# Temperature Sensitive Semi-IPN Microspheres from Sodium Alginate and *N*-Isopropylacrylamide for Controlled Release of 5-Fluorouracil

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**ABSTRACT:** Semi-interpenetrating network (IPN) of sodium alginate (NaAlg) and *N*-isopropylacrylamide (NIPAAm) microspheres were prepared by water-in-oil (w/o) emulsification method. The microspheres were encapsulated with 5-fluorouracil (5-FU) and release patterns carried in 7.4 pH at temperatures of 25 and 37°C. The semi-IPN microspheres were characterized by Fourier transform infrared spectroscopy (FTIR). Differential scanning calorimetry (DSC) and scanning electron microscopic studies were done on the drug-loaded microspheres to confirm the polymorphism of 5-FU and surface morphology of microspheres. These results indicated the molecular level dispersion of 5-FU in the semi-IPN microspheres. Particle size and size distribution were studied by laser light diffraction technique. Microspheres exhibited release of 5-FU up to 12 h. The swelling studies were carried in 1.2 and 7.4 pH buffer media at 25 and 37°C. Drug release from NaAlg-NIPAAm semi-IPN microspheres at 25 and 37°C confirmed the thermosensitive nature by *in vitro* dissolution. The micro domains have released in a controlled manner due the presence of NIPAAm in the matrix. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 2820–2829, 2008

**Key words:** sodium alginate; *N*-isopropylacrylamide; semi-IPN; 5-fluorouracil; thermoresponsive microspheres

## INTRODUCTION

Polymeric microspheres are important in controlled release (CR) applications, which have received a greater attention in recent years as the effective drug-delivery devices, in biomedical engineering.[1–3] Among the various polymers used, the natural polymers are widely used in drug release systems due to their biocompatibility, biodegradation, and nontoxicity upon *in vivo* administration.[4] The widely used natural polymers for the CR of drugs are chitosan, sodium alginate (NaAlg), cellulose derivatives, and guar gum.[3,5] Among these, NaAlg is an anionic linear polysaccharide with 1,4-linked D-mannuronic acid (M) and L-guluronic acid (G) residues either as blocks of the same units or as a random sequence of the two sugar residues.[6,7] The polymer forms a three-dimensional hydrogel network, due to the preferential acetal linkages by chemical reaction with glutaraldehyde in the presence of acid, resulting in the formation of homogeneous hydrogels.[3] The characteristic properties of these hydrogels, including favorable mechanical strength and porosity are dependent upon M:G ratio, type of crosslinker used (chemical or ionic), concentration, and viscosity of the initial alginate solution. NaAlg has carboxylic acid groups that exert interactions through hydrogen bonds, ion–ion, and dipole–ion with other polymers.[8]

Poly(*N*-isopropylacrylamide) (PNIPAAm) is one the most widely studied temperature-responsive polymers in CR application of short-lived drugs. The PNIPAAm in aqueous solution exhibits a thermoresponsive phase transition at 32°C.[9,10] Utilizing this property, PNIPAAm was widely used in solute separation,[11] concentrating dilute solutions, immobilizing enzymes[12], and in regulating the drug release.[9] Below this temperature, the polymer is hydrophilic and

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soluble, but above LCST it is hydrophobic and gets collapsed. Hydrogels composed of PNIPAAm and its copolymers exhibit discrete and reversible volume change in response to infinitesimal changes in temperature. Thus, it acts as a better candidate material for use in drug delivery due to its effective CR characteristics for many drugs under the influence of temperature. It has been reported that incorporation of hydrophilic/hydrophobic comonomers into PNIPAAm hydrogels will change its swelling characteristics as well as other physicochemical properties, including mechanical strength and solute diffusivity.[13–19] The phase transition temperature of PNIPAAm matrix occurs at or near the physiological temperature, i.e., 37°C.[20] PNIPAAm also exhibits extensive swelling in an aqueous media in response to small changes in temperature. However, PNIPAAm chains are able to hydrate giving extended structures in water when the temperature is below LCST, but will form compact structures due to dehydration when heated at temperatures above LCST. Since the critical temperature of collapse for PNIPAAm is 32°C, the polymer can be used as a drug delivery system PNIPAAm has the sharpest swelling transition among the class of thermosensitive alkylacrylamide polymers, which is attributed to its delicate hydrophobic–hydrophilic balance,[21] which changes abruptly across a small temperature gradient due to its cooperative stabilization.

5-Fluorouracil (5-FU) is an antimetabolic drug, used extensively in cancer chemotherapy[22] and is an effective drug in inhibiting the progression of disease such as proliferative vitretinopathy. 5-FU is an antineoplastic agent of extensive use in clinical chemotherapy for the treatment of solid tumors. Among the antineoplastics drugs, 5-FU is one of the most widely used drugs in the treatment of breast cancer,[23,24] gastric cancer[25], and pancreatic cancer.[26] As a consequence of its extensive use in the treatment of a great variety of tumors, 5-FU has been chosen in different CR formulations. The present research is an effort to develop semi-interpenetrating network (IPN) of NaAlg-NIPAAm microspheres with improved temperature sensitivity over small temperature cycles between 25 and 37°C. Microspheres were prepared by water-in-oil emulsification method using tween-80 as a surfactant by varying the amount of glutaraldehyde (GA), 5-FU, and NIPAAm. The prepared formulations were examined for the CR of 5-FU. To optimize the performance of stimuli-sensitive systems, a thorough understanding of the phase transitions is essential. Therefore, in this study, parameters like % encapsulation efficiency, drug loading, effect of crosslinking agent, etc., have been studied *in vitro* in terms of drug release characteristics of the microspheres developed.

## EXPERIMENTAL

### Materials and methods

Sodium alginate (low viscosity), potassium persulfate, light paraffin oil, glutaraldehyde (25% aqueous solution) were purchased from s.d. Fine Chemicals, Mumbai, India. *N*-isopropyl acrylamide was purchased from Aldrich, Milawaukee, WI. Tween-80 was purchased from Sigma Chemical, USA. 5-FU was purchased from MP Biochemicals, Eschwege, Germany.

