol)-tethered poly(propeline furmarate) and its modification with cell adhesion peptides.[19] Shinoda et al. have synthesized a novel type of amphiphilic biodegradable copolymer from L-aspartic acid and L-lactide.[20]

Chitin is the abundant polymer found in nature after cellulose. Chitosan, poly[β-(1,4)-amino-2-deoxy-β-D-glucose], is obtained from chitin by a deacetylation process. This is a biocompatible material and widely used in the biomedical purposes. Kulkarni and co-workers synthesized crosslinked chitosan with poly(ethylene glycol) and reported the production of a pH-independent swelling product.[21] Hu et al. provided the results on the formation process and mechanism of chitosan-poly(acrylic acid) nanospheres.[22] Honma et al. prepared the blends of PCL-chitin and PCL-chitosan with compositional gradients by using dissolution/diffusion procedure.[23] Yu and co-workers reported chitosan and characterized a crosslinked chitosan gel using a dialdehyde korizac glucomann as crosslinking agent.[24] Qu et al. obtained chitosan and acrylic acid (AA) polymer by laser-induced thermal polymerization of components and reported an insoluble ladder-type polymer, which can be used in dentistry.[25] Recently, the blends of chitosan, a naturally derived polysaccaride, with a synthetic polymer PCL were used for tissue engineering applications by Madihally and coworkers.[26]

Recently extensive work is published using chitosan and other biocompatible polymeric materials to produce scaffolds for tissue engineering and to use these materials as a potential carrier for drug release.[26–34] Some of these materials were prepared as blends of chitosan and PCL, chitosan and poly (L-lactide), or poly(L-lactic acid), chitosan, and wollastonite.[27–30] As chitosan is soluble in aqueous media it is difficult to interrelate the cell-growth or drug-release activities of these biomaterials to chitosan or the other blending substances.

Therefore, in this work, we described the preparation and characterization of a new biodegradable polymeric gel containing chitosan and PCL in the presence of a crosslinking agent. The Fourier transform infrared (FTIR) spectral analysis, morphological and thermal characterization of these gels were done. AA was added to some of these gels during preparation process to obtain a stable material for various applications. Swelling behavior of chitosan/PCL and chitosan/PCL/AA gels in different compositions was studied and the results were compared. Lidocaine (LD) was used as a model drug for the investigation of release behavior of the new chitosan/PCL/AA gels. LD is a local anesthetic drug and widely used IV for short-term management of life-threatening ventricular arrhythmias.[35]

MATERIALS AND METHODS

Materials

Chitosan (MW 350,000, ~ 85% deacetylated) and polycaprolatone (MW 80,000) were purchased from Aldrich Chemical Company, whereas AA was supplied from Fluka. *N*,*N*′-dicyclohexylcarbodiimide (DCC), Aldrich, was used as crosslinker. Its melting point range and density are 34–35°C and 0.92 g mL^{-1}, respectively. Cartsan DBTDL, stannous dilaurate (T-12) was used as a catalyst. This was a product of Cincinnati Milacron Chemicals. LD was supplied from Doga Ilac Company, Turkey. Acetic acid (98%) and glacial acetic acid were obtained from Aldrich Chemical Company.

Characterization

FTIR spectra were obtained using a Perkin-Elmer spectrum one model spectrometer at room temperature. Spectra were recorded from 400–4000 cm^{-1} using KBr pellets.

The scanning electron microscope (SEM; JEOL-JSM 5600) was used to study the morphology of chitosan-PCL gels. The morphological analysis of the pure components and the crosslinked gels at various compositions were performed after being coated with gold in a sputtering device.

Freeze Drier system, Lyphlock 6, a Labconco Model was used to lyophilize the gels to form porous scaffolds.

Thermal properties of the Chitosan/PCL gels were analyzed by scanning calorimeter (Pyris 1 DSC Perkin-Elmer). Samples of 5–20 mg were placed into aluminum differential scanning calorimeter (DSC) pans and were scanned at a heating rate 20°C min^{-1} under a stream of nitrogen from 0 to 200°C. The reported T_g values were based on the second run.

TABLE I
The Composition and Swelling Properties of the Hydrogels

Sample identity	Chitosan (wt %) in the original mixture	Crosslinker (wt %) in the original mixture	Swelling medium	Equilibrium mass swelling
Hydrogel-1	67	0.83	Water	40
Hydrogel-2	67	0.67	Water	91
Hydrogel-3	67	0.41	Water	180
Hydrogel-4	50	0.70	Water	95
Hydrogel-5	50	0.70	PBS[a]	48
Hydrogel-6	25	1.50	Water	17
Hydrogel-7	50	1.50	Water	53
Hydrogel-8	75	1.50	Water	58
Hydrogel-9	25	1.50	PBS[a]	16
Hydrogel-10	50	1.50	PBS[a]	32
Hydrogel-11	75	1.50	PBS[a]	42
Hydrogel-12	67	3.00	Water	26
Hydrogel-13	50 (Chitosan) 25 (acrylicacid)	0.37	Water	14
Hydrogel-14	50 (Chitosan) 25 (acrylicacid)	0.37	PBS[a]	6

[a] pH = 7.4.

The temperature was taken as the initial onset of the change of slope in the DSC curve.

LD release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophometer at 262 nm.

