Carbohydrate Research 344 (2009) 699–706

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Synthesis and characterization of chitosan-based pH-sensitive semi-interpenetrating network microspheres for controlled release of diclofenac sodium

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ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form 27 December 2008
Accepted 13 January 2009
Available online 19 January 2009

Keywords:
Chitosan
Microspheres
pH-Sensitive
Anti-inflammatory drug
In vitro release

ABSTRACT

pH-Sensitive semi-interpenetrating networks (IPNs) based on chitosan (Cs) and acrylamide-grafted hydroxyethylcellulose (AAm-g-HEC) were prepared in the form of microspheres (MPs) by emulsion-crosslinking technique using glutaraldehyde (GA) as a crosslinker. Diclofenac sodium (DS) drug was successfully encapsulated into IPN microspheres by varying the ratio of Cs and AAm-g-HEC, % drug loading, and amount of GA. DS encapsulation of up to 83% was obtained as measured by UV spectroscopy. MPs with average particle sizes in the range of 188–310 μm were obtained. MPs were characterized by Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), and Differential scanning calorimetry (DSC). Diffusion coefficients (D) of water transport through the microspheres were determined using an empirical equation. In vitro release of DS from these matrices has been investigated in pH 1.2 and 7.4 media.

The pH-sensitivity of hydrogel is an important factor in designing polymers for controlled drug release in the gastrointestinal tract (GIT). The pH of the GIT varies from pH 1 to 3 in the stomach and increases to approximately 7–8 in the colon.[1,2] The pH-sensitivity of hydrogels is due to the presence of weakly acidic and/or basic functional groups on the polymer backbone. Particularly, synthetic polymers like poly(methyl methacrylate),[3] poly(acrylic acid),[4] poly (*N*,*N*-*iso*-propylacrylamide),[5] and natural polysaccharides, such as chitosan,[6] have been used as pH-sensitive drug delivery systems. Chitosan, a polyaminosaccharide, is a partially deacetylated polymer of *N*-acetyl glucosamine and is usually prepared from chitin. Chitosan and chitin are natural polysaccharides found in a wide range of natural sources such as crustaceans, fungi, and insects.[7] Chitosan has many biomedical applications over polysaccharides due to its non-toxic, biocompatible, and biodegradable nature.[8–10] The crosslinked chitosan can be used as a pH-sensitive hydrogel that swells in acidic solutions due to protonation of free amino groups. Chitosan hydrogels have been widely used in controlled release (CR) of drugs in stomach via oral route.[11–13] Hydroxyethylcellulose (HEC) is a non-ionic polymer with little surface activity in solution and is compatible with a wide range of surfactants and salts.The mechanical strength of HEC can be improved by modifying it through grafting with acrylamide (AAm). Copolymers and terpolymers containing AAm offer a number of advantages, which include high permeability to both hydrophobic and water-soluble solutes and increased mechanical strength, depending upon copolymer composition and crosslink density.[14–16]

Diclofenac sodium (DS), one of the most useful non-steroidal anti-inflammatory drugs (NSAIDs) is a practically insoluble compound in acidic solution (pK_a 4.0), however, it dissolves in intestinal fluid. It has a short half-life in plasma (1–2 h). The daily dose varies between 75 and 200 mg/person, given in 3 or 4 divided portions depending on the route of administration. The most common adverse effects of the drug are gastritis, peptic ulceration, hypersensitivity reactions, and depression of renal functions.[17–20] Because of the short biological half-life and associated adverse effects, it is considered as an ideal model drug for controlled drug delivery. Recently, a number of studies have described oral release formulations of DS using chitosan matrix that has been reticulated covalently or ionically due to the amino groups of the biopolymer.[21–29] In this study, we report the synthesis of AAm-g-HEC to produce the IPN microspheres with Cs crosslinked by GA for the CR of DS. The microspheres formed have been characterized by variety of techniques to understand their drug release characteristics and morphological as well as chemical interactions.

1. Experimental

1.1. Materials

Chitosan from crab shells with 85% deacetylation degree was obtained from Sigma–Aldrich. Diclofenac Sodium was obtained

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0008-6215/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.carres.2009.01.011

from Bio-ethicals, Hubli, India. Hydroxyethylcellulose (medium molecular weight), acrylamide, acetic acid, ceric ammonium nitrate (CAN), light liquid paraffin oil, *n*-hexane, glutaraldehyde (25% solution), and Tween-80 were purchased from LOBA Chemical, Mumbai, India. All the chemicals were used without further purification.

1.2. Synthesis of acrylamide grafted onto hydroxylethylcellulose

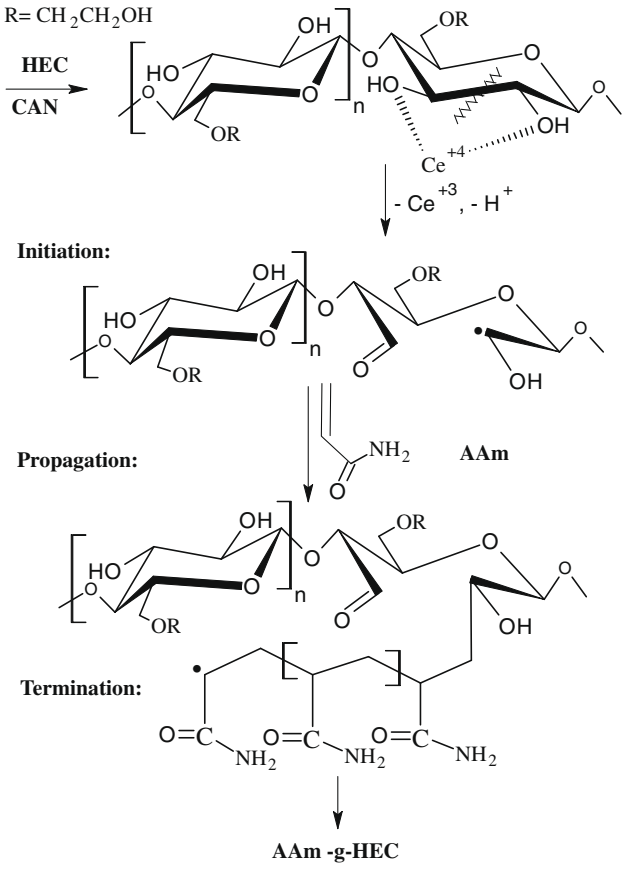
Acrylamide grafted onto hydroxyethylcellulose, hereafter designated as (AAm-g-HEC), was prepared by free-radical polymerization. A 2% aqueous solution of HEC was prepared by dissolving the polymer in distilled water under constant stirring in 250 mL three-necked round-bottomed flask overnight. To this, a solution of 0.14 mol of acrylamide (AAm) was added and stirred for 1 h at 70 °C. The initiator solution containing 5.47×10^{-4} mol of CAN was added dropwise to the above mixture. Free radical polymerization was carried out under continuous purging of nitrogen gas with a constant stirring at 70 °C for 5 h. The reaction mixture was then cooled and a pinch of hydroquinone was added to quench the reaction. The grafted copolymer that was obtained was precipitated in acetone and was washed with aqueous acetone to remove the monomer residues and the byproduct poly acrylamide homopolymer. The solid copolymer was dried in an electrically controlled oven at 40 °C before further use. Samples of grafted copolymers of varying compositions were synthesized by the same procedure as mentioned above. The % grafting efficiency was calculated by

