Carbohydrate Polymers 85 (2011) 164–172

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Al^{+3} ion cross-linked and acetalated gellan hydrogel network beads for prolonged release of glipizide

Sabyasachi Maiti[a,*], Somdipta Ranjit[a], Ranjit Mondol[a], Somasree Ray[a], Biswanath Sa[b]

[a] Gupta College of Technological Sciences, Division of Pharmaceutics, Ashram More, G.T. Road, Asansol 713301, West Bengal, India
[b] Department of Pharmaceutical Technology, Centre for Advanced Research in Pharmaceutical Sciences, Jadavpur University, Kolkata 700032, West Bengal, India

ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 15 January 2011
Accepted 3 February 2011
Available online 3 March 2011

Keywords:
Gellan gum
Hydrogel bead
Ionotropic gelation
Entrapment efficiency
Swelling
Drug release

ABSTRACT

Considering relatively fast drug release rate of Ca^{+2}/gellan beads in phosphate buffer solution, a novel glipizide-loaded bead system was developed through ionotropic gelation of gellan with trivalent Al^{+3} ions and covalent cross-linking with glutaraldehyde (GA). Following GA-treatment, spherical Al^{+3}/gellan beads contracted leaving wrinkles on bead surface. A maximum of 97.67% drug entrapment efficiency was achieved; however GA-treatment reduced the same by 11.89%. All the beads released only 10% drug in acidic medium in 2 h; however it was found 38–47% for Al^{+3}/gellan beads and only 15% for GA-treated beads in weakly alkaline medium. The drug release did correlate well with their swelling behaviors. The anomalous drug transport mechanism shifted to super case II transport for GA-treated beads, where the polymer relaxation phenomenon was dominant. The drug was relatively stable, amorphous in the beads. Thus both GA-treated and -untreated Al^{+3}/gellan beads could be useful carriers for the controlled oral delivery of glipizide.

1. Introduction

In the design of pharmaceutical dosage forms, naturally occurring polysaccharides are being preferred over their synthetic counterparts because they are nontoxic, biodegradable, freely available, and less expensive (Bhardwaj, Kanwar, Lal, & Gupta, 2000). Over the last few years, a great deal of attention has been paid to the development of polysaccharide-based hydrogel beads through ionotropic gelation technique useful as potential carriers in controlled drug delivery. One of the advantages of this technique is that the drug encapsulation in the beads could be achieved in an eco-friendly aqueous environment (Patil, Kamalapur, Marapur, & Kadam, 2010). In addition, multiunit systems avoid the vagaries of gastric emptying and different transit rates through the gastrointestinal tract, thereby, drugs release more uniformly and prevents exposure to a high drug concentration, when compared to single unit dosage form on chronic dosing (Davis, Hardy, Taylor, Whalley, & Wilson, 1984; Follonier & Doelkar, 1992).

The anionic biopolymer sodium alginate (Das & Senapati, 2008; Kikuchi et al., 1999; Yegin et al., 2007) has been investigated in detail for its unique nature of forming hydrogel beads with divalent calcium ions. However, calcium alginate beads erode/disintegrate rapidly in simulated intestinal fluids leading to quick release of the loaded drugs (Acarturk & Takka, 1999). Further, the gel strength of alginate beads was improved by the formation of polyelectrolyte complex with positively charged amines such as poly-L-lysine (Ueng et al., 2000), polyethyleneimine (Halder, Maiti, & Sa, 2005), and chitosan (Anal & Stevens, 2005) for the controlled delivery of drugs. But the results were not so encouraging. Chitosan is at the origin a neutral polysaccharide whose amine functional group can be protonated in acidic pH conditions and become one of the little polysaccharides which are positively charged. The ability of chitosan to form hydrogel beads in presence of negatively charged sodium tripolyphosphate has also been investigated for controlled drug delivery (Aydin & Akbuğa, 1996; Srinatha, Pandit, & Singh, 2008). However, higher porosity of sodium tripolyphosphate/chitosan beads led to rapid release of drug in acidic dissolution medium (Gupta & Ravi, 2000). Even, the pectin beads have some drawbacks due to their rapid *in vitro* drug release (Bodmeier, Oh, & Pramar, 1989; Sriamornsak, Puttipipatkhachorn, & Prakongpan, 1997). Thus it was evident that none of the polymeric bead systems seems suitable as an oral controlled release system.

Gellan gum is a water soluble linear anionic polysaccharide produced as a fermentation product by a pure culture of *Pseudomonas elodea* (Doner & Douds, 1995; Kang, Veeder, Mirrasoul, Kaneko, & Cottrell, 1982). The native gellan is partially esterified with L-glycerate and acetate (Kuo, Mort, & Dell, 1986) but the commercial gellan is a de-esterified form of native gellan, obtained by alkali treatment (Morris, 1995). However, both forms have similar linear

* Corresponding author. Tel.: +91 9474119931; fax: +91 341 2314604.
E-mail address: sabya245@rediffmail.com (S. Maiti).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.02.010

structure made up of repeating units of a tetrasaccharide, composed of β-D-glucose, β-D-glucuronic acid and α-L-rhamnose residues in the molar ratio of 2:1:1 (Izumi, Kikuta, Sakai, & Takezawa, 1996; Jansson, Lindberg, & Sandford, 1983).

According to Food and Drug Administration (FDA), gellan gum may be safely used as a direct food additive for human consumption as long as its use is in accordance with 21 CFR 172.665. Due to its ability to form hydrogel beads in presence of divalent cations, particularly Ca^{+2} ions, gellan gum has been investigated for the controlled release of various kinds of drugs (Agnihotri, Jawalkar, & Aminabhavi, 2006; Babu, Sathigari, Kumar, & Pandit, 2010; Kedzierewicz, Lombry, Rios, Hoffman, & Maincent, 1999; Patil, Sharma, Nimbalkar, & Pawar, 2006; Rajinikanth & Mishra, 2007). Most of the *in vitro* drug release studies have been conducted either in acidic or alkaline dissolution media. Gellan gum gels are stable in low pH solution but swell in weakly basic solutions and calcium gellan allowed releasing the drug rapidly in phosphate buffer (El Fattah, Grant, Gabr, & Meshali, 1998).

Chemically, glipizide is 1-cyclohexyl-3-[[p-[2-(5-ethylpyrazinecarboxamido) ethyl] phenyl] sulfonyl] urea. It is an oral hypoglycemic agent, which is a commonly prescribed drug for the treatment of patients with type II diabetes mellitus (Verma & Garg, 2004). The oral absorption is uniform, rapid and complete with a bioavailability of nearly 100% (Jamzad & Fassihi, 2006). Since it is absorbed throughout the gastrointestinal tract and shows a very high bioavailability, a well developed formulation would help to increase its therapeutic efficacy. Moreover, it has a short biological half-life of 3.4 ± 0.7 h and requires administration in 2–3 doses of 2.5–10 mg/day (Sweetman, 2005). Thus, glipizide is an ideal candidate for incorporation into a controlled release device.

In a study by Reddy and Tammishetti (2002), the efficiency of divalent and trivalent metal ions to form durable carboxymethyl guar gum beads was compared. They reported that due to higher valency, the cross-linking rate for trivalent aluminum (Al^{+3}) ions with carboxymethyl guar gum was faster than divalent calcium (Ca^{+2}) ions. The optimum salt concentration that provided maximum bovine serum albumin retention in the beads was found to be much lower for Al^{+3} ions. Furthermore, the beads cross-linked with Al^{+3} ions released the protein over a longer duration in simulated intestinal fluid, than those cross-linked with Ca^{+2} ions. Thus, the faster cross-linking rates with Al^{+3} ions could be very useful to load higher percentage of drug into the beads; otherwise the loaded drug would escape into cross-linking medium with time. This is why the trivalent Al^{+3} ion was used instead of Ca^{+2} ion for the preparation of gellan hydrogel network beads for prolonged release of glipizide.

