Novel Thermo-Responsive Semi-Interpenetrating Network Microspheres of Gellan Gum-Poly(*N*-isopropylacrylamide) for Controlled Release of Atenolol

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ABSTRACT: Thermoresponsive microspheres of gellan gum-poly(*N*-isopropylacrylamide), i.e., GG-P(NIPAAm) semi-interpenetrating polymer networks (semi-IPNs) have been prepared by ionic crosslinking and used to study the controlled release (CR) of atenolol (ATL), an antihypertensive drug. Interaction of the drug with polymers was studied by Fourier transform infrared (FTIR) spectroscopy. Differential scanning calorimetry (DSC) was used to confirm the polymorphism and molecular level dispersion of ATL. Scanning electron microscopy (SEM) indicated spherical nature and smooth surfaces of the microspheres with some debris attached on their surfaces. Mean particle size measured by laser light diffraction ranged between 34 and 76 μm. Equilibrium swelling performed at 25°C and 37°C in pH 7.4 phosphate buffer exhibited thermoresponsive nature of the polymers. *In vitro* drug release performed at 25°C and 37°C indicated temperature-dependency of ATL release, which was extended up to 12 h. *In vitro* release profiles at both the temperatures confirmed thermoresponsive nature of the polymers giving pulsatile trends. The % cumulative release data have been fitted to an empirical equation to estimate transport parameters and to understand the nature of drug release. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1832–1841, 2010

Key words: gellan gum; thermoresponsive; controlled drug release; interpenetrating network; microspheres

INTRODUCTION

The success of any medical treatment depends not only on pharmacodynamic activity of the drug, but also on the availability of active drug at the site of action. To improve the efficacy of a pharmacotherapy, the time release delivery systems have been widely explored, wherein drug is incorporated in a polymeric matrix and be released in a controlled manner. For this purpose, a variety of biodegradable polymers in various dosage forms have been developed and tested for a variety of drugs.[1] Among these, pH or temperature-responsive hydrogels have been widely explored, since both these parameters can be easily manipulated under physiological conditions.[2–5] In the prior-art, several hydrogels have been prepared by utilizing *N*-isopropylacrylamide (NIPAAm). For instance, Hoare and Pelton[6] prepared a series of temperature-responsive PNIPAAm-based microgels containing carboxylic acid functional groups via copolymerization with methacrylic acid and acrylamide, which are selectively hydrolyzed under optimized conditions to offer carboxylic acid functionality.[7] Recently, semi-interpenetrating polymer networks (semi-IPNs) with NIPAAm as the main backbone of the network have been prepared with several biopolymers like chitosan, sodium alginate, cellulose, etc.[8,9] Advantages of such systems over other delivery systems have been described elsewhere.[10]

Gellan gum (GG) is a naturally occurring carbohydrate polymer that can be used in combination with NIPAAm to form the semi-IPN matrix for encapsulating a drug molecule. GG is a linear anionic polysaccharide obtained from extracellular secretion of the microorganism, *Pseudomonas elodea*.[11,12] The presence of acetyl groups interferes in its ion bonding ability. On the other hand, commercially available GG is a deacetylated product obtained by treatment with alkali.[13] Because of the presence of free carboxylate groups, GG is anionic in nature and thus, would undergo ionic gelation in the presence of mono and divalent cations. Its affinity towards divalent cations is much stronger than monovalent cations.[12] The mechanism of gelation involves the formation of double helical junction zones followed by

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the aggregation of double helical segments to form a three-dimensional network by complexation with cations and hydrogen-bonding with water.[14] GG has been widely used in food and *in situ* gelling ophthalmic preparations[15] as well as in developing *in situ* gelling oral CR formulations.[16,17] In the prior-art, Quigley and Deasy[18] have developed CR bead formulations of GG for sulfamethizole by hot-extrusion method into a chilled ethyl acetate solvent. GG beads containing salbutamol sulfate have also been investigated for CR applications.[19] Recently, Agnihotri et al.[20] and Schild[21] developed the GG beads by ionotropic gelation containing a mixture of calcium and zinc ions for CR of cephalexin, an antibiotic drug.

In continuation of our earlier work[1,2,10] on semi-IPN polymers for CR applications, we present here the development of thermoresponsive semi-IPNs of GG with poly(*N*-isopropylacrylamide), i.e., GG-P(NIPAAm) for investigating temperature-responsive controlled release of a model antihypertensive drug like atenolol (ATL), which is a cardioselective β-adrenoreceptor blocking agent, used in the treatment of hypertension, angina pectoris, arrhythmias, and myocardial infarction.[22] Atenolol is phenylacetamide [(4-2′-hydroxy-3′-isopropyl-aminopropoxy) phenylacetamide] [see structure in Fig. 1(a)], which is relatively polar and has been reported to be subjected to extensive hepatic first-pass metabolism following its oral administration due to its short biological half-life of 4 h. Being a hydrophilic drug, its administration as conventional tablet has been reported to exhibit fluctuations in plasma drug levels, resulting either in the manifestation of side effects or reducing the drug concentration at receptor site.[23] Many attempts have been made in the literature to regulate its release using CR systems based on the hydrophilic polymers,[24,25] osmotic pumps, etc.[23] However, due to the incomplete intestinal absorption, its systemic bioavailability is about 50–60%. Therefore, there is a need to develop formulations based on hydrogels that are responsive to temperature fluctuations. To meet out these requirements, we have selected P(NIPAAm) that exhibits[21] temperature-dependent volume phase transition showing its lower critical solution temperature (LCST) at 32°C. Thus, at the temperature below LCST, the polymer absorbs water and swells whereas, above the LCST, a drastic shrinkage would take place.

