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Research Article

Novel pH- and Temperature-Responsive Blend Hydrogel Microspheres of Sodium Alginate and PNIPAAm-*g*-GG for Controlled Release of Isoniazid

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Abstract. This paper reports the preparation and characterization of novel pH- and thermo-responsive blend hydrogel microspheres of sodium alginate (NaAlg) and poly(*N*-isopropylacrylamide)(PNIPAAm)-*grafted*-guar gum (GG) *i.e.*, PNIPAAm-*g*-GG by emulsion cross-linking method using glutaraldehyde (GA) as a cross-linker. Isoniazid (INZ) was chosen as the model antituberculosis drug to achieve encapsulation up to 62%. INZ has a plasma half-life of 1.5 h, whose release was extended up to 12 h. Fourier transform infrared spectroscopy was used to confirm the grafting reaction and chemical stability of INZ during the encapsulation. Differential scanning calorimetry was used to investigate the drug's physical state, while powder X-ray diffraction confirmed the molecular level dispersion of INZ in the matrix. Scanning electron microscopy confirmed varying surface morphologies of the drug-loaded microspheres. Temperature- and pH-responsive nature of the blend hydrogel microspheres were investigated by equilibrium swelling, and *in vitro* release experiments were performed in pH1.2 and pH7.4 buffer media at 37°C as well as at 25°C. Kinetics of INZ release was analyzed by Ritger–Peppas empirical equation to compute the diffusional exponent parameter (n), whose value ranged between 0.27 and 0.58, indicating the release of INZ follows a diffusion swelling controlled release mechanism.

KEY WORDS: blend hydrogel microsphere; graft copolymer; isoniazid; pH sensitive; temperature sensitive.

INTRODUCTION

Polymer-based drug delivery devices are of particular interest in biomedical area, since these maintain the required narrow therapeutic window for drugs of short plasma time, thereby avoiding the toxicity issues. The residence time of the drug also gets enhanced at the target site, thus improving the overall pharmacokinetics of the drug as well as patient compliance (1,2). In biomedical research, pH- and temperature-sensitive hydrogels that are cross-linked three-dimensional hydrophilic polymer networks, which absorb large quantity of water or biological fluid without being dissolved, have been widely explored (3,4). Hydrogels not only protect the encapsulated drug from the harsh gastrointestinal conditions but also help to control the drug release by minimizing the overdose related side effects such as ulcer, nausea, burning sensations, etc. (5). Response to physiological signal in the body is the most interesting characteristic of the hydrogels, since these change volume and shape reversibly in response to various external physiological conditions (6–8). In order to tune the properties of hydrogels, graft copolymerization, introduction of responsive functional groups, etc. have been tried to produce smart hydrogels in drug delivery area (9).

In this work, we have chosen three biodegradable polymers *viz*., sodium alginate (NaAlg), guar gum (GG), and poly (*N*-isopropylacrylamide) (NIPAAm), as single matrix delivery devices for controlling the release of INZ. Of these, GG is a non-ionic plant polysaccharide having many advantages such as low cost, widespread availability, and biodegradability. GG-based controlled release (CR) formulations have been widely explored in clinical trials (10,11). On the other hand, PNIPAAm is a thermotropic polymer exhibiting lower critical solution temperature (LCST) and hydrogels containing NIPAAm swells in aqueous media at a temperature lower than 32°C due to the formation hydrogen bond between water molecules and the hydrophilic –CONH– groups of the polymer side chains. Water molecules take special orientations around the hydrophobic isopropyl groups to form "ice-berg" structures to decrease free energy of the system due to negative entropic contributions. When temperature is increased above the LCST, hydrogel structure gets disrupted, and hydrophobic groups of the polymer side chains become free (12,13). The resulting hydrophobic interactions between isopropyl groups of hydrogel lose a large amount of water and thereby change its volume dramatically. This property has been used in developing thermo-sensing delivery devices (14).

Hydrogels of PNIPAAm often exhibit poor strength, and hence, it is necessary to graft these by copolymerizing with GG (15). Even though this was useful in theophylline release,

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but due to PNIPAAm's slow response rate, it is necessary to blend the graft copolymer (16,17) of PNIPAAm-*g*-GG with NaAlg, another naturally occurring polysaccharide, obtained from the marine brown algae. It is comprised of a linear chain of 1,4-linked *β-D*-mannuronic acid and *α-L*-guluronic acid. Due to its polyelectrolyte nature as well as good film forming ability, NaAlg has been widely explored in biomedical area as a drug carrier (18,19). The presence of carboxylic acid groups in NaAlg makes it a pH-sensing device (20), but the fast dissolution of the drug from the NaAlg hydrogel at higher pH is a major limitation. To overcome this, NaAlg has been blended with GG to make it a pH-sensitive device in the CR of proteins (21).

In this work, NIPAAm is grafted onto GG and blended with NaAlg to obtain thermo- and pH-sensitive hydrogel microspheres for the CR of INZ, an antimycobacterial agent, for the first-line therapy of tuberculosis (TB). The half-life of INZ varies from 0.5 to 1.6 h, and its daily dosage for effective therapy is 5 mg/kg body weight (22). Therapeutic plasma concentration of INZ can be achieved by administering it through orally, intramuscularly, or intravenously. However, the drug undergoes metabolism in liver by a process called acetylation. In order to maintain the therapeutic concentration of INZ for a prolonged period, dosage frequency should be increased. However, the overdosages of INZ can cause symptoms such as nausea, vomiting, dizziness, slurring of speech, blurring of vision, and visual hallucinations (including bright colors and strange designs) within 30 min to 3 h after its ingestion (23,24). In order to avoid these complications and to control the release of INZ as well as to minimize its toxic side effects, we thought of developing thermo- and pH-sensitive hydrogel microspheres of PNIPAAm-*g*-GG with NaAlg as a blend matrix to achieve the CR of INZ in both acidic and alkaline pH media. The drug-loaded formulations have been characterized by Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), SEM, and X-ray diffraction (XRD) techniques. Temperature and pH sensitivity of the blend hydrogel microspheres were assessed from the equilibrium swelling studies in order to correlate their release profiles in acidic and alkaline media. The present work is a novel approach in the sense that no such devices have been developed before in the literature of pharmaceutics for the CR of INZ.