### Synthesis of NaAlg -NIPAAm semi-IPN microspheres

NaAlg and NIPAAm microspheres were synthesized in two steps. First, *in situ* polymerization of NIPAAm using potassium persulfate ($K_2S_2O_8$) as an initiator and crosslinking of the mixture with glutaraldehyde in w/o emulsion media as described before.[27] In brief, 2% aqueous solution of NaAlg was prepared by dissolving NaAlg in water overnight under constant stirring. Passing nitrogen gas for 30 min degassed the solution. To this solution, different amounts of NIPAAm were added and stirred thoroughly for 1 h. The initiator solution containing 50 mg of $K_2S_2O_8$ was added to the above mixture and stirred for 1 h at 40°C under a vacuum pressure of 10 Torr.

The final polymerization mixture was emulsified into liquid paraffin to form water-in-oil (w/o) emulsion at 400 rpm speed using Eurostar (IKA Labortechnik, Germany) high-speed stirrer for 30 min in a separate 500-mL beaker containing 100-mL of light liquid paraffin oil, 2% (w/v) of Tween-80, 1 mL of 0.1*M* HCl, and a required amount of GA. The microspheres formed were filtered, washed repeatedly with hexane and water mixture to remove the oil as well as excess amount of surfactant and the unreacted GA. The microspheres were dried under vacuum at 40°C and stored in a desiccator before further analysis. Different formulations and their composition are given Table I.

### Fourier transform infrared spectroscopy (FTIR)

FTIR spectral measurements were performed using Nicolet spectrophotometer (Model Impact 410, USA) to confirm the crosslinking of the NaAlg-NIPAAm matrix. The IPN particles were finely ground with KBr to prepare the pellets under a hydraulic pressure of 392.2 dynes/$m^2$ and spectra were scanned between 400 and 4000 $cm^{-1}$.

### Differential scanning calorimetry (DSC) studies

DSC curves of the placebo NaAlg-NIPAAm microspheres, plain drug, and drug-loaded microspheres

**TABLE I**
**Results of % Encapsulation Efficiency and Mean Particle Size of Different Formulations**

| Formulation code | Amount of NIPAAm (wt %) | Amount of 5-FU (wt %) | Crosslinking agent (GA in mL) | % Encapsulation efficiency | Mean particle size (μm) ± SD |
|---|---|---|---|---|---|
| 1 | 10 | 5 | 2.5 | 72.4 ± 1.5 | 318 ± 2.1 |
| 2 | 10 | 5 | 5 | 64.1 ± 1.6 | 295 ± 5 |
| 3 | 10 | 5 | 7.5 | 58.0 ± 2.1 | 281 ± 5 |
| 4 | 10 | 10 | 5 | 74.7 ± 1.8 | 305 ± 5 |
| 5 | 10 | 15 | 5 | 77.1 ± 0.4 | 328 ± 5 |
| 6 | 20 | 5 | 5 | 79.7 ± 1.9 | 313 ± 7 |
| 7 | 30 | 5 | 5 | 84.2 ± 1.8 | 363 ± 6 |
| 8 | – | 5 | 5 | 53.5 ± 2.4 | 174 ± 4 |

were recorded using Rheometric Scientific differential scanning calorimeter (Model-DSC SP, UK). The analysis was performed by heating the samples at the rate of 10°C/min under inert atmosphere.

### Particle size analysis

Particle size of the microspheres was measured by particle size analyzer (Mastersizer 2000, Malvern Instruments, UK). About 500 mg of the microspheres were transferred to a dry sample holder and stirred vigorously to avoid the agglomeration of particles during measurements. For the measurement of sizes of different formulations/batches, the sample holder was cleaned by vacuum. The particle size was also measured using an optical microscopy.

### Scanning electron microscopic (SEM) studies

The SEM images of semi IPN microspheres were recorded using a JSM 6400 scanning electron microscope (SEM) (Japan) at the required magnification. Working distance of 39 mm was maintained and the acceleration voltage used was 50 kV with the secondary electron image as a detector.

### Estimation of drug loading and encapsulation efficiency

Specific amount of dry microspheres were vigorously stirred in a beaker containing 10 mL 7.4 pH phosphate buffer solution to extract the drug from the microspheres of semi-IPN. The solution was then filtered and assayed by a UV spectrophotometer (model Anthelie, Secomam, Dumont, France) at the fixed $\lambda_{max}$ value of 270 nm. The results of % 5FU loading and encapsulation efficiency were calculated using eqs. (1) and (2). These results are compiled in Tables I and II, respectively.

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

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

### Swelling studies

Equilibrium swelling of the NaAlg-based semi IPNs prepared using three different crosslink densities as well as three different amounts of NIPAAm was studied in 1.2 and 7.4 pH buffer by mass uptake measurements with time. To perform equilibrium swelling experiments, microspheres were soaked in water, several of them were removed from the swelling bottles at different time intervals and blotted carefully (without pressing hard) to remove the surface-adhered water. The microspheres were then weighed ($w_1$) on an electronic microbalance (Mettler, AT 120, Switzerland) accurate to ±0.00,001 g. The microspheres were then dried to a constant weight ($w_2$) in an oven maintained at 60°C for 5 h. The swelling experiments were repeated thrice for each sample and the average values were used in data analysis. The standard deviations (S.D.) in all cases were <5%. The weight % water uptake was calculated as:

$$\%\,\text{Water uptake} = \left(\frac{\text{Weight of swollen beads } (w_1) - \text{Weight of dry beads } (w_2)}{\text{Weight of dry beads}(w_2)}\right) \times 100 \quad (3)$$

Dynamic swelling and deswelling of the microspheres was measured by using a light microscope. The changes in diameter were monitored precisely with an ocular microscope under the plane-polarized light at room temperature in gastric and intestinal pH conditions. Liquid droplets were removed by using blotting papers and again fresh media was added.