The present study deals with an investigation to prepare microspheres from the semi-IPNs of GG with P(NIPAAm), which are used to study the CR of ATL. Microspheres were prepared by modified emulsion technique using calcium chloride solution as the crosslinking agent with an aim to achieve pulsatile release of the drug depending on the temperature. FTIR was used to study the drug interaction

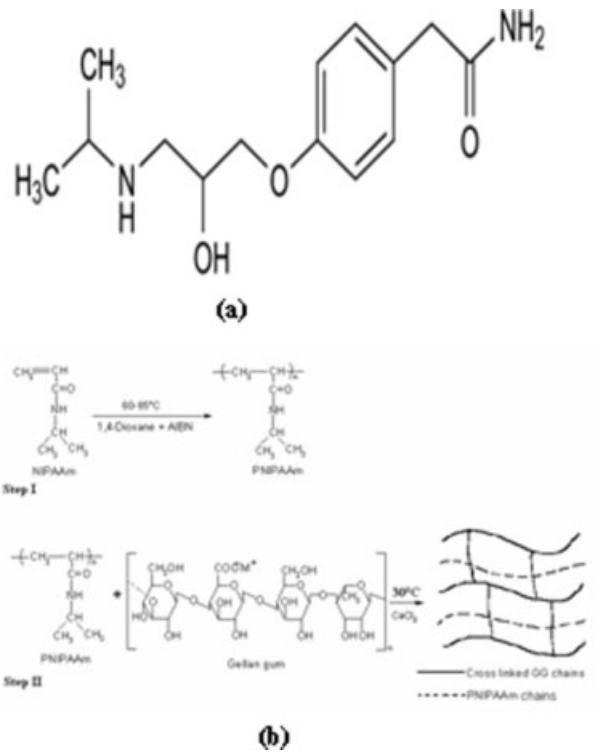

Figure 1 (a) Chemical structure of atenolol and (b) schematic representation of the synthesis of semi-IPN polymers.

with the polymers, whereas differential scanning calorimetry (DSC) was used to understand the polymorphism of the drug-loaded matrices. Scanning electron microscopy (SEM) was used to investigate surface morphology of the microspheres. Equilibrium swelling and temperature-responsive release of ATL-loaded microspheres have been investigated at 25°C and 37°C in phosphate buffer (pH 7.4). *In vitro* drug release data have been analyzed using an empirical equation to understand the release profiles[26] and the drug release patterns have been discussed in terms of drug loading, P(NIPAAm) content of the matrix as well as temperature.

MATERIALS AND METHODS

Materials

The drug ATL and deacetylated gellan gum (Gelrite®) (Merck, NJ) were procured, respectively, from Medreich Sterilab and Eros Pharma (Bangalore, India). NIPAAm monomer and azobisisobutyronitrile (AIBN) were obtained from Aldrich Chemical Company (Milwaukee, WI). Analytical reagent grade isooctane, calcium chloride, and Span 80® were procured from s.d. fine chemicals (Mumbai, India). Double-distilled water was used throughout the research work. All the chemicals were used without further purification.

Synthesis of poly(*N*-isopropylacrylamide)

A 4 g of NIPAAm, 0.5% AIBN and 1,4-dioxane (monomer : solvent ratio, 1 : 3, w/v) were placed in a three-necked round-bottom flask (50 mL) fitted with a mechanical stirrer, reflux condenser, and a nitrogen inlet. The mixture was flushed with oxygen-free nitrogen for 10 min. The flask was immersed in a thermostatic oil bath maintained at 60–65°C for 2 h under inert nitrogen atmosphere. Polymerization was stopped by cooling the reaction mixture to ambient temperature (30°C). The viscous product was precipitated in diethyl ether/hexane mixture and then reprecipitated using 1,4-dioxane in diethyl ether/hexane mixture to ensure complete removal of residual monomer. It was then dried in a vacuum oven at 40°C for 24 h. Polymer molecular weight was measured by GPC performed at SICART, Anand (Gujarat, India), using Perkin–Elmer (Milwaukee, WI), Series-200 GPC equipped with a RI detector using THF solvent at 30°C and found to be 1700.

Preparation of semi-IPN microspheres and drug loading

The crosslinked GG-PNIPAAm semi-IPN microspheres were prepared by emulsion-crosslinking method.[27] Briefly, 0.3 g of GG was dissolved in 20 mL of distilled water with continuous stirring until the homogeneous solution was formed. Then, accurately weighed amounts of PNIPAAm (0.3 g) and ATL (0.15 g) were dissolved in the polymer solution. This solution was slowly added to a flask containing 75 g of isooctane and 1.5 g of Span-80 with constant stirring using a mechanical stirrer at 1000 rpm speed for 10 min. To this w/o emulsion, 10 mL of aqueous solution consisting 8% calcium chloride was added and allowed to react with the dispersed globules for 20 min. The microspheres produced were collected by filtration in vacuum and washed with 20 mL of deionized water; microspheres were then dried under vacuum at 40°C for 12 h and stored in a desiccator before analytical testing. Placebo microspheres were prepared in a similar manner for control studies during characterization. Thus, by using the ionic gelation, we have prepared seven formulations by varying GG : P(NIPAAm) ratio and by varying drug loading. The formulation codes are assigned as given in Table I.

Estimation of drug content and encapsulation efficiency

Estimation of ATL content was done according to the method adopted earlier.[28] Microspheres of known weights were soaked in 50 mL of pH 7.4 phosphate buffer for 30 min and sonicated using a probe sonicator (Branson digital sonifier, Kanagawa, Japan) for 10 min to break the microspheres, thus facilitating the extraction of ATL. The solution was centrifuged using a tabletop centrifuge (Eppendorf 5810) to remove polymeric debris. The amount of ATL present in phosphate buffer medium was determined by UV spectrophotometry (UV-1650 PC, Shimadzu, Duisburg) at 226 nm wavelength using the calibration curve constructed from the series of ATL solutions with standard concentrations. The % drug loading and % encapsulation efficiency were calculated as:

$$\%\text{Drug loading} = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}}\right) \times 100 \quad (1)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Drug loading}}{\text{Theoretical loading}}\right) \times 100 \quad (2)$$

TABLE I
Results of % Encapsulation Efficiency and Mean Size of Semi-IPN Microspheres

Formulation codes	P(NIPAAm) : GG	% ATL	% Encapsulation efficiency	Mean particle size (μm)
F1	70 : 30	25	56	34
F2	70 : 30	50	64	42
F3	50 : 50	25	61	46
F4	50 : 50	50	67	53
F5	30 : 70	25	66	58
F6	30 : 70	50	69	69
F7	0 : 100	25	71	76

Particle size measurements

Particle size was measured by laser light diffraction technique (Mastersizer 2000, Malvern, UK). Sizes of the completely dried microspheres of different formulations were measured by dry sample technique using a dry sample adapter. Completely dried particles were placed on the sample tray and an inbuilt vacuum; the compressed air system was used to suspend the particles. Laser obscuration range was maintained between 1 and 2% to record the volume-mean diameter (V_d). After measurement of particle size of each sample, dry sample adapter was cleaned thoroughly to avoid any cross contamination.