EXPERIMENTAL

Materials and Methods

Isoniazid drug and Span-80 were purchased from Loba Chemicals, Mumbai, India. Sodium alginate, guar gum, *N*-isopropylacrylamide, analytical reagent grade glutaraldehyde solution 25% (*v*/*v*), *n*-hexane, and light liquid paraffin oil were all purchased from s.d. fine Chemicals, Mumbai, India. All the other chemicals were used without further purification.

Synthesis of Poly(*N*-Isopropyl Acrylamide)-*g*-Guar Gum

The graft copolymer of GG and *N*-isopropylacrylamide was prepared by free radical polymerization as described before (15). Briefly, 2 g of GG was dispersed in 150 mL of deionized water, which was allowed to hydrate and then dissolved by stirring overnight in a 250-mL round bottom flask. In another flask, 4 g of *N*-isopropylacrylamide was dissolved in 20 mL of water, which was added to the GG solution and stirred for 1 h. To this solution, 10 mL of 0.05 mol/L of ceric ammonium nitrate was added as an initiator to carry out polymerization at 60°C by continuously purging nitrogen gas for 6 h in a water bath under constant stirring. After complete polymerization, sufficient amount of acetone was added to precipitate the graft copolymer and washed several times with methanol to remove the homopolymer. The polymer was dried under vacuum (60 mm Hg) at 40°C for overnight. Under this condition, complete evaporation of acetone and methanol was achieved, thereby ruling out the probability of trace amount of acetone and methanol in the graft copolymers. The total mass of the copolymer was taken to calculate the percent grafting efficiency:

$$\%\ \text{Grafting efficiency} = \left(\frac{\text{Mass of graft copolymer}}{\text{Mass of(NIPAAm + GG)}}\right) \times 100 \quad (1)$$

Preparation of Blend Hydrogel Microspheres

Blend hydrogel microspheres of NaAlg and PNIPAAm-*g*-GG were prepared by water-in-oil (w/o) emulsion cross-linking method (24). Briefly, varying amounts of NaAlg and PNIPAAm-*g*-GG were dissolved in separate beakers, each containing 10 mL of double-distilled water with pH6.8. Both solutions were mixed to get 20 mL of 2% (*w*/*v*) polymer solution. About 40 mg of INZ was dissolved in the polymer solution, stirred for 10 min, and added slowly to light liquid paraffin oil (100 mL) containing 2 mL Span®-80 under constant stirring at 450 rpm speed using a Eurostat high-speed stirrer (IKA Labortechnik, Staufen, Germany) for 10 min. To this w/o emulsion, 5 mL of GA containing few drops of 0.5 N HCl was added slowly and stirred for another 3 h to obtain the hardened microspheres. The suction pump connected with Buckner funnel was used to filter the hardened microspheres through Whatman no. 40 filter paper under normal tap suction pump. Surface-adhered oil was removed by washing with *n*-hexane, and the unreacted GA was deactivated by adding 0.1 M glycine solution. The microspheres were air-dried at 40°C for 24 h and stored in a desiccator until further use.

Fourier Transform Infrared Spectra

FTIR spectra were taken on a Nicolet (Impact 410, Milwaukee, Wisconsin, USA) spectrophotometer to confirm grafting, cross-linking as well as to investigate the chemical stability of INZ in the microspheres. FTIR spectra of the plain GG, PNIPAAm-*g*-GG, NaAlg, the placebo microspheres (placebo F3), INZ-loaded microspheres (F3), and plain INZ were all taken on the KBr grounded powder and pelleted by applying a pressure of 600 kg/cm^2. FTIR spectral scanning was done in the range between 4,000 and 500 cm^{-1} at 2 cm^{-1} resolution.

Differential Scanning Calorimetry

DSC (Rheometric Scientific, Surrey, UK) was performed on placebo microspheres (placebo F3), INZ-loaded microspheres

(F3), and pristine INZ. For DSC measurements, a sample weighing about 2–3 mg was heated from 10°C to 400°C at the heating rate of 10°C/min in a nitrogen atmosphere (flow rate, 20 mL/min).

X-ray Diffraction

Crystallinity of INZ after encapsulation was assessed by XRD recorded for the placebo microspheres (placebo F3), INZ-loaded microspheres (F3), and pristine INZ using an X-ray diffractometer (Bruker Model D8 Advance, Germany). Scanning was done up to 2θ of 80º.

Scanning Electron Microscopy

SEM images were taken on plain NaAlg microspheres, NaAlg and PNIPAAm-*g*-GG blend hydrogel microspheres, as well as plain PNIPAAm-*g*-GG microspheres prepared by cross-linking with 5 mL of GA and loaded with 10% (*w*/*w*) INZ. Microspheres were sputtered to form a gold coating of 10 nm thickness to make them conducting and were placed on a copper stub. Scanning was done using a JEOL model 6390 LA, Japan instrument available at the Sophisticated Test and Instrumentation Center, Cochin University, Kochi, India. At least two different resolutions (magnification factor, ×) were used for each formulation.

Estimation of Drug Loading and Encapsulation Efficiency

INZ content was estimated in distilled water by grinding 10 mg of the microspheres to get the powder using an agate mortar, extracting for 6 h at 25°C in 50 mL water and sonicated for 1 h. The solution was centrifuged to remove polymeric debris and washed twice to completely extract the INZ. The clear supernatant solution was analyzed by UV spectrophotometer (Model Anthelic, Secomam, Ales, France) at the fixed λ_{max} value of 263 nm, which is the characteristic value for the INZ. The results of percent INZ loading and percent encapsulation efficiency (% EE) were calculated using the following equations:

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

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

Equilibrium Swelling

To assess the temperature-dependent swelling, equilibrium swelling of INZ-loaded NaAlg and PNIPAAm-*g*-GG blend hydrogel microspheres was investigated in a phosphate buffer (pH7.4) solution at 25°C and 37°C, respectively. Similarly, the pH-dependent swelling was assessed by performing the equilibrium swelling studies in stomach acidic (pH1.2) as well as intestinal alkaline (pH7.4) conditions at the physiological temperature of 37°C. Equilibrium swelling of all the blend hydrogel microspheres was determined gravimetrically by measuring the extent of swelling in the respective medium. To ensure complete equilibration, samples were allowed to swell for 12 h in an orbital incubator (S150, UK). Excess surface-adhered liquid was removed by blotting with soft tissue paper, and the swollen microspheres were weighed accurately to ±0.01 mg using an electronic balance (Mettler, Model AT 20, Switzerland). The blend hydrogel microspheres were then dried in an oven at 60°C for 5 h until there was no change in the dry mass of the samples. The percent equilibrium swelling was calculated as:

$$\%\ \text{Equilibrium swelling} = \left(\frac{M_s - M_d}{M_d}\right) \times 100 \quad (4)$$

