Synthesis and Characterization of Poly(*N*-Vinyl-2-Pyrrolidone) Grafted Sodium Alginate Hydrogel Beads for the Controlled Release of Indomethacin

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ABSTRACT: Graft copolymers of sodium alginate (NaAlg) with *N*-vinyl-2-pyrrolidone were prepared using azobisisobutyronitrile as initiator. The graft copolymers (NaAlg-*g*-PVP) were characterized with Fourier transform infrared spectroscopy, elemental analysis, and differential scanning calorimetry. Polymeric hydrogel beads of NaAlg and NaAlg-*g*-PVP were prepared by crosslinking method using glutaraldehyde (GA) as a crosslinker in the hydrochloric acid catalyst (HCl) and these beads were used to deliver anti-inflammatory drug, indomethacin (IM). Chemical stability of the IM after encapsulation into beads was confirmed by FTIR. Preparation conditions of the NaAlg-*g*-PVP beads were optimized by considering the percentage entrapment efficiency, particle size, swelling capacity and their release data. *In vitro* release studies were performed in simulated gastric fluid (pH 1.2) for the initial 2 h, followed by simulated intestinal fluid (pH 7.4) for 4 h. Effects of GA concentration, exposure time to GA, drug/polymer (d/p) ratio, and concentration of HCl on the release of IM were discussed. It was observed that IM release from the beads decreased with increasing GA concentration and exposure time. IM release also decreases with increasing d/p ratio and HCl concentration. The highest IM release was obtained to be 77% for beads crosslinked with 0.027*M* GA. Swelling experiments were also performed to compute molecular mass between crosslinks of the beads. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 481–493, 2008

Key words: graft copolymer; controlled release; drug delivery systems; indomethacin; hydrophilic polymers

INTRODUCTION

Conventional oral drug administration does not usually provide rate controlled release or target specificity. In many cases, conventional drug delivery provides sharp increases of drug concentration at potentially toxic levels. Following a relatively short period at the therapeutic level, drug concentration eventually drops off until re-administration.[1] Today new methods of drug delivery are possible: desired drug release can be provided by rate controlling membranes or by implanted biodegradable polymers containing dispersed medication.

Polymers have played a major role in the development of controlled release systems. Especially, much research on the application of biocompatible and biodegradable polymers has been carried out.[2] Natural polymers such as chitosan, guar gum, sodium alginate, cellulose etc. are often preferred over synthetic polymers due to their nontoxic, low cost, ease of availability, and biodegradability characteristics.[3,4] However, to control the release patterns, many attempts have been made to use the synthetically modified natural polymers. Among many methods of modifying structure of polymers, graft copolymerization is an easier method, which makes the derived polymer as attractive biomaterials in controlled release application. For example, polyacrylamide-grafted chitosan hydrogel microspheres,[5] acrylamide-grafted-xanthan gum matrix tablets,[6] acrylamide grafted dextran, and chitosan microspheres,[7] were prepared for the controlled release of the various drugs.

Beads or microspheres are one of the multi-particulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. They can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance.[8]

Alginate is a naturally occurring polysaccharide obtained mainly from brown algae belonging to the *Phaeophyceae* and composed of two monomeric units, β-D-mannuronic acid (M) and α-L-guluronic acid (G).[9] It has been used not only in controlled release applications of drugs[10–12] or pesticides[13–15] but also used in the biotechnology industry as a thickening agent, a gelling agent, and a colloidal stabilizer.[16] Alginate

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salts are known to form a reticulated structure when in contact with calcium ions or glutaraldehyde and this characteristic has been used to produce sustained release particulate systems for a variety of drugs, proteins and even cells.[9,17] The graft polymerization of NaAlg with various vinyl monomers is an effective method to improve the properties of the NaAlg.

N-vinyl-2-pyrrolidone (*N*-VP) is a hydrophilic and nonionic monomer, the polymerization of which is easily initiated through radicals, thermal or photo irradiation.[18] Poly(*N*-vinyl-2-pyrrolidone) (PVP) is a polymer with great potential applications in different biomedicines. The principal reason for successful PVP application is its excellent biocompatibility with living tissues and extremely low cytotoxicity.[19] *N*-VP has also a high gelling nature and possesses good complexing ability. In the crosslinked form, PVP forms three-dimensional high water absorbing hydrogels.[20] Biocompatible polymeric hydrogels has been effectively used for controlled release systems. Many worker have carried out grafting reactions of *N*-VP onto silica,[21] polypropylene film,[22] gelatin,[23] and low density polyethylene.[24] However, grafting of hydrophilic *N*-VP onto NaAlg and controlled release application of it has not been studied up to now.

The objective of this study is to evaluate the performance of newly developed NaAlg-*g*-PVP beads containing indomethacin (IM) to achieve a controlled drug release profile suitable for oral administration. IM, a nonsteroidal anti-inflammatory drug, has been successfully used in the treatment of soft tissue problems associated with trauma, osteoarthritis and rheumatoid arthritis.[25] However, drug therapy with this agent is known to cause several adverse effects and the frequency and the severity of the adverse effects are well correlated with the plasma concentration of the drug. Clinical study revealed that conventional dose-dumping indomethacin capsules induce several adverse effects like epigastic pain, peptic ulcer, vertigo, headache.[26] Because of short biological half-life and associated adverse effects, it is considered as an ideal candidate for controlled drug delivery. Controlled release of IM maintain adequate therapeutic plasma level of drug avoiding peaks and troughs[27] and thereby, minimize the emergence of adverse effects and increase patient compliance by reducing the frequency of administration.

In one of our previous studies,[28] acrylamide (AAm) was grafted onto poly(vinyl alcohol) (PVA) and PVA/NaAlg and PVA-*g*-AAm/NaAlg blend beads were prepared in various blend ratios with GA as a crosslinking agent and used for release of diclofenac sodium under *in vitro* conditions. In the present study, *N*-VP monomer was grafted onto NaAlg and then NaAlg-*g*-PVP beads were prepared in various preparation conditions using GA as a crosslinker in the HCl catalyst. Particle size, bead yield, entrapment efficiency, equilibrium swelling degree (ESD) of the beads, and IM release rate were investigated at 1.2 and 7.4 pH values. The effects of *N*-VP grafting, extent of crosslinking, and drug/polymer ratio on IM release from the beads were studied and discussed.

EXPERIMENTAL

Materials

Sodium alginate with a viscosity 3500 cps (2% solution, 25°C) and IM were purchased from Sigma Chemical Co (Louis, USA). *N*-VP was supplied from Fluka Chemie AG (Buchs, Switzerland) and purified by vacuum distillation at 2 mmHg and 65°C. Glutaraldehyde (25% v/v) solution, azobisisobutyronitrile (AIBN), Na_2HPO_4, NaH_2PO_4, hydroquinone, HCl, and ethanol were all supplied from Merck (Darmstadt, Germany) and were used as received.