$$\%\ \text{Grafting effciency} = \left(\frac{W_1 - W_0}{W_2}\right) \times 100 \qquad (1)$$

where W_0, W_1 and W_2 denote weights of hydroxyethylcellulose, grafted copolymer, and monomer, respectively. The proposed reaction mechanism is presented in Scheme 1.

1.3. Preparation of semi-IPN MPs and drug loading

Semi-IPN microspheres of Cs and different amounts of AAm-g-HEC (15%, 35%, 45%) were prepared by emulsion-crosslinking method using GA as a crosslinking agent. Weighed amounts of Cs and AAm-g-HEC were dissolved in 2% acetic acid solution with continuous stirring for 24 h to obtain a homogeneous solution. Required amount of diclofenac sodium was dispersed in the above polymer solution and allowed to stir overnight. This solution was emulsified into light liquid paraffin in the presence of 1% Tween-80 using mechanical stirrer equipped with a three-blade propeller at 500 rpm for 10 min. Then, a mixture of different quantities of GA and 1 mL of 5 N HCl was added slowly to this w/o emulsion to harden the microspheres, and stirring was continued for 2 h. The MPs thus produced were separated by filtration and washed repeatedly with *n*-hexane followed by water to remove the paraffin oil and excess of the crosslinking agent. Totally, 12 formulations were prepared by varying three parameters, that is, amount of AAm-g-HEC, drug loading, and amount of crosslinking. To understand the variables, formulation codes are assigned as given in Table 1. For example, the formulation code, P-*xyz* refers to three variables namely, *x*—represents three amounts of AAm-g-HEC numbered as 0, 1, 2, and 3 for 0%, 15%, 30%, and 45% AAm-g-HEC in IPN, *y*—represents three amounts of drug loadings numbered as 1, 2, and 3 for 15%, 30%, and 45% of drug in the microspheres, *z*—represents three amounts of crosslinking numbered as 1, 2, and 3 for 3 mL, 6 mL, and 9 mL of GA added. The formation of semi-IPN structure is schematically shown in Scheme 2.



Scheme 1. Proposed reaction mechanism for grafting of acrylamide AAm onto hydroxyethylcellulose HEC using ceric ion initiation.

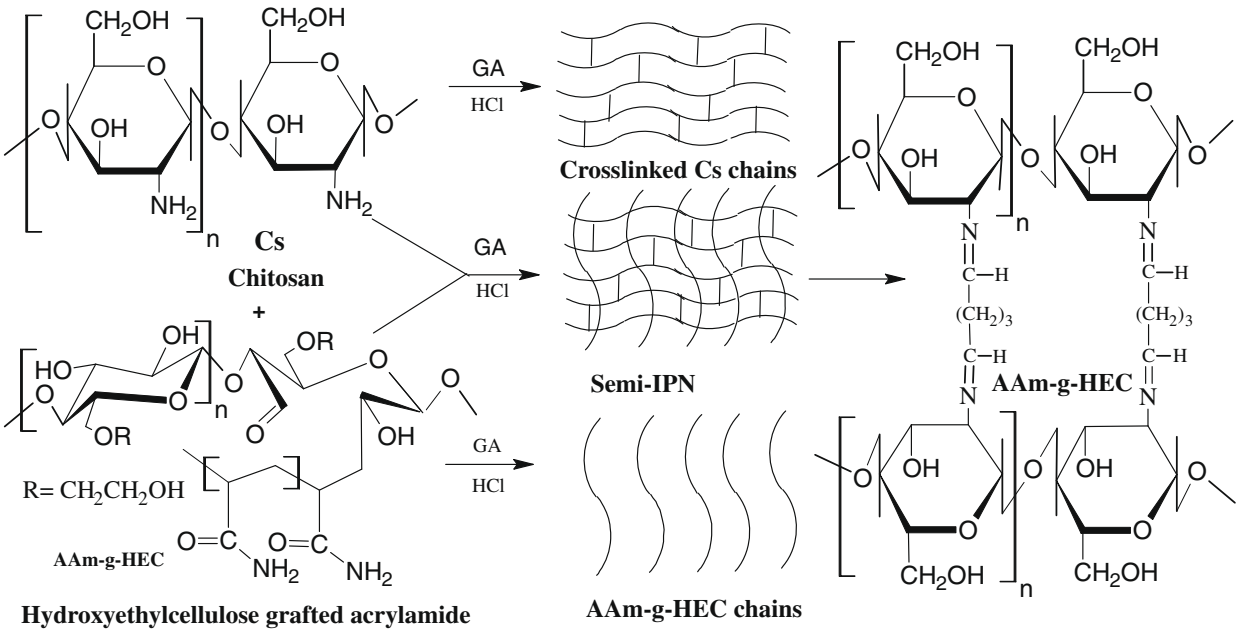
1.4. Estimation of percent drug loading and encapsulation efficiency

The amount of DS loaded in the microspheres was estimated by grinding ~10 mg of the microspheres to powder using an agate mortar, extracted for 24 h with 50 mL of buffer solution pH 7.4 at 50 °C. The solution was centrifuged to separate the suspended polymer particles which are washed twice to extract the drug completely into the centrifugate. The clear supernatant centrifugate was diluted with the buffer solution and was analyzed by UV-spectrophotometer (Shimidzu, Japan) at λ_{max} of 278 nm. These data were collected in triplicate, but the average values (standard errors

Table 1
Results of percent encapsulation efficiency and mean size of the microspheres at 6 mL GA and stirring speed of 600 rpm

Formulation code[a]	% AAm-g-HEC in microspheres	% Diclofenac sodium loaded	% Encapsulation efficiency	Mean particle size (μm)
P-012	—	15	60	188
P-022	—	30	62	190
P-032	—	45	63	192
P-112	15	15	66	220
P-122	15	30	72	224
P-132	15	45	76	230
P-212	30	15	70	260
P-222	30	30	73	264
P-232	30	45	77	265
P-312	45	15	80	305
P-322	45	30	82	308
P-332	45	45	83	310

[a] P-312 refers to Formulation with three parameters, three AAm-g-HEC compositions, three DS loadings and three crosslinking amount.