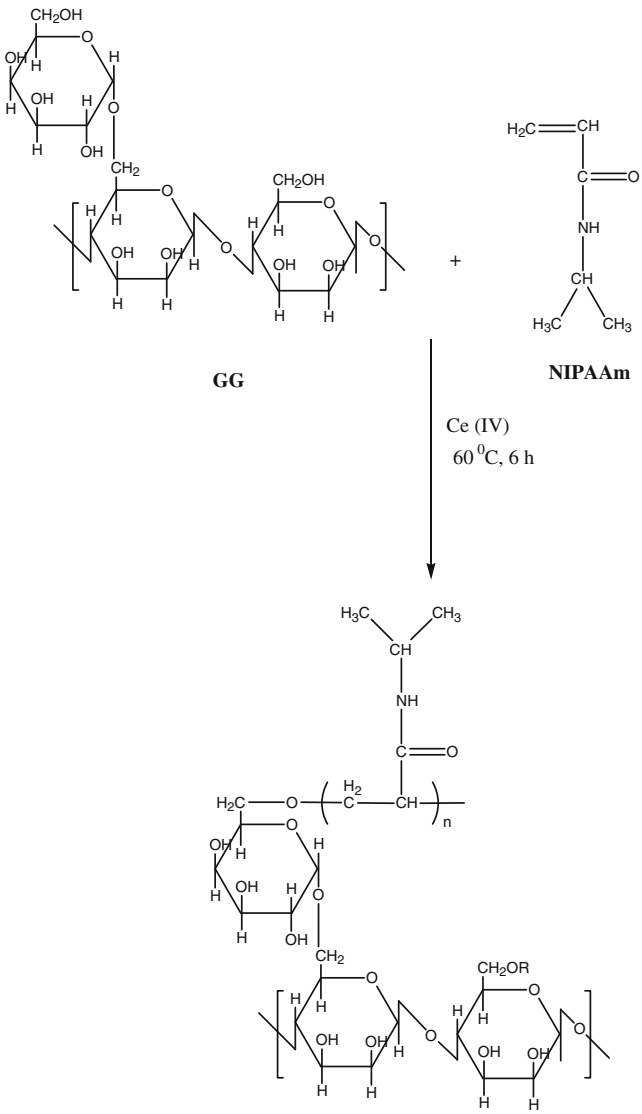
where M_s and M_d are masses of the swollen and dry microspheres, respectively.

In Vitro Drug Release

Dissolution studies from the blend hydrogel microspheres equivalent to 10 mg of INZ were investigated in a USP apparatus-I dissolution tester (Dissotest, LabIndia, Mumbai, India). In order to assess the pH sensitivity of the blend hydrogel microspheres, dissolution studies were performed in pH1.2 as well as in pH7.4 dissolution media at 37°C. To assess the response of blend hydrogel microspheres to temperature, drug release was monitored at 25°C as well as at 37° C in pH7.4 dissolution media. The microspheres were placed back in the basket that was immersed in 500 mL of the dissolution medium maintained at the respective temperature and at 100-rpm paddle stirring speed. A 5-mL aliquot was withdrawn at different time intervals and filtered through a 0.45-mm filter. Dissolution medium was replenished with another 5 mL of fresh dissolution media to maintain the sink condition. The concentration of INZ was determined by UV spectrophotometer (Secomam, model Anthelie, Ales, France) at the λ_{max} of 263 nm.

RESULTS AND DISCUSSIONS

Graft copolymerization of NIPAAm with GG with NIPAAm was achieved by Ce(IV) catalyzed free radical polymerization (see Scheme 1) using ceric ammonium nitrate as an initiator. The ceric ion reacts with GG to form GG–ceric complex. The chelate complex formed with –OH group of GG decomposes to generate the free radical site, thereby facilitating the grafting reaction to occur at the active site of GG with the incoming NIPAAm monomer (11). The grafting efficiency calculated using Eq. 1 was found to be 86%. The INZ drug was successfully encapsulated in the blend hydrogel microspheres of PNIPAAm-*g*-GG and NaAlg by cross-linking the matrix with GA. However, toxicity due to the unreacted GA can be reduced by deactivating free aldehydic groups by repeatedly washing the microspheres with glycine solution. The free aldehydic group of unreacted GA was converted to imine group after reacting with amine group of glycine. The negative Brady's test (24,25) confirmed the absence of unreacted GA, suggesting extremely low concentrations of GA, indicating their safe applications.



Scheme 1. Schematic representation of the synthesis of PNIPAAm-*g*-GG

Encapsulation Efficiency

The % EE values for the blend hydrogel microspheres as calculated from Eq. 2 included in Table I suggest that plain PNIPAAm-*g*-GG hydrogel microspheres entrapped small amount of INZ exhibiting only 38% EE. On the other hand, plain NaAlg microspheres showed a somewhat higher % EE of 46% because of the linear polymeric chain of NIPAAm grafted onto GG making a loose network. Hence, more of the INZ might have leached out of the matrix during the preparation of the microspheres. However, the blend hydrogel microspheres entrapped higher amount of INZ than the hydrogels of individual components of the blend due to the increase in the rigidity of the matrix that retains more of INZ particles, thus resulting in high % EE of the blend microspheres. The strong hydrogen-bonding interaction between $-COO^-$ of NaAlg and N–H group of PNIPAAm-*g*-GG creates more cross-linking sites in the network. Such type of hydrogen bonding interactions are observed from a shift of C=O stretching frequency at 1,744 cm^{-1} in the FTIR spectrum of the blend compared to the plain polymer components. Such rigid microspheres may not leach out the drug as easily compared to the loose network. Hence, more of INZ particles are entrapped in the matrix, but for compositions of PNIPAAm-*g*-GG with NaAlg ranging from 25% to 75% *w*/*w*, a decline in EE is