Synthesis of the graft copolymer of NaAlg with *N*-VP

The grafting reactions were carried out under nitrogen atmosphere in 250 mL three-necked flask equipped with a reflux condenser, a stirrer and a gas inlet system, immersed in a constant temperature bath. In a typical reaction, NaAlg (1.0 g) was dissolved in distilled water (50 mL) at room temperature with constant stirring. The required amount of *N*-VP was mixed with NaAlg solution. The mixture was immediately placed into the water bath adjusted to the polymerization temperature (70°C) and stirred bubbling with a slow stream of nitrogen for 30 min. Then AIBN at required concentration in 2 mL acetone was added slowly to the reaction mixture and the total volume of the reaction mixture was made up to 100 mL with distilled water. A continuous supply of nitrogen was maintained throughout the reaction period. The grafting reactions were carried out for varying period of time intervals (1–4 h). At the end of the predetermined polymerization time, the reaction was terminated by adding 2 mL saturated solution of hydroquinone. The products were precipitated in an excess of acetone, separated by filtration and then extracted with ethyl alcohol to remove the homopolymer (PVP) for 48 h. After complete removal of PVP, the pure graft copolymer was dried at 40°C under vacuum to a constant weight. The grafting parameter including grafting yield (GY) was determined from the elemental analysis using with a LECO CHNS-932 C-, H-, N- analyzer (USA).

Viscometric measurements

Viscosities of the copolymers solutions were determined using an Ubbelhode viscometer in 0.2*M*

aqueous NaCl solution thermostated at 25°C. The intrinsic viscosity [η] of polymer solutions was calculated as:

$$[\eta] = \lim_{C \to 0} \left(\frac{\eta - \eta_0}{\eta_0 C} \right) \quad (1)$$

where η_0, η, and C are the solvent viscosity, solution viscosity, and concentration (g/dL) of the polymer, respectively. Intrinsic viscosity was determined by extrapolating the linear portion of the reduced viscosity versus concentration plot to the zero concentration. From the values of [η], the viscosity average molar mass, $\overline{M}_\eta$ was calculated using the Mark Houwink-Sakurada (MHS) relation:

$$[\eta] = k(\overline{M}_\eta)^\alpha \quad (2)$$

The values of the MHS parameters, k and α were taken as 7.97×10^{-3} mL/g and 1.0 respectively, from the literature.[29]

Preparation of the NaAlg and NaAlg-*g*-PVP beads

NaAlg or NaAlg-*g*-PVP containing IM in various drug/polymer ratios were prepared and stirred to form homogenous solution for 12 h. The polymer solution containing IM was then added drop wise into water containing glutaraldehyde and HCl using peristaltic pump (Masterflex, L/S Digital Economy Drive, USA). The formed beads were then removed from the crosslinking solution at selected time intervals of 5, 15, 30 min and were washed with water repeatedly to remove the adhered glutaraldehyde and acid; the beads were then dried completely in oven at 40°C. Unloaded beads were prepared in a similar way without IM to determine ESD. To determine the size of beads, ten samples of the completely dried beads from different formulations were selected and their sizes were measured using an electronic digital caliper (Mitutoyo IP.65, Japan). Standard deviations from the average values were calculated.

Equilibrium swelling study of the beads

The ESD of the crosslinked empty beads was determined by measuring gravimetrically the extent of their swelling in HCl solution at pH 1.2, in buffer solution at 7.4 and in distilled water at 27, 37, and 47°C. To ensure complete equilibration, the samples were allowed to swell for 24 h. The excess surface-adhered liquid drops were removed by blotting, and the swollen beads were weighed using electronic balance (Precisa XB 220A, USA). The beads were then dried in an oven at 40°C till to constant weight. The percent ESD was calculated as follows:

$$\text{Equilibrium Swelling Degree (\%)} = \frac{(M_s - M_d)}{M_d} \times 100 \quad (3)$$

where M_s and M_d are mass of swollen beads and mass of dry beads, respectively.

Determination of IM content of the beads

The known mass of beads were crushed in an agate mortar with a pestle and then polymeric powder was refluxed with pH = 7.4 phosphate buffer solution for 4 h to ensure the complete extraction of IM from the beads. After that, the absorbance of the buffer solution containing the extracted amount of IM was taken at a wavelength of 265 nm in a UV spectrophotometer (Unicam UV2-100, UK) using pure phosphate buffer as a blank. The percentage of entrapment efficiency was then calculated as:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Practical IM loading}}{\text{Theoretical IM loading}} \times 100 \quad (4)$$

Fourier transform infrared measurements

Fourier transform infrared measurements (FTIR) spectra of the grafted, ungrafted NaAlg and NaAlg-*g*-PVP bead were taken with a Jasco FTIR-480 plus spectrometer (Japan). FTIR spectra were taken in the wavelength region 400–4000 cm^{-1} at ambient temperature.

Differential scanning calorimetry

The thermal analysis was performed with a differential scanning calorimeter (DSC) (PerkinElmer, Sapphire DSC, USA). The sample weights ranged from 5 to 8 mg. The samples were heated from 30 to 300°C at a heating rate of 10°C/min. The intercept point of the slopes was taken as glass-transition temperature (T_g).

Scanning electron microscope

Scanning electron microscope (SEM) photographs were taken with JSM 5600 Scanning Microscope (Japan) to examine the morphology and surface structure of the beads at the required magnification at room temperature. The beads were deposited on brass hold and sputtered with a thin coat of gold under vacuum. Acceleration voltage used was 20 kV with the secondary electron image as a detector.

TABLE I
Elemental Analysis, *N*-VP Grafting (%), Intrinsic Viscosity, and Viscosity Average Molar Mass of Polymers

Polymer	Reaction time (h)	C%	H%	N%	Grafting yield (%)	[η]	M_v
NaAlg	–	–	–	–	–	7.2239	90,638
NaAlg-*g*-PVP_1	1	36.37	4.69	1.74	14.90	5.6982	71,495
NaAlg-*g*-PVP_2	2	36.72	5.05	1.86	15.85	5.5698	69,883
NaAlg-*g*-PVP_3	3	36.72	5.98	1.93	17.17	4.9388	62,000
NaAlg-*g*-PVP_4	4	37.34	4.89	2.13	18.12	4.8342	60,654

In vitro drug release

The *in vitro* drug release from the beads was studied in 250 mL conical flasks containing pH 1.2 HCl solution and incubated in a shaking water-bath (Medline BS-21, Korea) at 37°C, with a speed of 100 rpm. At the end of 2 h, IM release medium was changed to 7.4 from pH = 1.2. Four milliliter of the solution was withdrawn at specific time intervals and IM content was determined by UV spectrophotometer at 265 nm. Equal volume of fresh HCl or phosphate buffer solution was replaced into the release medium to maintain constant volume. Experiments were performed in triplicate to minimize the variational error. Standard deviations from the average values were calculated.