Fourier transform infrared spectral studies

FTIR spectra were taken (Spectrum GX, Perkin–Elmer) to confirm the conversion of NIPAAm to P(NIPAAm) and also to investigate the chemical interactions between drug and polymer. Samples were crushed with KBr to get pellets under a pressure of 300 kg/cm^2. FTIR spectra of NIPAAm,

P(NIPAAm), placebo microspheres, pure ATL, and ATL-loaded microspheres were scanned from 500 to 4000 cm^{-1}.

Differential scanning calorimetric analysis

Differential scanning calorimetry (DSC7, Perkin–Elmer) was performed on placebo microspheres, pure ATL, and drug-loaded microspheres. Samples were heated from ambient temperature (30°C) to 400°C at the heating rate of 10°C/min in a nitrogen atmosphere (flow rate, 10 mL/min).

Scanning electron microscopic studies

Scanning electron microscopy (Jeol, JSM-840A, Japan, Indian Institute of Science, Bangalore) was used to examine the shape and surface morphology of the semi-IPN microspheres before and after drug loading. Microspheres were dusted onto double-sided tape on an aluminum stub that was coated with gold using cold sputter coater (JEOL coater) to achieve a thickness of 400 Å. Samples were then imaged using 25 kV electron beam.

Equilibrium swelling studies

Equilibrium swelling of the microspheres was investigated in phosphate buffer (pH 7.4) solution at 25°C and 37°C up to 24 h till the attainment of equilibrium swelling in an incubator (New Brunswick Scientific Innova 4230, MN). Adhered liquid droplets on the surface of particles were removed by blotting with soft tissue papers; the swollen microspheres were weighed on a electronic balance (A and D Weighing, Model GR-120) to an accuracy of ±0.01 mg. From the equilibrium weight gain data, W_1 of the sample, water uptake, Q was calculated by measuring the dry weight, W_2 of the particles using the equation:

$$Q = \left(\frac{\text{Weight of swollen particles } (W_1) - \text{Weight of dry particles } (W_2)}{\text{Weight of dry particles } (W_2)} \right) \times 100 \quad (3)$$

In vitro drug release studies

In vitro drug release from different formulations was investigated in a phosphate buffer solution of pH 7.4 (without enzymes). Microspheres (25 mg) were suspended in 1 mL phosphate buffer of pH 7.4 and placed within the dialysis membrane bag. Samples within the membrane were kept in conical flasks containing 50 mL of phosphate buffer solution of pH 7.4 as the dissolution medium and flasks were shaken at 50 rpm speed in an incubator (New Brunswick Scientific Innova 4230, MN). For pulsatile release study, incubation temperature was changed for every 2 h initially, starting from 25 to 37°C. The amount of drug released was determined by withdrawing each time 3 mL aliquots at the selected time intervals. The volume withdrawn was replaced with an equal volume of fresh and prewarmed phosphate buffer solution at 37°C. Samples were analyzed by UV spectrophotometer (UV-1650 PC, Shimadzu, Duisburg) at λ_{max} of 221 nm using phosphate buffer blank solution. These studies were performed in triplicate for each sample and average values were used in data analysis as well as graphical display.

RESULTS AND DISCUSSION

Preparation and characterization of microspheres

Preparation of GG microspheres by emulsification technique involved shear dispersion of aqueous GG : P(NIPAAm) mixture in a continuous organic phase by high-speed impeller rotation. The globules formed were thermodynamically unstable, but readily coalesced during microscopic examination, which required higher surfactant concentration (2%) to reduce interfacial tension developed at the surface of globules. Surfactant migrated to the surface of GG-ATL globules after addition to the reaction mixture. This provided additional stability to the globules that was crucial for the formation of small and discrete microspheres. Similarly, calcium chloride solution was sheared into globules when it was added to organic phase under continuous agitation. Unlike the extrusion method, there was no immediate contact between GG-ATL globules and calcium chloride solution. The initiation of crosslinking reaction between GG and calcium chloride was dependent merely on the chance of collision between these globules.

Gellan gum is an anionic deacetylated exocellular polysaccharide and its aqueous solution is known to form hydrogels when warmed to body temperature (37°C) in the presence of cations.[29] The strategy for ensuring hydrogelation in the present work is similar to that used earlier for the preparation of beads as well as microspheres from ionic polymers such as alginate, chitosan, etc.[30] Gellan gum contains carboxylate side groups in glucuronyl residues, and hence, it can be crosslinked by inducing ionic gelation with cations. Shi et al.[8] studied the CR of water-soluble indomethacin drug from the temperature-responsive alginate beads prepared by using calcium as a

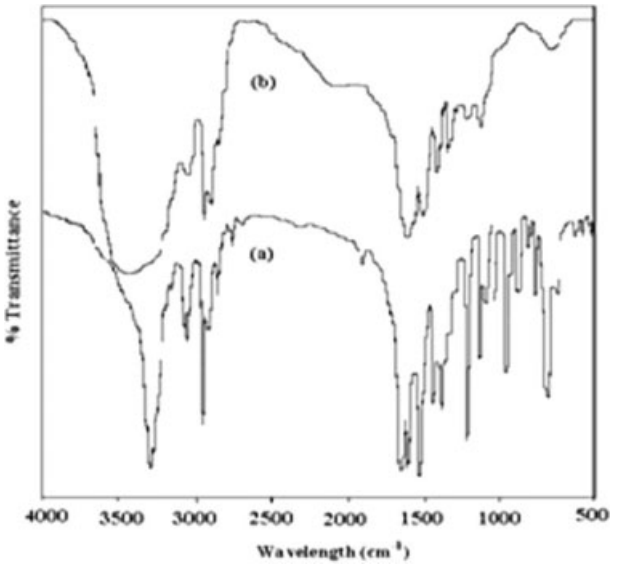

Figure 2 FTIR spectra of (a) NIPAAm and (b) P(NIPAAm).

counter-ion, wherein drug release was extended up to 7 h. These observations prompted us to use calcium as counterions for hydrogelation of GG to achieve easy encapsulation of ATL.