Table I. Formulation Details Along with Percent Encapsulation Efficiency (% EE)

Formulation codes	NaAlg (% *w*/*w*)	PNIPAAm-*g*-GG (% *w*/*w*)	INZ (mg)	GA (mL)	EE (%)
F1	100	0	40	5	46
F2	75	25	40	5	62
F3	50	50	40	5	58
F4	25	75	40	5	52
F5	0	100	40	5	38

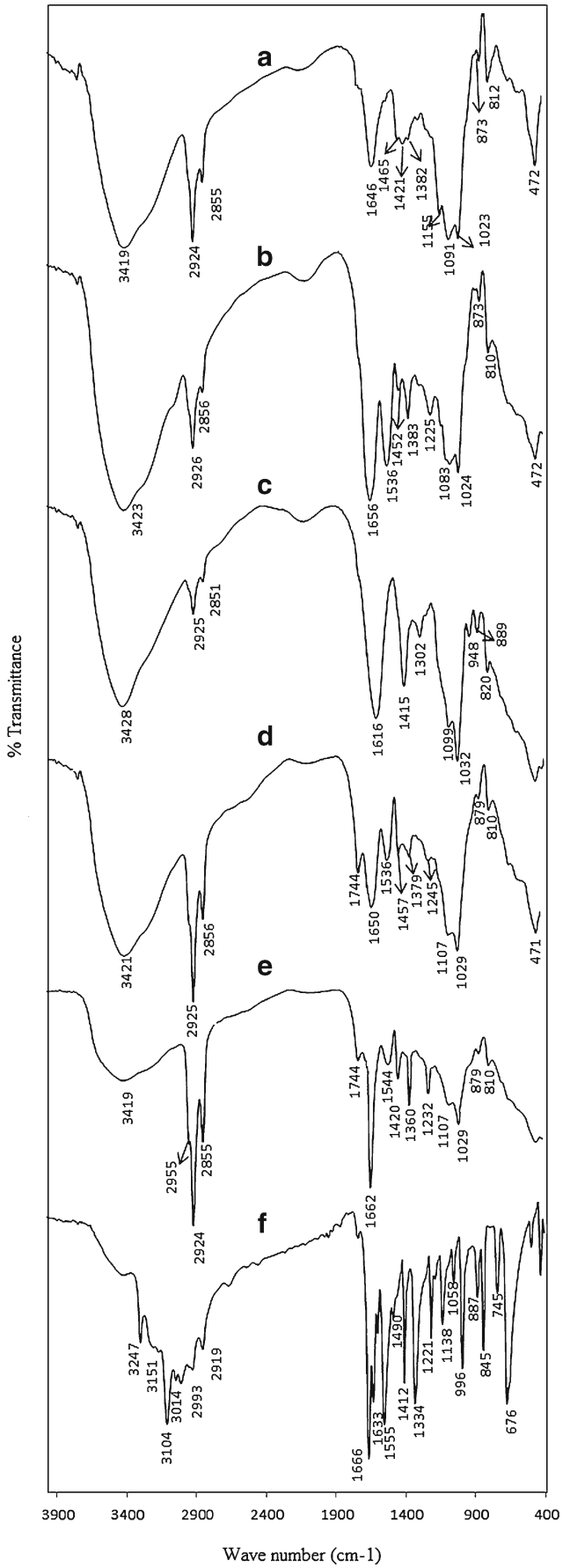


Fig. 1. FTIR spectra of **a** GG, **b** PNIPAAm-*g*-GG, **c** NaAlg, **d** placebo microspheres, **e** drug loaded microspheres (F3), and **f** pristine INZ

observed from 62% to 58% due to the loose network of PNIPAAm chains within the hydrogel matrix.

FTIR Spectral Analysis

The grafting reaction between GG and NIPAAm, blend polymer interactions as well as the chemical stability of INZ in the blend was studied using FTIR. Figure 1 depicts the FTIR spectra of GG (A), PNIPAAm-*g*-GG (B), NaAlg (C), placebo F3 (D), INZ-loaded F3 (E), and INZ drug (F). In the case of GG, a broad band due to O–H stretching vibrations is observed at 3,419 cm^{-1}. Two more peaks appeared at 2,924 and 2,855 cm^{-1} corresponding to asymmetric and symmetric stretching of C–H bonds, respectively. The peaks at ~1,000 to ~1,100 cm^{-1} are due to C–O stretching vibrations. In comparison to GG spectrum, PNIPAAm-*g*-GG spectrum showed a sharp new peak at 1,656 and at 1,536 cm^{-1} that are due to the stretching of C=O group and bending vibrations of N–H group of grafted PNIPAAm chain, respectively. The N–H stretching vibrations are observed as a shoulder peak at 3,236 cm^{-1} to the O–H stretching vibrations of GG as well as C–N stretching vibration that are observed at 1,452 cm^{-1}, confirming the grafting reaction (11) between GG and NIPAAm.

In the case of NaAlg, the band at 3,428 cm^{-1} for hydroxyl groups and at 1,616 and 1,415 cm^{-1} for asymmetric and symmetric stretching vibrations of $–COO^-$ are evident. The blend hydrogel microspheres showed a shift in asymmetric stretching vibration frequency from 1,616 cm^{-1} of $-COO^-$ to 1,744 cm^{-1}, but symmetric stretching frequency of 1,415 cm^{-1} has disappeared. This is because the hydrogen bonding interaction between N–H and $-COO^-$ groups makes the C=O bond stronger by disturbing the symmetry of carboxylate ion, which confirms the strong hydrogen bond interaction between the constituents of the blend (9,26,27). In the case of INZ, peaks between 3,100–3,000 cm^{-1} and 2,990–2,900 cm^{-1} correspond to respective asymmetric and symmetric stretching vibrations of the C–H bond, while the N–H stretching vibration is observed at 3,247 cm^{-1}. The bands at 1,633 and 1,555 cm^{-1} are due to asymmetric and symmetric stretching vibrations of C=N group, while the stretching frequency of C=O is observed at 1,666 cm^{-1}. Asymmetric and symmetric vibrational frequencies for ring C=C groups are observed at 1,602 and 1,412 cm^{-1}, respectively, while the C–N stretching frequency has appeared (28) at 1,333 cm^{-1}. In the FTIR spectra of INZ-loaded blend hydrogel microspheres, the bands that are present initially in INZ have also appeared, showing a small shift in the frequency and intensities, but the bands of INZ are not prominent in the drug-loaded microspheres due to the merging of bands of INZ in the broad bands of the polymer matrix, suggesting the chemical stability of INZ after encapsulation.