### *In vitro* release

*In vitro* release studies have been carried out by the dissolution experiments by using Tablet dissolution tester (LabIndia, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 25 and 37°C under 100 rpm speed. Drug release from the microspheres was studied in intestinal (7.4 pH phosphate buffer) fluids. At regular intervals of time, aliquot samples were withdrawn and analyzed using the UV spectrophotometer (Model Anthelie, Secomam, Dumont, France) at the fixed $\lambda_{max}$ value of 270 nm.

## RESULTS AND DISCUSSION

### FTIR

Figure 1 displays the FTIR spectra of plain NaAlg (curve a), uncrosslinked NaAlg-NIPAAm semi-IPN (curve b), and crosslinked NaAlg-NIPAAm semi-IPN microspheres (curve c). The spectra of plain NaAlg shows a characteristic broad peak appearing at 3456 $cm^{-1}$, which corresponds to O—H stretching vibrations of NaAlg. The absorption bands at 1620 and 1417 $cm^{-1}$ are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups, respectively. The bands appearing at 1320 (C—O), 1130 (C—C), 1090 (C—O), and 1020 (C—O—O), 952 (C—O) $cm^{-1}$ are attributed to saccharide structure.[28] Uncrosslinked semi-IPN shows amide I band at 1634 $cm^{-1}$ consisting of C=O stretch of NIPAAm and amide II band at 1542 $cm^{-1}$ representing the N—H bending vibrations. During the crosslinking of IPN membrane, GA has reacted with —OH groups of NaAlg through the formation of ether linkages. Hence, the appearance of a peak at 1248 $cm^{-1}$ in the FTIR spectra of the crosslinked membrane confirms the formation of ether linkage. The intensity of a band due to polymeric O—H stretching vibrations in the crosslinked semi-IPN membranes has decreased due to the utilization of some of OH groups during the crosslinking reaction. This was also supported by the presence of a sharp high intensity peak at 1008 $cm^{-1}$ due to $—CH_2$ group of alkyl chain as a result of crosslinking. The acetal ring formation is a further test of crosslinking of hydroxyl groups of the polymer with aldehydic groups of GA, which is shown by the peak at 1248 $cm^{-1}$.

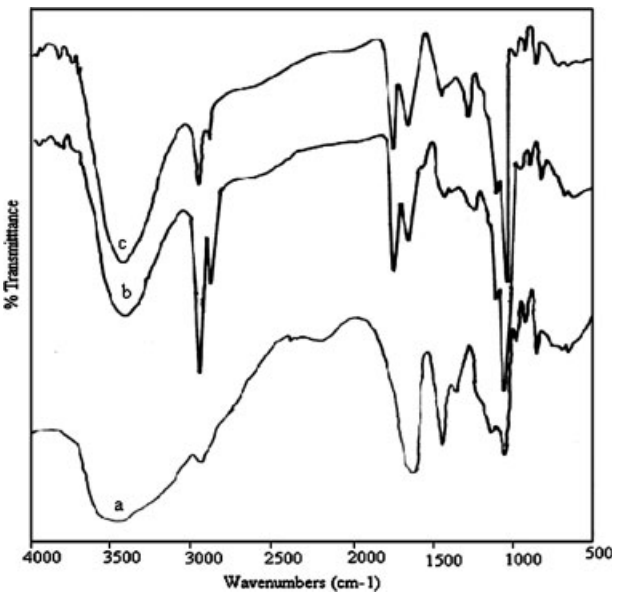


**Figure 1** FTIR spectra of (a) Plain NaAlg microspheres, (b) uncrosslinked semi-IPN of NaAlg-NIPAAm microspheres, and (c) crosslinked semi-IPN of NaAlg-NIPAAm microspheres.

**TABLE II**
**Release Kinetics Parameters of Different Formulations [Eq. (4)]**

| Formulation code | $k$ | $n$ | Correlation coefficient, $r$ |
|---|---|---|---|
| 1 | 0.016 | 0.362 | 0.9675 |
| 2 | 0.026 | 0.354 | 0.9753 |
| 3 | 0.053 | 0.371 | 0.9767 |
| 4 | 0.027 | 0.342 | 0.9777 |
| 5 | 0.015 | 0.348 | 0.9605 |
| 6 | 0.052 | 0.359 | 0.9612 |
| 7 | 0.054 | 0.549 | 0.9767 |
| 8 | 0.014 | 0.379 | 0.9867 |

### DSC

DSC tracings of the drug-loaded NaAlg-NIPAAm microspheres, 5-FU, and plain NaAlg-NIPAAm microspheres are displayed in Figure 2. The onset-melting peak of 5-FU was observed at 285.16°C. However, no characteristic peak of 5-FU was observed in the DSC curves of the drug-loaded microspheres, suggesting that drug is molecularly dispersed in the polymer matrix.

### SEM studies

SEM image of the few MPs are shown in Figure 3. The microspheres (MPs) are spherical without forming agglomeration and their surfaces are smooth. However, the polymeric debris seen around some particles are probably due to the typical method of particle production used (i.e., simultaneous particle production and formation of semi IPNs). However, the MGs produced by NaAlg and NIPAAm did not show any effect on the surface properties.