RESULTS AND DISCUSSION

Synthesis and characterization of the graft copolymer of NaAlg with *N*-VP

Graft copolymerization of NaAlg with *N*-VP was obtained using AIBN. The elemental analysis results and GY were presented in Table I. As it is seen from the Table I, GY (%) increased with the increase in reaction time. Presence of nitrogen in the grafted NaAlg and its increasing with the increasing of the reaction time confirm the grafting reaction. Figure 1 shows a possible reaction between NaAlg and *N*-VP. Similar graft copolymerization of *N*-VP has been reported by Gao et al.[23] They studied grafting of *N*-VP onto gelatin with four initiator systems which are AIBN, potassium persulfate, peroxide-ferrous, and cerium ammonium nitrate and they found that initiating ability of the AIBN is better than other initiators in the graft polymerization. Graft polymerization studies using AIBN initiator onto carboxymethyl cellulose were also investigated in the earlier study.[30]

FTIR spectra of NaAlg (A) and grafted NaAlg (B) were shown in Figure 2. The spectra of NaAlg showed the peaks around 3447, 2930, 1606, 1417, and 1033 cm^{-1}, indicating the stretching of O—H, aliphatic C—H, COO^-(asymmetric), COO^-(symmetric), and C—O—C, respectively, [Fig. 2(A)]. The spectra of the NaAlg-*g*-PVP showed the peaks around 3526, 2945, 1673, 1429, and 1290 cm^{-1}, indicating the stretching of O—H, aliphatic C—H, COO^-(asymmetric) bonded to N atom, C—N, and bending of C—N, respectively, which confirmed the grafting of the monomer [Fig. 2(B)].[20,30]

FTIR spectral data were also used to confirm the crosslinking of NaAlg-*g*-PVP matrix and chemical stability IM in the grafted NaAlg beads. Figure 3 compares FTIR spectra of empty bead (A), IM (B), and IM loaded NaAlg-*g*-PVP bead (C). The crosslinking process with GA provided a shift of lower intensity of COO^- stretching peak at 1673 and 1609 cm^{-1} to a higher wavenumbers (1747 and 1669 cm^{-1}) [Figs. 2(B) and 3(A)].[31] IM has shown characteristic bands between 3400 and 2500 cm^{-1} due to aromatic C—H stretching and carboxylic acid O—H stretching. The chemical structure of IM has displayed in Scheme 1. The bands at 1716 and 1691 cm^{-1} are due to C=O stretching. IM also has shown characteristic bands due to aromatic C=C, O—CH_3 deformation and C—O stretching plus O—H deformation at around 1591, 1480, and 1230 cm^{-1}, respectively.[32] When the drug is incorporated into the crosslinked NaAlg-*g*-PVP bead, along with all the characteristic bands of the empty NaAlg-*g*-PVP bead, additional bands have appeared due to the presence of IM in the matrix which further indicates the chemical stability of the drug in the grafted NaAlg matrix.

The results of viscosity measurements along with the viscosity average molecular masses were also presented in Table I, assuming the k and α values do not change with the grafting. The observed decreases in intrinsic viscosity and molecular mass of the NaAlg can be attributed to the grafting. Intrinsic viscosity of a polymer is a measure of its hydrodynamic volume in solution, which in turn, depends on its molar mass and structure, the nature of the solvent and temperature of the medium. In general, the intrinsic viscosity of polysaccharide increases with grafting. This behavior has been observed for starch, carboxymethyl cellulose and guar gum.[33] However, opposite behavior was reported for xanthan and sodium alginate. Similarly, intrinsic viscosity and molecular mass of the NaAlg decreased with the grafting of NaAlg. Table I also reflected that the

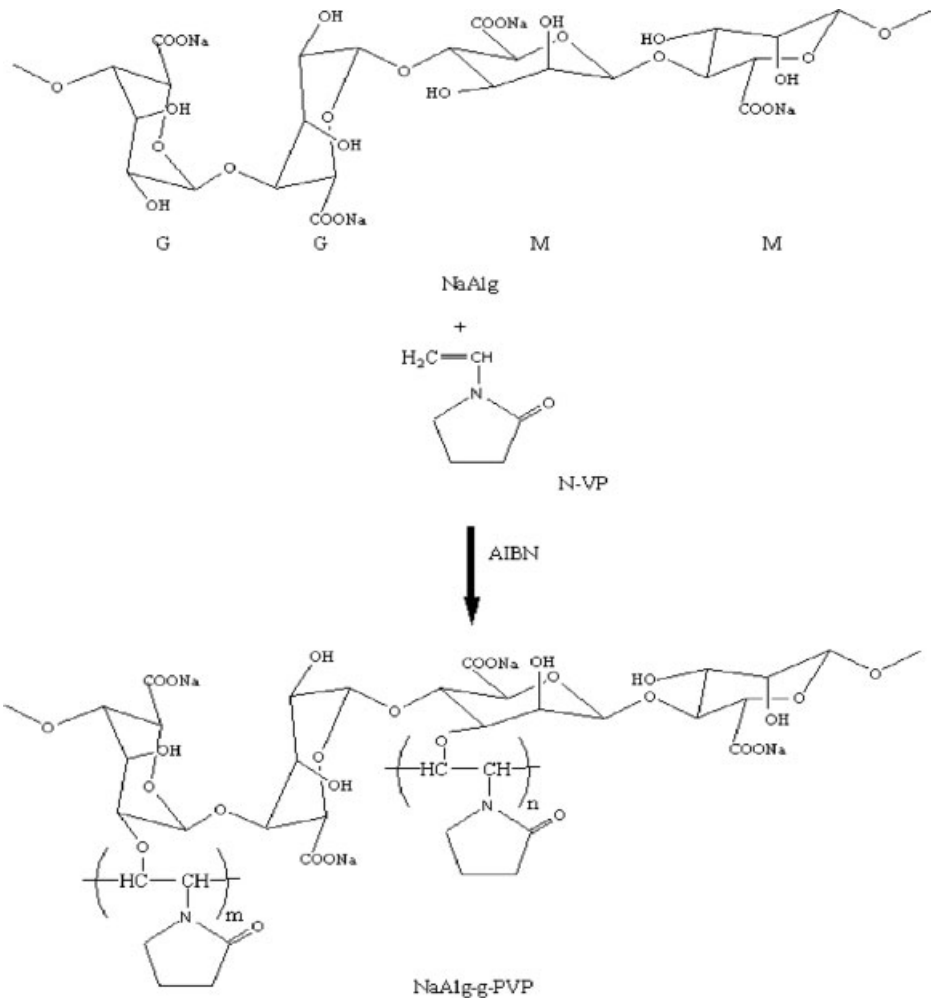


Figure 1 Postulated grafting reaction mechanism of NaAlg with *N*-VP.

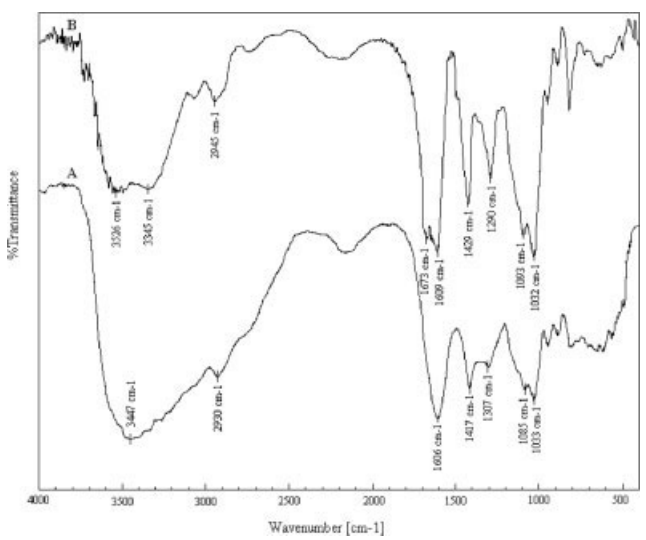


Figure 2 FTIR spectra of NaAlg (A) and NaAlg-*g*-PVP (B).