DSC Analysis

DSC thermograms of (a) placebo microspheres, (b) INZ-loaded microspheres (F3), and (c) pure INZ are used to understand the physical state of INZ within the blend hydrogel microspheres (see Fig. 2). For pristine INZ, a sharp peak at

172°C is observed representing its melting point (24). In the case of placebo blend hydrogel microspheres, transitions are observed at 109°C, 204°C, and 259°C, of which the observed broad endothermic peak at 109°C is due to the loss of moisture, while the two peaks at 204°C and 259°C correspond to endothermic transitions of the polymers. Thermograms of INZ-loaded blend hydrogel microspheres have shown all the peaks that are present in the thermograms of placebo blend hydrogel microspheres, but no peak is observed corresponding to INZ, indicating a molecular-level dispersion of INZ in the polymer matrix.

SEM Analysis

SEM images of the plain NaAlg hydrogel microspheres, blend hydrogel microspheres (F3), and plain PNIPAAM-*g*-GG hydrogel microspheres at two different magnifications are shown in Fig. 3. From the images (Fig. 3a), it is evident that plain NaAlg microspheres have spherical shapes with smooth surfaces, but for plain PNIPAAm-*g*-GG hydrogel microspheres, even though the particles are spherical, some agglomeration was observed with a rough surface, depicting the rigidity of the matrix as shown in Fig. 3c. The morphology of the blend hydrogel microspheres (Fig. 3b) of plain NaAlg and plain PNIPAAm-*g*-GG hydrogel microspheres showed uneven shapes, often with agglomeration. Because of the presence of PNIPAAm-*g*-GG in the blend, microspheres showed rigid surface morphology compared to the plain NaAlg microspheres.

XRD Analysis

XRD diffractograms of placebo blend hydrogel microspheres (A), INZ-loaded blend hydrogel microspheres (F3) (B), and pristine INZ (C) presented in Fig. 4 suggest the loss of crystallinity of INZ after its formulation. For instance, diffraction patterns of INZ showed many intense peaks between 2θ of 12° and 20° that are characteristics of its crystalline nature. However, these peaks have disappeared in INZ-loaded blend hydrogel microspheres, but only the peaks observed in placebo polymer are seen. The XRD profiles depend on the crystal size, but in the present study, for all the INZ-loaded matrices, the characteristic peak of INZ could overlap with the noise of the coated polymer itself. Further, the loaded INZ is amorphous, so it is difficult to measure, at the detection limit of the instrument, the crystal size, indicating that INZ is dispersed molecularly in the blend hydrogel microspheres.

Equilibrium Swelling

The variations in percent equilibrium swelling of the matrices with the change in temperature as well as pH of the medium calculated from Eq. 4 are presented in Table II. A decline in equilibrium swelling is observed for formulations F1 to F5 at all temperatures and pH ranges, suggesting the effect of PNIPAAm-*g*-GG content in the blend matrix on the swelling capacity of the hydrogel microspheres. This is because of the grafting of synthetic PNIPAAm polymer onto a natural polymer like GG, converting the matrix rigid. However,

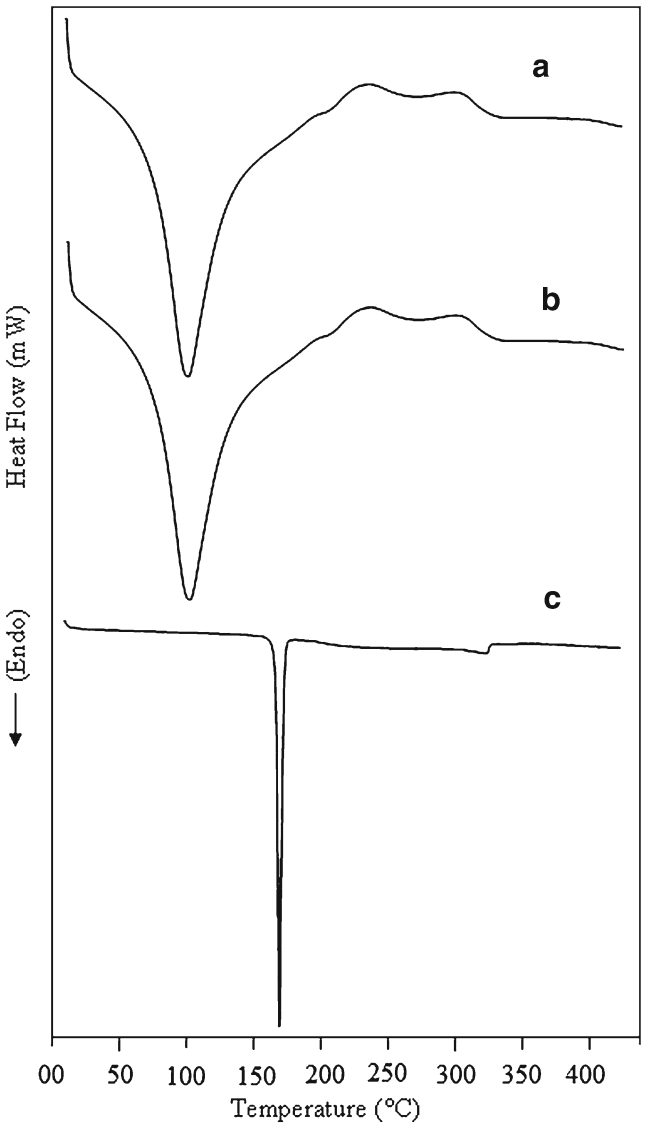


Fig. 2. DSC thermogram of **a** placebo microspheres, **b** drug loaded microspheres (F3), and **c** pristine drug (INZ)

hydrogen bonding interactions between the blend components could also make the matrix tougher as reflected on their swelling patterns.

