JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Novel interpenetrated polymer network microbeads of natural polysaccharides for modified release of water soluble drug: in-vitro and in-vivo evaluation

Raghavendra V. Kulkarni[a], Srinivas Mutalik[b], Banappa S. Mangond[a] and Usha Y. Nayak[b]

[a]Department of Pharmaceutics, BLDEA's College of Pharmacy, BLDE University Campus, Bijapur and [b]Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India

Keywords
diltiazem hydrochloride; interpenetrated network microbeads; pharmacokinetics; sodium alginate; tamarind seed polysaccharide

Correspondence
Raghavendra V. Kulkarni, BLDEA's College of Pharmacy, BLDE University Campus, Bijapur 586 103, Karnataka, India.
E-mail: pharma_75raghu@yahoo.com

Received June 23, 2011
Accepted November 21, 2011

doi: 10.1111/j.2042-7158.2011.01433.x

Abstract

Objectives The objective of this work was to prepare novel interpenetrating polymer network (IPN) microbeads of tamarind seed polysaccharide and sodium alginate for controlled release of the water soluble drug, diltiazem hydrochloride.

Methods The diltiazem-Indion 254® (a cation exchange resin) complex was prepared and the resulting complex was entrapped within IPN microbeads prepared by ionotropic gelation and covalent crosslinking. Microbeads were characterized by scanning electron microscopy, differential scanning calorimetry, thermogravimetric analysis, X-ray diffraction and Fourier transform infrared spectroscopy (FTIR) analyses, and evaluated for swelling, in-vitro release and preclinical pharmacokinetics.

Key findings The unformulated drug showed complete dissolution within 60 min, while drug release from diltiazem-ion-exchange resin complex was extended for 2.5 h but IPN microbeads extended the release for longer period. The ionically crosslinked microbeads released the drug for 6 h, while dual crosslinked microbeads extended the release for 9 h. The microbeads containing a higher amount of glutaraldehyde released the drug very slowly. The results of in-vivo pharmacokinetics of pure drug and drug-loaded IPN microbeads showed that the microbeads demonstrated prolonged release supporting the findings of in-vitro studies.

Conclusions Prepared IPN microbeads showed prolonged in-vitro and in-vivo release for diltiazem, indicating that this IPN would be a versatile delivery system for water soluble drugs.

Introduction

Recently, development of natural polymer-based multiparticulates like microspheres, microcapsules and microbeads has been a focussed research area for investigators. These multiparticulates can easily pass through the gastrointestinal tract, spread evenly on a larger area of the gastrointestinal tract, avoid exposure to higher drug concentrations and release the drugs in a sustained/controlled pattern.[1] Polysaccharides are widely used in oral controlled drug delivery systems to achieve the desired drug release pattern.[2] The natural polysaccharides, besides having many advantages, show some limitations such as hydration, microbial contamination, reduction in viscosity on storage, etc. These limitations can be conquered following crosslinking, blending or through the formation of interpenetrated polymer networks (IPN).[3] IPN happens to be a blend of two polymers in a network form, at least one of which is synthesized and/or crosslinked in the immediate presence of the other.[4,5] Nowadays, many natural polymers are being used for preparing IPNs.[6,7] The IPNs used for preparing drug delivery systems have an ability to deliver drugs at a constant rate over an extended period. IPNs possess improved mechanical properties because they have denser network structures. In such networks, the extent of crosslinking controls the drug release behaviour.[8,9]

Tamarind seed polysaccharide (TSP) is the seed kernel of *Tamarindus indica*, which is indigenous to India and South East Asia. It is composed of (1→4)-β-D-glucan backbone substituted with side chains of α-D-xylopyranose and β-D-galactopyranosyl (1→2)-α-D-xylopyranose linked (1→6) to glucose residues. The glucose, xylose and galactose units are

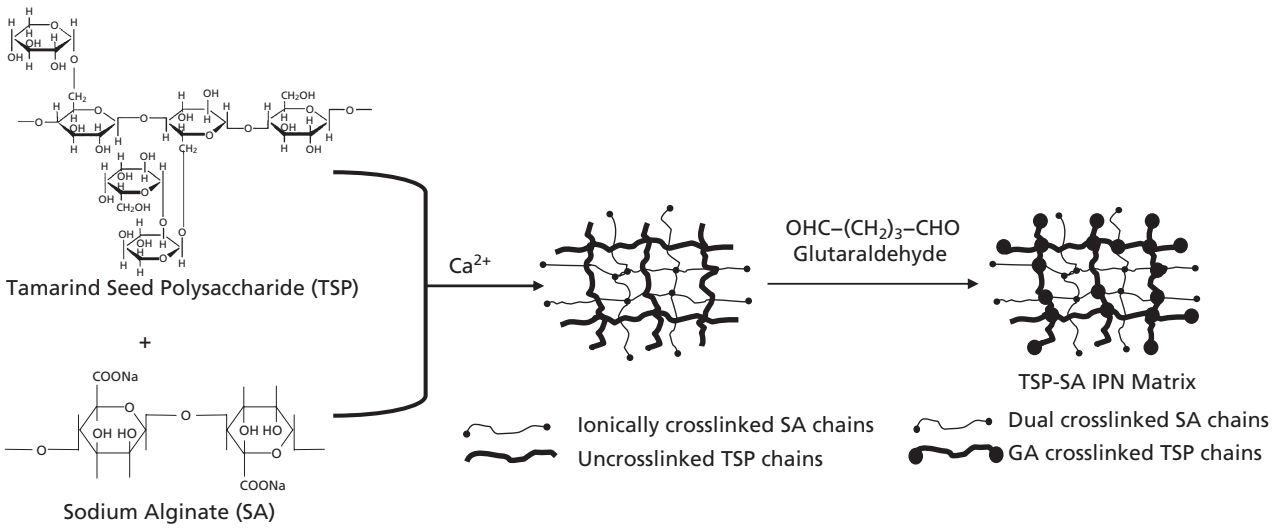

Figure 1 Schematic figure of the interpenetrated polymer network.

present in the ratio of 2.8 : 2.25 : 1.0. (Figure 1).[10,11] The TSP is noncarcinogenic, mucoadhesive and biocompatible. It possesses high viscosity and broad pH tolerance. It is used as a stabilizer, thickener, gelling agent and binder in the food and pharmaceutical industry.[12] Recently, TSP has been used as a carrier for drug delivery applications.[13,14] Sodium alginate, a natural polysaccharide, is composed of 1,4-linked-β-D-mannuronic acid and α-L-guluronic acid residues, and used as a gelling agent in the food industry. It undergoes gelation in the presence of multivalent cations in aqueous media. The ionic gelation takes place due to the exchange of sodium ions with multivalent cations. Sodium alginate can be crosslinked covalently using glutaraldehyde, which is very useful for modified release of drugs.[15–17]