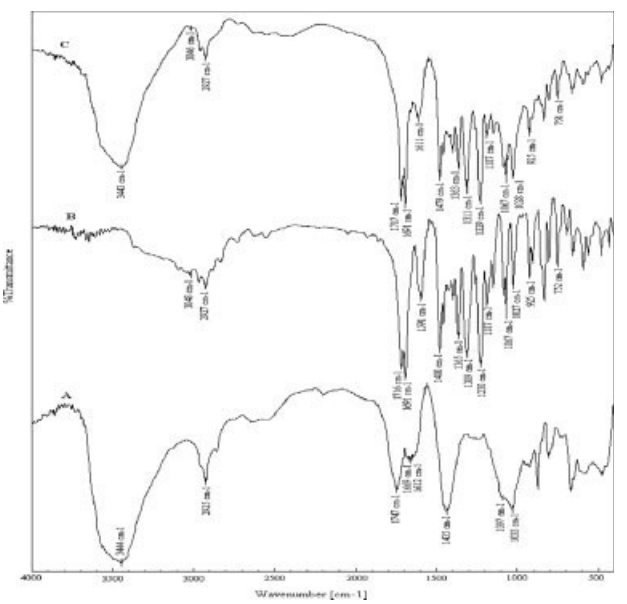
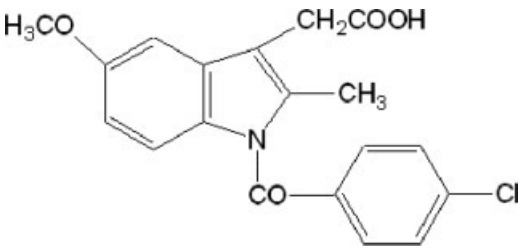


Figure 3 FTIR spectra of results of empty NaAlg-*g*-PVP bead (A), indomethacin (B) and drug loaded NaAlg-*g*-PVP bead (C).



Scheme 1 Chemical structure of Indomethacin.

intrinsic viscosity decreases with the increasing of GY due to reduction of hydrodynamic volume. Similar findings were also reported in the study of Tripathy et al.[34] They have prepared polyacrylamide-grafted sodium alginate and indicated that intrinsic viscosity of the grafted polymers decreases from 6.75 dL/g (conversion % = 83.76) to 6.00 dL/g (conversion % = 85.88) with the increasing cerium ammonium nitrate concentration from 1.003×10^{-4} to 3.009×10^{-4} mol, respectively.

DSC analyses were performed to understand the thermal behavior of the graft copolymers and results were illustrated in Figure 4. As it is reflected from the Figure 4(a) that temperature of the end point of the endotherm peak shifted to lower temperatures with the grafting of *N*-VP monomer. T_g value of NaAlg-*g*-PVP copolymer (56°C) was lower than that of NaAlg (93°C). It is attributed that grafted chains might act as internal plasticizer. Similar observations were also found in the literature.[35] DSC analyses also showed the presence of crosslinking. After crosslinking with GA, endothermic peak of NaAlg-*g*-PVP bead has shifted to a higher temperature value indicating the crosslinking reaction [Fig. 4(b)]. Hence, polymer matrix is more rigid and thereby shifts the endothermic peak to higher temperature. Melting point of the IM has been found 155°C from thermogram of the IM and IM loaded beads.

SEM study

SEM photographs of a single NaAlg and NaAlg-*g*-PVP beads taken at 50× magnification were shown in Figure 5. As it is seen from the Figure 5, both of the beads are almost spherical in shape and show roughness in surface.

Particle size, entrapment efficiency, and yield value evaluation of beads

The results of bead diameter, entrapment efficiency (%), and bead yield (%) were shown in Table II. As can be seen from the Table II, the beads formed have particle sizes ranging from 1.20 ± 0.01 to 1.98 ± 0.02 mm in diameter. The size of the beads changed with *N*-VP grafting, drug/polymer (m/m) ratio, crosslinking concentration and exposure time and percentage of HCl catalyst. In general, the diameter of the NaAlg-*g*-PVP beads is larger than that of NaAlg bead. Moreover, an increase in crosslinking concentration, time and catalyst percentage causes a decrease in diameters of the beads. With an increase in these parameters, the beads with smaller size were produced, probably due to the formation of a more rigid network as a result of increased crosslink density. Similar results with gellan gum and poly(vinyl alcohol) hydrogel microspheres have been found in the literature.[36] As it is also seen from the Table II, as the d/p ratio increases from 1/4 to 1/1, the diameter of the bead also increases from 1.30 ± 0.02 to 1.98 ± 0.002, respectively, due to the increase of IM amount.

The percentage of entrapment efficiency and bead yield may change depending on the preparation conditions. The results of percentage of entrapment efficiency and bead yield are presented in Table II. As it is seen from the table, values of entrapment efficiency and bead yield are quite high. These values

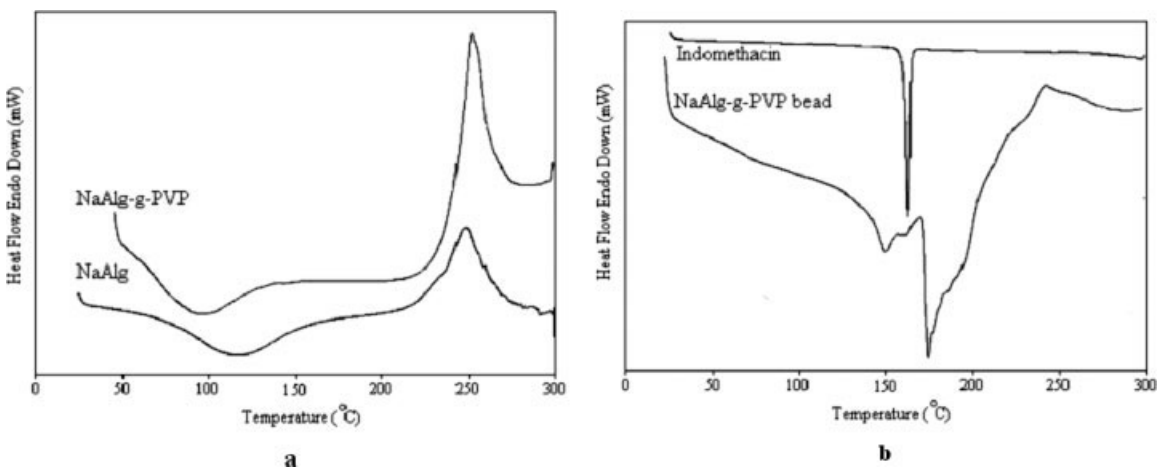


Figure 4 DSC results of NaAlg and NaAlg-*g*-PVP (a), indomethacin and indomethacin loaded NaAlg-*g*-PVP bead (b).