Particle size and size distribution were analyzed by dynamic laser light diffraction technique using a Mastersizer-2000, Malvern, UK. Volume mean diameter of the microspheres produced by taking different ratios of GG : P(NIPAAm) are summarized in Table I. Particle size analysis of semi-IPN microspheres containing ATL showed that mean diameter of the microsphere was affected by the GG : P(NIPAAm) ratio as well as drug loading. By increasing the ratio of GG in the microspheres, an increase in the size of microspheres was observed, which could be attributed to the formation of bigger droplets with increasing concentration of GG during the emulsification step. For all the formulations, with varying amount of drug in the microspheres, particle size also increased. For instance, in case of 30% GG-containing microspheres, particle size increased from 34 to 42 μm (formulations F1 and F2). Similar trends are observed for all other formulations (see Table I). This may be due to the size of emulsion being influenced by the presence of ATL in addition to the feed composition ratio of PNIPAAm and GG.[31] Notice that 25 wt % of drug-loaded GG microspheres (formulation F7) have a maximum size of 76 μm.

To study the effect of drug concentration, we have selected two concentrations viz., 25 and 50 wt % during ionic crosslinking of the matrix. The results of % encapsulation efficiency included in Table I increased with increasing drug loading as well as the amount of GG in the microspheres. On the other hand, the effect of GG for formulations containing 30, 50, and 70 wt % GG and 25 wt % ATL (F1, F3, and F5), the encapsulation efficiencies is 56, 61, and 66%, respectively.

FTIR study

FTIR was used to confirm the completion of polymerization from NIPAAm monomer to P(NIPAAm) polymer. Figure 2 compares FTIR spectra of (a) NIPAAm and (b) P(NIPAAm). In case of NIPAAm, a band observed at 3298 cm^{-1} is due to N—H stretching vibrations, whereas the bands at 1363 and 1245 cm^{-1} are due to C—N stretching vibrations. The bands at 2970 and 3072 cm^{-1} are attributed to C—H stretching vibrations, whereas that observed at 1658 cm^{-1} is due to C=O stretching vibration. The band at 1619 cm^{-1} is due to C=C stretching vibrations, whereas that at 1065 cm^{-1} indicates C—O stretching vibrations. In case of PNIPAAm, the peaks observed at 2975 and 1651 cm^{-1} are attributed to stretching vibrations of C—H and C=O, respectively; a strong and distinct band due to C=O group and the absence of monomer peaks confirm the successful polymerization.

To investigate the possible chemical interactions of ATL with the hydrogel, we have analyzed: (a) pure ATL, (b) ATL-loaded microspheres, and (c) placebo microspheres by FTIR. These spectra displayed in Figure 3 for pure ATL showed characteristic bands due to different functional groups of ATL. The band appearing at 3356 cm^{-1} is due to O—H/N—H stretching vibrations, whereas those at 2924 and 2963 cm^{-1} are due to aliphatic C—H stretching

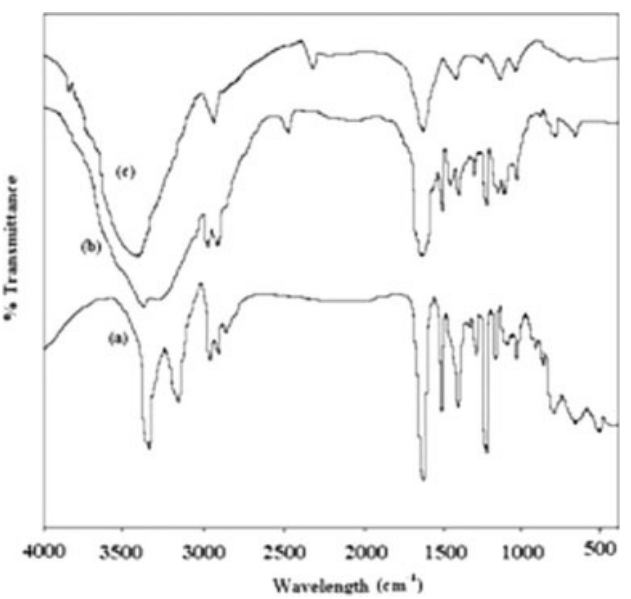

Figure 3 FTIR spectra of (a) Pristine ATL, (b) ATL-loaded microspheres, and (c) Placebo microspheres.

vibrations. The band at 3175 cm^{-1} is due to aromatic C—H stretching vibrations, whereas those appearing at 1637 and 1583 cm^{-1} are attributed to primary amide bond stretching and aromatic C=C stretching vibrations, respectively. The N—H bending vibrations are seen at 1516 cm^{-1}, whereas the bands at 1180 and 1092 cm^{-1} are due to C—O—C stretching vibrations of the ether linkages. The C—N stretching vibrations are seen at 1087 cm^{-1}, whereas the band appearing at 1243 cm^{-1} is due to aromatic C—O stretching vibrations.

In case of placebo microspheres, a broad peak appearing at 3444 cm^{-1} is due to the presence of hydroxyl groups of glucopyranose ring that are hydrogen-bonded. The bands appearing at 1633 and 1548 cm^{-1} are due to asymmetric and symmetric stretchings of the carboxylate group. The bands at 1159 and 1023 cm^{-1} are due to ether and hydroxylic C—O stretchings. The bending vibration of C—H has appeared at 890 cm^{-1}, whereas intense bands appearing at 2967, 2925, and 2859 cm^{-1} are attributed to aliphatic C—H stretching vibrations. The band observed at 3358 cm^{-1} is due to N—H stretching vibrations, whereas the bands at 1154 and 1128 cm^{-1} are due to C—N stretching vibrations.

Spectra of ATL-loaded microspheres are not characteristically different from the spectra of placebo microspheres. When drug is incorporated into crosslinked microspheres along with other characteristic bands of the crosslinked semi-IPN matrix, additional bands have appeared due to the presence of ATL in the matrix. It can be seen that some bands of ATL are not prominent in drug-loaded microspheres due to identical stretching of placebo microspheres as well as that of drug-loaded microspheres at the same wave number. The peaks appearing at 3356, 2970, 2924, 1636, 1512, 1461, 1299, 1245, and 1041 cm^{-1} for ATL also appeared in ATL-loaded microspheres, indicating the chemical stability of ATL in the formulations, which further confirms that ATL has not undergone any chemical change during formulation.