The literature on drug delivery systems report that IPNs of sodium alginate-poly(vinyl alcohol), sodium alginate-egg albumin and sodium alginate-carrageenan have been used for the controlled release of prazosin hydrochloride, cefadroxil and betamethasone acetate, respectively.[18–20] However, hitherto there have been no reports on IPNs of TSP for controlled drug delivery application. The preparation of IPN microbeads of TSP and sodium alginate is essentially important because this IPN contains two crosslinked polymers in a network form to give a 3-D network structure. This produces more free volume for the loading of drugs and improves mechanical strength of the matrix.

Diltiazem hydrochloride is widely used in the treatment of angina pectoris and hypertension. It has got a short half-life of 3.5 h and is administered three to four times a day.[21] The ion-exchange resins (IER) form a reversible complex with diltiazem. The benefits of using diltiazem-IER complex include: reducing the bitter taste of the drug, facilitating the development of a sustained-release dosage form, providing uniform drug absorption and increasing the stability by protecting the drug from hydrolysis. In addition, entrapping the diltiazem-IER complex within an IPN matrix can modify the release rate of the drug.[22]

The objective of the study was therefore to develop and evaluate novel IPN microbeads using TSP and sodium alginate by ionotropic gelation and covalent crosslinking methods for the modified release of diltiazem. The prepared IPN microbeads were characterized by Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray diffraction (XRD) studies, and scanning electron microscopy (SEM), and evaluated for in-vitro drug release and in-vivo pharmacokinetics in Wistar rats.

Materials and Methods

Materials

Diltiazem hydrochloride and IER (Indion 254) were obtained as gift samples from Strides Arcolab Ltd. (Bangalore, India) and Ion Exchange India (Pvt) Ltd. (Mumbai, India), respectively. TSP was received as a gift sample from Encore Natural Polymers (Pvt) Ltd. (Ahmedabad, India). Sodium alginate, glutaraldehyde (25% v/v), calcium chloride, sodium hydroxide, conc. hydrochloric acid, methanol (HPLC) and acetonitrile (HPLC) were purchased from S.D. Fine Chemicals (Mumbai, India). Double distilled water was used throughout the study. All other chemicals were used without further purification.

Preparation of tamarind seed polysaccharide

Two hundred millilitres of cold distilled water was added to 20 g TSP to prepare a slurry. The slurry was poured into

Table 1 Details of nine formulations of microbeads

Ingredients		ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9
Sodium alginate (% w/v)		3.0	2.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0
Tamarind seed polysaccharide (% w/v)		1.0	2.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0
Diltiazem-ion-exchange resin complex*		40	40	40	40	40	60	40	40	40
Crosslinking agents	$CaCl_2$ (% w/v)	5	5	5	10	15	15	10	10	10
	Glutaraldehyde*	–	–	–	–	–	–	5	10	15

*% w/w of dry polymer. ST1–ST9, microbead formulations.

800 ml boiling distilled water. The solution was boiled for 30 min with constant stirring using a Rotamantle (2RML Q-19A, Remi, Mumbai, India). The resulting solution was kept overnight so that most of the proteins and fibres settled out. The solution was centrifuged at 5000 rev/min for 20 min (R-8C DX Centrifuge, Remi, Mumbai, India). The supernatant was separated and poured into twice the volume of absolute ethanol with continuous stirring. The precipitate was washed with absolute ethanol, diethyl ether, petroleum ether and then dried at 50°C under vacuum. The dried polymer was powdered and stored in an air-tight container.

Preparation of diltiazem-resin complex

The resins (48 μ) were washed with 200 ml de-ionized water and methanol (2 × 50 ml) to remove impurities. The activation of IER was done by treating alternatively three times with 1 M NaOH and 1 M HCl (60 ml each) and washing after each treatment with de-ionized water. Finally, the IER were washed with de-ionized water until the elute was neutral. The activated IER was vacuum dried at 50°C to constant weight. To prepare diltiazem-resin (diltiazem-IER) complex, an accurately weighed quantity of diltiazem and IER were added to 100 ml distilled water and stirred until equilibrium was attained. The diltiazem-IER complex was filtered and washed with de-ionized water repeatedly to remove uncomplexed drug. The diltiazem-IER complex was dried in a hot air oven at 40°C for 12 h and stored in a desiccator. The particle size of the dried diltiazem-IER complex was 64 μ and drug loading on diltiazem-IER complex was found to be 74.32%.

Preparation of interpenetrated polymer network microbeads

Accurately weighed quantities of TSP, sodium alginate and diltiazem-IER complexes were added to distilled water and mixed uniformly to obtain a homogeneous solution. Twenty microlitres of the solution was extruded in the form of droplets into an aqueous solution of $CaCl_2$ using a 25-ml hypodermic syringe and needle (Gauge # 23). After incubating in $CaCl_2$ solution for 15 min, the microbeads were separated and dried at 40°C for 10 h. The microbeads were covalently crosslinked by treating with different concentrations of glutaraldehyde and 1 M HCl for 30 min at 50°C. The microbeads were filtered and washed repeatedly with distilled water and the washings were tested with Brady's qualitative reagent (2,4-dinitrophenyl hydrazine) for complete removal of the unreacted glutaraldehyde. The IPN microbeads were dried at 40°C for 10 h and stored in a closed container. The formulation details for microbeads ST1–ST9 are given in Table 1.

Scanning electron microscopic studies

The shape and surface morphology of the microbeads was determined using SEM. The microbeads were sputter coated with platinum using a sputter coater (Edwards S150, Sussex, UK). The coated beads were observed under SEM (JEOL, JSM-6360, Kyoto, Japan) at the required magnification at room temperature.

Measurement of bead size

The average size of the IPN microbeads was determined using a Digimatic micrometer (MDC-25S Mitutoyo, Tokyo, Japan) with an accuracy of 0.001 mm. A total of 100 beads per batch were measured and average size was calculated.