Scheme 2. Schematic representation of synthesis of semi-IPN.

<3%) were considered in calculating the percent drug loading and encapsulation efficiency. These were calculated as follows:

$$\% \text{ Drug loading} = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}}\right) \times 100 \quad (2)$$

$$\% \text{ Encapsulat ion efficiency} = \left(\frac{\text{Actual drug loading}}{\text{Theoretical drug loading}}\right) \times 100 \quad (3)$$

These data for various formulations are presented in Tables 1 and 2.

1.5. Swelling studies

Equilibrium water uptake by the microspheres was determined by measuring the extent of swelling of the matrix in phosphate solutions having pHs 1.2 and 7.4. To ensure complete equilibration, samples were allowed to swell for 24 h to obtain equilibrium at 37 °C. Excess surface adhered liquid drops were removed by blotting, and the swollen microspheres were weighed on an electronic microbalance. The hydrogel microspheres were then dried in an oven at 60 °C for 5 h until there was no change in the weight of the dried mass of the samples. The percent equilibrium water uptake (Q) was calculated as

$$Q = \left(\frac{W_\infty - W_0}{W_0}\right) \times 100 \quad (4)$$

where W_∞ is the mass of swollen MPs and W_0 is the mass of dry MPs.

Drug release from the crosslinked hydrogel depends upon the extent of water penetration into the matrix. In order to understand the molecular transport of water into crosslinked microspheres, dynamic swelling studies were carried out by the microscopic technique.[30] The change in diameter of the microspheres in gastric and intestinal pH conditions was monitored as a function of time. Experiments were performed in triplicate, but average values were considered for data treatment and calculations.

Table 2
Results of percent encapsulation efficiency and mean size of the MPs with different amounts of GA for MPs containing 30% AAm-g-HEC and 30% drug at stirring speed of 600 rpm

Formulation code	Crosslinking agent (GA in ml)	% Encapsulation efficiency	Mean particle size (μm)
P-221	3	82	284
P-222	6	73	264
P-223	9	60	240

1.6. In vitro release studies

In vitro release of diclofenac sodium from IPN microspheres was carried out in simulated gastric fluid of pH 1.2 for the initial 2 h and then in simulated intestinal fluid of pH 7.4, until completion of dissolution maintained at 37 °C. The experiments were carried out using the USP-I dissolution tester (Dissotest, Lab India, Mumbai). Into 500 mL of the dissolution fluids was introduced 100 mg of each sample and stirred at 100 rpm. At regular intervals of time, aliquots of 5 mL were withdrawn and analyzed for diclofenac sodium at λ_{max} value of 278 nm using a UV-spectrophotometer (Shimidzu, Japan). Dissolution medium was maintained at constant volume by replacing the samples with a fresh dissolution medium.

1.7. Fourier transform infrared (FTIR) spectral studies

FTIR spectra of plain Cs, MPs of Cs, and Cs/AAm-g-HEC were measured using Shimadzu-1800S spectrophotometer at 2 cm^{-1} resolution with 64 scans over the spectral range from 4000 to 400 cm^{-1}. All the samples were crushed with potassium bromide and pellets were obtained by applying a pressure of 600 kg/cm^2 using FTIR pellet maker.

1.8. Differential scanning calorimetry (DSC) studies

For the differential scanning calorimetric (DSC) measurements, a Perkin–Elmer DSC-7 operating in a dynamic mode was employed. Nitrogen gas was used as an inert gas at a flow rate of 20 mL/min. Each sample (80 mg) of (a) plain diclofenac sodium, (b) drug-loaded microspheres, and (c) placebo microspheres was placed in aluminum pan. An empty aluminum pan was used as a reference, and a heating/cooling rate of 10 °C/min was applied throughout the study with scan ranges between 0 and 300 °C.

1.9. Scanning electron microscopic (SEM) studies

SEM micrographs of the microspheres were measured using a JEOL model JSM-840A scanning electron microscope (Japan), and micrographs were taken at the required magnification. A working distance of 33.5 mm was maintained, and the acceleration voltage used was 10 kV with the secondary electron image (SEI) as a detector.

1.10. Particle size measurements

Particle size of the MPs was measured using optical microscopy (Swift Prior transmitted light microscope, Instrument UK). About 200 mg of MPs was dispersed into 100 mL of methanol and was stirred under sonication for 2 min to remove agglomerations of MPs. For measurement of sizes of different formulations, the sample holder was cleaned with distilled water followed by acetone to prevent cross contamination. The mean particle size (μm) was recorded, and these results are included in Tables 1 and 2.

2. Results and discussion

2.1. Preparation and characterization of microspheres

Graft copolymerization of HEC with AAm was carried out by Ce(IV)-catalyzed free radical polymerization. The complex formed with the –OH groups of HEC at the C-2 and C-3 positions decomposes to generate the free radical site, facilitating the grafting to occur at the active site of HEC with the incoming acrylamide monomer. The proposed reaction mechanism is shown in Scheme 1. The grafting efficiency was found to be 95%. The reaction was carried out at 70 °C for 6 h. The Ce(IV) toxicology has been investigated, and the results indicated that Ce(IV) salts are not biologically stable in the aqueous media.[31] Therefore, cerium species that freely circulate in the blood as colloidal compounds or protein complexes are likely to contain Ce(III). It is also reported that these ions are non-cytotoxic. The safety of glutaraldehyde (GA) was evaluated, and it has been proven that it is non-carcinogenic and safe.[32] The diclofenac sodium-loaded IPN microspheres of Cs and AAm-g-HEC were prepared by crosslinking with GA. The percent encapsulation efficiency ranged between 60 and 83. However, percent encapsulation efficiency showed a dependence on percent drug loading, polymer composition, and extent of crosslinking.

The percent encapsulation efficiency of plain Cs microspheres ranged from 60% to 63%, but for the remaining formulations, it ranged from 66% to 83%. Such smaller values are due to a lesser soluble drug in the polymer solution, thus incorporating a lesser amount of DS into microspheres.

