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Formulation and *In Vitro* Characterization of Sodium Alginate-Gellan Beads of Glipizide

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Abstract

Glipizide beads of sodium alginate alone and in combination with gellan were prepared by ionotropic gellation method. The prepared beads were evaluated for their physico-chemical characteristics like particle diameter, surface morphology, encapsulation efficiency, swelling and gelling rate and *in vitro* drug release characteristics. The effect of various formulation variables like polymer concentration (sodium alginate, and proportion of gellan in beads prepared by a combination of sodium alginate and gellan), drug loading, drying conditions, crosslinking agent concentration and curing time on *in vitro* dissolution of the prepared beads were evaluated. The results showed that both the diameter and the encapsulation efficiency of beads proportionally were increased with the increase in polymer concentration. In case of beads containing both sodium alginate and gellan, the mean diameter and the encapsulation efficiency were higher than the corresponding beads containing only alginate, and both were increased with an increase in proportion of gellan. The prepared beads were spherical in shape and were successful in sustaining drug release for 8 hours. Incorporation of gellan caused a significant decrease in drug release. The drug release followed a biphasic profile, in all cases, characterized by an initial phase of high drug release followed by a phase of moderate release. Further, the kinetic treatment of the drug release data revealed the prevalence of matrix diffusion kinetics.

Keywords: Glipizide, sodium alginate, gellan, controlled release, beads

Introduction

Over the past two decades, hydrogel polymers have attracted a great deal of attention for use as potential carriers in controlled and site-specific delivery of drugs

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(Alhaique, *et al.*, 1996 ; Risbud, *et al.*, 2000; Soppimath, *et al.*, 2002). Hydrogels are hydrophilic and three-dimensional network structures having the natural propensity to absorb large quantity of water or biological fluids and they resemble biological tissues. The ability of hydrogels to swell in the presence of water or biological fluids regulates the release of encapsulated drugs. By controlling the degree of swelling due to crosslinking makes them potential carriers of drugs for controlled release (CR) applications (Kudela, *et al.*, 1989; Hoffman, 2002). In view of their greater advantages than the synthetic polymers, the biocompatible polymers such as chitosan, sodium alginate, gellan gum, guar gum, etc have been widely used by researchers.

Alginate is a water soluble linear polysaccharide extracted from brown seaweed and is composed of alternating blocks of 1-4 linked α-L-guluronic (G) and β-D-mannuronic acid (M) residues. Alginate can be ionically cross linked by the addition of divalent cations in aqueous solution. It was reported that alginate is non toxic and biodegradable when given orally (Mumper *et al.*, 1994). It has been proposed that gelation takes place by forming an egg-box junction to associate the metal ions with the GG blocks of alginate chain (Grant *et al.*, 1973). Alginate has been used in food additives as a stabilizer and gelling agent and, also in pharmaceutical formulations as a viscosity enhancer, film former, polymer for sustaining drug release, antacid agent and wound protectant. In addition, it has been utilized for the immobilization of micro organisms, cells and enzymes by taking advantage of its gelling property. In the past two decades, alginate gel bead, which is a spherical gel prepared by dropping sodium alginate solution into calcium chloride solution, has received considerable attention as an alternative oral drug delivery vehicle for controlled-release preparations. Calcium-induced alginate gel beads (Alginate-Ca) have been developed in recent years as a unique vehicle for drug delivery. Alginate-Ca is rapidly formed by gelation of alginic acid in the presence of calcium ions and is able to incorporate some compounds such as drugs or polysaccharides in the gel matrix (Gaserod, *et al.*, 1998 ;Rajinikanth, *et al.*, 2003).

Gellan is a linear heteropolysaccharide produced aerobically by the bacterium *Auromonas (Pseudomonas) elodea*, renamed as *Sphingomonas paucimobilis* (Crescenzi, 1995; Miyazaki, *et al.*, 2001). The polysaccharide is one of a series of eight structurally close related bacterial polymers and consists of a linear structure of a repeating tetrasachharide unit of glucose, glucuronic acid and rhamnose in a molar ratio of 2:1:1. Gellan has been predominantly used in development of prolonged release ocular dosage forms, alone and in combination with sodium alginate (Rozier, *et al.*, 1989; Sanzgiri, *et al.*, 1993; Balasubramaniam and Pandit, 2003; Balasubramaniam *et al.*, 2004). Further, gellan has also been used for prolonging release of drugs from beads and microspheres, meant for oral delivery of drugs (Quigley and Deasy, 1992; El-Fattah *et al.*, 1998; Agnihotri and Jawalkar, 2005; Agnihotri *et al.*, 2006; Balasubramaniam *et al.*, 2006; Patil *et al.*, 2006).