Estimation of drug entrapment efficiency

The drug entrapment efficiency (DEE) was determined by a soaking method. An accurately weighed quantity of IPN microbeads was soaked in 100 ml phosphate buffer (pH 7.4) for complete swelling at 37°C. The beads were then crushed, the solution was gently heated for 3 h and centrifuged to separate the polymeric debris. The supernatant solution was analysed for the drug content using UV-visible spectrophotometer (Model Pharmaspec UV-1700, Shimadzu, Japan) at 236 nm. The DEE was calculated using the following equation:

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

Fourier transform infrared spectroscopy

FTIR spectra of the samples were recorded using a FTIR spectrophotometer (Nicolet, Model Magna 550, USA). The

samples were crushed with potassium bromide to get thin pellets under a pressure of 600 kg and were scanned between 450 and 4000 cm^{-1}.

Thermal analysis

The thermal analysis of samples was performed using a microcalorimeter (DuPont-9900, USA). TGA was performed on sodium alginate, TSP and IPN microbeads under a dynamic nitrogen atmosphere flowing at a rate of 50 ml/min and at a heating rate of 10°C/min in the temperature range of 0–400°C.

DSC analysis of the diltiazem, drug-free ST9 beads and drug-loaded ST9 beads was performed by heating the samples from 0–300°C at a heating rate of 10°C/min under an argon atmosphere.

X-ray diffraction studies

The XRD analysis of diltiazem, drug-free ST9 beads and drug-loaded ST9 microbeads was performed to investigate the crystallinity of the entrapped drug. The study was done using a Philips, PW-171, X-ray diffractometer with Cu-NF filtered Cu$K\alpha$ radiation in the 2θ range 0–70°. The powder X-ray diffractometer was attached to a digital graphical assembly and computer with Cu-NF 25 KV/20 mA tube as a Cu$K\alpha$ radiation source.

Swelling studies

The swelling behaviour of the IPN microbeads was determined by mass uptake studies. The microbeads were incubated in phosphate buffer solution of pH 7.4 at 37°C. The microbeads were separated from the buffer at different time intervals using a stainless steel grid and blotted carefully to remove the excess surface liquid. The accurate weight of the swollen beads was recorded using an electronic microbalance (Model BL-220H, Shimadzu, Kyoto, Japan) with an accuracy of 0.001 mg.

In-vitro drug release studies

The in-vitro drug release study was performed using United States Pharmacopeia dissolution testing apparatus (Electrolab TDT-06P, Mumbai, India). The drug release was measured in 900 ml acidic medium (pH 1.2) for the first 2 h and later in alkaline medium (pH 7.4 phosphate buffer) until the end of dissolution studies at 37.0 ± 0.5°C and 50 rev/min. At different time intervals, 5-ml samples were withdrawn and replaced with the same volume of fresh solution. The samples were filtered through a 0.45-μm membrane filter and the quantity of drug released was estimated using a UV/visible spectrophotometer at 236 nm.

In-vivo evaluation in Wistar rats

The ST9 IPN microbeads that showed better in-vitro performance were subjected to pharmacokinetic evaluation in Wistar rats. The rats (220–250 g) were kept four per cage with free access to food and water. Rats were fasted overnight and were divided into two groups ($n = 6$). Group I was orally administered plain diltiazem (15 mg/kg; in 0.5% w/v carmellose solution) whilst group II was orally administered IPN microbeads containing an equivalent amount of diltiazem (15 mg/kg; in 0.5% w/v carmellose solution). Blood samples were collected at different time intervals from the orbital sinus into heparinized tubes and plasma was separated by centrifuging (C24 Centrifuge, Sigma, Germany) the blood at 3000 rev/min for 15 min. The plasma samples were stored at −72°C until analysis of diltiazem by high performance liquid chromatography (HPLC). Animal experimental protocols were approved by the institutional ethics committee (IAEC/KMC/07/2007–2008).

High performance liquid chromatography estimation of diltiazem in plasma

The plasma concentrations of diltiazem were determined by following the reported method.[23] A Shimadzu (Japan) HPLC system (Model: LC2010CHT) equipped with a UV/vis detector (SPD-10A), two pumps (LC-10 AD), a system controller (CBM-20A Prominence) and LC solution software was used. A C_{18} BDS Hypersil phenyl column (Thermo, Runcorn, UK; size: 4.6 × 250 mm; i.d. 5 μ; pore size: 100 Å) was used. The mobile phase used was methanol : acetonitrile : 0.04 M ammonium bromide : triethylamine (24 : 31 : 45 : 0.1, v/v/v/v; adjusted to pH 7.4 with acetic acid). After the preparation, the mobile phase was passed through a membrane filter (0.45 μm). The column oven was maintained at 25°C and the vials containing the samples were maintained at 4°C during the analysis. The analysis was carried out in isocratic mode at a flow rate of 1.5 ml/min. The volume of injection was 50 μl and detection wavelength was 236 nm.

For the extraction of diltiazem from plasma, *tert*-butylmethylether (1.2 ml) was added to 0.2 ml plasma samples and the resulting mixture, after stirring for 2 min, was centrifuged at 10 000 rev/min for 10 min. The organic layer (1 ml) was mixed with 0.2 ml 0.01 M hydrochloric acid and stirred for 2 min. Exactly 50 μl of the aqueous layer was injected into the HPLC system. The calibration curve was obtained by plotting peak area of diltiazem vs theoretical concentration. The calibration curve of diltiazem was linear ($r^2 > 0.9990$) within the range of 10–1000 ng/ml. During the analysis of the samples, the standard and sample solutions were injected and the chromatograms were recorded. The diltiazem concentration in the plasma samples was determined using the calibration curve. There was no interference from the blank plasma of rat in diltiazem analysis and diltiazem

showed a retention time of 6.3 min. Different pharmacokinetic parameters were calculated by using PK Solutions 2.0 software.

Statistical analysis

Statistical analysis of different parameters (area under the curve (*AUC*), maximum plasma concentration (C_{max}), time for maximum plasma concentration (T_{max}), half life ($t^1/_2$), elimination rate constant (K_e)) of the pharmacokinetic study was performed using the Kruskal–Wallis test followed by Dunn's post-hoc test. The pharmacokinetic parameters obtained with the treatment of plain diltiazem were compared with those obtained with IPN beads of diltiazem. The *P*-values less than 0.05 were considered as statistically significant.