Preparation of chitosan-PCL gels

The hydrogels were prepared using the method described in the literature.[26] The stock solution of chitosan was prepared in 0.5*M* acetic acid. For this purpose, 3.9 g chitosan powder was first suspended in 90 mL of distilled water by stirring with fast agitation for 30 min at 50°C. The resulting suspension of chitosan was heated by vigorous stirring in an oil bath for 25 min at 120°C. Acetic acid was added to the stirred solution to obtain a 0.5*M* acid solution. Then the volume of the chitosan solution was completed to 100 mL by adding distilled water. PCL (1.8 g) was dissolved in 100 mL of glacial acetic acid by stirring at room temperature until homogenous solution was obtained. The PCL solution was added to the chitosan solution drop by drop under continuous stirring. Two drops of catalyze T-12 and 48 mg of crosslinker, DCC, were added into this mixture to obtain crosslinked hydrogel. The preparation composition and swelling properties of this sample is shown in Table I as hydrogel-1. The yield was found 89% of the initial substances. The crosslinked product was poured into the Teflon petri dishes of 2 cm diameter and subsequently evaporated in the hood at room temperature until a concentrated gel was obtained. All samples were prepared by using the same procedure, but at different mixing ratios of the original materials. The compositions of various gels were indicated in Table I. The chemical structures of the chitosan, PCL, and crosslinker are given in Figure 1(a).

Preparation of chitosan-PCL-AA gels

Chitosan-PCL mixtures were prepared in acetic acid using the same procedure described above. Certain amount of AA was added to this mixture to obtain 25% of AA in this solution. Crosslinker (DCC) and the catalyst were also added into this mixture. The crosslinked product was poured into the Teflon petri dishes and subsequently evaporated in the hood at room temperature to obtain the concentrated gel. AA containing hydrogels were included in Table I as samples hydrogels 13 and 14.

The chemical structure of chitosan/PCL/AA is given by Wu earlier.[36] The original chitosan was fully acrylated and the amino and amide groups were converted to imides. PCL is grafted to AA. Figure 1(b) represent the formula of PCL-*g*-AA/chitosan.[36]

Lyophilization

The concentrated gel products were first frozen for 2 h at −80°C. Then the frozen gels were lyophilized within a freeze drier for 6 h at −50°C to obtain the porous scaffold. The lyophilized freeze-dried samples were used in the cell growth and drug release experiments.

Swelling of the gels

A fundamental relationship exists between the swelling of a polymer in a solvent and the nature of the

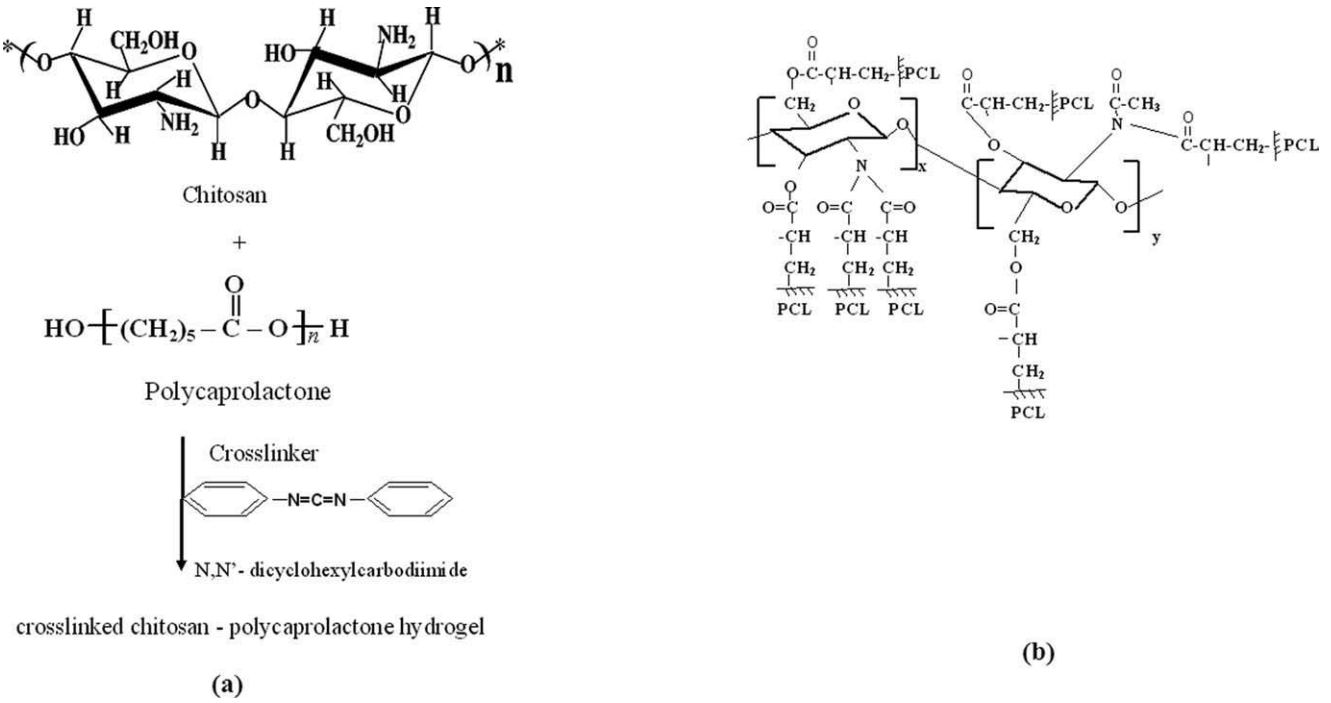

Figure 1 Chemical structures of (a) Chitosan/PCL gel (b) Chitosan/PCL/AA gel.

polymer and the solvent. The swelling properties of gels were determined by immersing dried test samples to swell in distilled water (pH = 5.6) and in phosphate buffered saline solution (PBS; pH = 7.4) in a temperature-controlled bath at 25 ± 0.1°C. To reach the equilibrium degree of swelling, the gels were immersed in distilled water or in PBS saline for 2 days. The equilibrium mass swelling and mass swelling percentages were calculated by using the following equations,

$$\text{Equilibrium mass swelling} = (m_\infty - m_o)/m_o \quad (1)$$

$$\text{Mass swelling}\ (\%) = (m_t - m_o)/m_o \times 100 \quad (2)$$

where m_o is the mass of the dry gel and m_t and m_∞ are the masses of the swollen gel at time t and at equilibrium, respectively. A sample of the chitosan/PCL/AA (50/25/25) containing 0.70% (w) crosslinker was dried in vacuum oven at 50°C until a constant weight of the gel was reached. The known amount of chitosan/PCL/AA dry gel film was immersed in distilled water and in PBS and kept at room temperature for several hours. The gels were removed from the solution and wiped gently with a tissue paper and weighted quickly. Thus, the hydrogel was weighed in the swollen state and equilibrium mass swelling was calculated using eq. (1). The equilibrium mass swelling values were compared for various compositions of the gel and different amounts of the crosslinker used in the gel.