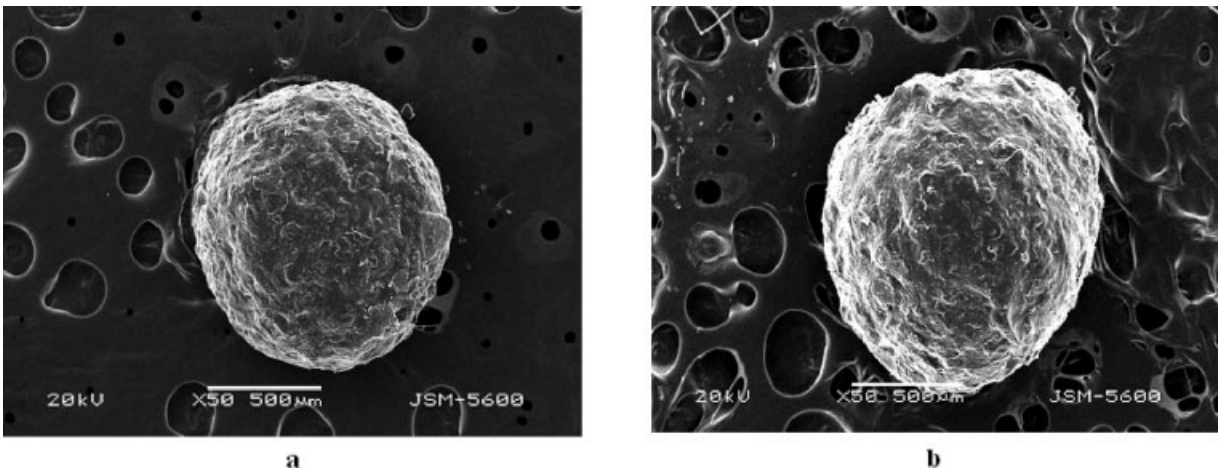


Figure 5 SEM photographs of IM loaded NaAlg (a) and NaAlg-*g*-PVP bead (b) with magnification of ×50.

increased slightly with the increasing of concentration of GA, exposure time and concentration of HCI. Percentage of entrapment efficiency and yield value of NaAlg-*g*-PVP beads were found to be higher than that of pure NaAlg beads. High percentage of entrapment efficiency of the NaAlg-*g*-PVP beads shows that prepared NaAlg-*g*-PVP polymer has good property for the entrapment of drug. When the *N*-VP is grafted, polymer traps more IM molecules and entrapment efficiency increases.

Effect of *N*-vinyl-2-pyrrolidone on the IM release

To understand the release of IM from crosslinked NaAlg-*g*-PVP beads *in vitro* release, a study was carried out in gastric and intestinal pH conditions at 37°C. For this purpose, NaAlg-*g*-PVP_2 graft copolymer (GY = 15.85%) was chosen for bead preparation because of very similar GY values. Figure 6 displays the effect of *N*-VP grafting on cumulative IM release of beads. From the Figure 6, it is observed that release rate of IM is much higher for the NaAlg-*g*-PVP beads than that of NaAlg beads at pH 7.4. The highest cumulative IM release obtained at the end of 6 h was 57% for NaAlg-*g*-PVP beads with 1/4 drug/polymer ratio. On the other hand, the highest cumulative IM release obtained was 43% for NaAlg beads with 1/4 drug/polymer ratio. This result is quite expected since the presence of PVP increases hydrophilic character of polymeric matrix. The results obtained are consistent with the swelling results. Equilibrium swelling experiments were performed in distilled water, in HCl and in buffer solutions of different pH values (pH: 1.2 and 7.4) for various empty bead formulations and were presented in Table III. As can be seen from the table, generally, ESD increases with grafting of *N*-VP on to NaAlg and with the increasing of temperature for all of the parameters. When the swelling degree increases, amorphous regions produce free volumes that are suitable for penetration of the liquid molecules to the bead and then the diffusion of the drug to the external medium. Therefore, cumulative release of IM increases with the grafting of *N*-VP. Similar observations were found in the previous studies.[28,37] Controlled release of ibuprofen from microgels of sodium alginate-acrylic acid was studied by Ramesh Babu et al.[38] and they found that percentage cumulative

TABLE II
Entrapment Efficiency, Yield (%), and Bead Diameter for the IM Loaded Beads

Code	Polymer	Concentration of GA (*M*) and HCl (*M*)	Exposure time to GA (min)	Drug/polymer ratio	Yield (%)	Entrapment efficiency (%)	Bead diameter (mm)
A_1	NaAlg-*g*-PVP	0.027 + 0.1	5	1/4	83.00	96.54 ± 1.24	1.45 ± 0.02
A_2	NaAlg-*g*-PVP	0.054 + 0.2	5	1/4	84.32	97.71 ± 1.13	1.30 ± 0.02
A_3	NaAlg-*g*-PVP	0.081 + 0.3	5	1/4	87.41	98.90 ± 0.62	1.25 ± 0.02
B_1	NaAlg-*g*-PVP	0.054 + 0.2	5	1/2	85.14	98.25 ± 1.00	1.60 ± 0.02
B_2	NaAlg-*g*-PVP	0.054 + 0.2	5	1/1	86.84	96.27 ± 1.42	1.98 ± 0.02
C_1	NaAlg-*g*-PVP	0.054 + 0.1	5	1/4	81.73	92.52 ± 1.95	1.35 ± 0.02
C_2	NaAlg-*g*-PVP	0.054 + 0.05	5	1/4	80.71	90.75 ± 1.26	1.41 ± 0.01
D_1	NaAlg-*g*-PVP	0.054 + 0.2	15	1/4	82.36	90.71 ± 1.36	1.23 ± 0.01
D_2	NaAlg-*g*-PVP	0.054 + 0.2	30	1/4	83.41	95.51 ± 1.49	1.20 ± 0.01
E	NaAlg	0.054 + 0.2	5	1/4	82.86	89.32 ± 1.74	1.24 ± 0.02

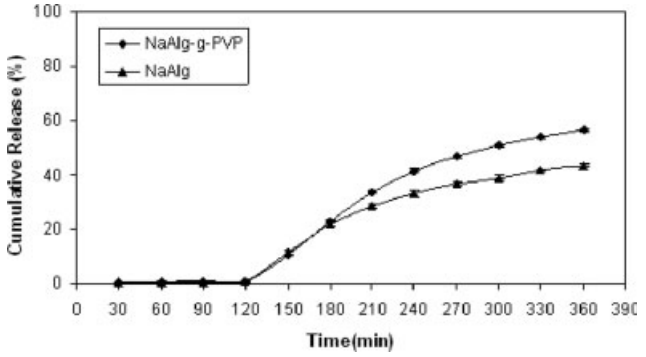


Figure 6 Effect of *N*-VP grafting on IM release. d/p: 1/4, concentration of GA: 0.054*M*, exposure time to GA: 5 min, concentration of HCl: 0.2*M*.

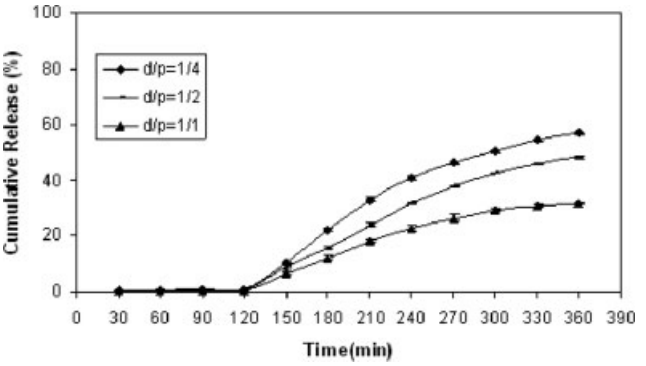


Figure 7 Effect of drug/polymer ratio on IM release. Concentration of GA: 0.054*M*, exposure time to GA: 5 min, concentration of HCl: 0.2*M*.

release increases with the increasing composition of acrylic acid.