Differential scanning calorimetric study

DSC thermograms of (a) pure ATL, (b) placebo microspheres, and (c) ATL-loaded microspheres are presented in Figure 4. The crystallinity of ATL and melting temperature (T_m) of the polymer were determined. Placebo microspheres have shown an endothermic peak at 250°C, indicating melting temperature of the polymer, whereas ATL-loaded microspheres showed an endothermic peak at 245°C. Such a slight decrease in melting temperature could be due to small physical and morphological changes taking place in the microspheres after the loading of ATL in the matrix. For pure ATL, an endothermic peak appeared at 157°C due to the melting of ATL, but this peak did not appear in the ATL-loaded microspheres, indicating the amorphous dispersion of ATL in the matrix.

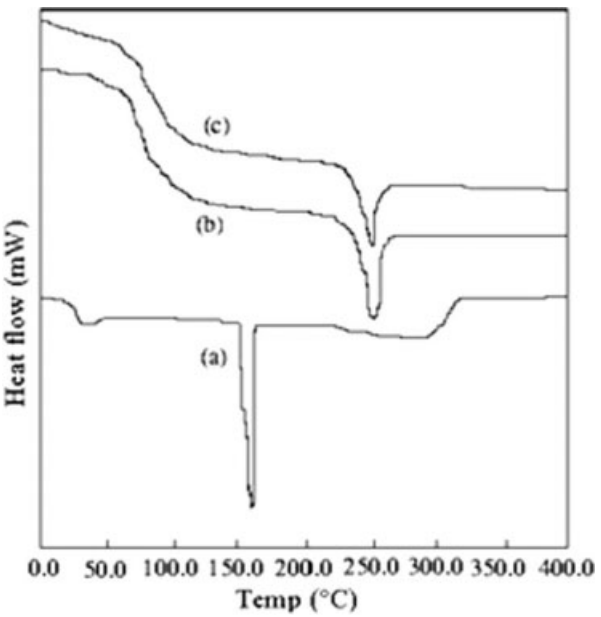

Figure 4 DSC thermograms of (a) Pristine ATL, (b) Placebo microspheres, and (c) ATL-loaded microspheres.

Scanning electron microscopic studies

SEM images of (a) placebo single particle taken at 2000×, (b) placebo group of particles at magnification of 1000×, (c) ATL-loaded particles at 850×, and (d) ATL-loaded group of particles at 600× magnification are displayed in Figure 5. Microspheres are somewhat spherical, but with agglomerations; however, their surfaces are smooth without any porous structure. Polymeric debris is seen around some particles due to the method used in particle production (i.e., simultaneous particle production and formation of semi-IPN). In case of microspheres, particles are adhered to each other, but no difference in the morphology of the placebo and drug-loaded microspheres can be observed. Hence, the loading of ATL in semi-IPN microspheres did not cause significant change in their morphology.

Equilibrium swelling studies

Equilibrium swelling results studied in pH 7.4 buffer media presented in Table II do not suggest any considerable influence on swelling of microspheres for plain GG; however, for the remaining formulations, equilibrium swelling is quite different. For all formulations, as expected, swelling is higher at 25°C than at 37°C, but it is least for plain GG, which was not affected greatly by temperature changes. With

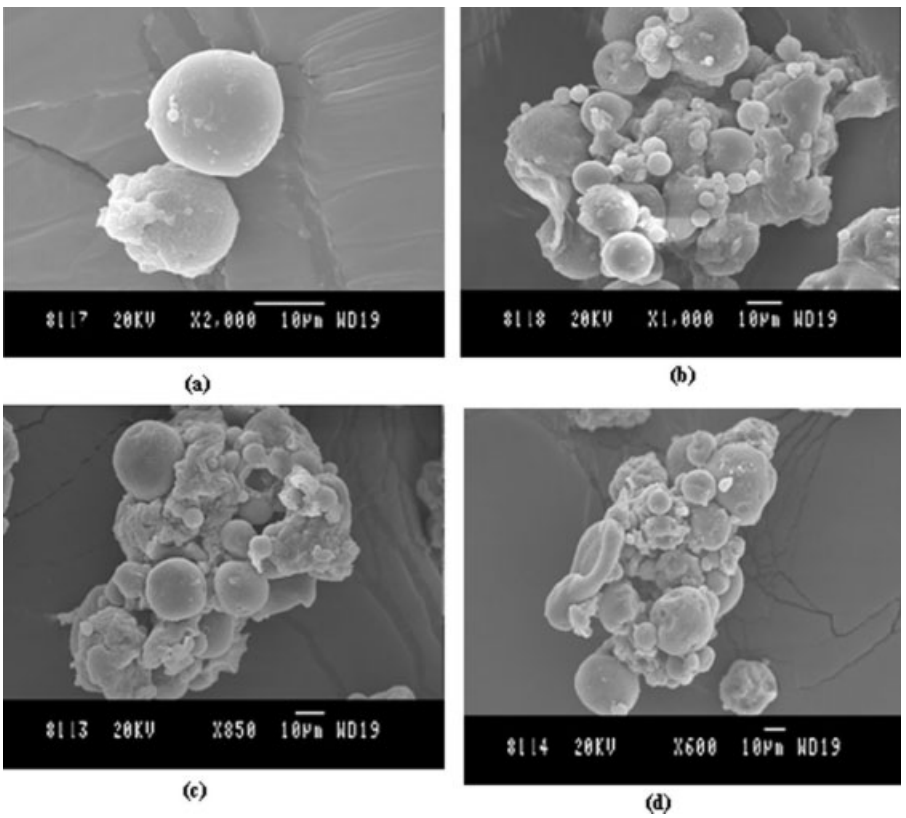

Figure 5 SEM images of microspheres: (a) Placebo single particle at 2000×, (b) Placebo group of particles at 1000×, (c) ATL-loaded particles at 850×, and (d) ATL-loaded group of particles at 600× magnifications.

decreasing amount of P(NIPAAm) of the matrix from 70 to 30% (i.e., formulations F1, F3, and F5), equilibrium swelling decreased from 162 to 103% at 25°C, but at 37°C, swelling decreased from 69 to 53% due to the collapse of PNIPAAm chains at which hydrogels are in a hydrophobic state.[32] Probably, intermolecular hydrogen bond formed between —COOH in GG and —CONH— in PNIPAAm shown by FTIR spectral shifts from 3444 cm^{-1} in placebo to 3356 cm^{-1} in drug-loaded formulations as shown in Figure 3.