Cell growth experiments

Lyophilized hydrogel samples were used *in vitro* cell culture experiments, which were performed using L929 mouse fibroblasts to determine the effects of this polymeric gel on cell attachment and the growth properties of cell on chitosan/PCL and chitosan/PCL/AA hydrogels were studied.

For this purpose, the biopolymers cut into 10-mm diameter and 1-mm thick films were exposed to UV light in a sterile hood for 1 h for sterilization before the experiments. Before cell seeding, the biopolymers were incubated with 0.5 mL of culture medium (DMEM/F12 Ham's medium with 10% fetal calf serum (FCS) and penicillin-streptomycin, Biological Industries). For cell seeding, the cells growing on cell culture plates were suspended using trypsin-EDTA solution (Biological Industries) and counted using a hemocytometer. A 100 μL of cell suspension containing 250,000 cells was placed onto the biopolymers, and these were subsequently placed into six-well plates. The cells were allowed to attach to the materials for 2 h. The growth of the cells on tissue culture polystyrene was observed by placing the 100 μL cell suspension directly into the wells. At the end of this incubation period, 2 mL of culture medium was added to each well and cells were grown in a CO_2 incubator for another 72 h.

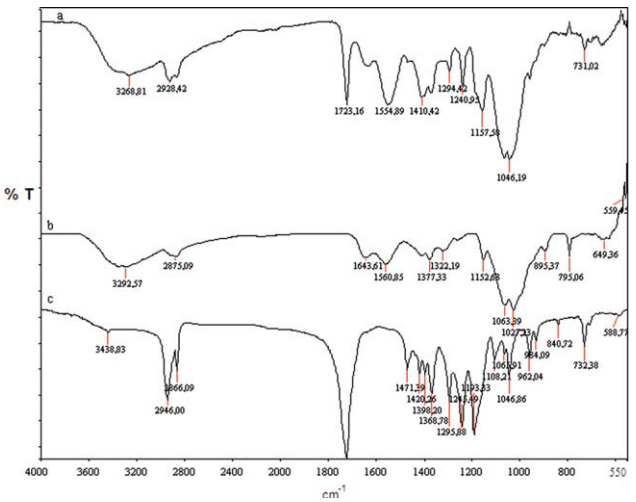

Figure 2 Fourier transform infrared (FTIR) spectrum (a) Chitosan/PCL (50/50) (hydrogel-7), (b) pure chitosan, (c) pure PCL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Cell viability experiments

The amount of cells present on the surfaces of the materials was quantified using a neutral red uptake assay.[37] Cells grown on the above materials were washed once with sterile PBS and incubated in sterile PBS containing 2% FCS and 0.001% neutral red (Sigma, St. Louis, MO) for 2 h at 37°C in the CO_2 incubator, during which the dye is internalized into viable cells by an active process. This was followed by a brief wash with 4% formaldehyde in PBS, which enabled the cells to be fixed. The internalized dye was solubilized in 1 mL of 50% EtOH, 1% acetic acid in H_2O. The amount of entrapped dye was measured spectrophotometrically at 540 nm, and the absorbance is proportional to the number of viable cells in each well.

Drug loading and release experiments

The dry hydrogels chitosan/PCL/AA (50/25/25) containing 0.70% (w) crosslinker were equilibrated in 1000 ppm (mg/L) of LD prepared in phosphate buffer at pH 7.4 at room temperature for 2 days. After incubation, the polymer gels were removed from the solution and rinsed with buffer solution. The LD release experiments were carried out by transferring previously incubated drug gels in a vessel containing 10 mL of phosphate buffer at pH 7.4 at 37 ± 0.1°C at a constant shaking rate (5 rpm). At various times aliquots of 2 mL were drawn from medium to follow LD release and placed again into the same vessel so that the liquid volume was kept constant. LD release was determined spectrophotometrically at 262 nm. The amount of the percentage release of LD at pH 7.4 was calculated from the following equation:

$$\% \text{ Release} = (W_t/W_{\text{total}}) \times 100 \quad (3)$$

where W_t is the weight of released drug in water at any time, and W_{total} is the initial total weight of the drug taken by the gel system.

RESULTS AND DISCUSSIONS

Characterization of the gels

FTIR spectrum of Chitosan/PCL (50/50) (wt/wt) (hydrogel-7) gel (a) is compared with pure chitosan (b), and pure PCL (c), shown in Figure 2. The characteristic peaks of the stretching of carbonyl groups (—C=O) occurring at 1730 cm^{-1} and (—C—O) ester groups at 1296 and 1245 cm^{-1} are seen in the spectrum of pure PCL. The carbonyl stretching band was observed to shift to the lower frequency, 1723 cm^{-1}, from 1730 cm^{-1} but the ester group existed almost