Although much effort has been put in understanding the sustained drug release properties of calcium gellan beads, no studies have been reported so far that describes the formation of drug-loaded gellan beads in presence of trivalent metal ions. In this study, we have prepared both *in situ* acetalated and trivalent Al^{+3} ion-induced gellan hydrogel network beads, and evaluated their properties with special emphasis being given here to *in vitro* drug release characteristics by mimicking *in vivo* pH-conditions. The drug–polymer interaction was evaluated by FTIR spectroscopy. The physical state of drug in the gellan beads was examined through DSC and XRD analysis.

2. Experimental

2.1. Materials

Glipizide was a gift from Micro Labs Limited, Hyderabad, India. Gelrite gellan gum was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Glutaraldehyde aqueous solution (25%, v/v) was procured from Merck Specialities Pvt Ltd., Mumbai, India. Tween 80 was supplied by Loba Chemie Pvt. Ltd., Mumbai, India. All other reagents were of analytical grade and used without further purification.

2.2. Preparation of glipizide-loaded gellan beads

Glipizide (30%, w/w) was dispersed uniformly into 10 ml 1.25% (w/v) aqueous solution of gellan gum. The dispersion (pH 6.0) was added dropwise through 21-gauze flat-tipped needle into slightly agitated 100 ml of 1%, 3% and 5% (w/v) aluminum chloride ($AlCl_3$) solution containing 0.08% (w/v) Tween 80. Following an incubation period of 10 min, the beads were isolated by filtration, washed with double distilled water (3×50 ml) and air-dried. The beads incubated in 5% $AlCl_3$ solution for 10 min, were transferred into 50 ml of water containing 4.5% (v/v) glutaraldehyde (GA) solution (pH 2.0) and were maintained at 50 °C for 2 h. The cross-linked beads were removed and washed with distilled water repeatedly to remove non-reacted GA. The complete removal of un-reacted GA was confirmed by the negative test of the washings with Brady's qualitative reagent (2,4-dinitrophenylhydrazine). The same procedure was adopted for the preparation of blank beads (without drug).

2.3. Characterization of blank gellan beads

The interaction between gellan gum and trivalent Al^{+3} ions, and the introduction of acetal linkage were evaluated by Fourier transform infrared (FTIR) spectroscopy. FTIR spectra of pure gellan gum, GA-treated and -untreated blank Al^{+3}/gellan bead, and GA-treated gellan gum were recorded in PerkinElmer IR Spectrophotometer (Spectrum RX1, UK) from 4000–600 cm^{-1} using KBr pellets. The pellets were made by applying pressure of 125 kg/cm^2 to a mixture of gum and sample (1:20) for 10 min in a hydraulic press (KP, Kimaya Engineers, India).

2.4. Estimation of drug entrapment efficiency

Accurately weighed, 10 mg of dried beads were crushed with a mortar-pestle and were transferred into 100 ml of pH 7.4 phosphate buffer solution. After 12 h, the suspension was filtered and the samples were analyzed with a spectrophotometer (UV1, Thermo Spectronic, UK) at λ_{max} 276 nm. Higher time was allowed to affect the maximum drug release. All samples were analyzed in triplicate. The drug entrapment efficiency (%) was calculated using the equation: Entrapment efficiency (%) = (actual drug content/theoretical drug content) × 100.

2.5. Bead size analysis

The size of dried glipizide-loaded beads was measured using an optical microscope (Olympus Model HB, India). A standard stage micrometer was used to calibrate the optical micrometer. The mean diameter was calculated using 50 beads, randomly selected from each formulation.

2.6. Scanning electron microscopy (SEM)

The dried drug-loaded beads were examined under a scanning electron microscope (JEOL-JSM-6360, serial No: G5/IL/42/08, JEOL Datum Ltd., Japan). SEM photographs of uncoated samples were taken at an acceleration voltage of 10 kV. The samples were placed on NEM TAPE adhesive paper (Nisshinem. Co. Ltd.) and photographs were taken.

2.7. In vitro drug release study

The drug release from the beads was studied using paddle-type dissolution rate test apparatus (VDA-6D, Veego Instruments Corporation, Mumbai, India). Accurately weighed, 50 mg of dried beads was placed in 900 ml of pH 7.4 phosphate buffer solution and maintained at 37 ± 0.5 °C. The paddle was rotated at 50 rpm. Ten milliliters of aliquot was withdrawn from the dissolution medium at specified time intervals and the same volume of fresh medium was replenished immediately. The samples were analyzed spectrophotometrically (UV1, Thermo Spectronic, UK) at 276 nm, without filtration and further dilution. The *in vitro* drug release study of the samples was carried out in pH 1.2 KCl/HCl buffer solution under the same experimental condition for 2 h and the beads were subsequently transferred into 900 ml pH 7.4 phosphate buffer solution and the study was continued for another 6 h. Cumulative percentage of drug released in the respective dissolution medium was plotted as a function of time. Each sample was tested and analyzed in triplicate.

2.8. Swelling study

The swelling behaviors of GA-treated and -untreated blank beads were studied in both pH 1.2 KCl/HCl buffer solution and pH 7.4 phosphate buffer solution. Samples of known weight were exposed to 50 ml of the swelling medium and allowed to swell for 1.5 h. The swollen beads were periodically removed, blotted with tissue paper and weighed (Metler Toledo, AB 204-S, Switzerland). The swelling ratios were calculated as follows: Swelling ratio = (final weight − initial weight)/initial weight.

2.9. Drug release kinetics

The *in vitro* drug release data up to 60% was fitted into Korsmeyer–Peppas Eq. (1) (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983):

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where M_t/M_∞ is the fractional solute release at time t, k is a constant which incorporates the structural and geometric characteristics of the device, and n is diffusion exponent. The mechanism of drug release from spherical polymeric devices may be Fickian diffusion when the value of $n = 0.43$ or less, anomalous (non-Fickian) transport when the value of n lies between 0.43 and 0.85, and case II transport when $n = 0.85$. An exponent value of n greater than 0.85, signifies super case II transport mechanism (Ritger & Peppas, 1987).

2.10. Drug–polymer interaction study through FTIR spectroscopy

FTIR spectra of pure drug, physical mixture of drug and polymer, GA-treated and -untreated formulations were recorded in PerkinElmer IR Spectrophotometer (Spectrum RX1, UK) using KBr pellets. The changes in characteristic peaks of pure glipizide were noted in the physical mixture as well as in the formulations.

2.11. Differential scanning calorimetry (DSC)

DSC thermograms of pure drug, physical mixture of drug and polymer, and GA-treated and -untreated beads containing drug were recorded using PerkinElmer instrument (Pyris-Diamond TG/DTA, Osaka, Japan). Each sample (2–4 mg) was accurately weighed into a 50 μl aluminum pan in a hermetically sealed condition. The measurements were performed in an atmosphere of nitrogen (150 ml/min) between 32 °C and 250 °C at a heating rate of 15 °C/min. Platinum crucible with alpha alumina powder was used as reference.

2.12. X-ray diffractometry (XRD)

XRD patterns of pure glipizide, physical mixture of glipizide and excipients, glipizide-loaded GA-treated and -untreated beads were traced in a wide angle X-ray diffractometer (Ultima III, model: D/Max 2200, Rigaku Corporation, Japan) using Cu-Kα radiation source. The instrument was set up with the tube voltage of 40 kV, current of 30 mA and scanning rate of 5°/min, over a diffraction angle (2θ) range of 10–30°.