The percent encapsulation efficiency increased with increasing amount of the AAm-g-HEC in the microspheres. For microspheres containing 15, 30, and 45 wt % AAm-g-HEC and 30 wt % DS with 6 mL GA, encapsulation efficiencies were 66%, 73%, and 80% for formulations P-122, P-222 and P-322, respectively (Table 1). However, the microspheres crosslinked with 3, 6 and 9 mL of GA, encapsulation efficiencies were, 82%, 73% and 60% for formulations, P-221, P-222 and P-223, respectively (Table 2). Such a decreasing trend is due to increasing crosslink density, whereby the microspheres will become rigid, reducing the free volume spaces within the polymer matrix. The size of particles depends on the amount of percent AAm-g-HEC content, drug present, and extent of GA used for crosslinking. In general, the size of particles ranged from 188 to 310 μm (Tables 1 and 2). The particle size of plain Cs is smaller than that of Cs/AAm-g-HEC MPs. For instance, as the amount of AAm-g-HEC increased from 15% to 45%, particle size increased from 220 to 310 μm. This can be explained by the fact that at higher amounts of the AAm-g-HEC, the viscosity of polymer solution increased, thereby producing bigger droplets during emulsification which were later hardened in the presence of GA. With increasing amount of drug in the MPs, particle size also increases (Table 1).This is attributed to the fact that drug molecules might have occupied the free volume spaces within IPN matrix, thereby hindering the inward shrinkage of the polymer matrix.[33] However, amount of crosslinking has a significant effect on the particle size (Table 2). For instance, for the MPs containing 30% drug and 30% AAm-g-HEC, with increasing crosslinking, by adding 3–9 mL of GA, the particle size decreased from 284 to 240 μm for formulations P-221 to P-223. This is attributed to the fact that with increasing amount of GA in the IPN matrix, the shrinkage of particles had taken place, thereby reducing their sizes.

2.2. FTIR spectral studies

Figure 1 shows the FTIR spectra of (a) plain HEC and (b) AAm-g-HEC. As can be seen, in the spectrum of HEC, a broad band at 3440 cm^{-1} is due to O–H stretching vibrations. The O–H bending is seen at 1355 cm^{-1}. Aliphatic C–H stretching and bending vibrations are indicated by bands at 2929 and 1429 cm^{-1}, respectively. The C–O–C stretching vibration is present at 1145 cm^{-1}, while bands at 1020 and 1050 cm^{-1} indicate the presence of C–O stretching vibrations. In case of AAm-g-HEC, all the peaks observed in HEC had appeared. A new shoulder band that appeared at ~3200 cm^{-1} and a sharp peak around ~1665 cm^{-1} correspond to –NH and C=O stretching vibration, respectively, thereby confirming grafting reaction. The new peak at ~1560 cm^{-1} corresponding to C–N bending vibration further supports the grafting reaction. Figure 2 shows the FTIR spectra of (a) plain Cs, (b) Cs/AAm-g-HEC, and (c) placebo microspheres. In case of plain Cs, a broad band that appeared at ~3450 cm^{-1} corresponded to the amine N–H symmetric stretching vibration, which might be due to deacetylation of chitosan. Another broad band appearing around ~1073 cm^{-1} indicates the C–O stretching vibration of chitosan. Three bands observed at 1650, 1594, and 1379 cm^{-1} indicate amide-I, amide-II, and amide-III, respectively. Peaks observed at ~2845 and ~2919 cm^{-1} are typical of C–H stretching vibrations. The observed peaks at ~1380 and ~1410 cm^{-1} are assigned to CH_3 symmetrical deformation mode.[34,35]

In case of placebo microspheres, all the bands of both Cs and AAm-g-Dx were observed in addition to a new band observed at 1560 cm^{-1}, which was formed due to imine bonds (C=N) as a result of crosslinking reaction between amino groups in Cs and aldehyde groups in GA.[36,37] A reaction leading to the formation of crosslinks is depicted in Scheme 2.

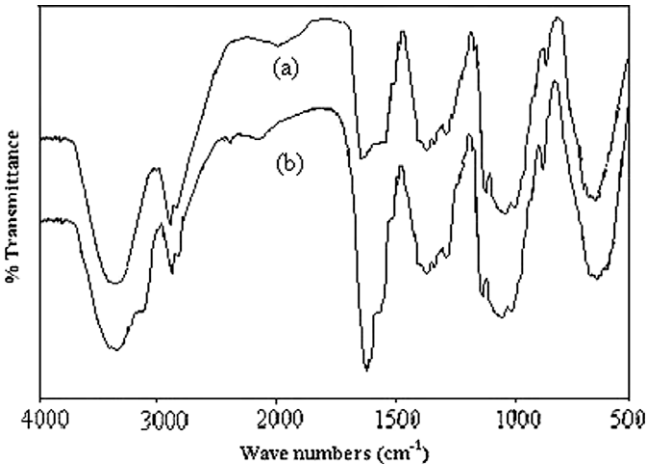


Figure 1. FTIR spectra of (a) plain HEC and (b) AAm-g-HEC.

2.3. DSC studies

DSC thermograms of (a) plain DS, (b) drug-loaded microspheres, and (c) placebo microspheres are displayed in Figure 3. The DSC thermogram of plain DS showed three endothermic peaks. The first medium endothermic peak at 50 °C was due to water loss. The second and third sharp peaks at 104 and 250 °C are due to polymorphism and melting of DS. In case of placebo microspheres, broad peaks were observed at 88 and 195 °C, respectively, due to endothermic and exothermic transition. In the case of drug-loaded microspheres, all the peaks observed in placebo microspheres are noticed, but there was no peak corresponding to DS, indicating the amorphous dispersion of DS in the polymer matrix.

2.4. SEM studies

SEM micrographs of (a) single repaglinide-loaded microspheres and (b) group of repaglinide-loaded microspheres taken at 200 and 40 magnifications are shown in Figure 4. In all cases, the microspheres were almost spherical in nature and aggregated with rough surfaces. This phenomenon may be due to the type of polymers.