At higher P(NIPAAm) content of the matrix, reduced swelling is observed. Since NIPAAm contains hydrophilic amido (—CONH) group in addition to hydrophobic isopropyl groups, the latter would facilitate intermolecular hydrogen bond interactions with water molecules at lower temperature, i.e., below the gel transition temperature. Thus, water penetrating into GG-P(NIPAAm) hydrogels will be in a bound state at lower temperature, which would gain enthalpy at higher temperature facilitating hydrophilic group of NIPAAm to establish

TABLE II
Results of Equilibrium Swelling at Different Temperatures and *n* Values of eq. (4) Along with Correlation Coefficients, *r*, for Various Formulations

Formulation codes	Equilibrium swelling (*Q*) at		*n*	r^a	*n*	r^a
	25°C	37°C	25°C		37°C	
F1	162	69	0.33	0.98	0.73	0.99
F2	168	72	0.40	0.96	0.69	0.99
F3	129	53	0.44	0.95	0.65	0.98
F4	134	58	0.46	0.98	0.64	0.97
F5	103	63	0.52	0.99	0.60	0.96
F6	107	68	0.46	0.94	0.49	0.97
F7	96	99	0.58	0.98	0.57	0.96

[a] *r* Values were estimated at 95% confidence limit.

intramolecular hydrogen bonds. However, hydrophobic interaction of isopropyl group of P(NIPAAm) will increase. These two effects make water inside the hydrogel to change from the bound state to a free state, thereby inducing ATL release from the hydrogel matrix with a rapid decrease in equilibrium swelling of hydrogels at the gel transition temperature.

In vitro release studies

In vitro cumulative release of ATL performed in pH 7.4 media at 25°C and 37°C for 12 h displayed in Figure 6(A,B), respectively, suggests that formulations containing higher amount of ATL (i.e., 50 wt %) displayed higher release rates than those containing smaller amounts of ATL (i.e., 25 wt %). This could be attributed to higher drug concentration gradient between the microspheres and the dissolution medium, thus increasing the ATL release rate. It is observed that those formulations that contained 50 : 50 ratio of P(NIPAAm) to GG exhibited lesser release rates than those containing 30 : 70 of P(NIPAAm) : GG at 37°C due to the collapse of semi-IPN matrix. At 25°C (in the swollen state), the release rate and the total amount of drug release were considerably higher than that observed at 37°C (in a collapsed state). Equilibrium swelling of P(NIPAAm) holds a linear relationship with their release profiles. At 37°C, the network is collapsed thus, exhibiting a lesser tendency to water uptake with a decrease in drug diffusion rates.[31]

Prolonged and slow release rates are observed for formulations containing 25 wt % of ATL at 25°C and 37°C, due to the large free volume of the matrices through which, lesser amount of ATL would transport. Notice that for all the ATL-loaded formulations, nearly 80–90% ATL was released in about 12 h, depending on the nature of the matrix as well as the amount of ATL. The release of ATL from semi-IPN microspheres is understood to be controlled by three processes: (1) release from the surface of microspheres, (2) diffusion through the swollen rubbery matrix, and (3) release due to polymer erosion. Encapsulated drug inside the microspheres will act as inert filler occupying the free volume spaces of the swollen hydrogel, thus creating tortuous paths for water molecules to permeate through, but the degree of tortuosity of diffusion path lengths depends on the volume fraction of the filler.[33]

The cumulative release versus time plots of the microspheres containing different amounts of P(NIPAAm) at 25°C and 37°C displayed in Figure 7(A,B), respectively, show that drug release increased with increasing amount of P(NIPAAm) at 25°C, but at 37°C, the precipitation of P(NIPAAm) in the hydrogel matrix might be responsible for controlled release of encapsulated ATL. The cumulative pulsatile release is displayed as a function of time for formulations F1, F3, F5, and F7 at 25°C and 37°C. The systems of this study are sensitive to changes in temperature. For instance, at 25°C (in the swollen state), release rate and total amount of drug released are considerably higher than those at 37°C (in a collapsed state). Drug molecules entrapped inside the semi-IPN matrix will diffuse out due to quick hydration of microspheres in the swollen state. In contrast, at 37°C, network structure is in a collapsed state and exhibits a lesser tendency for water uptake, giving decrease in drug diffusion. Figure 8 displays pulsatile cumulative release trends of ATL from semi-IPN microspheres under varying temperature cycles. Thus, the pulsatile "on–off" release of ATL is mainly due to the presence of P(NIPAAm), since these have the propensity to exhibit a transition from swollen to a collapsed state above 32°C. Such a swelling/collapse transition alters the permeability characteristics

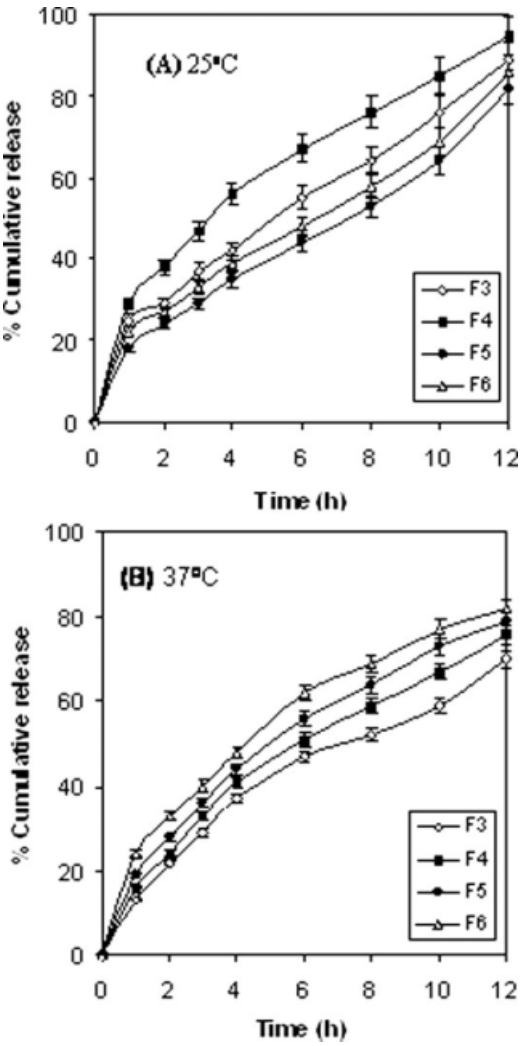

Figure 6 *In vitro* cumulative release profiles of formulations containing different drug loadings (A) at 25°C and (B) at 37°C.