2.13. Statistical analysis

The differences in drug entrapment efficiency and drug release rates of the beads were evaluated by one-way ANOVA: single factor, using Microsoft Excel 2002 software. Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Formation of gellan hydrogel beads

Gellan gum is a water soluble anionic polysaccharide and its gelling ability with divalent Ca^{+2} ions is well known for the preparation of drug-loaded beads. Chemically, the gelation and aggregation occurs via ionic-linking between divalent Ca^{+2} ions and two carboxylate groups belonging to glucuronic acid molecules in the gellan chains (Kanesaka, Watanabe, & Matsukawa, 2004). This mechanism is quite similar to that of calcium alginate gel formation (Rees, 1970). In this study, we observed that it was possible to devise self-standing, isolatable hydrogel beads in aqueous gelling medium containing different concentration of trivalent Al^{+3} ions. Preliminary investigation revealed that it was very hard to prepare beads with a gellan concentration above 1.25%. Higher viscosity of gellan dispersion made its extrusion difficult into the gelation medium at a concentration above 1.25%.

The formation of Al^{+3}/gellan beads could be attributed to the ionic-linking between Al^{+3} ion and carboxylate groups of glucuronic acid moiety of the gellan gum. Aluminum ions have an extra positive charge compared to Ca^{+2} ions thus each aluminum ion was able to bind to one more carboxylate from the gellan molecules. In addition, both the cation size and supramolecular structure of tetrasaccharide unit may also favor subsequent coordination of Al^{+3} ion with oxygen atoms of hydroxyl groups located in gellan chains in close vicinity of the cation. This may cause additional strengthening of the Al^{+3}/gellan reticulates. The involvement of coordination bonds has also been proposed for calcium alginate gel (Khromova, 2006).

In the FTIR spectrum of Gelrite gellan gum (Fig. 1a), a broad band, which appeared at 3426.35 cm^{-1} could be attributed to the O–H stretching of hydroxyl groups of glucopyranose ring. The peak at 880.48 cm^{-1} was due to C–O–C skeletal of glycosidic linkages. The stretching vibration of $–CH_2$ groups and hydroxyl C–O–H was observed at 2976.74 cm^{-1} and 1076.33 cm^{-1}, respectively. The band appearing at 1561.03 cm^{-1} indicated –C=O stretching of acetate. The stretching of C–O–C linkage between acyl carbon and C-6 of glucose in Gelrite appeared at 1298.41 cm^{-1}, and the band at 1369.71 cm^{-1} was due to $–CH_3$ bending of acetate. As Gelrite is in its low esterified form in which the acetate groups do not interfere with helix aggregation during gel formation, the stretching and bending vibration due to acetate functionality could be expected. The peaks corresponding to –C=O stretching of carboxylate groups of Gelrite appeared at 1415.59 cm^{-1} (Agnihotri & Aminabhavi, 2005). In the spectrum of GA-untreated blank beads (Fig. 1b), the

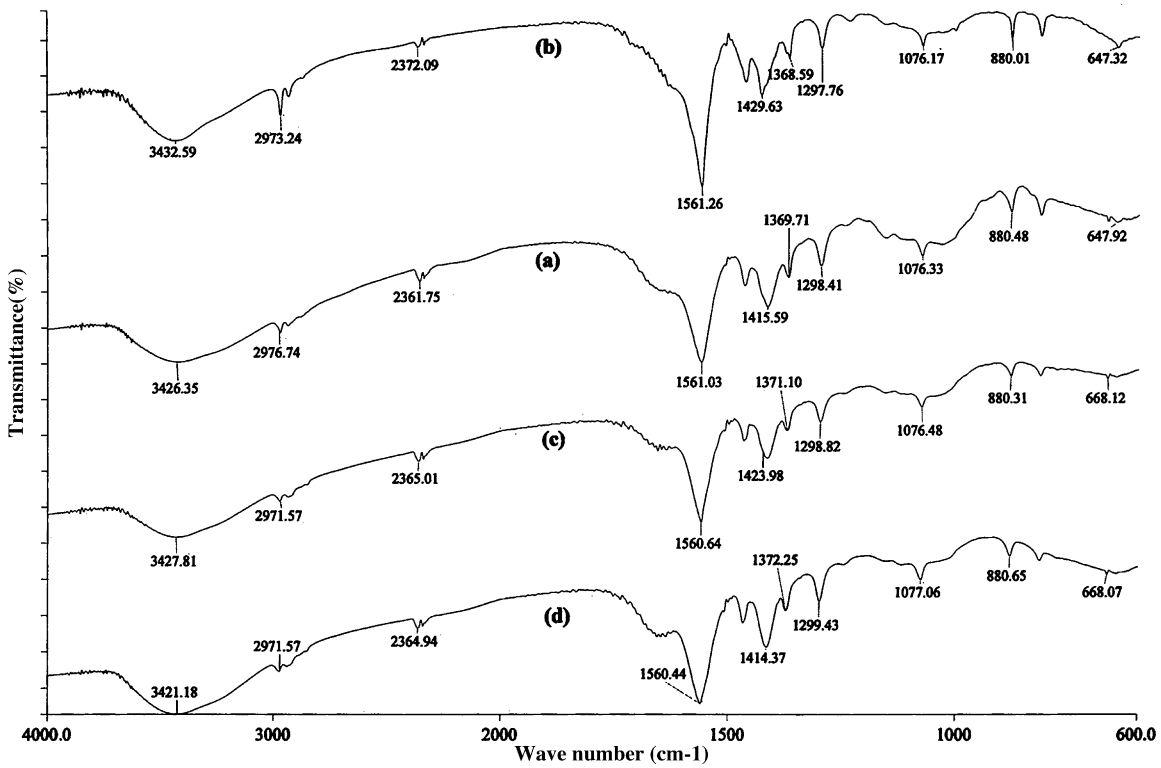

Fig. 1. FTIR spectra of (a) gellan gum; (b) blank Al^{+3}/gellan bead; (c) GA-treated blank Al^{+3}/gellan bead; and (d) GA-treated gellan gum.

same peak shifted to a higher absorption frequency 1429.63 cm^{-1}, and this indicates ionic interaction between $–COO^-$ and Al^{+3} ions. In the IR spectra of GA-treated blank beads (Fig. 1c) and GA-treated gellan gum (Fig. 1d), the ethereal stretching appeared at 1298.82 cm^{-1} and 1299.43 cm^{-1}, respectively (Mundargi et al., 2010). Such ethereal stretching vibrations were also evident at marginally lower wave numbers in the IR spectra of Gelrite and GA-untreated beads. This suggested that there was a contribution from GA that gave birth to acetal linkages *in situ*. Moreover, the COO– peak shifted to a higher wave number 1423.98 cm^{-1} for the GA-treated beads. In contrast, the same was observed at a lower frequency (1414.37 cm^{-1}) for the films than that observed for GA-treated and -untreated beads. Hence, it was believed that GA-treatment induced covalent linkages in addition to ionic linkages.

When the gellan dispersion comes in contact with $AlCl_3$ solution, Na^+, K^+, H^+ ions in the droplets are substituted by Al^{+3} ions thus yielding a spherical gel particle. The gelation starts instantly from the periphery of the droplets and the circular boundary constituting the border of ion exchange between Na^+, K^+, H^+ and Al^{+3} ions, progressed inwards with time and finally disappeared. Soon the process of gelation or curing could be visually detected as the appearance of a translucent spherical figure, followed by contraction of the figure (Yotsuyanagi, Ohkubo, Ohhashi, & Ikeda, 1987). Based on the visual observation, the time required for complete disappearance of the border was 10 min and this was considered the time of curing.