Glipizide is an oral hypoglycemic drug belonging to sulfonyl urea class. It is well absorbed in the GI tract and has a plasma half-life of 2-4 hours, which makes it a

candidate for sustained release formulations. Some reported sustained release formulations of glipizide that have been reported include bioadhesive microspheres (Garcia and Ghali, 2001), osmotic controlled delivery systems etc (Varma and Garg, 2004). Thus, an attempt has been made in the present study to develop beads of Glipizide (a model of poorly water soluble drug) containing sodium alginate alone and in combination with gellan. The prepared beads were evaluated for different physical parameters and their drug release characteristics are reported.

Materials and Methods

Materials

Glipizide was purchased from Transchem Limited (Mumbai, India). Gellan gum (Gelrite®) was purchased from Sigma-Aldrich (Santiago, California, USA) and sodium alginate low viscosity grade (Keltone LV) from ISP International Corporation (Wayne NJ). All other chemicals and reagents used were of analytical grade and were procured commercially.

Preparation of Beads

Required quantities of sodium alginate either alone or in combination with gellan (Table 1) was added to 50 ml of water (when gellan was used, water was heated to 80°C with 0.125 % sodium citrate) and stirred on a magnetic stirrer until dissolved. Required quantities of glipizide was dispersed in 5-10 ml of methanol and the drug dispersion was added slowly to the alginate solution under stirring. Stirring was continued after complete addition until a uniform dispersion was obtained. The resultant dispersion was dropped through an 18G syringe needle into 150 ml of calcium chloride solution (varying concentrations), which was kept under stirring condition to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. The beads were then separated by filtration, after appropriate curing. They were washed with water and isopropanol and dried at room temperature or in an oven at 40°C, or under vacuum.

Particle size analysis and morphology

Particle size of the prepared beads were determined using an optical microscope (Carl Zeiss, Germany) fitted with a stage and an ocular micrometer. 20 beads were measured for calculating mean diameter of microspheres. The shape and surface morphology of various batches were determined by scanning electron microscopy (Super III A model, International Scientific Instruments, Militpas, CA, USA).

Determination of encapsulation efficiency

Dried beads equivalent to 20 mg of glipizide was dissolved in pH 6.8 phosphate buffer by soaking the beads for 2 hours, followed by sonication for 30 minutes. The resulting solution was filtered through a G2 glass filter. An aliquot of the filtrate was suitably diluted and the drug content was determined spectrophotometrically at 226 nm. Encapsulation efficiency was calculated as the percentage (w/w) of the theoretical drug content. Results were based on triplicate determination.

Table 1. Formulation variables of the prepared glipizide beads

Batch Code	Sodium alginate (% w/v)	Gellan (% w/v)	Glipizide (% w/v)	Calcium chloride (% w/v)
GP_1	1	-	1	5
GP_2	2.5	-	1	5
GP_3*	3.5	-	1	5
GP_4	5	-	1	5
GP_5	3.5	-	0.5	5
GP_6	3.5	-	2	5
GP_7	3.5	-	1	2.5
GP_8	3.5	-	1	7.5
GP_9	3.25	0.25	1	5
GP_{10}	3	0.5	1	5
GP_{11}	2.75	0.75	1	5
GP_{12}	2.5	1	1	5

* Beads of the same composition were prepared and dried under vacuum or oven dried at 40°C. In all other cases beads were air dried

In vitro release studies

The release studies were conducted using USP 24 dissolution apparatus I using 900 ml of pH 6.8 phosphate buffer as the dissolution medium maintained at 50 rpm and 37±0.5°C. At pre-determined time intervals 5ml samples were withdrawn and replaced with an equal volume of pre-warmed phosphate buffer. The withdrawn samples were diluted suitably and spectrophotometrically analyzed for glipizide content at 226 nm.