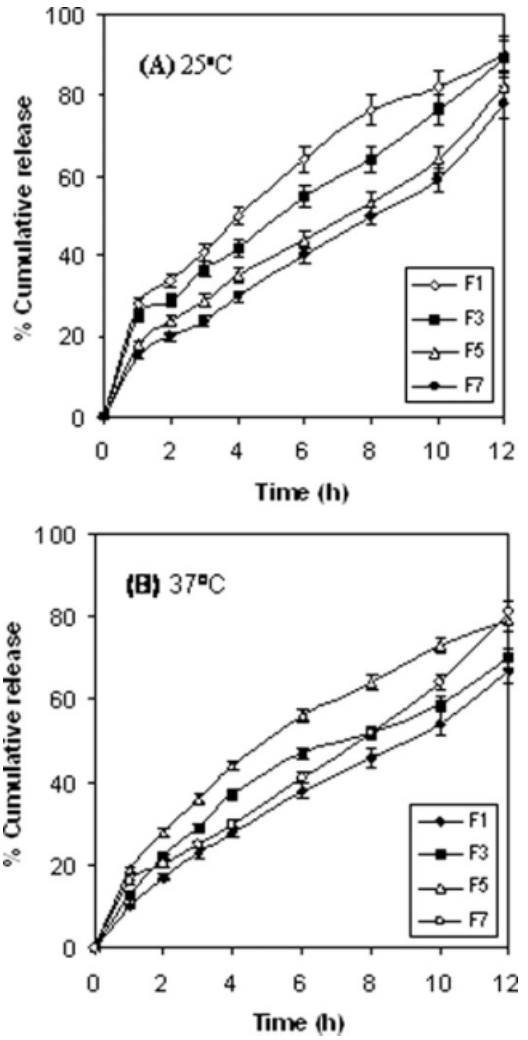

Figure 7 *In vitro* cumulative release profiles of formulations containing different amounts of P(NIPAAm) (A) at 25°C and (B) at 37°C.

of the network, and hence, matrices of this study are able to switch the release of ATL in a "on–off" cycle.

It may be noted that P(NIPAAm) hydrogel by itself may not be the desired matrix for biomedical applications because of its high glass transition temperature ($T_g = 47$°C) and its rigid network structure, but its desirable phase transition temperature in three-dimensional network occurs around physiological temperature[34] of 37°C. In addition, P(NIPAAm) has the propensity to exhibit greater swelling in aqueous medium due to small changes in temperature. This tendency[35] is apparent in aqueous solutions of P(NIPAAm) at the LCST near 32°C. The P(NIPAAm) chains are, therefore, able to hydrate giving extended structures in water when the solution temperature falls below its LCST, but will form compact structures due to the dehydration when heated at temperatures above LCST. Since the critical temperature of collapse for P(NIPAAm) is 32°C, the polymer can be potentially used as a drug delivery device. Moreover, P(NIPAAm) has the sharpest swelling transition among the class of thermoresponsive polymers that makes it suitable to develop CR devices. These observations further confirm the observations made in the previous literature. For instance, Ju et al.[36,37] studied the temperature dependency of swelling of alginate/P(NIPAAm) hydrogels. Park and Choi[38] prepared the IPN hydrogel beads composed of alginate and P(NIPAAm) to investigate the temperature-modulated release of indomethacin.

Release kinetics

Cumulative release data up to 60% of release versus time profiles have been fitted to the empirical equation[26]:

$$\frac{M_t}{M_\infty} = Kt^n \tag{4}$$

where M_t/M_∞ is the fraction of drug released at time t, K is kinetic rate constant, and n is diffusional exponent characterizing the nature of drug release. For $n = 0.5$, drug diffuses and releases out of the polymer matrix following the Fickian diffusion. For $n > 0.5$, anomalous or non-Fickian type diffusion occurs. If $n = 1$, Case II release kinetics is operative. The estimated values of n along with the correlation coefficient, r, values both at 25°C and 37°C are presented in Table II. For all formulations, the values of n range between 0.33 and 0.60 and 0.49 and 0.73, respectively, at 25°C and 37°C, suggesting slight deviations from the Fickian mode of transport.

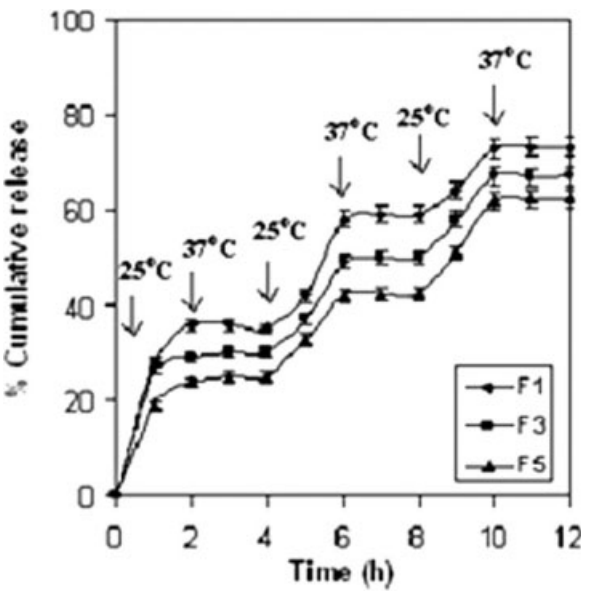

Figure 8 Pulsatile cumulative release profiles of ATL through formulations containing different amounts of P(NIPAAm) at 25°C and 37°C.