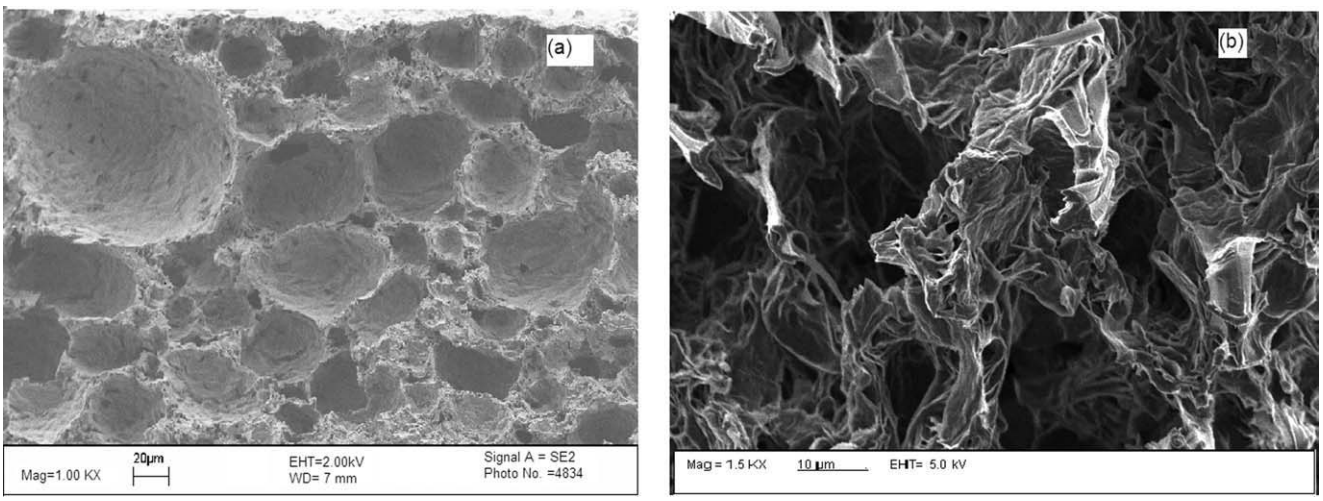

Figure 3 SEM micrographs of pure PCL, (a) surface, (b) cross-section.

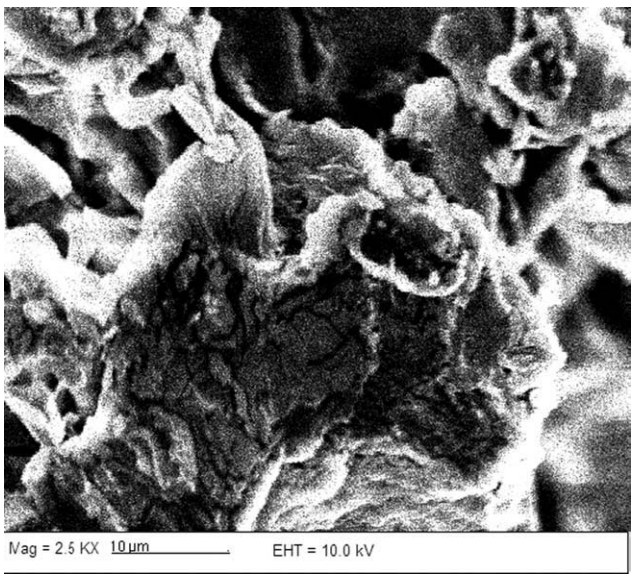

Figure 4 SEM micrograph of pure chitosan (cross-section).

at the same place (1295 cm^{-1}) in the spectrum of the gel. In the FTIR spectrum of pure chitosan [Fig. 2(b)], the characteristic band because of *N*—H bending vibration is observed at 1644 cm^{-1}, which also appeared in the gel. The other characteristic bands were also observed at 1064 and 1027 cm^{-1} for the secondary hydroxyl group (characteristic peak of —CH—OH in cyclic alcohols C—O stretch) and the primary hydroxyl group (characteristic peak of CH_2—OH in primary alcohols C—O stretch), respectively. These bands were also found in the spectrum of the gel shifted to narrow peak at 1067 and 1046 cm^{-1}, respectively.

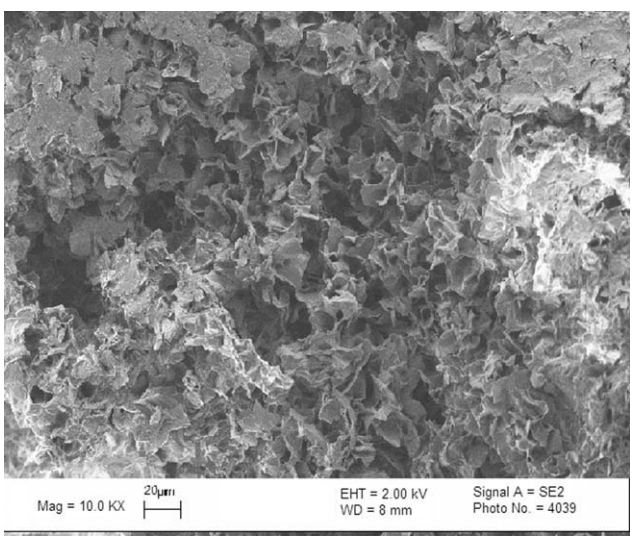

Figure 5 SEM micrograph of chitosan/PCL (25/75) (hydrogel-6) (cross-section).

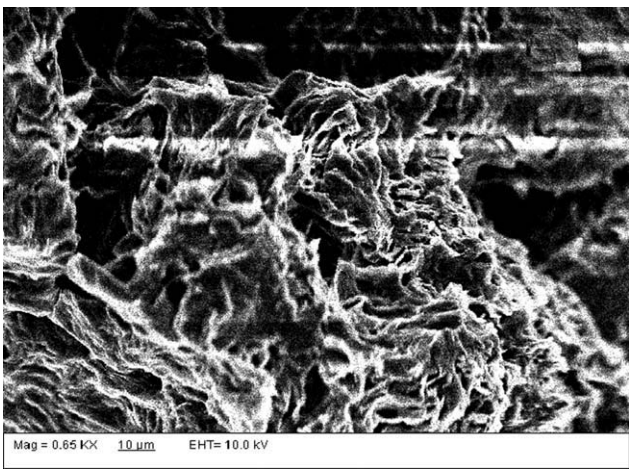

Figure 6 SEM microaph of chitosan/PCL (50/50) (hydrogel-7) (cross-section).