3.2. Size, morphology and drug entrapment efficiency

The effect of $AlCl_3$ concentration: 1%, 3%, and 5% (w/v), on the properties of beads such as their size, shape, and morphology, and drug entrapment efficiency was studied. When the sol droplets of gellan gum came into contact with salt solutions, the drops immediately gelled to shape spherical beads. While suspended in the salt solution, and immediately upon washing and isolation, all the beads look spherical having smooth surfaces (micrographs not shown). Even when air-dried to constant weight, the spherical nature of the beads did not change appreciably. The bead surface was periodically changed during drying operation otherwise they became a little flat on the side on which they sat. Scanning electron microscopy revealed that the dried beads were more or less spherical in shape at all concentrations of $AlCl_3$ (Fig. 2a–c). At all studied concentrations, the surface of the dried beads was smooth. The drug entrapment efficiency of the beads decreased from 97.67% to 83.05% with the increase in $AlCl_3$ concentration (Table 1). Such difference in drug entrapment efficiency was statistically significant ($p < 0.05$). Reddy and Tammishetti (2002) had the similar experiences. They reported that increase in $AlCl_3$ concentration from 0.08 M to 0.16 M decreased the bovine serum albumin entrapment efficiency of carboxymethyl guar gum beads from 85% to 76%. Another report indicated that higher concentration of calcium chloride (1.0–5.0 M) decreased the drug encapsulation efficiency of gellan beads (41.71–6.22%) in nearly proportional manner (Singh & Kim, 2005). It is assumed that as gelation proceeds water is expelled due to cationic cross-linking. Therefore, the higher the degree of cross-linking, the higher is the water loss. The expulsion of water will cause convective loss of drug molecules from the gellan beads during incubation into gelation medium (Ostberg, Lund, & Graffner, 1994).

It was found from the particle size data that the standard deviation was quite low for each of the formulations prepared under different conditions. As the beads were obtained by extruding the gellan solution through a 21-gauge flat-tipped needle into ionic cross-linking medium, they exhibited a mono-modal and narrow size distribution. However, the mean diameter of beads decreased from 1.18 ± 0.04 mm to 1.08 ± 0.04 mm as the $AlCl_3$ concentration was increased and this may be due to contraction of the beads (Table 1). A similar finding was reported by Hosny and Al-Helw (1998). They showed that an increase in $AlCl_3$ con-

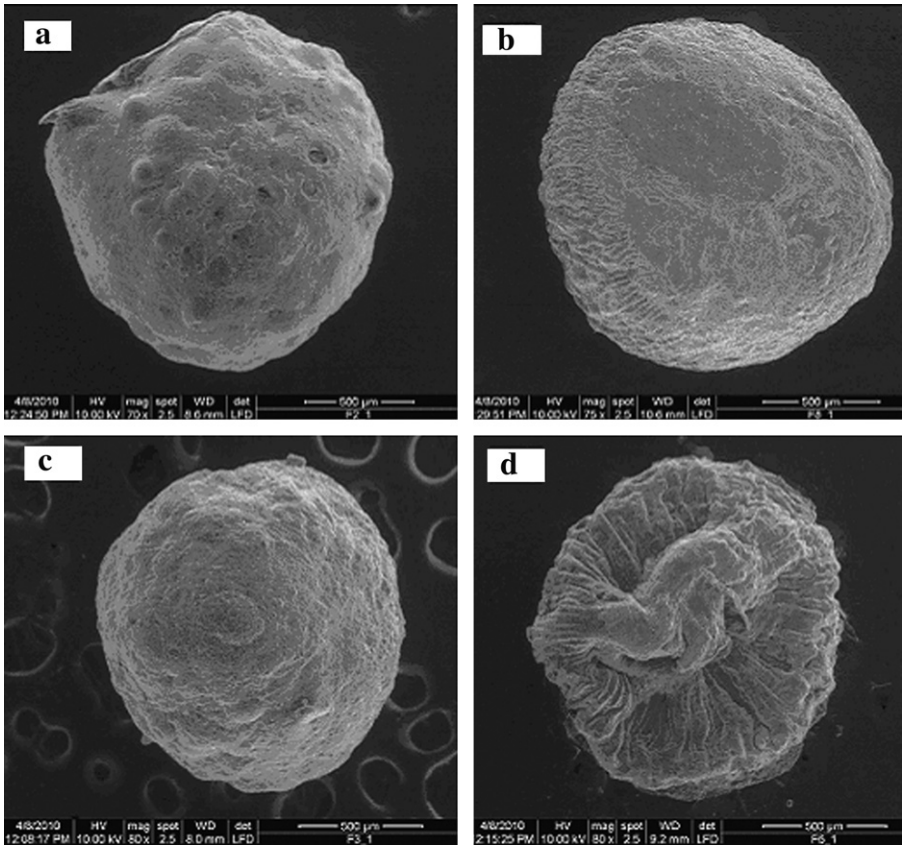

Fig. 2. Scanning electron micrographs of the glipizide-loaded gellan beads. Key: Concentration of $AlCl_3$: (a) 1%; (b) 3%; (c) 5%; and (d) GA-treated bead.

centration from 20% to 60% (w/v) decreased the mean diameter of diclofenac sodium-loaded aluminum carboxymethyl cellulose beads from 2.30 mm to 1.80 mm. Babu et al. (2010) found that the mean diameter of gellan beads decreased (2.80–2.40 mm) with increasing concentration of calcium chloride (0.1–0.4 M).

The effect of GA on the properties of beads was also studied. The surface of GA-treated beads appeared to be wrinkled and was rough in nature when observed under scanning electron microscope. Moreover, the diameter of beads reduced further (Table 1). This might be due to higher degree of cross-linking of the gellan matrix (Fig. 2d). The drug entrapment efficiency of GA-treated beads decreased in comparison to untreated beads (Table 1).

3.3. Swelling behaviors

The swelling studies were performed with the blank GA-treated and -untreated beads to understand the drug release kinetics. Following oral administration, the beads are presented to the gastric fluid and then emptied into intestinal fluid. The pH of the gastrointestinal tract varies from pH around 1.2 (gastric fluid) to pH around 7.4 (intestinal fluid). That is why solutions like the simulated gastric fluid (KCl/HCl buffer) at pH 1.2 and the simulated intestinal fluid (phosphate buffer) at pH 7.4 were the media of choice to study the swelling behaviors of the beads and then, the corresponding release kinetics of drug glipizide from the particles. *In vitro* drug release studies are usually carried out in a limited dissolution medium and hence, the drug release slows down after sometime due to continuous build-up of the concentration of drug in the bulk. At equilibrium, the carrier does not release its total drug load. However, under *in vivo* conditions, there is no concentration build-up in the bulk of the solution because once the drug dissolves; it is absorbed into the systemic circulation. Thus, *in vitro* drug release study must always be carried out under sink conditions. This was achieved by bathing the dissolving solid in fresh solvent from time to time (see Section 2.7).

To establish the reference parameters which should then help to understand the release kinetics of drug from the beads, the swelling study was conducted with blank beads in pH 7.4 phosphate buffer and pH 1.2 KCl/HCl buffer. To explain the drug release behaviors of the beads, the swelling study was conducted with blank beads in pH 7.4 phosphate buffer and pH 1.2 KCl/HCl buffer solutions for 1.5 h (Fig. 3). It was observed that the swelling tendencies of the beads decreased in either of the swelling medium with the increase in $AlCl_3$ concentration. The swelling ratios of the beads in weakly

Table 1
Effect of $AlCl_3$ concentration on drug entrapment efficiency, mean diameter, and drug release kinetics of gellan beads.