It is also observed from the figures that NaAlg-*g*-PVP beads have also demonstrated a almost zero release of IM in the acidic pH as compared to medium of pH 7.4 due to high solubility of IM in high pH. A pronounced difference is observed in the release data between pH of 1.2 and pH of 7.4 conditions, which can be also attributed to less swelling of alginate in acidic medium.[10] At acidic pH, IM release is zero for 2 h. At low pH values, the less swelling should reduce the matrix permeability and limit the drug diffusion. At this pH, alginate is protonated into insoluble form of alginic acid; this displays properties of swelling that explains low amount of the release. Moreover, IM exists in its acidic form in an acidic solution such as in gastric fluid and it is practically insoluble in stomach, but soluble in intestinal fluid and water.[32] At pH 7.4 a rapid increase of the release observed is up to 77%. The deprotonation of alginic acid causes disintegration of the bead systems, which speeds the release of IM as soluble ions.

TABLE III
Equilibrium Swelling Degree (%) of the Beads at Three Temperature

Kodu	T (°C)	Distilled water	pH = 1.2	pH = 7.4
A_1	27	482.70 ± 7.76	182.07 ± 0.20	2117.45 ± 3.08
A_2	27	227.18 ± 1.75	143.13 ± 1.13	541.32 ± 6.33
A_3	27	161.39 ± 2.58	120.17 ± 0.80	392.91 ± 5.66
C_1	27	248.45 ± 2.05	148.04 ± 0.73	566.65 ± 6.10
C_2	27	321.07 ± 1.80	166.21 ± 1.02	672.00 ± 8.53
D_1	27	195.94 ± 4.62	128.05 ± 0.52	503.41 ± 9.60
D_2	27	184.36 ± 3.40	119.59 ± 0.60	483.21 ± 1.94
E	27	142.02 ± 2.94	109.22 ± 1.51	383.31 ± 4.56
A_1	37	633.37 ± 2.29	192.22 ± 0.43	2272.90 ± 8.24
A_2	37	270.80 ± 4.80	152.33 ± 0.58	664.12 ± 6.56
A_3	37	186.80 ± 2.20	127.08 ± 1.17	436.63 ± 3.91
C_1	37	299.61 ± 2.42	175.30 ± 0.34	704.73 ± 3.67
C_2	37	409.04 ± 4.36	194.70 ± 1.00	780.53 ± 3.93
D_1	37	234.81 ± 2.25	136.03 ± 0.17	609.66 ± 2.12
D_2	37	211.28 ± 3.20	125.67 ± 0.65	577.95 ± 2.62
E	37	209.29 ± 8.28	117.41 ± 0.73	419.26 ± 5.12
A_1	47	841.05 ± 3.05	203.54 ± 0.68	2421.98 ± 10.25
A_2	47	328.09 ± 1.55	162.22 ± 0.08	856.26 ± 1.58
A_3	47	221.19 ± 4.87	135.58 ± 1.09	503.56 ± 2.34
C_1	47	369.30 ± 7.03	197.25 ± 2.09	906.08 ± 5.14
C_2	47	548.64 ± 5.13	235.10 ± 0.75	981.92 ± 10.05
D_1	47	281.77 ± 5.64	144.89 ± 1.11	781.77 ± 10.35
D_2	47	254.41 ± 1.22	131.45 ± 1.83	731.82 ± 6.73
E	47	271.43 ± 8.94	127.51 ± 0.72	452.13 ± 5.78

Effect of drug/polymer ratio on the IM release

Another parameter that affects the IM release from the beads is drug/polymer ratio. The effect of d/p ratio on IM release was shown in Figure 7. The figure illustrates that IM release from the NaAlg-*g*-PVP beads with 1/4 d/p ratio is higher than that of beads with 1/2 and 1/1 d/p ratio. The maximum IM release for the beads with 1/4 d/p ratio obtained is 57%. When the d/p ratio decreases from 1/1 to 1/4, IM content of the beads decreases as well. Lower IM content might lead to the easier penetrating of the liquid through the beads and then IM diffusion from the beads gains speed. In other words, while IM content of the beads decreases, a loose structure in the polymeric beads is formed and this loose structure causes the liquid to easily penetrate into the beads and eases the diffusion of the IM. Similar trends were obtained in the study of diclofenac sodium release from the PVA/NaAlg blend beads.[27] Similar observations were also found in the literature.[3–5,25]

Kumbar and Aminabhavi[4] studied controlled release of IM from polyacrylamide grafted chitosan microspheres. They have reported that drug release at lower loadings (<10%) is quicker than that of higher loading due to possibility of formation of a large pore volume, which might enhance the drug release. They have also reported that IM release were found to be 89% at 8 h for the microsphere prepared with 5 mL of GA and 10% drug loading.

In vitro release behavior of IM from the polystyrene microparticles was also investigated emulsion-

solvent evaporation method by Tamilvanan and Sa.[25] They have obtained that release of IM from 20% drug loaded microparticles was higher than that of 50% drug loaded microparticles. IM release was found to be 85% at the end of the 6 h. Compared with these studies, IM release from the NaAlg-*g*-PVP beads have better extended and controlled.

Effect of exposure time to GA and concentration of GA on the IM release

IM release from the beads or microspheres were subjected to a number of physical and chemical parameters including those related directly to the release medium, the release conditions (temperature, pH), preparation conditions and those resulting from the change in the characteristics of the beads. One of the most effective ways to change release rate of beads is to change crosslink density of the matrix by employing varying time of exposure to crosslinking agent and concentrations of the crosslinking agent. The effect of exposure time to GA on the release rate of IM has been investigated at exposure times namely 5, 15, and 30 min. The results were shown in Figure 8, which clearly indicates that with increasing exposure time to GA (5–30 min), the cumulative release decreases at pH of 7.4. The maximum IM release from the NaAlg-*g*-PVP beads, which were prepared with an exposure time of 5 min, was found to be 57% at the end of 6 h.

Another way to change the crosslink density of the bead is to change the concentration of GA. For this purpose, GA concentration was changed during the bead preparation from 0.027*M* to 0.081*M* and release results from these beads were presented in Figure 9. As it is seen from the figure, as the GA concentration increased from 0.027*M* to 0.081*M*, IM release decreased from 77 to 35% at pH 7.4.

The observed decreases in the cumulative release are due to the fact that increasing exposure time and

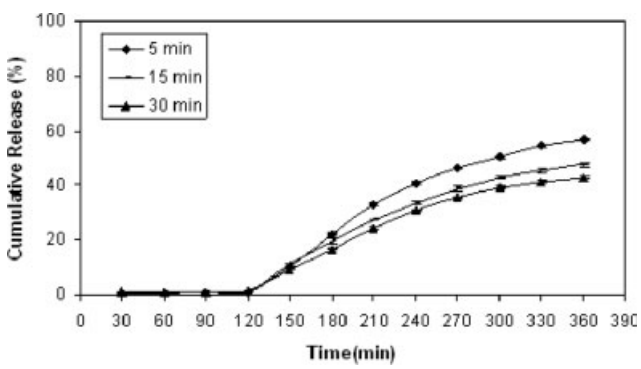


Figure 8 Effect of exposure time on IM release. *d*/*p*: 1/4, concentration of GA: 0.054*M*, concentration of HCl: 0.2*M*.