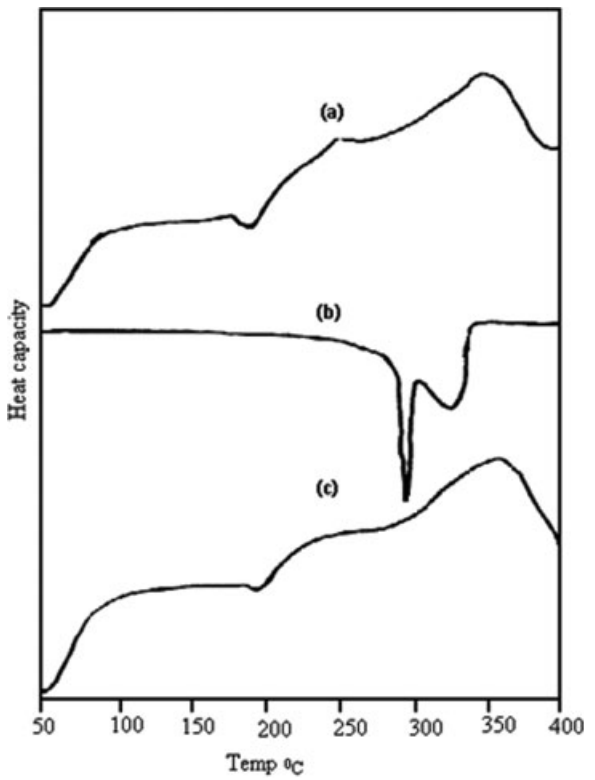


**Figure 2** DSC thermograms of (a) 5-FU loaded NaAlg-NIPAAm, (b) pure 5-FU, microspheres, and (c) plain NaAlg-NIPAAm microspheres.

## Particle size

Particle size and size distributions have been analyzed by laser light diffraction technique (Mastersizer-2000, Malvern, UK). Results of volume mean diameter of the microspheres produced by taking three different amounts of crosslinking agent are included in Table I. These results suggest that as the extent of crosslinking increases, the volume mean diameter decreased. On a population basis, particle size distribution is unimodal. Microspheres used in preparing the drug-loaded formulations were selected from a uniform size distribution range. A narrow size distribution of microspheres (see Fig. 4) was observed with particles ranging in size from 174 to 363 μm.

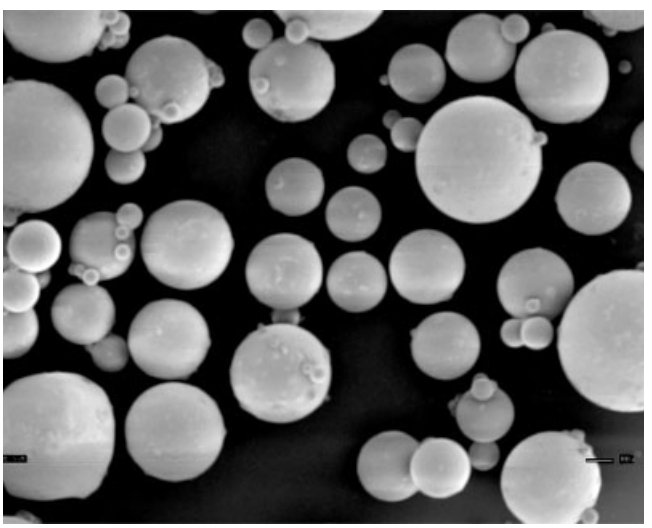

**Figure 3** SEM photograph of the microspheres.

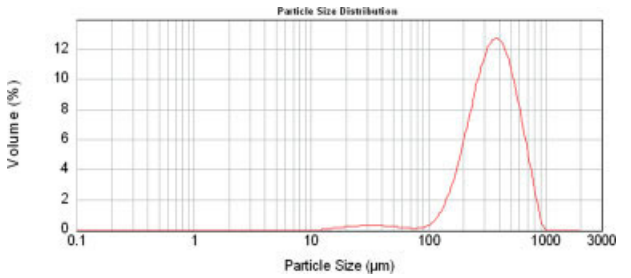


**Figure 4** Particle size distribution curve for NaAlg-NIPAAm microspheres. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

## Microscopic study

Particle size was also measured alternatively by optical microscopy. These results along with % encapsulation efficiency, % drug-loading, and mean particle size for different formulations is presented in Table I. The size of particles depends on the amount of drug present, % NIPAAm content, and extent of GA employed. Particles are generally spherical in shape with sizes ranging from 174 to 363 μm. Particle size of the plain NaAlg is smaller than those of NaAlg-NIPAAm microspheres. By increasing the NIPAAm content of the microspheres, size of the microspheres also increased from 295 to 363 μm for 5 wt % 5-FU-loaded microspheres. This can be explained on the basis of hydrodynamic viscosity concept, i.e., as the amount of NIPAAm in the microspheres increases, the interfacial viscosity of the polymer droplets in the emulsion also increases, because NIPAAm has more water-uptake capacity than NaAlg, which will hinder the breaking of the dispersed phase into smaller size particles during emulsification. As the NaAlg content increases, the size of microspheres decreased because of the availability of more free sites for crosslinking. On the other hand, with increasing amount of NIPAAm, the number of free sites available for crosslinking is less so that size of the microspheres will also increase with increasing NIPAAm content of the microspheres; for instance, as the amount of NIPAAm increases from 10 to 30%, the particle size has increased from 295 to 363 μm.

For all the formulations, with increasing amount of drug in the microspheres, particle size also

increased. For formulations containing 10 wt % NIPAAm and microspheres loaded with different amounts of drug, particle size has increased from 295 to 328 μm; a similar trend was also observed for all other formulations (see Table I). This is attributed to the fact that drug molecules might have occupied the free volume spaces within the IPN matrix, thereby hindering the inward shrinkage of the polymer matrix.[29] The 5 wt % 5-FU-loaded and 30 wt % NIPAAm-containing microspheres exhibited the maximum particle size of 363 μm. However, the extent of crosslinking has shown an effect on the particle size (see data in Table I). For microspheres containing 10 wt % NIPAAm and 5 wt % 5-FU with an increasing amount of GA from 2.5 to 7.5 mL, the particle size decreased from 318 to 281 μm. This is attributed to the fact that with increasing amount of GA in the semi-IPN matrix, shrinkage of the particles occurred, thereby reducing their size.[29,30]