Results and Discussion

Preparation of IPN microbeads

When a dispersion of TSP-sodium alginate containing diltiazem-IER complex was added to $CaCl_2$ solution, the ionic crosslinking occurred between two polymer strands of sodium alginate and different parts of the same polymer entrapping diltiazem-IER complex and un-crosslinked TSP. Further, these ionically crosslinked microbeads were treated with glutaraldehyde (a covalent crosslinking agent), which reacted with TSP and sodium alginate through the formation of acetal structures (between –CHO groups of glutaraldehyde and –OH groups of TSP and/or sodium alginate) leading to the formation of an interpenetrated network of TSP and sodium alginate (Figure 1).

Size and surface morphology

As evident from SEM, the microbeads were spherical in shape and the surface morphology was found to be rough and dense along with surface foldings (Figure 2). The size of microbeads varied from 986 to 1257 μm (Table 2). It may be noted that the size of dual crosslinked beads was smaller than the ionically crosslinked beads. This may have been attributed to rapid shrinking of the polymer network after treating the beads with glutaraldehyde. As the concentration of glutaraldehyde was increased, bead size decreased. This may have been due to the formation of a stiffer matrix at higher crosslinking densities. This is in agreement with earlier reports.[24] An increase in bead size was observed with an increase in the concentration of TSP. This may have been due to the formation of bigger droplets due to increase in the viscosity of the solution during extruding through a needle. On the other hand, as the amount of diltiazem-IER complex increased in the beads the size was increased because diltiazem-IER complex might have occupied the internal spaces between IPN matrix.[25]

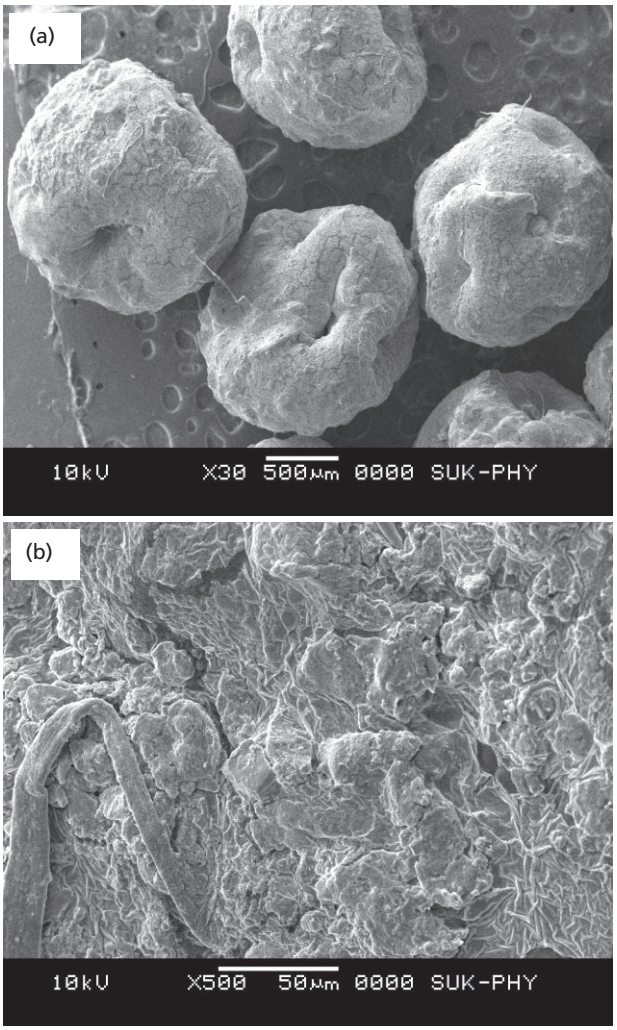

Figure 2 Scanning electron microscope photomicrographs of a group of interpenetrated polymer network microbeads. (a) Microbeads and (b) their surface morphology.

Drug entrapment efficiency

The IPN microbeads exhibited DEE values ranging from 78.15 to 92.15% (Table 2). Among the formulation variables, as the concentration of $CaCl_2$ increased, the DEE decreased. This may have been due to the displacement of resin-bound diltiazem by Ca^{2+} ions. At higher concentrations of $CaCl_2$, more Ca^{2+} ions diffused into the diltiazem-IER complex; consequently a higher amount of drug got displaced from the diltiazem-IER complex. This free drug then diffused out of the beads, which resulted in a decreased DEE.[26,27] On the other hand, DEE of the microbeads prepared by dual crosslinking was lower than those prepared by ionic crosslinking. In the case of dual crosslinked beads, the DEE was low at the lower concentration of glutaraldehyde because at this concentration the polymer network may have been

Table 2 Average size, drug entrapment efficiency, diffusion coefficients and release parameters of interpenetrated polymer network microbeads

Parameters	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9
Mean size (μm) ±SD	1166 ± 1.23	1209 ± 1.56	1257 ± 2.36	1128 ± 3.12	1063 ± 1.3	1093 ± 0.36	1103 ± 0.75	1042 ± 0.89	986 ± 0.87
Drug entrapment efficiency (%)	92.15 ± 0.05	88.94 ± 0.85	83.58 ± 0.45	86.97 ± 0.42	88.06 ± 0.96	90.42 ± 0.78	83.09 ± 1.23	81.87 ± 1.02	78.15 ± 0.56
n	0.51	0.47	0.45	0.46	0.59	0.57	0.78	0.83	0.89
r	0.91	0.97	0.96	0.90	0.94	0.97	0.93	0.90	0.89

Results are mean ± SD. Correlation coefficients, *r*; Diffusion coefficients, *D*; release parameters, *n*. ST1–ST9, microbead formulations.