2.5. Swelling studies

For the percent equilibrium water uptake for the plain Cs MPs, with increasing amount of DS, only a small increase in swelling was noticed from 200% to 204% (P-012 to P-032) at pH 1.2. However, in media having a pH 7.4 the percent in swelling ranged from 180% to 184%. The results show that in media having a pH of 1.2, the equilibrium swelling of MPs was found to be higher than in the media having a pH of 7.4, which can be attributed to the protonation of Cs amine groups that lead to structure relaxation due to the repulsion of the polymeric chains, and the dissociation of secondary interactions followed by swelling.[38] It is also noted that formulations containing higher amounts of AAm-g-HEC showed higher swelling rates than those formulations containing lesser amounts of AAm-g-HEC. As the amount of AAm-g-HEC in the matrices increased from 15% to 45%, equilibrium water uptake increased significantly from 188% to 239% (P-112 to P-332) at pH 1.2, while at pH 7.4, it increased from 200% to 322%. This is attributed to the extremely hydrophilic nature of AAm-g-HEC matrix, leading to higher water uptake. As the amount of GA in the matrices increased from 3 to 9 mL, equilibrium water uptake decreased significantly from 245% to 150% (P-221 to P-223) at pH 1.2, while at pH 7.4, it decreased from 295% to 170%. The reduction in water uptake may be due to the formation of a rigid network structure at higher extent of crosslinking.

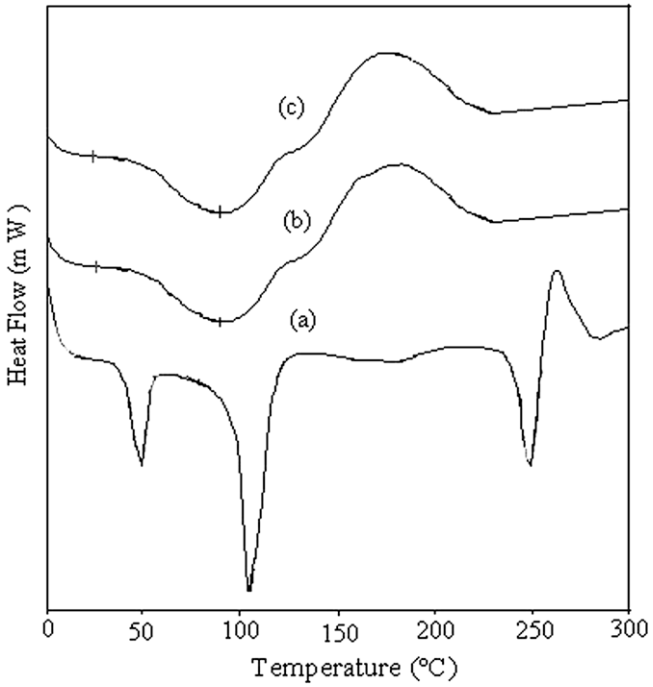


Figure 3. DSC thermograms of (a) plain diclofenac sodium (b) drug-loaded microspheres, and (c) placebo microspheres.

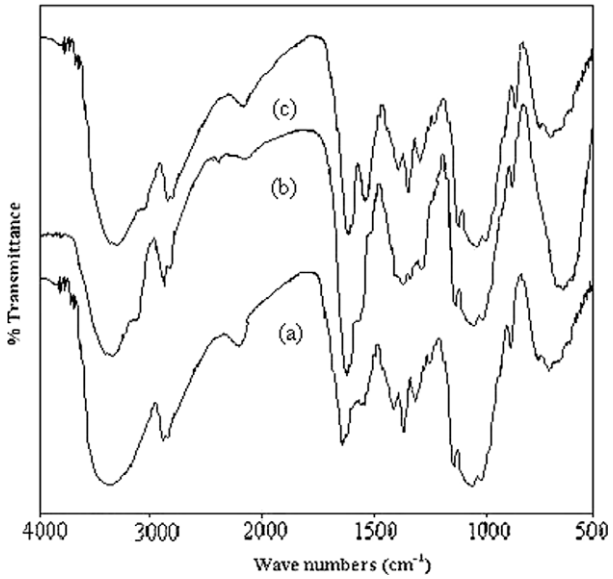


Figure 2. FTIR spectra of (a) plain Cs, (b) CS/AAm-g-HEC, and (c) placebo microspheres.

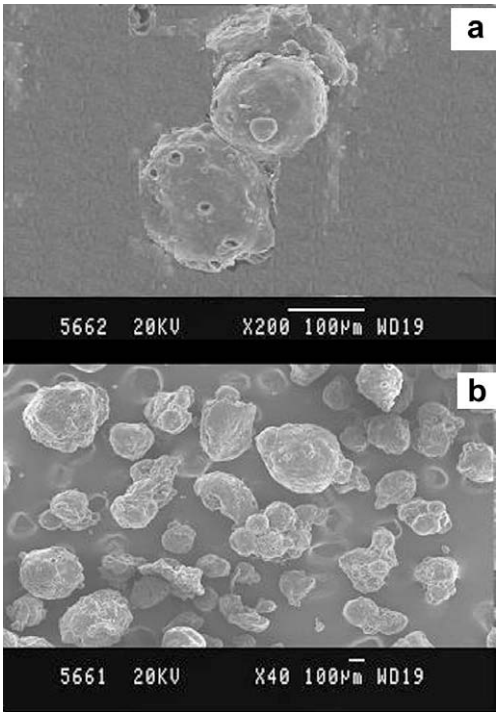


Figure 4. SEM micrographs of single repaglinide-loaded MP (a) and group of repaglinide-loaded MPs (b).

The dynamic swelling was determined from measured changes in the microsphere diameter, D_t, as a function of time using an optical microscope with a micrometer. Figure 5 shows the plot of normalized diameter, D_t/D_0 (where D_0 is initial diameter of the MPs), as a function of time for different amounts of GA added at pH 7.4, and pH 1.2. It is evident that the normalized diameter decreases with increasing amount of GA, which could be due to the rigid network formed at a higher amount of GA (P-221 to P-223).

In the present study, DS is dispersed in the almost spherically shaped microspheres; it is possible to model the transport process to calculate the diffusion coefficient, D. The diffusion process involves immersion of microspheres into the medium of interest followed by drastic absorption of the liquid by the polymer matrix. The sorption and desorption processes under simulated test conditions were described in mathematical models.[39,40]

Diffusion in spherically shaped matrices can be described by the following equation derived from Flick's second law using Laplace transformation as

$$\frac{M_t}{M_\infty} = 6\sqrt{\frac{Dt}{\pi^2}}\left(\frac{1}{\sqrt{\pi}} + 2\sum_{n=1}^{\infty} ierf\frac{nr}{\sqrt{Dt}}\right) - 3\frac{Dt}{r^2} \quad (5)$$

Here, M_t is the amount of liquid released at time t, and M_∞ is total amount of liquid in the microsphere. Because Eq. (5) is quite complicated to be solved, Baker and Lonsdale[41] have derived a simple equation appropriate for the present case, after using the following initial and boundary conditions:

$$t = 0, \quad r \prec R, \quad C = C_{in} \quad (6)$$

$$t \succ 0, \quad R = 0, \quad \partial C_{in}/\partial R = 0 \quad (7)$$

$$t \succ 0, \quad r = R, \quad C = C_{eq} \quad (8)$$

Here, r is the initial radius, R is the radius of the swollen microsphere, and C_{in} and C_{eq} are concentrations at the beginning and at the end of the diffusion process, respectively, so that