SEM evaluation

The SEM pictures in Figures 3–7 represent the morphology of pure PCL, pure chitosan, and chitosan/PCL gel, respectively. Figure 3(a,b), shows SEM micrographs of pure PCL. The cross-section of PCL in Figure 3(b) with more magnification shows the fibrous structure. The crystalline structure of PCL can easily be observed.

Figure 4 represents the morphological structure of pure chitosan. The structures of chitosan/PCL gel in different compositions are given in Figures 5–7 The porous and loose structure are observed in all gel micrographs. The crystalline structure as flakes seen between the holes comes from PCL. The diameters of the holes are about 25–60 µm. The homogenous

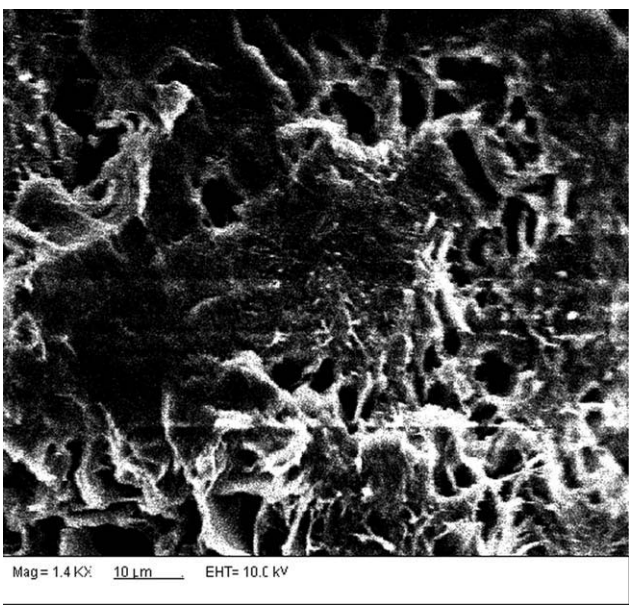

Figure 7 SEM micrograph of chitosan/PCL (75/25) (hydrogel-8) (cross-section).

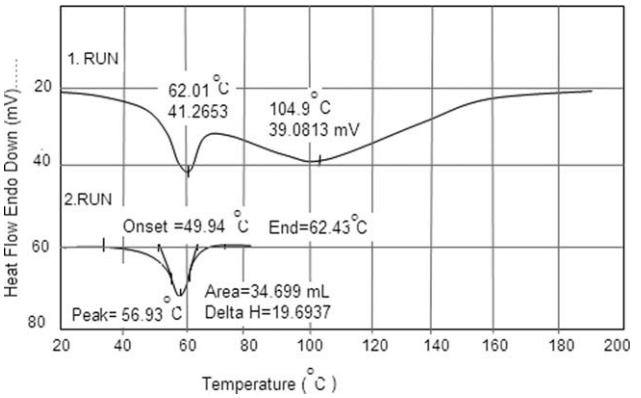

Figure 8 DSC Thermograms of chitosan/PCL(50/50) (hydrogel-7).

structure is seen in the composition of chitosan/PCL (25/75) (hydrogel-6). Comparing with the composition (75/25) of the crosslinked chitosan/PCL gel (hydrogel-8), fine dispersion and homogeneity of chitosan in the PCl matrix is clearly seen in crosslinked chitosan/PCL (25/75) gel.

DSC studies

PCL is the crystalline polymer and melts at 60°C and glass transition temperature of PCL is around −60°C. Chitosan is also a semicrystalline polymer and its degradation temperature is at 270°C before melting.[38] The melting point was obtained at 56.9°C for the chitosan/PCL (50/50) (hydrogel-7) as seen in Figure 8. The melting point of the gel shifted to the lower temperature when compared with the melting point of PCL. This decrease may be related to the lowering of the chemical potential of the crystalline PCL in the gel as reported in the literature.[26]

Swelling of the gels

The swelling percentage results of the gel are listed in Table I. The equilibrium mass swelling decreases with increasing crosslinker content (hydrogels 1–3). The mass swelling of the chitosan/PCL in PBS solution is lower than in the water for each sample. The mass swelling of AA samples (hydrogels 13 and 14) in water and in PBS solution was also small when compared with the other samples. The mass swelling also increases with increasing of the amount of chitosan in the gels. The swelling experiments were carried out with hydrogel (chitosan/PCL/AA) (50/25/25) containing 0.70% (w) crosslinker. The percentage of the swelling curves of the chitosan/PCL/AA gels were constructed and representative swelling curves at water and at 1000 ppm of LD solutions are shown in Figure 9. In the presence of LD, the equilibrium swelling of the chitosan/PCL/AA gel is lower than in water.

Diffusion

Diffusion in polymers is an important mechanism in pharmacy for the controlled release of drugs.[39–42] The swelling percentage of the chitosan/PCL/AA gels in aqueous LD solutions was used for the calculation of a certain diffusion characteristics. The following equation was used to determine the nature of diffusion of LD solution into hydrogels.[39]

$$F = M_t/M_\infty = kt^n \tag{4}$$

Here F is the fractional uptake, M_t/M_∞, where M_t is the amount of diffusant sorbed at time t, M_∞ is the maximum amount absorbed, k is a constant incorporating characteristics of macromolecular network system and the penetrant, n is the diffusional exponent, which is indicative of the transport mechanism. Equation (4) is valid for the first 60% of the normalized solvent uptake. For Fickian kinetics in which the rate of penetrate diffusion is rate limiting, $n = 0.5$, whereas values of n between 0.5 and 1 indicate the contribution of non-Fickian processes such as polymer relaxation.