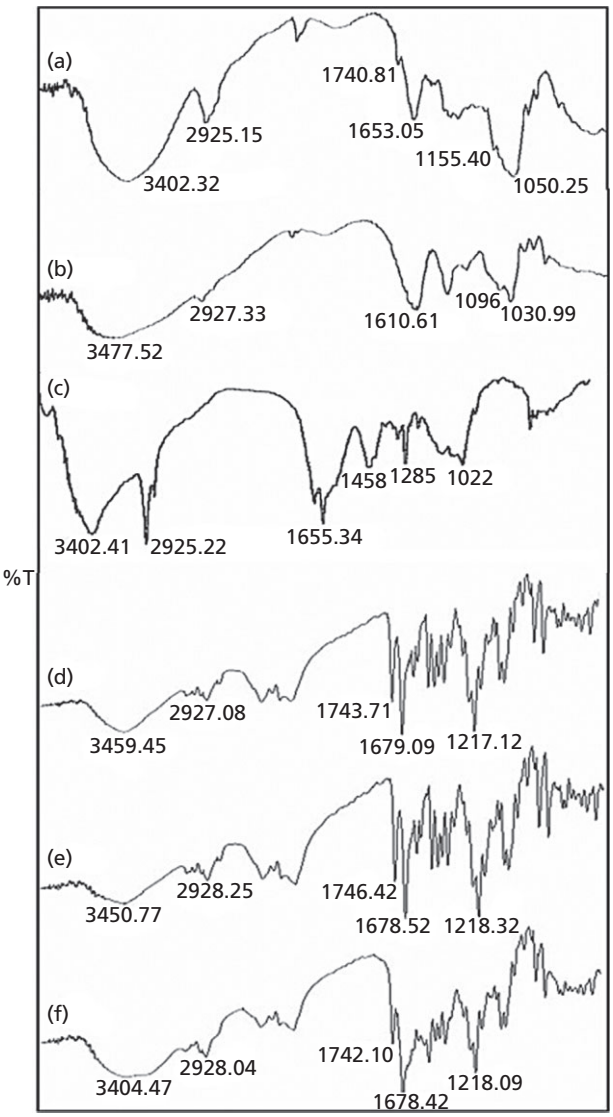

Figure 3 Fourier transform infrared spectra of the various formulations. (a) Tamarind seed polysaccharide; (b) sodium alginate; (c) placebo interpenetrated polymer network microbeads ST9; (d) diltiazem; (e) diltiazem-ion-exchange resin complex; and (f) drug-loaded ST9 interpenetrated polymer network microbeads.

loose-fitting and had larger pores due to deficient crosslinking, which may have allowed higher leakage of drug from the matrix leading to lower DEE; while, at the higher concentration of glutaraldehyde, the polymer network was rigid and leakage of drug from the matrix was low resulting in higher DEE.[28]

Fourier transform infrared spectroscopy

FTIR spectroscopy was used to confirm the IPN formation and drug stability within the prepared IPN matrix. Figure 3

depicts the spectra of TSP (a), sodium alginate (b), placebo IPN beads ST9 (c), diltiazem (d), diltiazem-IER complex (e) and drug-loaded IPN microbeads ST9 (f). The spectra of TSP (Figure 3a) exhibited a broad peak at ~3400 cm^{-1} corresponding to the associated –OH groups stretching vibrations. The peaks appearing at ~1740 cm^{-1} and ~1653 cm^{-1} were due to carbonyl (-HC = O) stretching; the peak appearing at ~2925 cm^{-1} was due to the C-H stretching (-CH_2 groups) of cyclic aldehyde; and the peaks appearing at ~1050 and 1155 cm^{-1} were due to the C-O stretching of alcoholic groups. In the case of sodium alginate (Figure 3b), the broad peak appearing at 3477 cm^{-1} corresponded to the associated –OH group stretching vibrations of hydroxyl groups; the peak appearing at 1610 cm^{-1} corresponded to the deformation of carbonyl group of sodium alginate; the peak appearing at 2927 cm^{-1} was due to the C-H stretching of cyclic aldehyde and the peaks that appeared at 1030 and 1096 cm^{-1} were due to the C-O stretching of alcoholic groups. In the spectra of IPN microbeads (Figure 3c), the peak at 3400 cm^{-1} was due to the –OH group stretching vibrations of –OH groups of the polysaccharides; a sharp peak appearing at 1655 cm^{-1} corresponded to the carbonyl functional groups of polysaccharides. The peak at 1458 cm^{-1} was due to symmetric stretching of the carboxylate groups, whereas the peak appearing at 1022 cm^{-1} represented the C-O-C stretching vibrations. The characteristic peak appearing at 1285 cm^{-1} corresponded to the formation of acetal structures, which appeared to be due to the reaction between the OH groups of TSP-sodium alginate and –CHO groups of glutaraldehyde. Glutaraldehyde reacts with the –OH groups of TSP and sodium alginate in the presence of each other to form a network through the formation of acetal structures. This could be further supported by the presence of a sharp high intensity peak at 2925 cm^{-1} due to –CH_2 groups of the alkyl chain formed by crosslinking. This was the evidence for crosslinking and IPN structure.

The spectrum of diltiazem (Figure 3d) showed the characteristic peaks at 3459 cm^{1} due to stretching vibration of –NH groups, the peak at 2927 cm^{-1} was due to aliphatic –CH stretching vibrations and peaks at 1743 and 1679 cm^{-1} that were due to two carbonyl groups present on the diltiazem and the peak at 1217 cm^{-1} was assigned to stretching vibrations of –CN. In the spectra of diltiazem-IER complex (Figure 3e) and drug-loaded IPN microbeads (Figure 3f), similar characteristic peaks related to diltiazem were observed with slight variations, indicating the chemical stability of diltiazem in the IPN matrix.

Thermal analysis

Figure 4 shows the typical TGA thermograms of TSP (a), sodium alginate (b) and placebo IPN beads ST9 (c). The

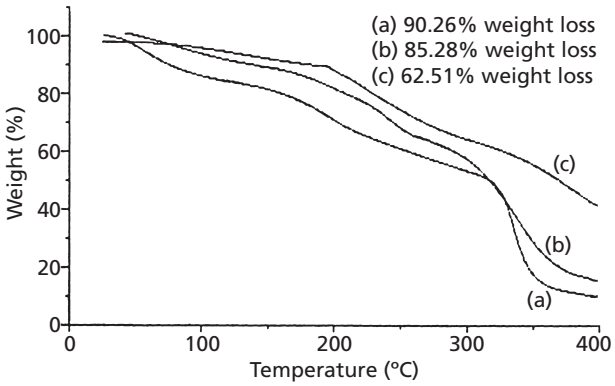

Figure 4 Thermogravimetric thermograms. (a) Tamarind seed polysaccharide; (b) sodium alginate; (c) placebo interpenetrated polymer network microbeads ST9.

decomposition of TSP started at 100°C and 17.26% weight loss was observed up to 100°C. This may have been due to the removal of free and bound water from the polymer matrix. Subsequent loss of 38.23% weight was seen between 100 and 310°C. Further, a 44.51% weight loss was observed between 310 and 400°C, and reached a value of 90.26% at 400°C. This could have been due to the decomposition of the polymer matrix. The decomposition of sodium alginate started after 150°C and 12.42% weight loss was observed up to 150°C, which was due to the loss of water from the polymer. A weight loss of 24.07% was observed between 150 and 262°C and reached a value of 85.28% at 400°C. In the case of IPN microbeads, we observed the decomposition of the matrix at the higher temperature (200°C). A 62.51% weight loss was observed at the end of 400°C. The weight loss in the case of IPN matrix was constant and residual mass was higher than TSP and sodium alginate polymers. This denoted that the thermal stability of the IPN matrix was higher than the TSP and sodium alginate polymers. In the case of IPN, the polymeric chains of two polymers were very closely associated and bonded. Hence, the thermal stability of IPN was higher than that of individual polymers suggesting the formation of an IPN.