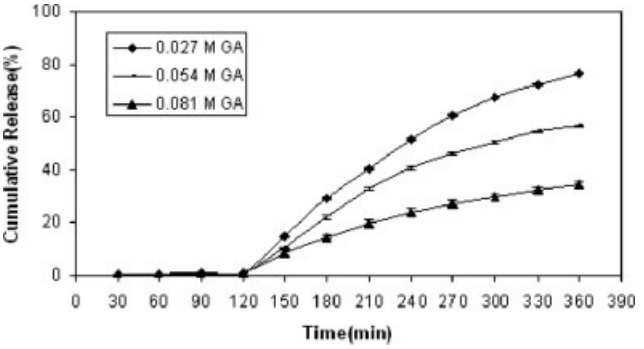


Figure 9 Effect of GA concentration on IM release. *d*/*p*: 1/4, exposure time to GA: 5 min, concentration of HCl: 0.2*M*.

concentration of GA result in an increase in crosslink density of the bead which gives rise to a compact network of the polymer. Consequently, the free volume reduces and penetration of water molecules and diffusion of IM molecules become difficult. IM release results were also supported by swelling measurements. IM release is significantly influenced by the ESD of the crosslinked polymeric beads. Drug release from the beads increased with increasing the ESD due to easy diffusion of drug molecules outside of the beads. As it is seen from the Table III, an increase in concentration and exposure time to GA decreases swelling percentage at all the temperature and pH values. The concentration of the GA in the of bead preparation solution were increased from 0.027*M* to 0.081*M*; ESD of the NaAlg-*g*-PVP beads in the pH 7.4 phosphate buffer medium at 37°C significantly decreased from (2272.90 ± 8.24)% to (436.63 ± 3.91)%. In the same way, the exposure time to the GA was increased from 5 to 30 min, ESD of the NaAlg-*g*-PVP beads in the pH 7.4 phosphate buffer at 37°C decreased from (664.12 ± 6.56)% to (577.95 ± 2.62)%, respectively, because of increase in crosslinking degree. Similar results were reported by many other workers.[3,39–41]

Kulkarni et al. studied controlled release of diclofenac sodium from crosslinked alginate beads.[40] They have reported that when the exposure to GA increased from 5 min to 10 min at 25 and 40°C, DS release significantly decreased.

Effect of HCl concentration on the IM release

The other parameter that affects the release of IM is concentration of HCl as a catalyst. Figure 10 represents the effect of catalyst concentration on the IM release with the other preparation conditions of bead to be taken constant. As it is seen from the Figure 10, when the concentration of HCl increases from 0.05*M* to 0.2*M*, cumulative IM release decreases

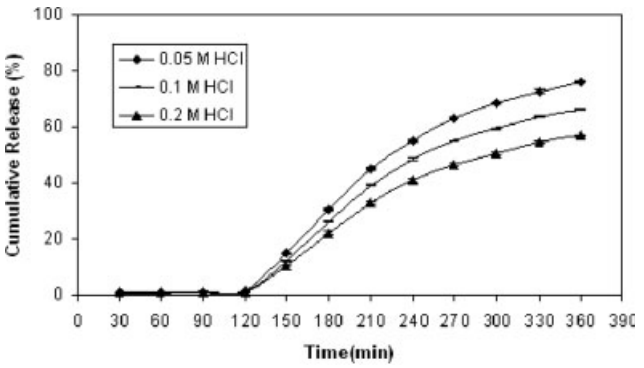


Figure 10 Effect of concentration of HCl on IM release. d/p: 1/4, concentration of GA: 0.054*M*, exposure time to GA: 5 min.

from 76 to 57%. This result is attributed to occurring dense network with the high HCl concentration. GA is a dialdehyde and their bonding to alginate is expected to occur in a planar two dimensional manner. Therefore, if there is an increase in the concentration of HCI, there is also an increase in the number of bounds between COO^- or OH groups of the grafted alginate and CHO group of GA. Consequently, the release of IM from the NaAlg-*g*-PVP beads decreases with an increase in the catalyst concentration. Swelling experiments are also correlated with the release results as reflected in Table III. The concentration of HCl in the bead preparation solution was increased from 0. 05*M* to 0.2*M*, ESD in the pH 7.4 phosphate buffer at 37°C decreased from (780.53 ± 3.93)% to (664.12 ± 6.56)%, respectively, due to formation of dense bead structure. In the previous study,[42] controlled release of IM was investigated from the NaAlg beads and same trend was obtained in the effect of the HCl concentration on the IM release. The highest cumulative IM release was obtained at the end of 6 h was 68% for alginate beads which were prepared with 0.05*M* HCl in that study.

Analysis of kinetic results

The phenomenon of solvent sorption by a polymeric bead depends mechanistically on the diffusion of water molecules into the gel matrix and subsequent relaxation of macromolecular chains of the bead.[43] The release data of all the systems have been further substantiated by fitting the fraction release data M_t/M_∞ to an empirical equation proposed by Peppas[44]

$$\frac{M_t}{M_\infty} = kt^n \tag{5}$$

where M_t is the amount of IM released at time t and M_∞ is the drug released at equilibrium time; k, a constant characteristic of the drug-polymer system; and n, the diffusional exponent which suggests the nature of the release mechanism. Fickian release is defined by an initial $t^{1/2}$ time dependence of the fractional release for slabs, cylinders, and spheres. Analogously, Case-II transport is defined by an initial linear time dependence of the fractional release for all geometries.[45] A value of $n = 0.5$ indicates the Fickian transport (mechanism), while $n = 1$ is of Case II or non-Fickian transport (swelling-controlled).[38] The intermediary values ranging between 0.5 and 1.0 are indicative of the anomalous transport.[3–5] Fickian transport occurs when the drug release is governed by drug diffusion. Non-Fickian or Anomalous diffusion occurs when the diffusion and relaxation rates are comparable. Drug release depends on two simultaneously rate processes, water migration into the device and drug diffusion through continuously swelling hydrogels is highly complicated.[45,46] Case II diffusion (relaxation-controlled transport) occurs when diffusion is very rapid compared with relaxation process. In Case II system, diffusion of water through the previously swollen shell is rapid as compared with the swelling-induced relaxation of polymer chains. Thus, the rate of water penetration is controlled by polymer relaxation and release of drug occurs as it diffuses out when the polymer swells by absorbing water.

The least-squares estimations of the fractional release data along with the estimated correlation coefficient values, r, are presented in Table IV. From these data, the n value ranged between 0.71 and 0.97, with correlation coefficient values of 0.99, indicating IM release from the beads display anomalous transport in which drug release is governed by coupling of drug diffusion and polymer relaxation processes.

Diffusion coefficient, D can be calculated for water absorption or drug release by beads using the equation as follow:[47]

$$D = \left(\frac{r\theta}{6M_\infty}\right)^2 \pi \tag{6}$$

where θ is slope of the linear portion of the plot of M_t/M_∞ versus $t^{1/2}$, r is radius of the beads and M_∞ is the maximum drug release. The data reported in Table IV show a relationship between the exposure time and concentration of GA, d/p ratio and concentration of HCl. The D value decreases from 23.47×10^{-13} cm^2/s to 3.24×10^{-13} cm^2/s with increasing GA concentration from 0.027*M* to 0.081*M*, respectively. An increase in exposure time to GA and concentration of HCl decreases diffusion coefficient which is also in agreement with release results (A_2, D_1, D_2 and A_2, C_1, C_2). The D values also increase with decreasing d/p ratio (A_2, B_1, B_2) which also supports IM release results.