The pH-dependent water uptake capacity of hydrogel microspheres at 37°C were assessed in 1.2 and 7.4 pH buffer solutions. The plain PNIPAAm-*g*-GG (F5) hydrogel microspheres showed 61% and 62% of equilibrium swelling at pH 1.2 and 7.4 buffer media, respectively, suggesting the negligible influence of pH on their swelling capacity. On the other hand, plain NaAlg hydrogel (F1) microspheres showed 331% and 422% of equilibrium swelling values at pH1.2 and 7.4 buffer media, respectively. Similarly, for formulations F2, F3, and F4, equilibrium swelling values of 242%, 162%, and 81% were observed at 1.2 pH, while 326%, 214%, and 112% at pH 7.4. These data suggest a strong pH-dependent swelling of the formulations due to the presence of acidic group of β-*D*-mannuronic acid and α-*L*-guluronic acid structural units of NaAlg that are protonated in acidic environment (pH1.2), but in

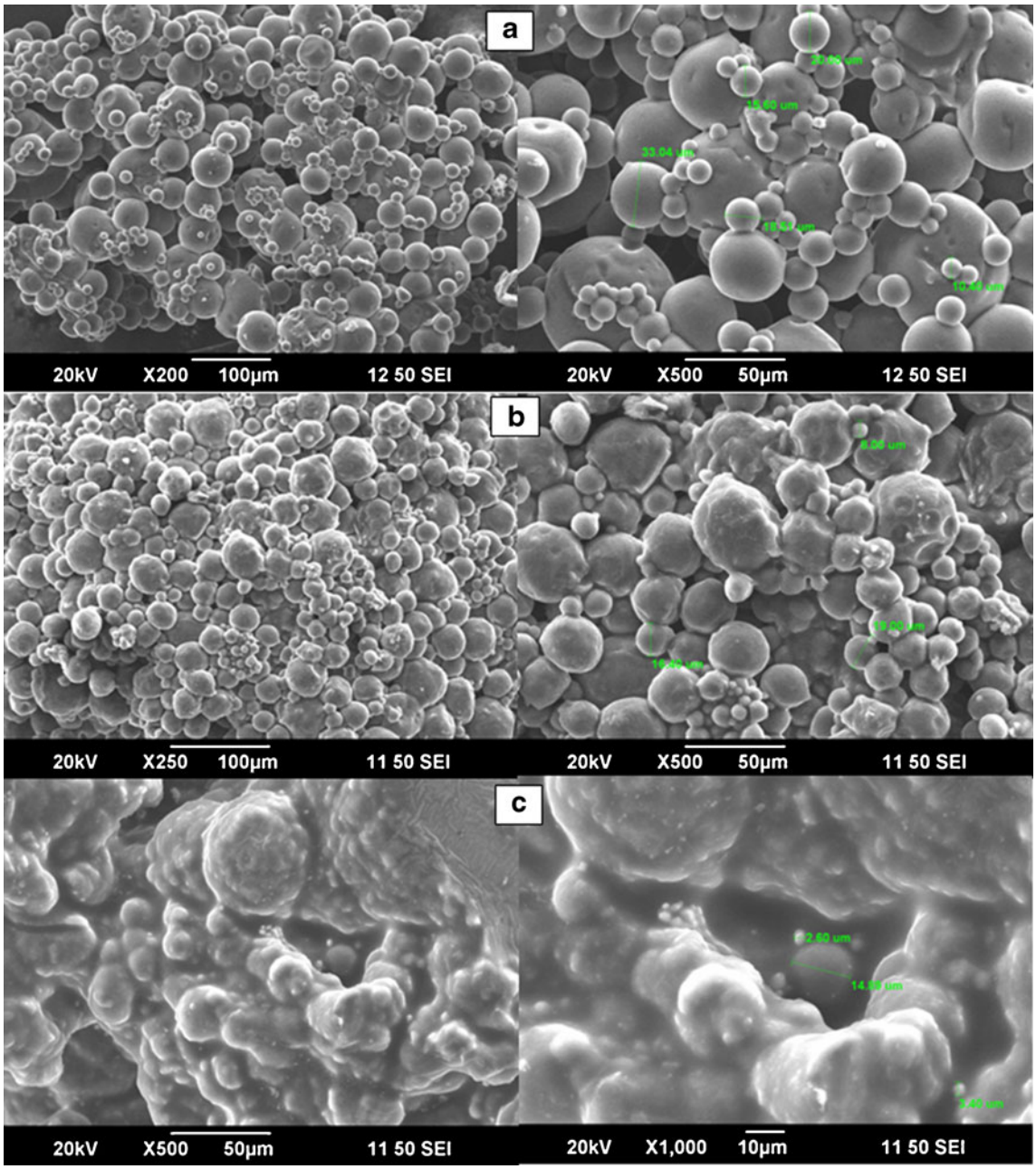


Fig. 3. SEM photograph of drug-loaded microspheres of **a** plain NaAlg hydrogel microspheres, **b** blend hydrogel microspheres, and **c** plain PNIPAAm-*g*-GG hydrogel microspheres

alkaline medium (pH7.4), the deprotonated $-COO^-$ group might exist. However, the repulsion among these groups made the matrix to swell in alkaline media.

Equilibrium swelling results studied in pH7.4 buffer at 25°C and 37°C presented in Table II influence the swelling of microspheres for plain NaAlg. Notice that, for all other formulations, equilibrium swelling is quite different. As expected, swelling is higher at 25°C than at 37°C, but is least for plain NaAlg hydrogel microspheres, which was not greatly affected by the changes in temperature. With increasing amount of PNIPAAm-*g*-GG in the matrix from 25% to 75% (*i.e.*, formulations F2, F3, and F4), equilibrium swelling decreased from 382% to 142% at 25°C, but at 37°C, swelling decreased from 326% to 112% due to the collapse of PNIPAAm chains at which hydrogels become hydrophobic in nature.