Figure 5 presents the DSC thermograms for diltiazem (a), drug-free ST9 beads (b) and drug-loaded ST9 beads (c). The drug-free IPN beads showed a sharp endothermic peak at 154°C and drug-loaded IPN beads showed an endothermic peak at 138°C. The shift of the endothermic peak towards the lower temperature may have been due to the formation of a loose network after drug loading. On the other hand, diltiazem showed a sharp endothermic peak at 223°C due to melting of the drug; but this peak was not observed in the drug-loaded beads. This suggested that the drug was uniformly dispersed in an amorphous form in the IPN matrix of TSP and sodium alginate.

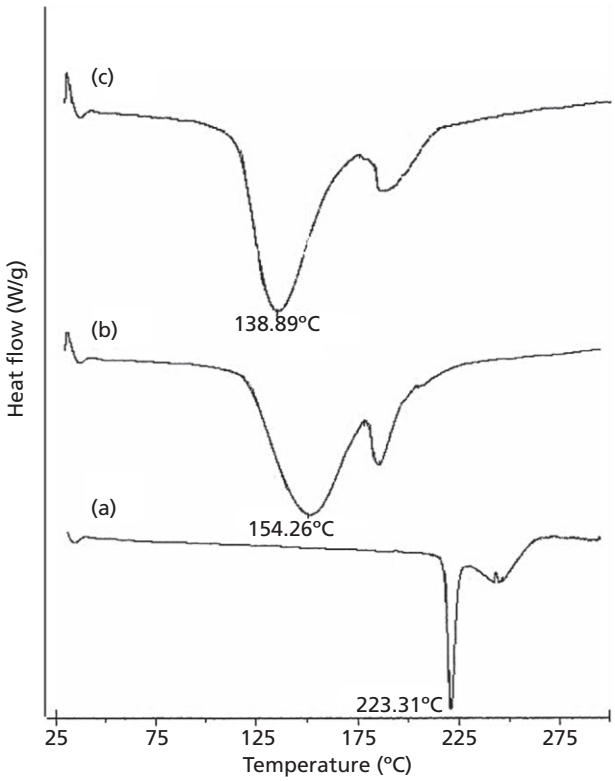

Figure 5 Differential scanning calorimetry thermograms. (a) Diltiazem; (b) drug-free ST9 interpenetrated polymer network microbeads; and (c) drug-loaded ST9 interpenetrated polymer network microbeads.

X-ray diffraction studies

The X-ray diffraction studies indicated that diltiazem showed characteristic intense peaks between the 2θ of 12° and 42° due to its crystalline nature; whereas drug-free and drug-loaded microbeads exhibited identical diffractograms and did not show any intense peaks between the 2θ of 12° and 42° (figure not shown). This suggested the amorphous dispersion of drug in the IPN matrix of TSP and sodium alginate.

Swelling studies

The release of entrapped drug from the IPN matrix depends upon its swelling behaviour. As the IPN swells, pores of the network open and release of the entrapped drug occurs. In the present application, the penetration of dissolution fluid into the IPN matrix together with displacement of the drug from diltiazem-IER complex by counter ions present in the dissolution medium and subsequent diffusion of the free drug out of swollen matrix were responsible for drug release. The swelling studies suggested that the extent of crosslinking had a considerable effect on swelling behaviour of the beads (figure not shown). We observed a decreased swelling rate at the higher concentration of glutaraldehyde.

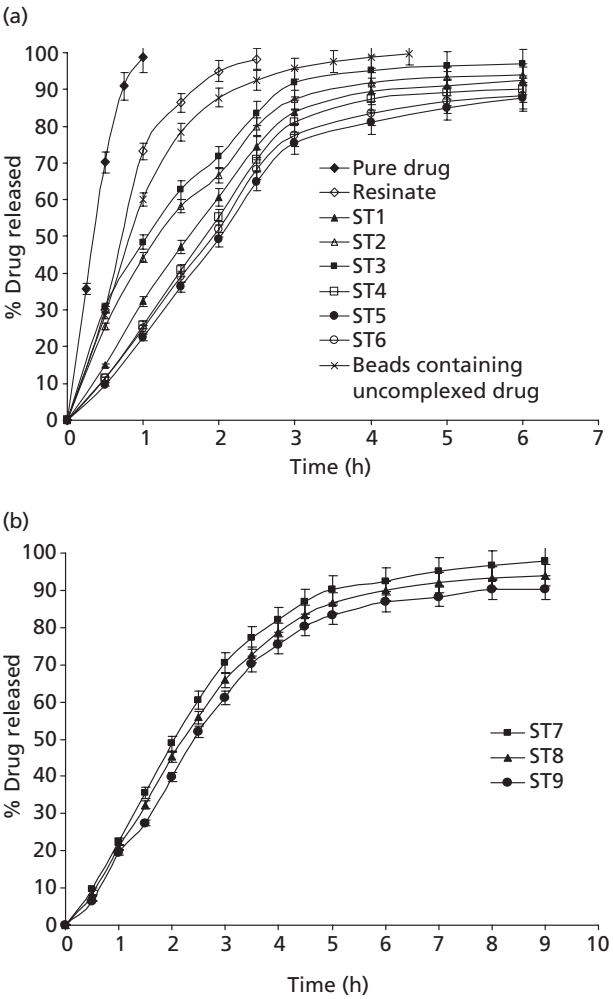

Figure 6 In-vitro drug release behaviour of crosslinked microbeads. (a) Ionically crosslinked microbeads; (b) dual crosslinked microbeads. Results are expressed as mean ± SD.

This may have been due to the formation of a stiffer network. At low crosslinking density, the polymer network was loosely packed with greater hydrodynamic free volume and could absorb a large amount of solvent leading to higher swelling. Swelling of dual crosslinked IPN beads (ST7–ST9) was less than that of ionically crosslinked beads (ST1–ST6).