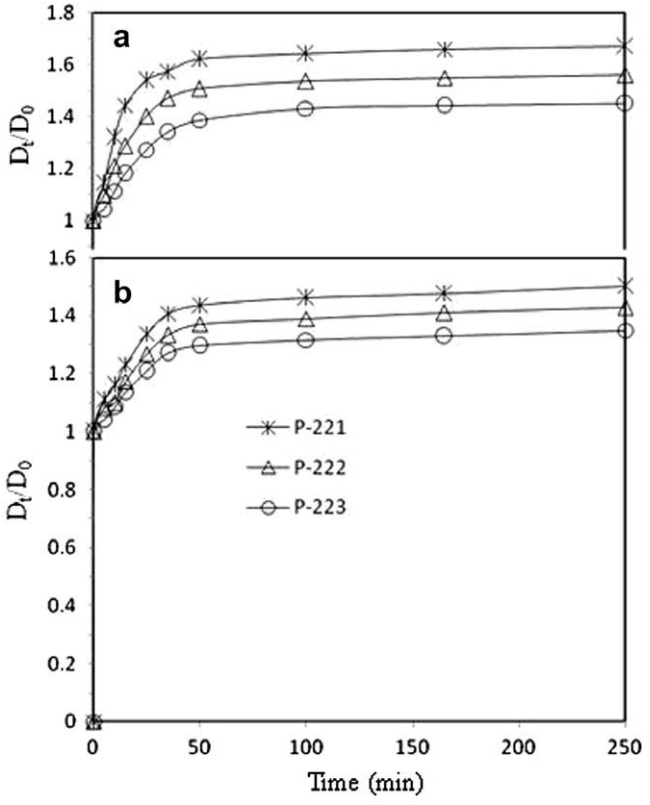


Figure 5. Plot of normalized diameter (D_t/D_0) versus swelling time, t is the effect of extent of crosslinking, (a) at pH 7.4, (b) at pH 1.2.

$$\frac{M_t}{M_\infty} = 6\sqrt{\frac{Dt}{\pi r^2}} - \frac{3Dt}{r^2}, \quad (9)$$

The above Eq. (9) is valid for the initial drug release (i.e., $0 \prec M_t/M_\infty \prec 0.4$).At short times, Eq. (9) can be approximated as

$$\frac{M_t}{M_\infty} = 6\left(\frac{Dt}{\pi r^2}\right)^{\frac{1}{2}} \quad (10)$$

The diffusion coefficient (D) of water absorption or drug release through microspheres can then be computed by using Eq. (11):

$$D = \left(\frac{r\phi}{6}\right)^2 \pi \quad (11)$$

Here, ϕ is slope of the linear portion of the plot of M_t/M_∞ versus $t^{1/2}$, r is initial radius of the microspheres, and M_∞ is maximum equilibrium swelling value.

The values of diffusion coefficient, D, thus calculated are found to depend on the extent of crosslinking agent added into the polymer matrix. The values of D decreased with increasing amount of GA in the MPs due to the fact that at higher crosslinking, free volume of the matrix will decrease, thereby hindering the small molecular diffusion through the MPs matrix. The D values in media having a pH of 7.4 are higher than those observed in media having a pH of 1.2. This indicates that in basic media, more solvent molecules will be transported through the hydrogel matrix than in the acidic media. This property correlates well with the drug release characteristics of the MPs developed. The crosslinking of polymer network generally leads to a reduction in molecular transport, with the reduced value of diffusion coefficient due to a decrease in crystallinity as a result of introduction of more number of junction points. In the present system, expectedly, the amount of liquid transported at any given time decreased with increasing crosslink density.

2.6. In vitro drug release

To understand the release of DS from the IPN microspheres of Cs and AAm-g-HEC, in vitro release experiments were carried out in simulated gastric fluid of pH 1.2 for the initial 2 h, then in simulated intestinal fluid of pH 7.4, for 10 h. When analyzing the release profile of drugs such as DS, the dependence of solubility on pH must be taken into account. In fact, DS presents a higher solubility in water as salt, but once the MPs are in artificial gastric solution (pH 1.2), DS is converted into its ionized form, which is well known to be practically insoluble in the stomach.[42,43] In contrast, when the MPs were immersed in a buffer solution of pH 7.4, drug reconversion into soluble salt depends on dissolution speed. The release profiles of DS from the different formulations in the pH-change systems are shown in Figures 6–8. From the figures, it is evident that the release rate of DS is low in all formulations for the first 2 h in acidic condition (pH 1.2). When the dissolution was changed to buffer solution of pH 7.4, an initial burst release followed by a slow release of DS from all formulations was observed.

The percent in vitro release time plots for the drug-loaded microspheres for formulations P-221, P-222 and P-223 are shown in Figure 6 to illustrate the effect of pH and crosslinking on in vitro release profiles. The formulation P-221 shows higher release rate than P-222, and similarly P-222 exhibits higher release rate than P-223. This is due to the formation of a more tightly crosslinked rigid network structure as the amount of crosslinking agent is increased from 3 to 9 mL.

Effects of polymer ratio in formulations P-022, P-122, P-222, and P-322 on release rates are presented in Figure 7. The percent in vitro release is higher in case of P-122 than in P-022. Similarly, P-222 exhibits higher release rates than P-122, and P-322 exhibits

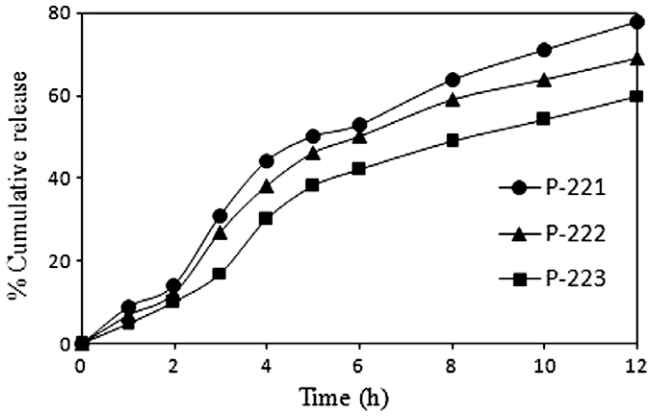


Figure 6. Effect of different crosslinkings on in vitro release profiles in the pH-change system for formulations P-221, P-222, and P-223.