In Vitro Release of Drug

Effect of Blend Ratio. The effect of blend ratio on percent cumulative release of INZ can be observed from the release profiles displayed in Fig. 5. The INZ, which is present on the surface of the hydrogel microspheres, is responsible for the initial burst release portion of the release profiles. Plain NaAlg hydrogel microspheres (F1) showed a higher release of INZ, but plain PNIPAAm-*g*-GG hydrogel microspheres (F5)

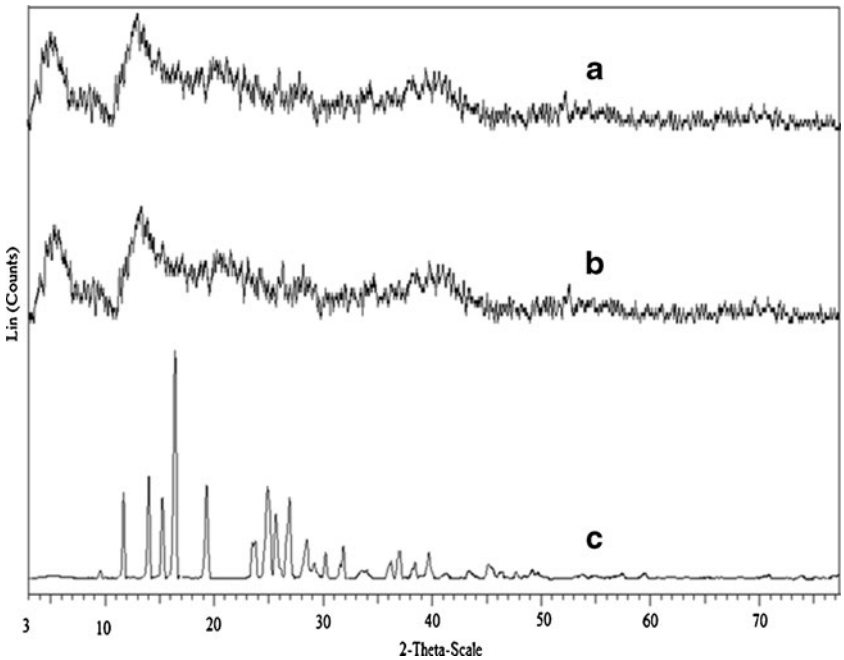


Fig. 4. XRD patterns of **a** placebo microspheres, **b** drug loaded microspheres, and **c** pristine INZ

showed the least release. The intermediary release profiles are observed from the blend microspheres, *viz*., F2, F3, and F4. It was found that release of pharmaceuticals from the hydrogels depend on their swelling characteristics. Due to the presence of synthetic PNIPAAm onto the GG backbone, the matrix becomes rigid, and thus, its response to the release media becomes slow. On the other hand, plain NaAlg hydrogel microspheres with higher swelling capacity showed higher release. However, a decrease in swelling capacity and release rates are observed for F2, F3, and F4 formulations, due to the rigid nature of PNIPAAm-*g*-GG blend component and the possible hydrogen-bonding interactions between $-COO^-$ of NaAlg and N–H group of PNIPAAm-*g*-GG.

Effects of pH. In vitro release experiments were performed in both gastric (pH1.2) and intestinal (pH7.4) conditions at 37°C. As expected from the swelling studies, formulations F1 to F4 show a pH-dependent release profiles except F5. At pH7.4, plain NaAlg hydrogel microspheres released 90% of INZ within 4 h, whereas at pH1.2, the release was about 48% due to the pH sensing carboxylate group of NaAlg. Increasing the PNIPAAm-*g*-GG content of the blend, a decline in the release profiles is observed. At pH1.2 and at 37°C, the blend hydrogel microspheres (F2, F3, and F4) showed smaller release rates than that in pH7.4 at 37°C. In pH1.2 at 37°C, the barriers restrict the release of INZ. One is due to less swelling capacity of NaAlg in pH1.2 buffer media and the other due to PNIPAAm-*g*-GG, whose *N*-isopropyl group is in a collapsed state at 37°C. On the other hand, in pH7.4 at 37°C, the blend matrix offers restriction to drug release due to the collapsed mass of PNIPAAm-*g*-GG, whereas NaAlg undergoes a quick swelling at pH7.4 buffer media. It is also concluded from the release profiles that plain PNIPAAm-*g*-GG hydrogel microspheres showed no pH-dependent release.

Effect of Temperature. In vitro cumulative release of INZ performed in pH7.4 media at 25°C and at 37°C, respectively, for 12 h displayed in Fig. 5, suggests that plain NaAlg hydrogel microsphere (F1) showed a temperature-independent release of INZ, whereas microspheres of PNIPAAm-*g*-GG (F2 to F5) showed a temperature-

Table II. Percent Equilibrium Swelling (%ES) Data Along with Estimated Parameters of Empirical Equation at Different Release Conditions

Formulation codes	pH1.2 at 37°C				pH7.4 at 37°C				pH7.4 at 25°C			
	ES (%)	*n*	*k*	*r*	ES (%)	*n*	*k*	*r*	ES (%)	*n*	*k*	*r*
		Eq. 5				Eq. 5				Eq. 5		
F1	331	0.58	0.493	0.991	422	0.46	0.648	0.957	418	0.47	0.642	0.960
F2	242	0.46	0.515	0.998	326	0.33	0.719	0.953	382	0.44	0.742	0.941
F3	162	0.44	0.513	0.996	214	0.32	0.733	0.960	268	0.57	0.763	0.920
F4	81	0.46	0.539	0.994	112	0.28	0.742	0.904	142	0.54	0.779	0.972
F5	62	0.48	0.535	0.998	61	0.27	0.593	0.930	86	0.46	0.630	0.976