Diffusion coefficients are important penetration parameters of some chemical species to polymeric systems. Using "n" and "k," the diffusion coefficient (D) of solvent in the matrix could be calculated using the following equation[43,44]:

$$\begin{aligned} k &= 4\,[D/\pi\, r^2]^n \\ 4\,D^n &= k(\pi\, r^2)^n \\ D^n &= (k/4)(\pi\, r^2)^n \end{aligned} \tag{5}$$

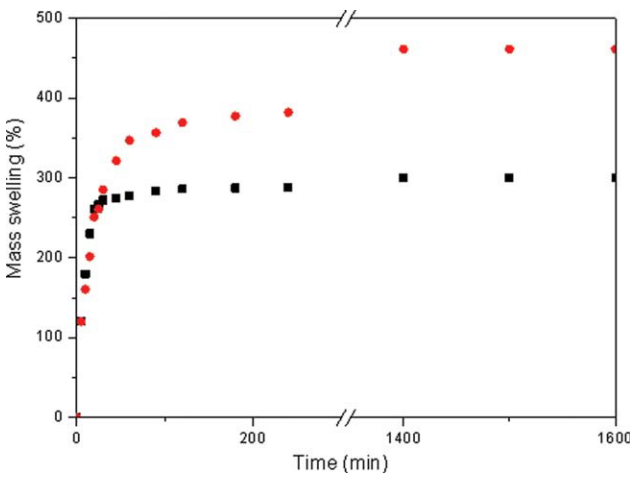

Figure 9 Percentage mass swelling as a function of time for hydrogel (chitosan/PCL/AA) (50/25/25) containing 0.70% (w) crosslinker: ● in water and ■ in 1000 ppm of LD solutions at 25 ± 0.1°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II
The Parameters of Diffusion of Water and the Aqueous Solution of LD into (chitosan/PCL/AA) (50/25/25) Containing 0.70% (w) Crosslinker

Solution	$k \times 100$	n	Swelling mechanism	$D \times 10^8$ ($cm^2\ sec^{-1}$)	[a] $D \times 10^8$ ($cm^2\ sec^{-1}$)
Water	1.5	0.50	Fickian	1.4	12.2
Lidocaine	2.9	0.47	Fickian	1.9	11.4

[a] Ref. 12.

where, "D" is the diffusion coefficient and "r" is the radius of gel disc.

From the plots of ln F versus ln t for the series of chitosan/PCL/AA gels in water and aqueous LD solutions, the exponents n and k values were calculated from the slope and intercept of the lines, respectively, and are presented in Table II. It can be clearly seen from the table that the number determining the type of diffusion (n) is 0.47 and 0.50, respectively. According to these results, one can see that the diffusion of water and LD into the chitosan/PCL gels is Fickian character. Diffusion coefficients of the chitosan/polycarolactone gels in water and in aqueous solutions of LD were calculated from the Figure 9 and the eq. (5) and given in Table II. Similar findings have been found with experiments of Taşdelen et al. and their diffusion coefficients were also given in Table II.[12] However, in our previous work, the diffusion coefficient of the poly(*N*-isopropylacrylamide/itaconic acid) copolymeric hydrogels in solution of LD is slightly higher than that of the chitosan/PCL/AA gels prepared in this work.

Absorption of water and LD solution

The water absorbed by the chitosan/PCL/AA hydrogel is quantitatively represented by the equilibrium water content (EWC),[45,46] where

$$\mathrm{EWC} = \frac{W_{\mathrm{eq}} - W_{\mathrm{o}}}{W_{\mathrm{eq}}} \tag{6}$$

Here, W_{eq} is the weight of the swollen gel at time t (equilibrium) and W_{o} is the weight of the dry gel at time 0. The EWC values of the hydrogels and equilibrium LD content were calculated as 0.82 and 0.75 for water and LD, respectively. All EWC values of the hydrogels (0.75–0.82) were greater than the percent content values of body (0.6 or 60%). Thus, the chitosan/PCL/AA gels exhibited fluid contents similar to those of living tissues.

Drug loading

For the investigation of drug uptake behavior of chitosan/PCL/AA gels, the uptake capacities of were determined by measuring the mass of adsorbate per unit mass of adsorbent (q_e). q_e values (mg g^{-1}) are calculated from the following equation.[47]

$$q_e = [(C_i - C)^* V_t]/m \tag{7}$$

C_i and C are the initial and equilibrium concentration of solution of adsorbate, V_t the volume of solution treated, and m is the mass of dry adsorbent in gram.

LD was used as a model drug for the investigation of controlled release behavior of hydrogels. LD adsorption capacity of the hydrogels used in this work was found to be 113.8 (mg LD/g dry gel).

Release behavior of hydrogels

The release profile of LD in the chitosan/PCL/AA gels in phosphate buffer at pH 7.4 at 37 ± 0.1°C is shown in Figure 10. The LD release increases rapidly at first and then gradually reaches the equilibrium value in ~ 24 h. Figure 11 shows the fractional LD release, expressed as M_t/M_∞, where M_t and M_∞ are the amounts of drug released at the times t and infinite, respectively, as a function of square root of time ($t^{1/2}$) for the hydrogels. In this figure, the drug release during the first stage could be influenced for

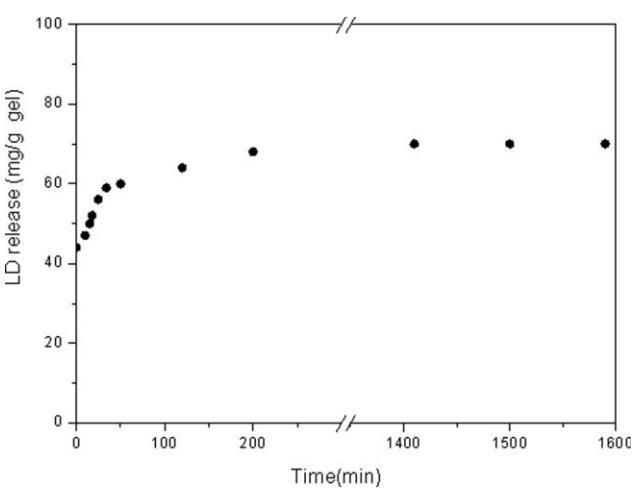

Figure 10 The release profiles of LD from hydrogel (chitosan/PCL/AA) (50/25/25) containing 0.70% (w) crosslinker in phosphate buffer at pH 7.4 at 37 ± 0.1°C.

the relaxation of polymer chains. Thus, the hydrogels show an initial non-Fickian behavior indicating similar rates of Fickian diffusion and polymer relaxation.[48]

Finally, it is found that 59% of LD is released from the chitosan/polycarolactone hydrogel in phosphate buffer at pH 7.4 at 37°C.