### Encapsulation efficiency

Three different concentrations of 5-FU i.e., 5, 10, and 15 wt % were loaded during crosslinking of the microspheres. Results of % encapsulation efficiency included in Table I show the increasing trends with increasing drug loading. Encapsulation efficiency of 53% was observed for plain NaAlg microspheres, but for the remaining formulations, it ranged from 58 to 84%. Such smaller values are due to the lesser soluble drug in the polymer solution, thus making a lesser amount of 5-FU to be incorporated into the microspheres. Notice that the % encapsulation efficiency increased with increasing amount of NIPAAm of the semi-IPN matrix. For microspheres containing 10, 20, and 30 wt % NIPAAm and 5 wt % 5-FU, encapsulation efficiencies were 64.1, 79.7, and 84.2%, respectively. For 10 wt % NIPAAm in the semi-IPN matrix, the results of extent of crosslinking on the size and encapsulation efficiency increased the crosslinking, but % encapsulation efficiency decreased(see Table I). For microspheres crosslinked with 2.5, 5, and 7.5 mL of GA, encapsulation efficiencies are 72.4, 64.1, and 58%, respectively. Such a decreasing trend is due to the increase in crosslink density, because the microspheres become rigid, thereby reducing the free volume spaces within the semi-IPN matrix, and hence, a reduction in encapsulation efficiency.

### Drug release kinetics

Drug release kinetics was analyzed by plotting the cumulative release data versus time by fitting to the empirical equation:[31]

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \tag{4}$$

Here, $M_t/M_\infty$ represents the fractional drug release at time $t$, $k$ is a constant characteristic of the drug-polymer system, and $n$ is an empirical parameter characterizing the release mechanism. Using the least squares procedure, we have estimated the values of $n$ and $k$ for all the nine formulations developed; these values are given in Table II. If $n = 0.5$, drug diffuses and releases out of the polymer matrix following a Fickian diffusion or Case I. For $n > 0.5$, anomalous or non-Fickian transport occurs. If $n = 1$, non-Fickian or Case II release kinetics is prevalent. The intermediary values of $n$ ranging between 0.5 and 1 indicate the anomalous transport.[31]

In the present research, the values of $k$ and $n$ show a dependence on the extent of crosslinking, % drug loading, and NIPAAm content of the semi-IPN matrix. Values of $n$ for microspheres prepared by using the varying amounts of NIPAAm (10, 20, and 30 wt %) keeping 5-FU (5 wt %) and GA (5 mL) constant have ranged from 0.354 to 0.549, producing a slight deviation from the Fickian mode of transport. The 5-FU loaded microspheres have the $n$ values ranging from 0.342 to 0.549 giving a shift from erosion type release to swelling-sustained non-Fickian transport. Correlation coefficients, $r$, obtained while fitting the release data fall in the range 0.967–0.986, but non-Fickian trends are due to a reduction in the regions of low microviscosity and closure of microcavities in the swollen state of the semi-IPN matrix. Similar findings have been observed elsewhere[32] wherein, the effect of different polymer ratios on the dissolution kinetics was investigated. The $n$ values for formulations containing different amounts of NaAlg, 5-FU, and GA are $<0.5$, which indicates the non-Fickian diffusion transport, i.e., slight deviation from the Fickian trend.

### Effect of NIPAAm content

The effect of NIPAAm content was studied at a constant loading of 5 wt % 5-FU. It was found that NaAlg produced almost 100% cumulative drug release in about 10 h, whereas NaAlg-NIPAAm microspheres produced up to 90% cumulative release in 12 h. Release of NaAlg-NIPAAm microspheres prepared with different amounts of NIPAAm are displayed in Figure 5(A). This could be due to the fact that during dissolution microspheres have systematically swollen with an increasing amount of NIPAAm due to the formation of loosely crosslinked network chains of NIPAAm. Thus, a relaxation-type response of the polymeric chains might be possible due to stresses induced by the surrounding solvent medium during the dissolution, resulting in an increase of chain dimension (radius of gyration) of the polymer; this will increase the molecular volume of hydrated polymer due to increased swelling of NIPAAm com-

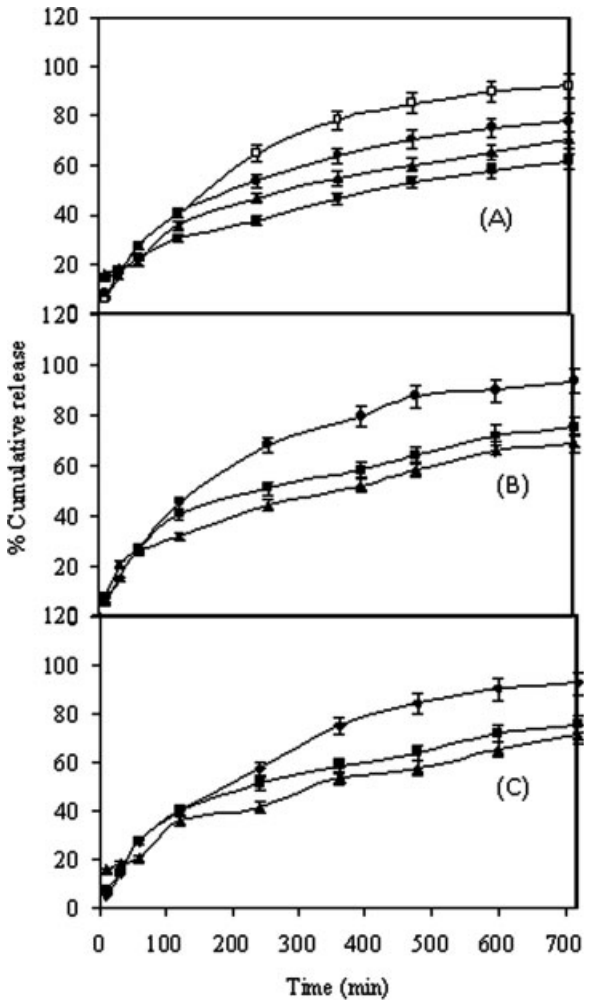


**Figure 5** % Cumulative release of 5-FU through NaAlg-NIPAAm microspheres containing different amounts of NIPAAm: (A): (□) pure NaAlg, (■) 10%, (▲) 20%, and (●) 30%, different amounts of 5-FU (B): (●) 15%, (■) 10%, and (▲) 5% and different amounts of GA (C): (●) 2.5 mL, (■) 5 mL, and (▲) 7.5 mL.

ponent of the semi-IPN, thereby reducing the void volume of the matrix. Notice that the nature of release profiles remains almost identical in all the formulations containing different amounts of GA, indicating that swelling of NIPAAm has established a linear relationship with the release profiles.