$AlCl_3$(% w/v)	Conc. of GA(% v/v)	Entrapment efficiency (%) ± SD, $n = 3$	Particle size($\times 10^3$ μm) ± SD	Korsmeyer–Peppas model		
				k	n	[a] r^2
1.0	0	97.67 ± 1.00	1.18 ± 0.04	0.0535	0.4493	0.9755
3.0	0	87.72 ± 1.17	1.13 ± 0.05	0.0273	0.5568	0.9756
5.0	0	83.05 ± 2.25	1.08 ± 0.04	0.0215	0.5635	0.9639
5.0	4.5	73.17 ± 2.32	1.02 ± 0.02	0.0003	1.2694	0.9845

[a] r^2 is correlation coefficient.

Table 2
Effect of $AlCl_3$ concentration on swelling ratio and drug release behaviors of glipizide-loaded gellan beads in pH 7.4 phosphate buffer and pH 1.2 KCl/HCl buffer solution.

$AlCl_3$(% w/v)	Conc. of GA(% v/v)	Drug release in alkaline medium, Mean ± SD		Drug release in acidic medium, Mean ± SD	Swelling ratio in 1.5 h, Mean ± SD	
		% Drug release in 2 h	$t_{50\%}$ (h)	% Drug release in 2 h	Alkaline medium	Acidic medium
1.0	0	46.67 ± 1.96	2.34 ± 0.14	9.99 ± 0.36	3.95 ± 0.12	1.61 ± 0.05
3.0	0	40.61 ± 1.55	3.27 ± 0.08	7.17 ± 0.05	3.42 ± 0.26	1.47 ± 0.14
5.0	0	37.90 ± 1.77	5.54 ± 0.36	6.21 ± 0.11	3.26 ± 0.25	1.12 ± 0.12
5.0	4.5	14.87 ± 0.71	6.27 ± 0.15	4.16 ± 0.11	1.29 ± 0.06	0.93 ± 0.14

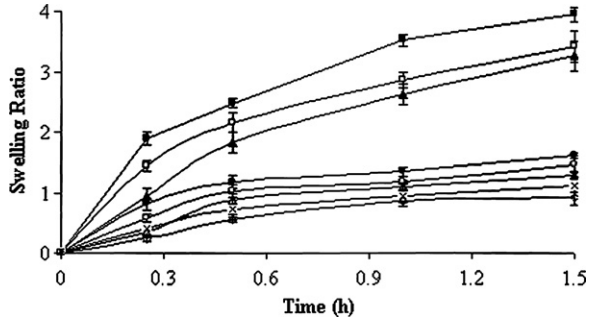

Fig. 3. Release profiles of glipizide-loaded gellan beads in pH 7.4 phosphate buffer solution. Key: Concentration of $AlCl_3$: (■) 1%; (□)) 3%; (▲) 5%; and (△) GA-treated Al^{+3}/gellan bead.

alkaline medium have been tabulated (Table 2), and it was estimated that gradual increase in $AlCl_3$ concentration to 5% reduced the swelling ratio by 17.47%. GA-treatment of the beads (prepared with 5% $AlCl_3$) suppressed the swelling ratio further by 60.43% in the same medium. On the other hand, the swelling of the beads was slower in the acidic medium than in weakly alkaline medium. In comparison to weakly alkaline medium, the swelling ratio of the beads in acidic medium became lower by 59.24%, 57.02%, and 65.64% at 1.5 h, respectively with their increasing $AlCl_3$ concentration (Table 2). However, the same was 27.90% lower for GA-treated beads in acidic medium. This was reflected in differences in cumulative percentage drug release of the beads as a function of $AlCl_3$ concentration.

3.4. Drug release kinetics

To modulate the drug release rate, the concentration of cationic cross-linker was varied. The three $AlCl_3$ concentrations studied within the range from 1% to 5% were 1%, 3% and 5%. *In vitro* drug release profiles of the beads in pH 7.4 phosphate buffer solution have been illustrated in Fig. 4. The drug release profiles of the beads were always higher at low salt concentration. The beads released their 46.67 ± 1.96%, 40.61 ± 1.55%, and 37.90 ± 1.77% drug load in 2 h, when the concentration of $AlCl_3$ was increased gradually (Table 2). In order to compare the drug release rate, a time point approach was adopted. The values of $t_{50\%}$ (i.e. time required for the release of 50% drug load) were calculated, and were 2.34 ± 0.14, 3.27 ± 0.08, and 5.68 ± 0.36 h, respectively in the order of their increasing $AlCl_3$ concentration (Table 2). The differences in their $t_{50\%}$ values were statistically significant ($p < 0.05$). In acidic medium, the drug release was 9.99 ± 0.36%, 7.17 ± 0.05%, and 6.21 ± 0.11% in 2 h, respectively with increasing metal ion concentration (Table 2) and resulted in a statistically significant difference in their drug release rate ($p < 0.05$). Keeping in mind the variable pH of gastrointestinal tract, the beads were exposed initially to pH 1.2 KCl/HCl buffer solution for 2 h and then to pH 7.4 phosphate buffer solution for another 6 h. *In vitro* drug release profiles of the beads have been illustrated in Fig. 5.

Subsequent exposure of the beads to phosphate buffer solution led to faster drug release (releasing their 25.18%, 22.61%, and 20.73% content in next 2 h, in accordance with increasing $AlCl_3$). In last 4 h, all the beads released about 34–39% drug in the weakly alkaline buffer solution. Thus it was evident that when the beads were put directly in weakly alkaline medium, the drug release was faster than when exposed successively from acidic to weakly alkaline medium. The Lewis acid character of Al^{+3} ions causes disruption of aluminum gellan gel matrix faster in phosphate buffer solution due to the chelating action of the phosphate ions. Dainty and his associates explained similarly the phenomena of faster drug release from calcium alginate gel matrix in phosphate buffer solution (Dainty, Goulding, Robinson, Sinpkins, & Trevan, 1986). The data in Table 2 indicated that the drug release from the beads was slower in acidic medium than in weakly alkaline dissolution medium at all preparative conditions. Moreover, the drug release could be extended up to 8 h or more for GA-treated and -untreated beads. The slower drug release in pH 1.2 KCl/HCl buffer solution from Al^{+3}/gellan beads could be attributed to the limited solubility of Al^{+3}/gellan beads into the acidic pH condition as well as the weakly acidic nature of the drug. The gellan beads contain carboxyl groups and the ionization of this group leads to hydrogel that would respond to changes in environmen-

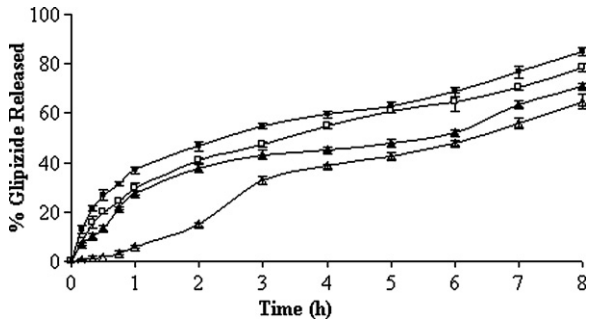

Fig. 4. Release profiles of glipizide-loaded gellan beads in pH 1.2 KCl/HCl buffer solution for 2 h and subsequently in pH 7.4 phosphate buffer solution for 6 h. Key: Concentration of $AlCl_3$: (■) 1%; (□) 3%; (▲) 5%; and (△) GA-treated Al^{+3}/gellan bead.