CONCLUSIONS

This study reports the development of novel type of semi-IPN matrices prepared by ionic crosslinking method using gellan gum and *N*-isopropylacrylamide for investigating controlled release of atenolol. The microspheres produced range in size from 34 to 76 μm. FTIR was used to confirm polymerization reaction as well as drug interactions with the semi-IPN. Thermal studies indicated molecular level dispersion of drug in the microspheres. Scanning electron microscopy indicated the formation of agglomerations due to the presence of gellan gum in the matrix and the microspheres produced are spherical. *In vitro* release experiments suggested that release rates are much faster at 25°C than at 37°C due to the collapse of P(NIPAAm) moiety; these data corroborate well with the equilibrium swelling results. Microspheres exhibited pulsatile drug release trends from 25°C to 37°C and the release of atenolol showed dependence on temperature, amount of drug loading, as well as the extent of P(NIPAAm) : GG ratio. Drug release followed Fickian to anomalous trend. Formulations of this study could have the potential as temperature-responsive delivery systems for other drugs as well.

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References

1. Soppimath, K. S.; Aminabhavi, T. M.; Kulkarni, A. R.; Rudzinski, W. E. J Control Release 2001, 70, 1.
2. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. J Control Release 2001, 75, 331.
3. Hoffman, A. S. Adv Drug Deliv Rev 2002, 54, 3.
4. Matsusaki, M.; Akashi, M. Biomacromolecules 2005, 6, 3351.
5. Lo, C. L.; Lin, K. M.; Hsiue, G. H. J Control Release 2005, 104, 477.
6. Hoare, T.; Pelton, R. Langmuir 2004, 16, 2123.
7. Zhang, Q.-S.; Zha, L.-S.; Ma, J.-H.; Liang, B.-R. J Appl Polym Sci 2007, 103, 2962.
8. Shi, J.; Alves, N. M.; Mano, J. F. Macromol Biosci 2006, 6, 358.
9. Schmaljohann, D. Adv Drug Deliv Rev 2006, 58, 1655.
10. Agnihotri, S. A.; Aminabhavi, T. M. Drug Dev Ind Pharm 2005, 31, 491.
11. Kang, K. S.; Veeder, G. T.; Mirrasoul, P. J.; Kaneko, T.; Cottrell, I. W. Appl Environ Microbiol 1982, 43, 1086.
12. Jansson, P. E.; Lindberg, B.; Sandford, P. A. Carbohydr Res 1983, 124, 135.
13. Kuo, M. S.; Mort, A. J.; Dell, A. Carbohydr Res 1986, 156, 173.
14. Grasdalen, H.; Smidsroed, O. Carbohydr Polym 1987, 7, 371.
15. Sanzgiri, Y. D.; Maschi, S.; Crescenzi, V.; Callegaro, L.; Topp, E. M.; Stella, V. J. J Control Release 1993, 26, 195.
16. Kubo, W.; Miyazaki, S.; Attwood, D. Int J Pharm 2003, 258, 55.
17. Miyazaki, S.; Aoyama, H.; Kawasaki, N.; Kubo, W.; Attwood, D. J Control Release 1999, 60, 287.
18. Quigley, K. J.; Deasy, P. B. J Microencapsul 1992, 9, 1.
19. El Fattah, E. A.; Grant, D. J.; Gabr, K. E.; Meshali, M. M. Drug Dev Ind Pharm 1998, 24, 541.
20. Agnihotri, S. A.; Jawalkar, S. S.; Aminabhavi, T. M. Eur J Pharm Biopharm 2006, 63, 249.
21. Schild, H. G. Prog Polym Sci 1992, 17, 163.
22. Hoffman, B. B. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed.; Hardman, J. G., Limbird, L. E., Eds.; McGraw Hill: New York, 2001.
23. Sastry, S. V.; Reddy, I. K.; Khan, M. A. J Control Release 1997, 45, 121.
24. Vázques, M. J.; Casalderrey, M.; Duro, R. Eur J Pharm Sci 1996, 4, 39.
25. Frishman, W. H.; Glasser, S. M. D.; Stone, P. M. D.; Prakash, C.; Deedwania, M. D.; Johnson, M.; Fakouhi, D. T. Am J Cardiol 1999, 83, 507.
26. Ritger, P.; Peppas, N. A. J Control Release 1987, 5, 37.
27. Wong, W.; Lee, H. Y.; Chan, L. W.; Heng, P. W. S. Int J Pharm 2002, 242, 233.
28. Agnihotri, S. A.; Aminabhavi, T. M. J Control Release 2004, 96, 245.
29. Crescenzi, V.; Dentini, M.; Coviello, T. In Novel Biodegradable Microbial Polymers; Dawes, E. A., Ed.; Kluwer Academic Publishers: The Netherlands, 1990; p 227.
30. Miyazaki, S.; Kawasaki, N.; Kubo, W.; Endo, K.; Attwood, D. Int J Pharm 2001, 220, 161.
31. Soppimath, K. S.; Aminabhavi, T. M. Eur J Pharm Biopharm 2002, 53, 87.
32. Moura, M. R.; Guilherme, M. R.; Campese, G. M.; Radovanovic, E.; Rubira, A. F.; Muniz, E. C. Eur Polym Mater 2005, 41, 2845.
33. Peppas, N. A.; Smolen, V. F., Eds. Bioavailability and the Pharmacokinetic Control of Drug Response; Wiley: New York, 1980.
34. Zhang, X. Z.; Yang, Y. Y.; Wang, F. J.; Chung, T. S. Langmuir 2002, 2013, 18.
35. Kuckling, D.; Hoffmann, J. M.; Plotner, D.; Ferse, K.; Kretschmer, H. J. P.; Adler, K.; Arndt, F.; Reichelt, R. Polymer 2003, 4, 4455.
36. Ju, H. K.; Kim, S. Y. Polymer 2001, 42, 6851.
37. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. J Appl Polym Sci 2002, 83, 1128.
38. Park, T. G.; Choi, H. K. Macromol Rapid Commun 1998, 19, 167.