### Effect of drug loading

Figure 5(B) shows the release profiles of 5-FU loaded microspheres of NaAlg-NIPAAm at different loadings of 5-FU. The formulations exhibited higher encapsulation efficiency in the range 72–77 due to higher solubility of 5-FU in the polymer solution, since it is soluble in water. Release data showed that formulations containing highest amount of 5-FU (15 wt %) displayed the highest (99%) release than those containing a small amount of 5-FU. On the other hand, those formulations containing lower amount of 5-FU have released only 90% of 5-FU. Thus, CR was observed for formulation containing lower amount of 5-FU, since its release from the microspheres was sustained by the diffusion mechanism.[33] With an increasing concentration of 5-FU in the microspheres, volume swelling of the semi-IPN matrix decreased due to the hydration effect, but increased hydrophobicity lowered the drug diffusion rate. Thus, release rates are slower at a lower amount of 5-FU due to the availability of more free void spaces through which a lesser number of drug molecules will transport. For all the 5-FU loaded formulations, the complete release of 5-FU was not found even after 600 min, but a complete release occurred at 720 min.

### Effect of crosslinking agent

The % cumulative release data versus time plots for varying amounts of GA (i.e., 2.5, 5.0, and 7.5 mL) at the fixed amount of 5-FU (5 wt %) are displayed in Figure 5(C). The % cumulative release is quite higher when small of GA (i.e., 2.5 mL) was used, whereas the release was lower at higher amount of GA (i.e., 7.5 mL) in the semi-IPN matrix. Therefore, the cumulative release is smaller at a lower amount of GA, because at higher concentration of GA, polymeric chains will be more rigid due to the contraction of microvoids as reported before.[27] This will decrease the swelling as well as % cumulative release of 5-FU through the microspheres. As expected, the drug release becomes slower at higher amount of GA, but it will be faster at lower amount of GA.

### Effect of temperature

Release profiles of 5-FU from semi-IPN NaAlg-NIPAAm microspheres prepared with different amounts of NIPAAm have been studied at two temperatures in the chosen dissolution medium, alternatively from 25 to 37 °C and *vice versa*. Drug release profiles exhibited drastic changes by variations in temperature from 25 to 37°C. It may be noted that drug was released slowly at 37°C, i.e., above the LCST, but the release was much faster at 25°C, i.e., below the LCST than at 37°C. This is due to the fact that at higher temperature, the surface of microspheres will shrink, causing the drug to migrate toward the surface of microspheres as seen by the initial burst effect during the dissolution experiments (Fig. 6). However, dense surfaces of the microspheres will prohibit the release of more amount of 5-FU. At lower temperatures, the already shrunken surface layer starts to reswell, which will allow the drug to be released after certain of time, depending

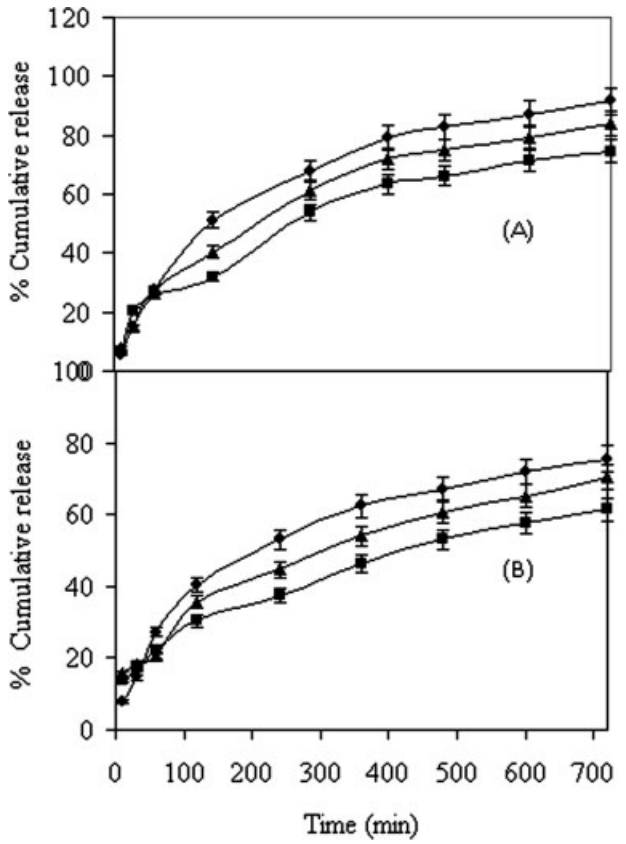


**Figure 6** % Cumulative release of 5-FU at 25°C (A) and 37°C (B) through NaAlg-NIPAAm microspheres containing different amounts of NIPAAm (A): (■) 10%, (▲) 20%, and (●) 30%.

upon the minimum time required for reswelling of the surface. Thus, the time required for drug release was accelerated as a result of cooling below LCST, which has further slowed down upon reheating. Microspheres are thus found to be sensitive to changes in temperature. At 25°C (in the swollen state), both release rate and total amount of drug release were considerably higher than those found at 37°C (in a collapsed state). Drug molecules entrapped inside the polymer network will diffuse out of the microspheres, since they quickly get hydrated in the swollen state. In contrast, at 37°C, the network structure is collapsed and exhibits a lesser tendency to uptake water or buffer solution, thereby leading to a decrease in drug diffusion rate.