In-vitro drug release studies

Figure 6 shows the in-vitro drug release behaviour of diltiazem from IPN microbeads in both simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). The release profiles suggested that the unformulated diltiazem showed complete dissolution within 1 h, while the diltiazem-IER complex extended the drug release up to 2.5 h. Further, IPN beads prepared with uncomplexed drug (plain diltiazem)

have shown drug release up to 4 h and those IPN beads prepared with diltiazem-IER complex exhibited drug release up to 9 h. In the case of IPN beads, the penetration of dissolution fluid was slow leading to slower displacement of drug from the diltiazem-IER complex by counter ions of the dissolution fluid. This process may have been responsible for the slow release of drug from IPN beads.[27]

The ionically crosslinked beads (ST1–ST6; Figure 6a) discharged the drug quickly and were capable of releasing drug only up to 6 h, but the dual crosslinked beads (ST7–ST9; Figure 6b) extended drug release for 9 h depending upon the formulation variables. The beads having a higher concentration of glutaraldehyde released the drug slowly as compared with the beads having a lower concentration of glutaraldehyde. This was due to the fact that at higher crosslinking, the free volume of the IPN matrix will decrease and this restricts the diffusion of drugs through the polymer network. It was noticed that as the initial drug loading was increased, the drug release was increased also.[29]

The release data was fitted to an empirical equation to understand the drug release mechanism.[30]

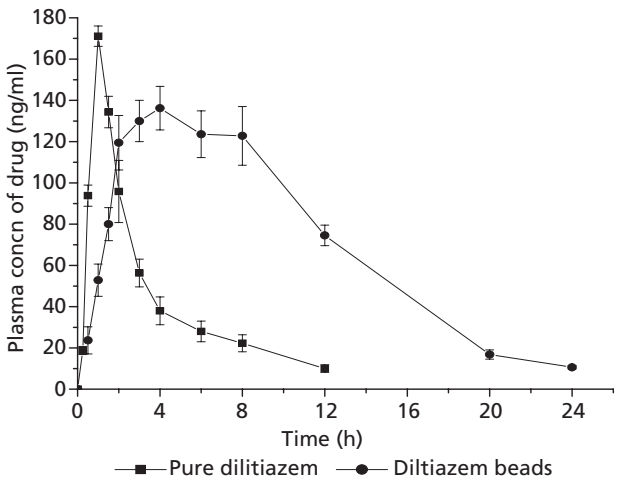

Figure 7 Plasma concentration–time profiles of pure diltiazem and interpenetrated polymer network microbeads in rats after oral administration. Results are expressed as mean ± SD; $n = 6$.

$$\frac{M_t}{M_\infty} = Kt^n \quad (2)$$

Where, M_t is the amount of drug released at time t, M_∞ is the total amount of drug loaded and n-values are the indication of the type of release mechanism. The n-values along with the correlation coefficients are given in Table 2. The results showed that the values of n increased with an increase in crosslinking. At higher crosslinking, an IPN matrix with reduced porosity was likely to be formed. This reduces the drug release. As a result, the drug release mechanism changes from diffusion controlled release to zero-order transport with an increased n-value.[31]

Assessment of pharmacokinetics in Wistar rats

The plasma concentration of diltiazem vs time curves are shown in Figure 7. All the pharmacokinetic parameters obtained with IPN beads were considerably different from those obtained with plain diltiazem (although not significantly different; $P > 0.05$) (Table 3). Plain diltiazem was rapidly absorbed after oral administration, which was indicated by a low value for T_{max} of 1 h. The observed low T_{max} value was in accordance with previous reports where plain diltiazem showed a T_{max} value of < 1 h.[23] Conversely, IPN beads showed a higher T_{max} value (≈ 4 h). The C_{max} value was low with IPN beads in comparison with plain drug. This may have been due to the slow absorption of diltiazem from IPN beads. The diltiazem was released slowly from IPN beads, leading to slow absorption and elimination. This was indicated by a higher elimination half-life value ($t^1/_2$) with IPN beads in comparison with that of plain diltiazem. This observation was further confirmed by the elimination rate constant values (K_e). IPN beads and plain diltiazem showed K_e values of 0.120 ± 0.009 and 0.210 ± 0.070/h, respectively. The IPN beads exhibited comparatively higher *AUC* values signifying the greater bioavailability of diltiazem from IPN beads than the plain diltiazem. This might have been due to the slow release of diltiazem from IPN beads up to ≈ 8 h. This observation revealed the prolonged release of diltiazem from

Table 3 Pharmacokinetic parameters for plain diltiazem and interpenetrated polymer network beads of diltiazem obtained from in-vivo studies in Wistar rats

Formulations	Pharmacokinetic parameters					
	C_{max} (ng/ml)	T_{max} (h)	$t^1/_2$ (h)	$AUC_{0\text{-}t}$ (ng/h/ml)	$AUC_{0\text{-}\infty}$ (ng/h/ml)	K_e (/h)
Plain diltiazem	171.16 ± 4.94	1.00 ± 0.00	3.55 ± 0.94	528.02 ± 35.02	581.00 ± 31.88	0.210 ± 0.070
Interpenetrated polymer network beads of diltiazem	136.33 ± 10.71	4.00 ± 0.00	5.79 ± 0.47	1690.1 ± 101.91	1778.16 ± 99.47	0.120 ± 0.009

Results are mean ± SD, $n = 6$. *AUC*, area under the curve; C_{max} , maximum plasma concentration; K_e, elimination rate constant; T_{max}, time for maximum plasma concentration; $t^1/_2$, half life.

IPN beads *in vivo* and supported the in-vitro drug release profile in the dissolution studies.

Conclusions

The IPN microbeads of TSP and sodium alginate were successfully prepared by a simple and viable method for the prolonged release of diltiazem. The microbeads were spherical with entrapment efficiency ranging from 78 to 92%. The IPN structure and stability of diltiazem in the IPN matrix was confirmed by FTIR and TGA analysis. The DSC and XRD studies confirmed the amorphous dispersion of the drug in the microbeads. Unformulated diltiazem showed rapid dissolution within 1 h, while drug release from diltiazem-IER complex was extended for 2.5 h. The ionically crosslinked beads discharged the drug quickly; whereas dual crosslinked beads extended the release of diltiazem up to 9 h. The in-vitro and in-vivo evaluation of IPN beads in Wistar rats showed slow and prolonged release of diltiazem, indicating the versatility of prepared IPN microbeads for controlled release of water soluble drugs.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was a part of M.Pharm project with financial assistance from the BLDEA's College of Pharmacy, Bijapur, India for Mr Banappa S. Mangond.