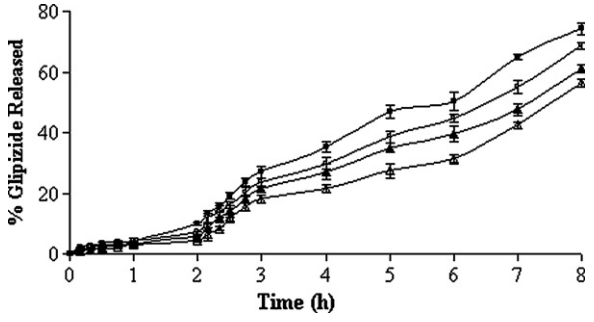

Fig. 5. Swelling behavior of the gellan beads in different dissolution media. Swelling study in pH 7.4 phosphate buffer: Key: Concentration of $AlCl_3$: (■) 1%; (□) 3%; (▲) 5%; and (△) GA-treated Al^{+3}/gellan bead. Swelling study in pH 1.2 KCl/HCl buffer: Key: Concentration of $AlCl_3$: (●) 1%; (○) 3%; (×) 5%; and (◊) GA-treated Al^{+3}/gellan bead.

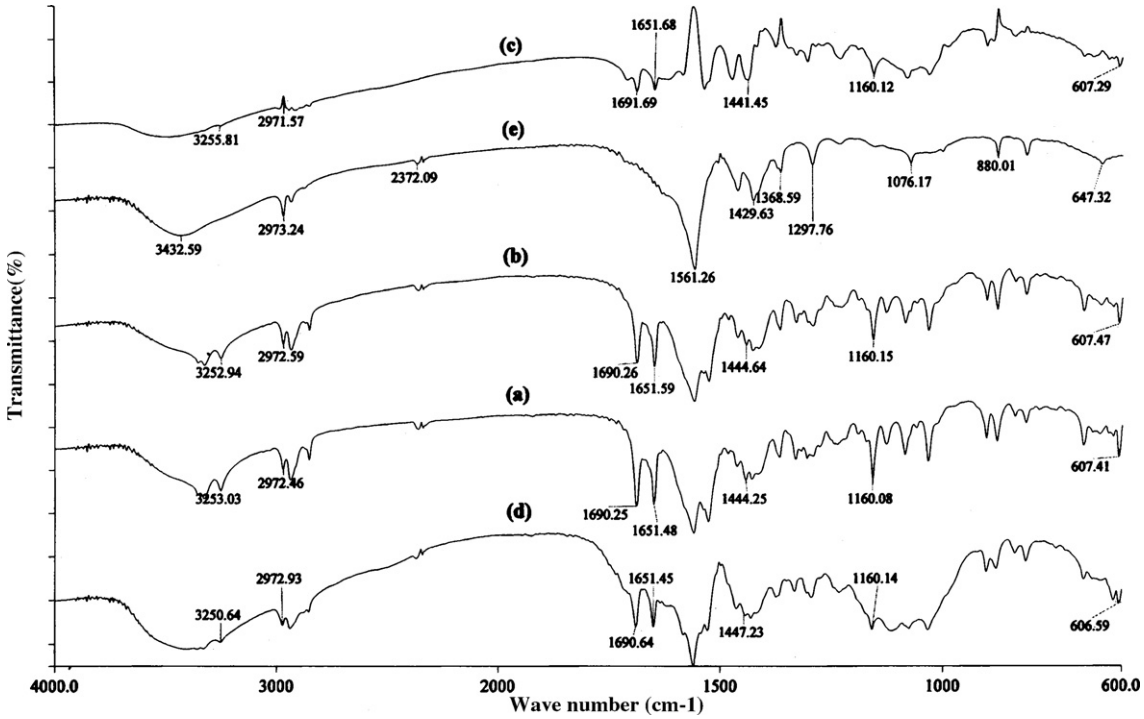

Fig. 6. FTIR spectra of (a) pure glipizide; (b) physical mixture of gellan and glipizide; (c) glipizide-loaded Al^{+3}/gellan bead; (d) GA-treated glipizide-loaded Al^{+3}/gellan bead; and (e) blank Al^{+3}/gellan bead.

tal pH. At higher pH values, the carboxyl functional groups of the beads undergo ionization and consequently, the osmotic pressure inside the beads increases resulting in higher swelling. The shift of the carboxyl groups to a unionized form which is thermodynamically favorable in the acidic environment; result in loss of the electrostatic repelling force and hence, lower swelling of the beads. However, the swelling depends upon the cross-link density. At low cross-link density, the hydrogel network is loose with a greater hydrodynamic free volume and can adsorb more of the solvent resulting in higher swelling. As aluminum ions are depleted by the ion exchange with sodium ions in the weakly alkaline dissolution medium, electrostatic repulsion between the carboxylate anions accelerates the swelling of the Al^{+3}/gellan gel beads and results in faster drug release in phosphate buffer solution. The faster drug release from calcium alginate matrix has been explained similarly (Kikuchi, Kawabuchi, Sugihara, & Sakurai, 1997). Thus, it was inferred that the pH-sensitive swelling of the beads was responsible for the faster or slower drug release in different dissolution medium.

The mechanical strength and permeability barrier of hydrogel network beads, prepared using 5% $AlCl_3$, was modified by covalent cross-linking with GA. Within a same time frame of 2 h, the GA-treatment of the beads prepared with 5% $AlCl_3$ suppressed the drug release by 60.77% in weakly alkaline dissolution medium (Fig. 4). The value of $t_{50\%}$ was 6.27 ± 0.15 h and thus comparatively higher for GA-treated beads.

GA-treatment of the beads (prepared with 5% $AlCl_3$), reduced the drug release in acidic medium by 33.01% in the same duration. When the GA-reinforced beads were transferred from acidic to weakly alkaline medium, they released 17.47% drug load in 2 h (Fig. 5). In addition to ionic cross-links, GA gave birth to covalent acetal linkages and slowed the drug release in both dissolution media than those having ionic linkages only. GA substituted part of the hydroxyl groups and the cross-linking causes a change in the polymer structure (Lindblad & Albertsson, 2005). It is noteworthy to mention that the beads released its load without any sign of disintegration either in acidic or alkaline media.

There are many mathematical models to explain the mechanism of drug release such as Hixson–Crowell, Higuchi model. But these models fail to explain the drug release that is due to swelling (upon hydration) along with gradual erosion of the matrix i.e. when more than one type of release phenomena could be involved. It was stated above that the Al^{+3}/gellan beads respond to environmental pH and exhibit different degrees of swelling in different dissolution medium (see discussion above in Section 3.3).

Therefore, to explain the drug release mechanism, the release data obtained for the beads in pH 7.4 phosphate buffer solution was fitted to the well known Korsmeyer–Peppas model (see Eq. (1) and discussion in Section 2.9) (Korsmeyer et al., 1983; Ritger & Peppas, 1987). Irrespective of $AlCl_3$ concentration, the values of diffusion exponent, n lies between 0.43 and 0.85 (Table 1). Thus it was suggested that the drug release from the Al^{+3} ion cross-linked beads followed anomalous transport mechanism i.e. the drug release occurs due to a coupling of Fickian diffusion and polymer relaxation (see Section 2.9). It is interesting to note that following GA-treatment, the drug release mechanism shifted to super case II transport because the value of diffusion exponent, n was 1.2694 (Table 1). For systems exhibiting super case II transport, the dominant mechanism for drug transport was polymer relaxation as the gels swells. This mechanism also explains the initial slow release where the polymer was not completely hydrated, resulting in an incomplete relaxation of the side chains. Insufficient hydration would lead to the creation of a channel/pore network difficult through which the free diffusion of the drug would be hindered. Upon complete hydration, the polymer began to swell with subsequent relaxation of the side chains.