## Swelling studies

To understand the drug release characteristics, it is important to investigate the swelling of microspheres. The nature and extent of interaction between the solvent molecules and the polymer chains are influenced by the polymer structure since the porosity of the microspheres and the nature of hydrophilic groups of the polymer play a major role.

**TABLE III**
**Equilibrium Swelling of Semi IPN Microspheres**

| | pH at 37°C | | Temperature (°C) at pH7.4 | |
|---|---|---|---|---|
| Formulation code | 1.2 | 7.4 | 25 | 37 |
| 1 | 83 | 147 | 174 | 147 |
| 2 | 71 | 137 | 150 | 137 |
| 3 | 60 | 123 | 132 | 123 |
| 6 | 59 | 129 | 152 | 129 |
| 7 | 48 | 122 | 146 | 122 |
| 8 | 119 | 231 | 227 | 231 |

The results of % equilibrium swelling of GA-crosslinked semi IPNs, measured in 1.2 and 7.4 pH media at 37°C and also in pH 7.4 at 25°C, for different NaAlg-based microspheres are reported in Table III. For the plain NaAlg microspheres, in 7.4 pH phosphate buffer, the equilibrium swelling of microspheres was higher than found in 1.2 pH media. The swelling increased from 122 to 231% in 7.4 pH media, while in 1.2 pH media, it increased from 48 to 119%. This behavior may be due to p*K*a of alginate, which was about 3.2 and 4 for guluronic and

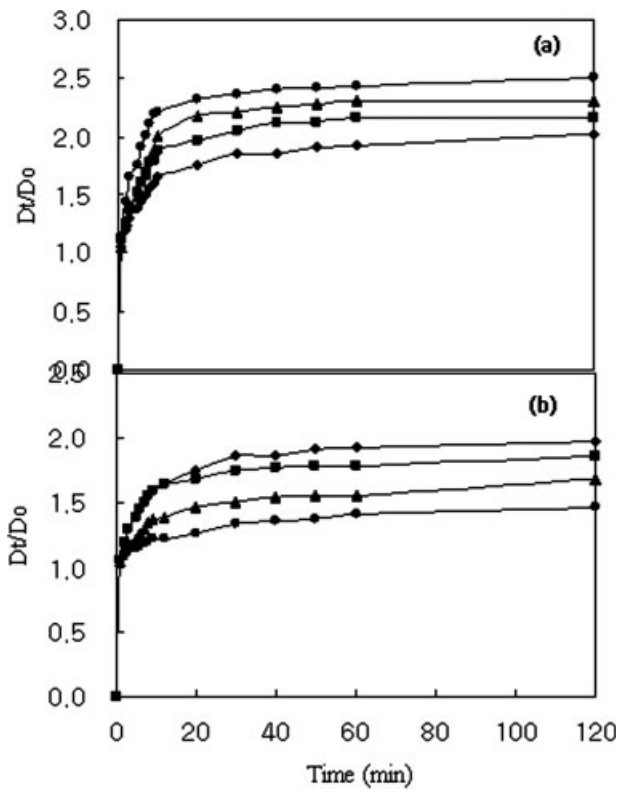


**Figure 7** Swelling studies of microspheres at 1.2 (A) and 7.4 (B) pH media at room temperature. Microspheres containing different amounts of NIPAAm (A): (▲) 10%, (■) 20%, (◆) 30%, and (●) pure NaAlg.

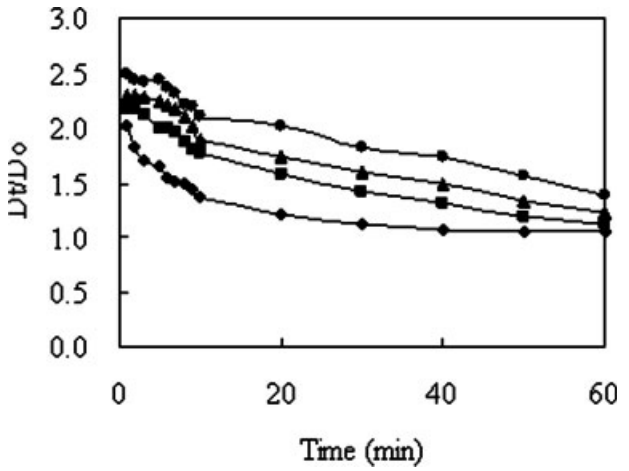


**Figure 8** Deswelling studies microspheres at room temperature. Symbols are same as mentioned in Figure 7.

mannuronic acids, respectively.[34] At a low pH region, most carboxylic acid groups in alginate were in the form of COOH. As the pH of the solution increased, COOH groups became ionized, and the resulting electrostatic repulsion caused the hydrogels to swell. The swelling of alginate microspheres showed drastic change with the pH, whereas that of the semi IPNs was sustained or a little decreased.