TABLE IV
The Results of *D*, *k*, *n*, and *r* Calculated from eqs. (5) and (6)

Code	$D \times 10^{13}$ (cm^2/s)	k (min^{-n}) $\times 10^3$	n	r	Diffusion mechanism
A_1	23.47	7.00	0.902	0.998	Non-Fickian
A_2	10.19	6.46	0.864	0.988	Non-Fickian
A_3	3.24	4.33	0.843	0.995	Non-Fickian
B_1	2.40	5.16	0.862	1.000	Non-Fickian
B_2	0.47	3.82	0.858	0.999	Non-Fickian
C_1	23.38	5.02	0.951	0.990	Non-Fickian
C_2	33.79	5.78	0.965	0.998	Non-Fickian
D_1	7.95	7.11	0.811	0.987	Non-Fickian
D_2	5.07	6.68	0.791	0.989	Non-Fickian
E	25.86	11.45	0.706	0.998	Non-Fickian

Molar mass between the crosslinks

Release of drug from the polymer matrix is a function of the extent of crosslinking. To understand the crosslinking of the polymer network, it is important to calculate the molar mass, M_c, between crosslinks of the network polymer. The magnitude of M_c significantly affects the physical and mechanical properties of crosslinked polymer and its determination has great practical significance. Equilibrium swelling is widely used to determine M_c. Flory and Rehner's equation in the following form was used to calculate M_c values.[48]

$$M_c = -\rho_p V_s \phi^{1/3}[\ln(1-\phi) + \phi + \chi\phi^2]^{-1} \quad (7)$$

The volume fraction, ϕ of the swollen polymer was calculated as follows:

$$\phi = \left[1 + \frac{\rho_p}{\rho_s}\left(\frac{M_a}{M_b}\right) - \frac{\rho_p}{\rho_s}\right]^{-1} \quad (8)$$

In the above equations, ρ_p and ρ_s are the densities of polymer and solvent, respectively; M_b and M_a, are the mass of polymer before and after swelling, respectively. V_s is the molar volume of the solvent. The interaction parameter, χ, was calculated using eq. (9), the procedure published by Aithal and Aminabhavi.[49]

TABLE V
Molecular Mass Between Crosslinks of the Beads of Different Compositions

Swelling temperature(°C)	Polymer	Concentration of GA (*M*) and HCl (*M*)	Exposure time to GA (min)	*N*	Φ	χ	M_c
27	NaAlg-*g*-PVP	0.027 + 0.1	5	−1.1526	0.2510	0.4950	3849
37	NaAlg-*g*-PVP	0.027 + 0.1	5	−1.1655	0.2405	0.4972	4442
47	NaAlg-*g*-PVP	0.027 + 0.1	5	−1.1806	0.2285	0.4988	5203
27	NaAlg-*g*-PVP	0.054 + 0.2	5	−1.1033	0.2990	0.5277	3073
37	NaAlg-*g*-PVP	0.054 + 0.2	5	−1.1156	0.2857	0.5278	3582
47	NaAlg-*g*-PVP	0.054 + 0.2	5	−1.1291	0.2721	0.5277	4204
27	NaAlg-*g*-PVP	0.081 + 0.3	5	−1.0727	0.3370	0.5512	2548
37	NaAlg-*g*-PVP	0.081 + 0.3	5	−1.0827	0.3237	0.5504	2936
47	NaAlg-*g*-PVP	0.081 + 0.3	5	−1.0940	0.3097	0.5491	3414
27	NaAlg-*g*-PVP	0.054 + 0.1	5	−1.1098	0.2919	0.6072	18018
37	NaAlg-*g*-PVP	0.054 + 0.1	5	−1.1447	0.2578	0.5912	25428
47	NaAlg-*g*-PVP	0.054 + 0.1	5	−1.1735	0.2343	0.5814	34526
27	NaAlg-*g*-PVP	0.054 + 0.05	5	−1.1327	0.2687	0.5992	27742
37	NaAlg-*g*-PVP	0.054 + 0.05	5	−1.1691	0.2376	0.5851	39544
47	NaAlg-*g*-PVP	0.054 + 0.05	5	−1.2140	0.2063	0.5717	59102
27	NaAlg-*g*-PVP	0.054 + 0.2	15	−1.0834	0.3227	0.5355	2556
37	NaAlg-*g*-PVP	0.054 + 0.2	15	−1.0934	0.3105	0.5357	2930
47	NaAlg-*g*-PVP	0.054 + 0.2	15	−1.1059	0.2961	0.5350	3427
27	NaAlg-*g*-PVP	0.054 + 0.2	30	−1.0713	0.3389	0.5294	2053
37	NaAlg-*g*-PVP	0.054 + 0.2	30	−1.0800	0.3272	0.5303	2330
47	NaAlg-*g*-PVP	0.054 + 0.2	30	−1.0900	0.3145	0.5306	2666
27	NaAlg	0.054 + 0.2	5	−1.0581	0.3583	0.5950	3307
37	NaAlg	0.054 + 0.2	5	−1.0698	0.3411	0.5901	3923
47	NaAlg	0.054 + 0.2	5	−1.0840	0.3220	0.5843	4740

$$\chi = [\phi(1-\phi)^{-1} + N\ln(1-\phi) + N\phi] \times [2\phi - \phi^2 N - \phi^2 T^{-1}(d\phi/dT)^{-1}]^{-1} \quad (9)$$

where N is as follows in eq. (10)

$$N = \left(\frac{\phi^{2/3}}{3} - \frac{2}{3}\right)\left(\phi^{1/3} - \frac{2\phi}{3}\right)^{-1} \quad (10)$$

and $d\phi/dT$ is the slope of the line obtained by plotting the volume fraction versus temperature (in Kelvin).

The M_c values were calculated from equilibrium swelling data at three different temperatures and presented in Table V. The M_c values increase with increasing temperature and varies in the range from 2053 to 59102. These data indicate that M_c values decrease with increasing amount of GA in the formulation and concentration of catalyst, since the network would become denser, which supports the IM release results. Similar type of results has also been reported elsewhere.[43,47]

CONCLUSIONS

IM included NaAlg-*g*-PVP hydrogel beads were successfully prepared with high entrapment efficiency and characterized with FTIR, SEM, DSC. IM release studies from the beads indicate that grafting of *N*-VP onto NaAlg polymers leads to an increase in the cumulative release of IM and the entrapment efficiency. It is observed that release of IM is much higher at high pH value compared to low pH value showing that the release system is interesting as a controlled release system for colon-specific drug delivery. Furthermore, IM release from the beads decreases with the increase of exposure time to GA, concentration of GA, drug/polymer ratio and concentration of HCl. ESD of all the formulations is in consistence with the release results. The data of molar mass between the crosslinks also indicate that an increase in the concentration of GA, exposure time to GA and concentration of HCl means a decrease in M_c values, hence NaAlg-*g*-PVP beads become denser structures. From the release data, all of the bead formulations display anamolous transport. Finally, IM release from the NaAlg-*g*-PVP beads depends on the preparation conditions. More effective controlled release formulations can be obtained by changing the preparation conditions.

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