3.5. Compatibility of drug in the beads

The drug–polymer interaction was studied by FTIR spectroscopy. IR stretching bands of pure glipizide (Fig. 6a) were observed at 3253.03 cm^{-1} (N–H stretching), 2972.46 cm^{-1} (C–H stretching of methylene group), 1690.25 cm^{-1} (C=O), 1651.48 cm^{-1} (–CONH), 1444.25 cm^{1} (substituted cyclohexane),

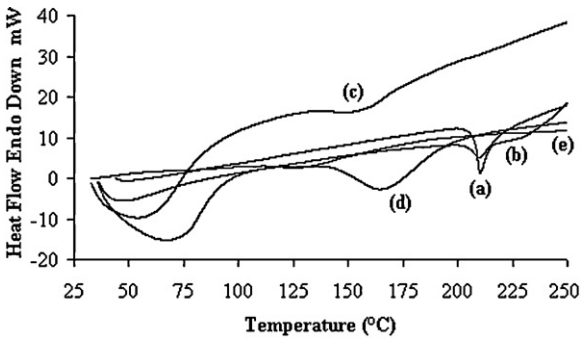

Fig. 7. DSC thermograms of (a) pure glipizide; (b) physical mixture; (c) glipizide-loaded Al^{+3}/gellan beads; (d) GA-treated Al^{+3}/gellan beads containing glipizide; and (e) blank Al^{+3}/gellan beads.

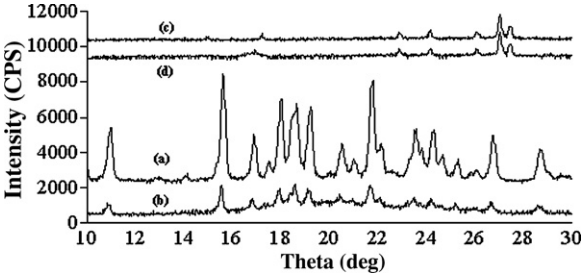

Fig. 8. X-ray diffraction patterns of (a) pure glipizide; (b) physical mixture; (c) GA-untreated beads; and (d) GA-treated beads.

1160.08 cm^{-1} (sulfate group), 607.41 cm^{-1} (disubstituted benzene) (Tiwari, Tiwari, Srivastava, & Rai, 2008). These bands were also identified in both the physical mixture (Fig. 6b) as well as drug-loaded beads (Fig. 6c and d) with minor differences in frequencies. This suggested that there was no interaction between drug and polymer.

The physical state of the drug was examined through DSC analysis. DSC traces of pure glipizide exhibited a sharp endothermic peak at 210.35 °C corresponding to its melting transition point (Fig. 7a). The physical mixture of drug and polymer did show weaker melting endotherm of glipizide (Fig. 7b). However, GA-treated and -untreated beads did not show any endothermic peak of drug close to its melting point (Fig. 7c and d). The early endothermic peaks probably resulted from the evaporation of water. XRD pattern of pure glipizide showed the important crystallographic characteristics of pure glipizide at different scattering angles from 10° to 30° with different signal intensities (Fig. 8a). The physical mixture also showed weak signals at the respective scattering angles to that observed with pure glipizide (Fig. 8b). However, the signal intensities were very weak for GA-untreated and -treated gellan beads (Fig. 8c and d). Thus, the thermal behavior coupled with the X-ray crystallographic data suggested that the degree of crystallinity of glipizide reduced and most of the drug existed in amorphous state and distributed homogenously in the beads.

4. Conclusion

Trivalent Al^{+3} ions could form spherical beads by ionotropic gelation with gellan polysaccharide. The concentration of gelling ions affected the properties of the network beads especially their *in vitro* drug release behaviors in different pH medium conditions. The drug release was slower in weakly alkaline media than in acidic media. GA-treatment of the beads led to chemical cross-linking and slowed the drug release. Both the GA-treated and -untreated beads were able to control the drug release for a prolonged period of time. The beads did not break down when the pH of the dissolution medium was altered from acidic to weakly alkaline, and released the drug for a prolonged period. The drug release mechanism of Al^{+3} ion cross-linked beads deviated from anomalous transport to super case II transport by further achieving GA-treatment. The stable, amorphous nature of the drug in the physical/chemical cross-linking gellan beads has also been identified in the beads. Thus, GA-treated and -untreated beads had the potential for oral controlled delivery applications which could not only minimize dosing frequency but also dose-related side effects.

Acknowledgments

The authors wish to thank all the management and faculty members of Gupta College of Technological Sciences, Asansol, West Bengal, India and the authority of Jadavpur University, Department of Pharmaceutical Technology, Kolkata, India, for their kind cooperation and facilities provided to carry out the present research work.

References

Acarturk, F., & Takka, S. (1999). Calcium alginate microparticles for oral administration: II effect of formulation factors on drug release and drug entrapment efficiency. *Journal of Microencapsulation*, *16*, 291–301.
Agnihotri, S. A., & Aminabhavi, T. M. (2005). Development of novel interpenetrating network gellan gum-poly (vinyl alcohol) hydrogel microspheres for the controlled release of carvedilol. *Drug Development and Industrial Pharmacy*, *31*, 491–503.
Agnihotri, S. A., Jawalkar, S. S., & Aminabhavi, T. M. (2006). Controlled release of cephalexin through gellan gum beads: Effect of formulation parameters on entrapment efficiency, size, and drug release. *European Journal of Pharmaceutics and Biopharmaceutics*, *63*, 249–261.
Anal, A. K., & Stevens, W. F. (2005). Chitosan-alginate multilayer beads for controlled release of ampicillin. *International Journal of Pharmaceutics*, *290*, 45–54.
Aydin, Z., & Akbuğa, J. (1996). Chitosan beads for the delivery of salmon calcitonin: Preparation and release characteristics. *International Journal of Pharmaceutics*, *131*, 101–103.
Babu, R. J., Sathigari, S., Kumar, M. T., & Pandit, J. K. (2010). Formulation of controlled release gellan gum macro beads of amoxicillin. *Current Drug Delivery*, *7*, 36–43.
Bhardwaj, T. R., Kanwar, M., Lal, R., & Gupta, A. (2000). Natural gums and modified natural gums as sustained-release carriers. *Drug Development and Industrial Pharmacy*, *26*(10), 1025–1038.
Bodmeier, R., Oh, K.-H., & Pramar, Y. (1989). Preparation and evaluation of drug-containing chitosan beads. *Drug Development and Industrial Pharmacy*, *15*, 1475–1494.
Dainty, A. L., Goulding, K. H., Robinson, P. K., Sinpkins, I., & Trevan, M. D. (1986). Stability of alginate-immobilized algal cells. *Biotechnology and Bioengineering*, *28*, 210–216.
Das, M. K., & Senapati, P. C. (2008). Furosemide-loaded alginate microspheres prepared by ionic cross-linking technique: Morphology and release characteristics. *Indian Journal of Pharmaceutical Sciences*, *70*, 77–84.
Davis, S. S., Hardy, J. G., Taylor, M. J., Whalley, D. R., & Wilson, C. G. (1984). A comparative study of the gastrointestinal transit of a pellet and tablet formulation. *International Journal of Pharmaceutics*, *21*, 167–177.
Doner, L. W., & Douds, B. D. (1995). Purification of commercial gellan to mono gellan cation salts results in acute modification of solution and gel forming properties. *Carbohydrate Research*, *273*, 225–233.
El Fattah, E. A., Grant, D. J., Gabr, K. E., & Meshali, M. M. (1998). Physical characteristics and release behavior of salbutamol sulfate beads prepared with different ionic polysaccharides. *Drug Development and Industrial Pharmacy*, *24*, 541–547.
Follonier, N., & Doelkar, E. (1992). Biopharmaceutical comparison of oral multiple unit and single unit sustained release dosage forms. *STP Pharma Sciences*, *2*, 141–158.
Gupta, K. C., & Ravi, K. M. N. V. (2000). Drug release behavior of beads and microgranules of chitosan. *Biomaterials*, *21*, 1115–1119.
Halder, A., Maiti, S., & Sa, B. (2005). Entrapment efficiency and release characteristics of polyethyleneimine-treated or-untreated calcium alginate beads loaded with propranolol–resin complex. *International Journal of Pharmaceutics*, *302*, 84–94.
Hosny, E. A., & Al-Helw, A. A-R. M. (1998). Effect of coating of aluminium carboxymethylcellulose beads on the release and bioavailability of diclofenac sodium. *Pharmaceutica Acta Helvetiae*, *72*, 255–261.
Izumi, Y., Kikuta, N., Sakai, K., & Takezawa, H. (1996). Phase diagrams and molecular structures of sodium-salt-type gellan gum. *Carbohydrate Polymers*, *30*(2–3), 75–218.
Jamzad, S., & Fassihi, R. (2006). Development of controlled release low dose class II drug-glipizide. *International Journal of Pharmaceutics*, *312*, 24–32.
Jansson, P. E., Lindberg, B., & Sandford, P. A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseuomonas elodea*. *Carbohydrate Research*, *124*, 135–139.