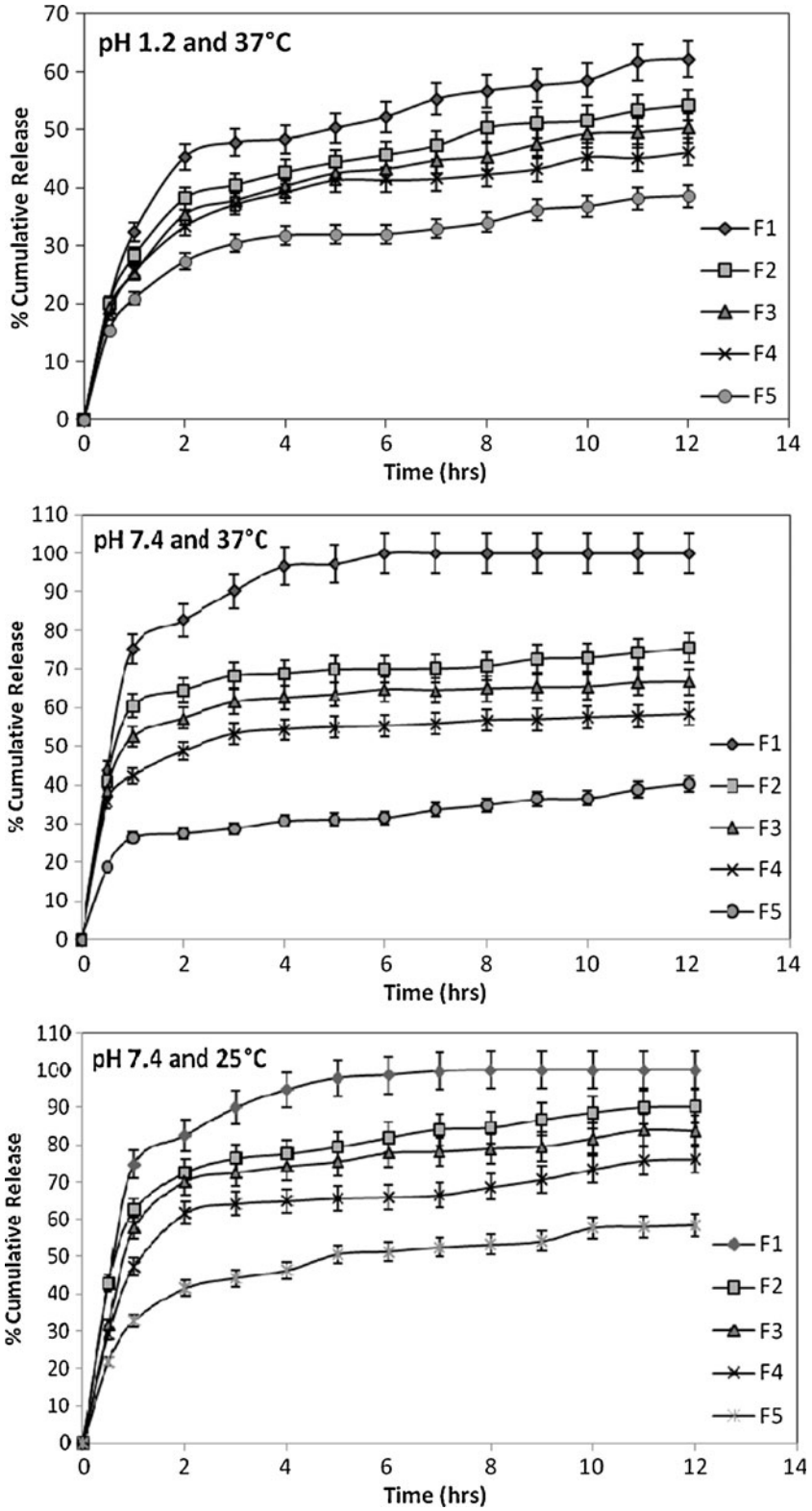


Fig. 5. *In vitro* release of INZ from blend hydrogel microspheres in pH1.2 and pH7.4 release media at 37°C and 25°C

dependent release at 25°C (in the swollen state). Release rates and the total amount of drug released are considerably higher than those at 37°C (in a collapsed state). Drug molecules entrapped inside the polymer matrix will diffuse out due to the quick hydration of microspheres in the swollen state. In contrast, at 25°C, the blend hydrogel microspheres are more hydrophilic due to the presence of water molecules that are oriented around the hydrophobic isopropyl groups to form "ice-berg" structure with a decrease in free energy of the system through a negative

entropic contribution, whereas at 37°C, free energy of the system increases with a positive entropy, which will disrupt the orientation of water molecules around *N*-isopropyl groups of the polymer side chain. The resulting hydrophobic interactions between isopropyl groups make the blend hydrogel to lose a large amount of water and hence, collapse in a network structure, exhibiting a lesser tendency for water uptake, giving a decrease in drug diffusion.

Release Kinetics

To determine the mechanism of drug release, the initial of percent drug release *vs.* time profiles have been fitted to the empirical equation (29):

$$M_t/M_\infty = kt^n \quad (5)$$

where M_t/M_∞ is fractional solute release, t is release time, k is a constant incorporating structural and geometrical characteristics of the delivery device, and n is diffusion exponent, characteristics of the release mechanism. For microspheres, $n<0.43$ indicates the drug release following the Fickian diffusion. If $n>0.85$, swelling-controlled drug release occurs. The intermediary values of n ranging between 0.43 and 0.85 are indicative of both diffusion and swelling-controlled drug release *i.e.*, anomalous-type release (30). The estimated values of n along with correlation coefficient, r, for all the formulations presented in Table II vary between 0.44 and 0.58 for formulations whose release study was performed in pH1.2 release media at 37°C. In pH7.4 release media at 37°C, the n value for F1 is 0.46 and for other formulations (F2–F5), it ranged between 0.33 and 0.27, indicating the anomalous and diffusion-controlled mechanism. However, at 25°C in pH7.4 release media, the n value varied between 0.44 and 0.57, suggesting the anomalous type of release.

CONCLUSIONS

This study reports the development of novel pH- and thermo-responsive blend hydrogel microspheres prepared by emulsion cross-linking method using the blends of sodium alginate and poly(*N*-isopropylacrylamide)-*g*-guar gum as the delivery matrix for the controlled release of isoniazid. Scanning electron microscopy indicated the formation of spherical microspheres; however, agglomerated microspheres with rough surfaces are observed due to the presence of PNIPAAm-*g*-GG in the blend. Encapsulation efficiency up to 62% was achieved by these matrices. FTIR confirmed the graft copolymerization as well as INZ interaction with the blend polymer system. DSC and XRD techniques confirmed the molecular-level uniform distribution of INZ within the blend hydrogel microspheres. Equilibrium swelling as well as *in vitro* release studies performed in pH1.2 and pH7.4 media at 25°C and 37°C confirmed the pH- and thermo-sensitive nature of the blend hydrogel microspheres. Drug release was extended up to 12 h. *In vitro* release kinetics was analyzed by Ritger–Peppas empirical equation to compute the diffusional exponent parameter (n), whose values suggest the diffusion and swelling controlled release mechanism.

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