Cell culture

Cell growth experiments were carried out using the chitosan/PCL gels described in this work. The chitosan/PCL hydrogels produced using low amounts of crosslinkers were not stable for cell growth experiments. Their structural integrity was lost in 3 days in PBS solution. The chitosan/PCl hyrogels synthesized by using 3% (*w*) crosslinker (hydrogel-12 in Table I) were not stable for cell growth experiments. The chitosan/PCL/acrylate gels were also used for cell culture experiments. The results of cell growth experiments are shown in Figure 12. Following 72-h incubation on different surfaces, L929 mouse fibroblasts have proliferated on pure PCL films, albeit less than cells, which were seeded on tissue culture plastic or glass. The amount of cells on chitosan/PCL (hydrogel-12 in Table I) or chitosan/PCL/acrylate (hydrogel-13 in Table I) gels were substantially lower than PCL, indicating that these biopolymers did not support cell attachment and growth.

CONCLUSIONS

In this study, the crosslinked chitosan/PCL gel was prepared and characterized. The FTIR spectral analysis, morphological and thermal characterization were performed. The swelling properties in water and PBS were studied. The cell culture experiments carried out with chitosan/PCL gels were not satisfactory because these gels were not stable in buffer solutions. Two stable hydrogels were prepared and used for cell growth experiments: (a) a chitosan/PCL stable hydrogel containing 67% chitosan was obtained by using 3% (*w*) crosslinker (hydrogel-12) and (b) a chitosan/PCL/AA terpolymer containing 50% chitosan (hydrogels 13 and 14). Cell growth experiments indicate that chitosan, a positively charged polysaccharide, is not suitable for cell proliferation studies. On the other hand, the drug release studies were successful and, 59% of LD was released from a chitosan/PCL/AA hydrogel in buffer solution at pH = 7.4 at 37°C.

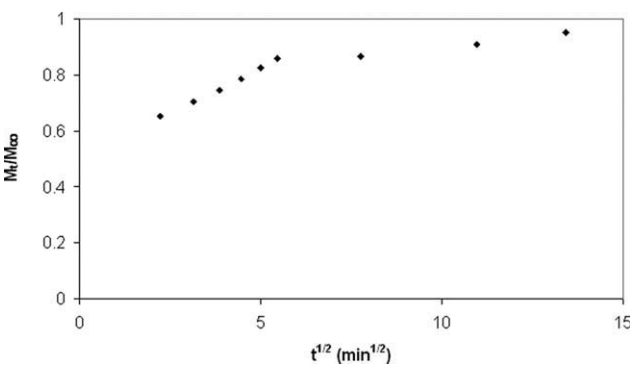

Figure 11 The fractional LD release from hydrogel (chitosan/PCL/AA) (50/25/25) containing 0.70% (w) crosslinker in phosphate buffer at pH 7.4 at 37 ± 0.1°C.

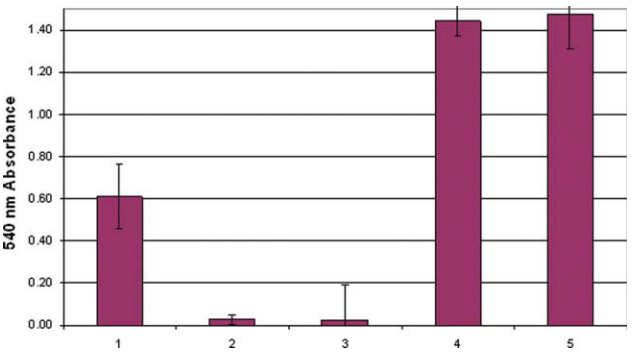

Figure 12 Cell viability assay by neutral red uptake. Cells grown on 1- PCL film, 2- Chitosan/PCL/acrylic acid (hydrogel 13), 3- Chitosan/PCL (hydrogel 12), 4-Cells grown on tissue culture plastic, 5- Cells grown on glass. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

References

1. Peppas, N. A.; Langer, R. Science 1994, 263, 1715.
2. Sahoo, S.; Sasmal, A.; Nanda, R.; Phani, A. R.; Nayak, P. L.; Carbohyd Polym 2010, 79, 106.
3. Bhattarai, N.; Gunn, J.; Zhang, M. Adv Drug Deliver Rev 2010, 62, 83.
4. Duan, K.; Honglin, C.; Huang, J.; Yu, J.; Liu, S.; Wang, D.; Li, Y. Carbohyd Polym 2010, 80, 498.
5. Kelner, A.; Schacht, E. J Control Release 2005, 101, 13.
6. Allen, C.; Han, J.; Yu, Y.; Maysinger, D.; Eisenberg, A. J Control Release 2000, 63, 275.
7. Kayaman-Apohan, N.; Karal-Yılmaz, O.; Baysal, K.; Baysal, B. M. Polymer 2001, 42, 4109.
8. Kayaman-Apohan, N.; Baysal, B. M. Macromol Chem Phys 2001, 202, 1182.
9. Porjazoska, A.; Kayaman-Apohan, N.; Karal-Yılmaz, O.; Cvetkovska, M.; Baysal, K.; Baysal, B. M. J Biomater Sci Polymer Ed 2002, 13, 1119.
10. Karal-Yılmaz, O.; Kayaman-Apohan, N.; Misirli, Z.; Baysal, K.; Baysal, B. M. J Mater Sci Mater Med 2006, 17, 213.
11. Porjazoska, A.; Karal-Yılmaz, O.; Baysal, K.; Cvetkovska, M.; Sirvanci, S.; Ercan, F.; Baysal, B. M. J Biomater Sci Polymer Ed 2006, 17, 323.
12. Tasdelen, B.; Kayaman-Apohan, N.; Guven, O.; Baysal, B. M. J Appl Polym Sci 2004, 91, 911.
13. Intveld, P. J. A.; Shen, Z. R.; Takens, G. A. J.; Dijkstra, P. J.; Feijen, J. J Polym Sci A Polym Chem 1994, 32, 1063.
14. Barrera, D. A.; Zylstra, E.; Lansbury, P. T.; Langer, R. Macromolecules 1995, 28, 425.