For the plain NaAlg microspheres, in pH 7.4 phosphate buffer at 25°C, the equilibrium swelling of microspheres was higher than found at 37°C media. In all cases, swelling decreased with increasing temperature gradually from 25 to 37°C. But in case of semi IPN microspheres, an increase in temperature would increase hydrophobicity of IPN microspheres due to dehydration of PNIPAAm moiety. The swelling decreased from 231% to122% at 37°C, when it was 25°C, it decreased from 227% to 146%. However, with increasing temperature, % decreased considerably, suggesting the matrix shrinkage due to the thermo responsive nature of PNIPAAm.[35]

Dynamic swelling data were obtained by monitoring the changes in diameter, $D_t$,of the spherical microspheres with a lapse of time by using an optical microscope. Figure 7 display the normalized diameter, $D_t/D_0$ as a function of time for the placebo microspheres of the (i.e., without 5-FU) different wt % NIPAAm in the matrix (i.e., formulations: 1, 2, and 3). The alginate hydrogel reached an equilibrium swelling state within 2 h, whereas the semi IPNs showed slow swelling kinetics. In the semi-IPN hydrogels, the swelling ratio decreased with the NIPAAm content in the microspheres. Figure 8 shows the deswelling kinetics of hydrogels at room temperature. The PNIPAAm gel was quickly shrunk during the deswelling process, whereas the swelling ratio of alginate did not change according to the temperature. In all the sample compositions, the deswelling rate of semi-IPN hydrogels was slower than that of prestaine NaAlg.

## CONCLUSIONS

Novel type of thermoresponsive 5-FU loaded NaAlg-NIPPAm semi-IPN microspheres were prepared by water-in-oil method using tween-80 as a surfactant. The microspheres prepared were characterized by DSC, which indicated a molecular distribution of 5-FU in the polymer matrix. The microspheres have exhibited a prolonged release of 5-FU over an extended period of time. The prepared microspheres have shown the thermoresponsive trends during *in vitro* drug release of 5-FU when dissolution experiments were performed at 25 and 37°C. The thermoresponsive nature of the semi IPN microspheres was supported by the swelling studies.

## References

1. Morkerver, R.; Meeussen, F.; Koningsveld, R.; Berghmans, H. Macromolecules 1998, 31, 2223.
2. Ramkission, C. G.; Lin, F.; Baudys, M.; Kin, S. W. J Control Rel 1999, 59, 287.
3. Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi T. M. J Control Rel 2004, 100, 5.
4. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. Euro J Pharm Biopharm 2000, 50, 27.
5. Mallikarjuna Reddy, K.; Ramesh Babu, V.; Sairam, M.; Subha, M. C. S.; Mallikarjuna, N. N.; Kulkarni, P. V.; Aminabhavi T. M. Design Monom Polym 2006, 9, 491.
6. Smidsrod, O. Faraday Discuss Chem Soc 1974, 57, 263.
7. Park, K.; Sharby W. S. W.; Park, H. In Biodegradable Hydrogels for Drug Delivery; Technomic Publishing: Lancaster, PA, 1993; pp 99–140.
8. Anlar, S.; Capan, Y.; Guven, O.; Gogus, A.; Dalkara, T.; Hincal, A. Pharm Res 1994, 11, 231.
9. Braze1, C. S.; Peppas, N. A. Macromolecules 1995, 28, 8016.
10. Maeda H.; Seymour, I. W.; Miyamoto, Y.; Bioconjugate Chem 1992, 3351.
11. Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. J Membr Sci 1991, 64, 283.
12. Gehrke, S. H.; Andrews, G. P.; Cussler, E. L. Chem Eng Sci 1986, 41, 2153.
13. Dong, L. C.; Hoffman, A. S. J Control Rel 1986, 4, 223.
14. Hoffman, A. S. J Control Rel 1987, 6, 297.
15. Ebara, M.; Aoyagi, T.; Sakai, K.; Okano, T. J Polym Sci Part A: Polym Chem 2001, 39, 335.
16. Muniz, E. C.; Geuskens, G. Macromolecules 2001, 34, 4480.
17. Hinrichs, W. L. J; Schuurmans-Nieuwenbroek, N. M. E.; Wetering, P.; Hennink, W. E. J Control Rel 1999, 60, 249.
18. Zhang, X. Z.; Zhou, R. X.; Cui, J. Z.; Zhang, J. T. Int J Pharm 2002, 235, 43.
19. Erbil, C.; Akpynar, F. D.; Uyanyk, N. Macromol Chem Phys 1999, 200, 2448.
20. Zhang, X. Z.; Yang, Y. Y.; Wang, F. J.; Chung, T. S. Langmuir 2002, 18, 2013.
21. Prange, M. M.; Hooper, H. H.; Prausnitz, J. M. AIChE J 1989, 35, 803.
22. Einmahl, S.; Zignani, M.; Varesio, E.; Heller, J.; Veuthey, J. L.; Tabatabay, C.; Gurny, R. Int J Pharm 1999, 185, 189.

23. Longley, D. B.; Harkin, D. P.; Johnston, P. G. Nature Rev Cancer 2003, 3, 330.
24. Earl, H.; Iddawela, M. Expet Rev Anticancer Ther 2004, 4, 189.
25. Dickson, J. L. B.; Cunningham, D. Eur J Gastroenterol Hepatol 2004, 16, 255.
26. Pasetto, L. M.; Jirillo, A.; Stefani, M.; Monfardini, S. Crit Rev Oncol Hematol 2004, 49, 135.
27. Babu, V. R.; Krishna Rao, K. S. V.; Sairam, M.; Naidu, B. V. K.; Hosamani, K. M.; Aminabhavi, T. M. J Appl Polym Sci 2006, 99, 2671.
28. Sartori, C.; Finch, D.; Ralph, B.; Gilding, K. Polymer 1997, 38, 43.
29. Soppimath, K. S.; Kulkarni, A. R.; Aminabhavi, T. M. Eur J Pharm Biopharm 2002, 53, 87.
30. Korsmeyer, R. C.; Peppas, N. A. J Membr Sci 1981, 9, 211.
31. Ritger, P. L.; Peppas, N. A. J Control Rel 1987, 5, 37.
32. Aminabhavi, T. M.; Naik, H. G. J Hazard Mater 1998, 60, 175.
33. Boundy, V.; Voute, N.; Pradeau, D.; Chaumeil, J. C. Int J Pharm 2002, 239, 13.
34. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. J Appl Polym Sci 2002, 83, 1128.
35. Coughlan, D. C.; Quilty, F. P.; Corrigan O. I. J Control Rel 2004, 98, 97.