Acknowledgements

Authors are thankful to the management of BLDE Association, Bijapur, India and Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India for their support in the completion of this project.

References

1. Halder A, Sa B. Preparation and in vitro evaluation of polystyrene-coated diltiazem-resin complex by oil-in-water emulsion solvent evaporation method. *AAPS PharmSciTech* 2006; 7: 46.
2. Tomida H *et al*. Polysaccharides as potential antioxidative compounds for extended-release matrix tablets. *Carbohydr Res* 2010; 345: 82–86.
3. Changez M *et al*. The effect of composition poly(acrylic acid)-gelatin hydrogel on gentamycin sulphate release in vitro. *Biomaterials* 2003; 24: 527–536.
4. Hsieh TT *et al*. Interpenetrating polymer networks of 2-hydroxyethyl methacrylate terminated polyurethane and polyurethane. *Polymer* 1999; 40: 3153–3163.
5. Kosmala JD *et al*. Preparation of interpenetrating networks of gelatin and dextran as degradable biomaterials. *Biomaterials* 2000; 21: 2019–2023.
6. Kulkarni RV, Sa B. Evaluation of pH-sensitivity and drug release characteristics of (polyacrylamide-grafted-xanthan)-carboxymethylcellulose-based pH-sensitive interpenetrating hydrogel beads. *Drug Dev Ind Pharm* 2008; 34: 1406–1415.
7. Kulkarni RV, Sa B. Novel pH-Sensitive interpenetrating network hydrogel beads of carboxymethylcellulose-(polyacrylamide-grafted-alginate) for controlled release of ketoprofen: preparation and characterization. *Curr Drug Deliv* 2008; 5: 256–264.
8. Desai NP, Hubbell JA. Surface physical interpenetrating networks of poly(ethylene-terephthalate) and poly(ethylene oxide) with biomedical applications. *Macromolecules* 1992; 25: 226–232.
9. Rokhade AP *et al*. Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine. *Carbohydr Polym* 2006; 65: 243–252.
10. Pal S *et al*. Carboxymethyl tamarind: synthesis, characterization and its application as novel drug delivery agent. *J Appl Polym Sci* 2008; 110: 392–400.
11. Picout DR *et al*. Pressure cell assisted solubilization of xyloglucans: tamarind seed polysaccharide and detarium gum. *Biomacromolecules* 2003; 4: 799–807.
12. Gidley MJ *et al*. Structure and solution properties of tamarind seed polysaccharide. *Carbohydr Res* 1991; 214: 299–314.
13. Zhang J *et al*. Preparation and characterization of tamarind gum/sodium alginate composite gel beads. *Iran Polym J* 2008; 17: 899–906.
14. Sumathi S, Ray AR. Release behaviour of drugs from tamarind seed polysaccharide tablets. *J Pharm Pharm Sci* 2002; 5: 12–18.
15. Blandino A *et al*. Glucose oxidase release from calcium alginate gel capsules. *Enzyme Microb Technol* 2000; 27: 319–324.
16. Bregni C *et al*. Alginate microspheres of *Bacillus subtilis*. *Ars Pharm* 2000; 41: 245–248.
17. Isiklan N. Controlled release of insecticide carbaryl from sodium alginate, sodium alginate/gelatin, and sodium alginate/sodium carboxymethyl cellulose blend beads crosslinked with glutaraldehyde. *J Appl Polym Sci* 2006; 99: 1310–1319.
18. Kulkarni RV *et al*. Interpenetrating network hydrogel membranes of sodium alginate and poly(vinyl alcohol) for controlled release of

prazosin hydrochloride through skin. *Int J Biol Macromol* 2010; 47: 520–527.
19. Kulkarni AR *et al*. In-vitro release kinetics of cefadroxil loaded sodium alginate interpenetrating network beads. *Eur J Pharm Biopharm* 2001; 51: 127–133.
20. Mohamadnia Z *et al*. pH-sensitive IPN hydrogel beads of carrageenan-alginate for controlled drug delivery. *J Bioact Compat Polym* 2007; 22: 342–356.
21. Shivkumar HN *et al*. Design and evaluation of pH-sensitive multi-particulate systems for chronotherapeutic delivery of diltiazem hydrochloride. *Ind J Pharm Sci* 2006; 68: 781–787.
22. Junyaprasert VB, Manwiwattanakul G. Release profile comparison and stability of diltiazem-resin microcapsules in sustained release suspensions. *Int J Pharm* 2008; 352: 81–91.
23. Choi JS, Han HK. Enhanced oral exposure of diltiazem by the concomitant use of naringin in rats. *Int J Pharm* 2005; 305: 122–128.
24. Soppimath KS *et al*. Chemically modified polyacrylamide-*g*-guar gum-based crosslinked anionic microgels as pH-sensitive drug delivery systems: preparation and characterization. *J Control Release* 2001; 75: 331–345.
25. Agnihotri SA *et al*. Controlled release of cephalexin through gellan gum beads: effect of formulation parameters on entrapment efficiency, size, and drug release. *Eur J Pharm Biopharm* 2006; 63: 249–261.
26. Halder A *et al*. Development and evaluation of polyethyleneimine-treated calcium alginate beads for sustained release of diltiazem. *J Microencapsul* 2005; 22: 67–80.
27. Halder A, Sa B. Entrapment efficiency and release characteristics of polyethyleneimine-treated or untreated calcium alginate beads loaded with propranolol-resin complex. *Int J Pharm* 2005; 290: 45–54.
28. Rokhade AP *et al*. Novel interpenetrating polymer network microspheres of chitosan and methylcellulose for controlled release of theophylline. *Carbohydr Polym* 2007; 69: 678–687.
29. Agnihotri SA, Aminabhavi TM. Development of novel interpenetrating network gellan gum-poly(vinyl alcohol) hydrogel microspheres for the controlled release of carvedilol. *Drug Dev Ind Pharm* 2005; 31: 491–503.
30. Ritger PL, Peppas NA. A simple equation for description of solute release: II Fickian and anomalous release from swellable devices. *J Control Release* 1988; 5: 37–42.
31. Toti US, Aminabhavi TM. Modified guar gum matrix tablet for controlled release of diltiazem hydrochloride. *J Control Release* 2004; 95: 567–571.