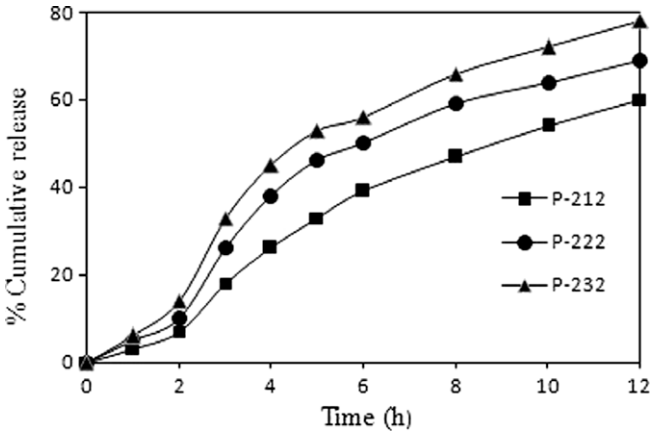


Figure 8. Effect of percent drug loading on in vitro release profiles of DS in the pH-change system for formulations P-212, P-222, and P-232.

higher release rates than P-222. The results can be explained by the fact that as the AAm-g-HEC content in the polymer matrix increases, swelling of the matrix increases due to the extremely hydrophilic nature of AAm-g-HEC, which in turn facilitates the penetration of water molecules into the loaded gel and subsequently enhances the amounts of DS released. The effect of percent drug loading on in vitro release profiles for formulations P-212, P-222, and P-232 is displayed in Figure 8. The formulation P-222 exhibits a higher release rate than P-212. Similarly, P-232 shows higher release rates than P-222. Thus, the release rates vary depending upon the amount of drug present in the matrices, that is, release is more for those formulations having higher amount of drug and vice versa. In phosphate buffer saline of pH 7.4, all formulations showed an initial fast release due to the dissolution of drug from the surface, but the release of drug was extended up to 12 h. The release data were investigated by fitting the cumulative fraction release data, M_t/M_∞, to an empirical equation:[44]

$$\frac{M_t}{M_\infty} = kt^n \tag{12}$$

Here, M_t and M_∞ represent the amount of DS released at time t and at infinite time, k is a constant characteristic of the drug-polymer system, and n is the diffusional exponent which suggests the nature of the release mechanism. The release parameters k and n were computed by applying the least-squares estimation method to the release data at 95% confidential limit. The values of k decrease with increasing crosslinking agent and increase with increasing ratio of the grafted copolymer and drug loading in the semi-IPN matrix. The k-values range between 0.181 and 0.427, indicating mild-type of interactions between the drug and the polymer matrices. Smaller values of k indicated the prolonged release of DS from the semi-IPN matrix. However, the values of n increased with increasing crosslinking agent and decreased with increasing ratio of the grafted copolymer and drug loading in the semi-IPN matrix. For instance, the n values of microspheres prepared by varying the amounts of GA, 3, 6, and 9 mL keeping DS and AAm-g-HEC (30%) constant ranged from 0.78 to 0.81, indicating that the drug release in the microspheres follows the anomalous transport, as was also suggested in earlier reports.[45,46] The n values for microspheres crosslinked with 3 mL of GA are smaller than those observed with 6 and 9 mL of GA. This is because of the loose crosslinking of the network matrix, thereby, leading to greater swelling. The values of n are higher for microspheres crosslinked with 9 mL of GA due to a tighter crosslinking of the semi-IPN matrix. Values of n for microspheres prepared by varying the amounts of AAm-g-HEC, 15, 30, and 45 wt % keeping DS (30%) and GA (6 mL) constant, ranged from 0.65 to 0.58, indicating that the drug release follows non-Fickian type. However, the DS-loaded microspheres, 15, 30 and 45 wt %, exhibited n values ranging from 0.42 to 0.39, suggesting that the drug release follows a Fickian type.

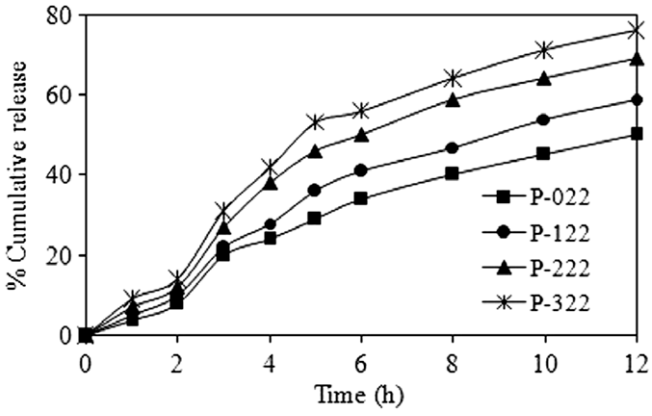


Figure 7. Effect of different blend compositions on in vitro release profile of DS in the pH-change system for formulation, P-022, P-122, P-222 and P-322.

3. Conclusions

DS was successfully encapsulated into the MPs and percent encapsulation efficiency up to 83% was noticed. The percent in vitro release rates depend upon the pH of the medium, AAm-g-HEC content, amount of crosslinker and extent of drug loading. Release rate was initially fast but it gradually became slow, which extended up to about 12 h. The values of diffusional exponent, n, calculated from the empirical equation have indicated the non-Fickian transport of drug through the matrices developed. Swelling studies of the microspheres have shown that with an increasing amount of AAm-g-HEC in the microspheres, water uptake increased. This effect is correlated with the release rates of drug though the microspheres contained different amounts of AAm-g-HEC. FTIR confirmed the formations of semi-IPN MPs. DSC has confirmed the amorphous dispersion of the drug in the polymer matrix.

References

1. Ogata, H.; Aoyagi, N.; Kaniwa, N.; Ejima, A.; Suzuki, K.; Ishioka, T.; Morishita, M.; Ohta, K.; Takagishi, Y.; Doi, Y.; Ogura, T. *J. Pharmacobio-dynam.* **1984**, *7*, 656–664.