15. Hrkach, J. S.; Ou, J.; Lotan, N.; Langer, R. Macromolecules 1995, 28, 4736.
16. John, G.; Tsuda, S.; Morita, M. J Polym Sci A Polym Chem 1997, 35 1901.
17. Ouchi, T.; Miyazaki, H.; Arimura, H.; Tasaka, F.; Hamada, A.; Ohya, Y. J Polym Sci Pol Chem 2002, 40, 1426.
18. Lavik, E. B.; Hrkach, J. S.; Lotan, N.; Nazarov, R.; Langer, R. J Biomed Mater Res 2001, 58, 291.
19. Jo, S.; Engel, P. S.; Mikos, A. G. Polymer 2000, 41, 7595.
20. Shinoda, H.; Asou, Y.; Suetsugu, A.; Tanaka, K. Macromol Biosci 2003, 3, 34.
21. Kulkarni, A.; Hukkeri, V. I.; Sung, H. W.; Liang, H. F. Macromol Biosci 2005, 5, 925.
22. Hu, Y.; Chen, Y.; Chen, Q.; Zhang, L.; Jiang, X.; Yang, C. Polymers 2005, 46, 12703.
23. Honma, T.; Zhao, L.; Asakawa, N.; Inoue, Y. Macromol Biosci 2006, 6, 241.
24. Yu, H.; Lu, J.; Xiao, C Macromol Biosci 2007, 7, 1100.
25. Qu, X.; Wrzyszczynski, A.; Pielichowski, K.; Pielichowski, J.; Adamczak, E.; Morge, S.; Linden, L. A.; Rabek, J. F. Macromol Sci Pure Appl Chem 1997, A34, 881.
26. Sarasam, A.; Sundararajan, V. M. Biomaterials 2005, 26, 5500.
27. Son, T.; Kim, D.; Kim, B.; Koo, K.; Park, Y.; Kim, J.; Lim, H. J Appl Polym Sci 2007, 104, 3057.
28. Chen, C.; Dong, L.; Cheung, M. K. Eur Polym Mater 2005, 41, 958.
29. Prabaharan, M.; Rodriguez-Perez, M. A.; de Saja, J. A.; Mano, J. F. J Biomed Mater Res Part B: Appl Biomater 2006, 81B, 427.
30. Zao, L.; Chang, J. Mater Sci: Mater Med 2004, 15, 625.
31. Yao, Z.; Zang, C.; Ping, Q.; Yu, L. L. Carbohydr Polym 2007, 68, 781.
32. Fukuda, J.; Khademhosseini, A.; Yeo, Y.; Yang, X.; Yeh, J.; Eng, G.; Blumling, J.; Wang, C. F.; Kohane, D. S.; Langer, R. Biomaterials 2006, 27, 5259.
33. Sinha, V. R.; Singla, A. K.; Wadhawan, S.; Kaushik, R.; Kumria, R.; Bansal, K.; Dhawan, S. Int J Pharm 2004, 274, 1.
34. Jin, J.; Song, M.; Hourston, D. J. Biomacromolecules 2004, 5, 162.
35. Dollo, G.; Estebe, J. P.; Le Corre, P.; Chevanne, F.; Le Verge, R. Eur J Phar Sci 2001, 13, 319.
36. Wu, C. S. Polymer 2005, 46, 147.
37. Borenfreund, E.; Puerner, J. A. J Tissue Cult Methods 1984, 9, 7.
38. Min, B. M.; Lee, S. W.; Lim, J. N.; You, Y.; Lee, T. S.; Kang, P. H.; Park, W. H. Polymer 2004, 45, 7137.
39. Crank, J. Mathematics of Diffusion; Oxford University Press: New York, 1970.
40. Peppas, N. A.; Franson, N. M. J Polym Sci Polym Phys Ed 1983, 21, 983.
41. Tasdelen, B.; Kayaman-Apohan, N.; Guven, O.; Baysal, B. M. Radiat Phys Chem 2004, 69, 303.
42. Tasdelen, B.; Kayaman-Apohan, N.; Guven, O.; Baysal, B. M. Radiat Phys Chem 2005, 73, 340.
43. Peppas, N. A. Pharm Acta Helv 1985, 60, 110.
44. Korsmeyer, R. W.; Peppas, N. A. In Controlled Release Delivery Systems; Roseman, T. J., Mansdorf, S. Z., Eds.; Marcel Dekker: New York, 1983; p 77.
45. Huglin, M. B.; Zakaria, M. B. J Appl Polym Sci 1986, 31, 457.
46. Karadag, E.; Saraydın, D.; Guven, O. Nucl Inst and Meth B 2004, 225, 489.
47. Sen, M.; Uzun, C.; Guven, O. Int J Pharm 2000, 203, 149.
48. Martellini, F.; Mei, L. H. I.; Balino, J. L.; Carenza, M. Radiat Phys Chem 2003, 66, 155.