Kanesaka, S., Watanabe, T., & Matsukawa, S. (2004). Binding effect of Cu^{2+} as a trigger on the sol-to-gel and the coil-to-helix transition processes of polysaccharide, gellan gum. *Biomacromolecules*, *5*, 863–868.

Kang, K. S., Veeder, G. T., Mirrasoul, P. J., Kaneko, T., & Cottrell, I. W. (1982). Agar-like polysaccharide produced by pseudomonas species: Production and basic properties. *Applied Environmental Microbiology*, *43*, 1086–1091.

Kedzierewicz, F., Lombry, C., Rios, R., Hoffman, M., & Maincent, P. (1999). Effect of the formulation on the in-vitro release of propranolol from gellan beads. *International Journal of Pharmaceutics*, *178*, 129–136.

Khromova, Y. L. (2006). The effect of chlorides on alginate gelation in the presence of calcium sulfate. *Colloid Journal*, *68*(1), 115–119.

Kikuchi, A., Kawabuchi, M., Sugihara, M., & Sakurai, Y. (1997). Pulsed dextran release from calcium-alginate gel beads. *Journal of Controlled Release*, *47*, 21–29.

Kikuchi, A., Kawabuchi, M., Watanabe, A., Sugihara, M., Sakurai, Y., & Okano, T. (1999). Effect of Ca^{2+}-alginate gel dissolution on release of dextran with different molecular weights. *Journal of Controlled Release*, *58*, 21–28.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983). Mechanism of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics*, *15*, 25–35.

Kuo, M. S., Mort, A. J., & Dell, A. (1986). Identification and location of L-glycerate, an unusual acyl substituent in gellan gum. *Carbohydrate Research*, *156*, 173–187.

Lindblad, M. S., & Albertsson, A. C. (2005). Chemical modification of hemicelluloses and gums. In S. Dumitriu (Ed.), *Polysaccharides: Structural diversity and functional versatility* (p. 502). New York: Marcel Dekker, Inc.

Morris, V. J. (1995). Bacterial polysaccharides. In A. M. Stephen (Ed.), *Food polysaccharides and their application* (pp. 341–375). New York: Marcel Dekker, Inc.

Mundargi, R. C., Shelke, N. B., Babu, V. R., Patel, P., Rangaswamy, V., & Aminabhavi, T. M. (2010). Novel thermo-responsive semi-interpenetrating network microspheres of gellan gum-poly (N-isopropylacrylamide) for controlled release of atenolol. *Journal of Applied Polymer Science*, *116*, 1832–1841.

Ostberg, T., Lund, E. M., & Graffner, C. (1994). Calcium alginate matrixes for oral multiple unit administration: IV. Release characteristics in different media. *International Journal of Pharmaceutics*, *112*, 241–248.

Patil, J. S., Kamalapur, M. V., Marapur, S. C., & Kadam, D. V. (2010). Ionotropic gelation and polyelectrolyte complexation: The novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review. *Digest Journal of Nanomaterials and Biostructures*, *5*, 241–248.

Patil, S., Sharma, S., Nimbalkar, A., & Pawar, A. (2006). Study of formulation variables on properties of drug-gellan beads by factorial design. *Drug Development and Industrial Pharmacy*, *32*, 315–326.

Rajinikanth, P. S., & Mishra, B. (2007). Preparation and in vitro characterization of gellan based floating beads of acetohydroxamic acid for eradication of *H. pylori*. *Acta Pharmaceutica*, *57*, 413–427.

Reddy, T., & Tammishetti, S. (2002). Gastric resistant microbeads of metal ion cross-linked carboxymethyl guar gum for oral drug delivery. *Journal of Microencapsulation*, *19*(3), 311–318.

Rees, D. A. (1970). Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. *Advances in Carbohydrate Chemistry and Biochemistry*, *24*, 267–332.

Ritger, P. I, & Peppas, N. A. (1987). A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release*, *5*, 37–42.

Singh, B. N., & Kim, K. H. (2005). Effects of divalent cations on drug encapsulation efficiency of deacylated gellan gum. *Journal of Microencapsulation*, *22*(7), 761–771.

Sriamornsak, P., Puttipipatkhachorn, S., & Prakongpan, S. (1997). Calcium pectinate gel coated pellets as an alternative carrier to calcium pectinate beads. *International Journal of Pharmaceutics*, *156*, 189–194.

Srinatha, A., Pandit, J. K., & Singh, S. (2008). Ionic cross-linked chitosan beads for extended release of ciprofloxacin: In vitro characterization. *Indian Journal of Pharmaceutical Sciences*, *70*, 16–21.

Sweetman, S. C. (2005). *Martindale the complete drug reference* (34th ed.). London: Pharmaceutical Press., pp. 324–348.

Tiwari, G., Tiwari, R., Srivastava, B., & Rai, A. K. (2008). Development and optimization of multiunit solid dispersion system of poorly water soluble drug. *Research Journal of Pharmacy and Technology*, *1*, 444–449.

Ueng, S. W. N., Lee, S.-S., Lin, S.-S., Chan, E.-C., Hsu, B. R.-S., & Chen, K.-T. (2000). Biodegradable alginate antibiotic beads. *Clinical Orthopaedics Related Research*, *380*, 250–259.

Verma, R. K., & Garg, S. (2004). Development and evaluation of osmotically controlled oral drug delivery system of glipizide. *European, Journal of Pharmaceutics and Biopharmaceutics*, *57*, 513–525.

Yegin, B. A., Moulari, B., Durlu-Kandilci, N. T., Korkusuz, P., Pellequer, Y., & Lamprecht, A. (2007). Sulindac loaded alginate beads for a mucoprotective and controlled drug release. *Journal of Microencapsulation*, *24*, 371–382.

Yotsuyanagi, T., Ohkubo, T., Ohhashi, T., & Ikeda, K. (1987). Calcium-induced gelation of alginic acid and pH-sensitive reswelling of dried gels. *Chemical and Pharmaceutical Bulletin*, *35*, 1555–1563.