2. Charman, W. N.; Porter, C. J. H.; Mithani, S.; Dressman, J. B. *J. Pharm. Sci.* **1997**, *86*, 269–282.
3. Bettini, R.; Chlombo, P.; Peppas, A. N. *J. Contr. Release* **1995**, *37*, 105–111.
4. Ramakisson-Ganorkar, C.; Liu, F.; Baudys, M.; Kim, S. W. *J. Contr. Release* **1999**, *59*, 287–298.
5. Tao, W.; Maher, T.; Sundaram, G. *J. Polym. Int.* **2004**, *53*, 911–918.
6. Tomlinson, E. et al. Plenum Press: New york and London, **1984**; p 199.
7. Shepherd, R.; Reader, S.; Falshaw, A. *Glycoconjugate J.* **1997**, *14*, 535–542.
8. Tozaki, H.; komoike, J.; Tada, C.; Maruyama, T.; Terabe, A.; Suzuki, T.; Yamamoto, A.; Moranishi, S. *J. Pharm. Sci.* **1997**, *86*, 560–563.
9. Paul, W.; Sharma, C . P. *STP. Pharma. Sci.* **2000**, *10*, 5–22.
10. Miyazaki, S.; Ishii, K.; Nadai, T. *Chem. Pharm. Bull.* **1981**, *29*, 3067–3069.
11. Illum, L. *Pharm. Res.* **1998**, *15*, 1326–1331.
12. Thacharodi, D.; Rao, K. P. *J. Chem. Tech. Biotechnol.* **1993**, *58*, 177–181.
13. Nakatsuka, S.; Andrady, A. L. *J. Appl. Polym. Sci.* **1992**, *44*, 17–28.
14. Downs, E. C.; Robertson, N. E.; Riss, T. L.; Plunkett, M. I. J. *J. Cell. Physiol.* **1992**, *152*, 422.
15. Dalal, P. S.; Narukar, M. M. *Int. J Pharm.* **1991**, *73*, 157.
16. leputure, P.; Hui, S. H.; Ropertson, A. *J. Appl. Polym. Sci.* **1973**, *17*, 3143.
17. Tapia, C.; Escobar, Z.; Costa, E., et al *Eur. J. Pharm. Biopharm.* **2004**, *57*, 65–75.
18. Gillman, A. G.; Nies, T. W.; Taylor, P. The Pharmacological Basis of Therapeutics; Pergamon Press: New York, NY, 1991.
19. Palomo, M. E.; Ballesteros, M. P.; Frutos, P. *Drug Dev. Ind. Pharm.* **1997**, *23*, 273–283.
20. Sallmann, A. R. *Am. J. Med.* **1986**, *80*, 29–33.
21. González-Rodríguez, M. L.; Holgado, M. A.; Sánchez-Lafuente, C.; Rabasco, A. .; Fini, A. *Int. J. Pharm.* **2002**, *232*, 225–234.
22. Vanessa, L. G.; Mauro, C. M. L.; Valfredo, T. F.; Rozângela, C. P. *Polímeros* **2005**, *15*, 6–12.
23. Al-Angary, A. A.; Al-Rahecm, A.; Al-Helw, M.; Al-Dardiri, M. M.; Mahrous, G. M. *Pharm. Ind.* **1998**, *60*, 629–634.
24. Pimwipha, P.; Nalena, P.; Nuanphun, C.; Nongnuj, M. *AAPS. Pharm. Sci. Technol.* **2007**, *8*, 1–11.
25. Kumbar, S. G.; Kulkarni, A. R.; Aminabhvi, T. M. *J. Microencapsul.* **2002**, *19*, 173–180.
26. Shu, X. Z.; Zhu, K. J.. *Int. J. Pharm.* **2000**, *201*, 51–58.
27. Açikgöz, M.; Kas, H. S.; Orman, M.; Hincal, A. A. *J. Microencapsul.* **1996**, *13*, 141–160.
28. Ko, J. A.; Park, H. J.; Hwang, S. J.; Park, J. B.; Lee, J. S. *J. Phram.* **2002**, *249*, 165–174.
29. Murata, Y.; Miyamoto, E.; Kawashima, S. *J. Contr. Release* **1996**, *38*, 101–108.
30. Robert, C. C. R.; Bun, P. A.; Peppas, N. A. *J. Appl. Polym. Sci.* **1985**, *30*, 301–306.
31. Jakupec, M. A.; Unfried, P.; Keppler, B. K. *Rev. Physiol. Biochem. P* **2005**, *153*, 101–111.
32. Garcia-Sellas, J.; Pascual, E.; Funes, E.; Pagan, J. A.; Lopez, J. D.; Negro, J. M.; Hernandez, J. *Allergologia et, Immunopathologia Madrid* **2003**, *31*, 63–69.
33. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. *Eur. J. Pharm. Biopharm.* **2002**, *53*, 87–89.
34. Peng, T.; Yao, K. D.; Chen, Y.; Goosen, M. F. *J. Polym. Sci. Polym. Chem. Ed.* **1994**, *32*, 591–596.
35. Sannan, T.; Kurita, K.; Ogura, K.; Iwakura, Y. *Polymer* **1978**, *19*, 458–459.
36. Kalsi, P. S.. *Spectroscopy of Organic Compounds*. In *6 Edn.*; New Age. Int. (P) Ltd: New Delhi, India, 2004. P 90.
37. Silverstien, R. M.; Bessler, R. G.; Morril, T. C. *4 Edn.*; Wiley: New York, 1981.
38. Kim, S. Y.; Cho, S. M.; Lee, Y. M.; Kim, S . J. *J. Appl. Polym. Sci.* **2000**, *78*, 1381–1391.
39. Crank, J. *The Mathematics of Diffusion*, 2nd ed.; Clarendon: Oxford, 1975; Daniel, W. W. *Biostatistics: A Foundation for Analysis in the Health Sciences*, 5th ed.; Wiley: New York, 1987.
40. Vergnaud, J. M. *Liquid Transport Processes in Polymeric Materials—Modeling and Industrial Applications*; Prentice Hall: Englewood Cliffs, NJ, 1991.
41. Baker, R. W.; Lonsdale, H. K. Controlled Release: Mechanisms and Rates. In *Controlled Release of Biologically Active Agents*; Tanquarry, A. C., Lacey, R. E., Eds.; Plenum Press: New York, 1974; pp 15–71.
42. Sheu, M.; Chou, H.; Kao, C.; Liu, C.; Sokoloski, T. D. *Int. J. Pharm.* **1992**, *85*, 57–63.
43. Kincl, M.; Vrečer, F.; Veber, M. *Anal. Chem. Acta* **2004**, *502*, 107–113.
44. Ritger, P. L.; Peppas, N. A. *J. Contr. Release* **1987**, *5*, 37–42.
45. El-Taher, M. A.; El-Haty, M. T.; Hussien, T. M. *Polish. J Chem.* **2001**, *75*, 79–91.
46. Korsmeyer, R. C.; Peppas, N. A. *J. Membr. Sci.* **1981**, *9*, 211–227.