Measurement of gelling rate

The gelling rate was determined, as described by Tateshita *et al.*, 1993. Briefly, 20 drops/particles were collected, after dropping the drug-polymer dispersion into calcium chloride solution, at appropriate time intervals and weighed after removal of surface moisture with filter paper. Results were expressed as weight loss, corresponding to gelation due to water loss during matrix formation.

Measurement of swelling rate

The prepared beads were placed on a watch glass and pH 6.8 phosphate buffer was added. The swelling rate was determined by periodically measuring the diameter of beads. Measurements were made using an optical microscope (Carl Zeiss,

Germany) fitted with a stage and an ocular micrometer. The experiments were conducted in triplicate.

Results and Discussion

Evaluation of beads

Increasing the concentration of sodium alginate beyond 5.0 % w/v, resulted in formation of agglomerates and in case of combination systems, increasing the concentration of gellan beyond 1% w/v resulted in gelation of the polymer-drug dispersion. The physicochemical properties of the various batches of the prepared beads are shown in Table 2.

Morphology and diameter of prepared beads

The prepared beads were spherical in shape with rough surface as revealed by the SEM images shown in Figure 1.

The diameter of different dried glipizide beads are shown in Table 2. The size of beads varied from 0.78 to 1.22 mm for different batches. The results indicated that as the amount of sodium alginate in alginate based beads and gellan, in alginate-gellan based beads was increased, the mean bead diameter was also progressively increased. The increase in bead diameter was observed to be higher for batches containing varying proportions of gellan (GP_9 to GP_{12}) in comparison to batches containing only sodium alginate (GP_3). This could be attributed to increase in micro-viscosity of the polymeric dispersion due to increasing concentration of gellan, which eventually led to formation of bigger beads. However, the mean diameter of prepared beads was marginally increased with an increase in drug loading, while an increase in calcium chloride concentration did not result in any change in the mean diameter of the particle.

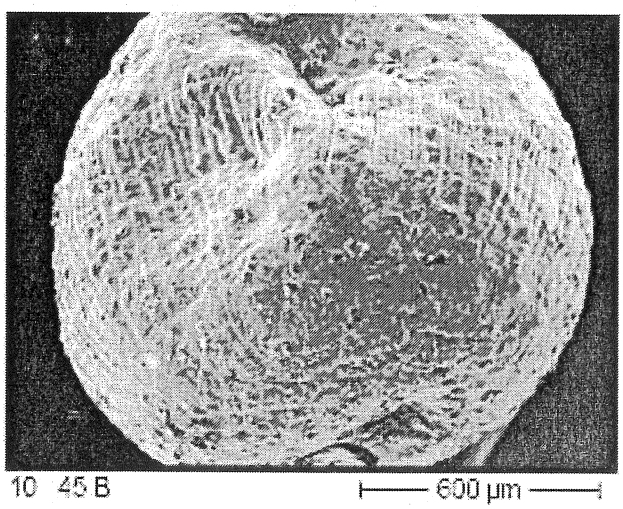


Fig. 1. SEM microphotograph of prepared glipizide beads

Table 2. Physicochemical characteristics of prepared glipizide beads

Batch code	Diameter of beads (mm) (** Mean ± SD)	Encapsulation efficiency (% w/w) (*Mean ± SD)
GP_1	0.78 ± 0.4	74.66 ± 1.92
GP_2	0.84 ± 0.4	79.36 ± 2.01
GP_3	0.93 ± 0.3	82.66 ± 1.89
GP_4	0.99 ± 0.6	86.11 ± 2.42
GP_5	0.89 ± 0.4	81.66 ± 2.12
GP_6	0.95 ± 0.3	83.88 ± 2.56
GP_7	0.94 ± 0.5	84.36 ± 2.45
GP_8	0.95 ± 0.6	84.11 ± 2.78
GP_9	1.01 ± 0.3	88.86 ± 1.08
GP_{10}	1.07 ± 0.4	90.88 ± 2.45
GP_{11}	1.15 ± 0.5	93.66 ± 2.51
GP_{12}	1.22 ± 0.4	95.71 ± 1.92

* n = 3; ** n = 20

Encapsulation efficiency

Encapsulation was found to be consistently higher for all the batches of the beads prepared. Alginate-gellan based beads (GP_9-GP_{12}) exhibited higher percentage of encapsulation than the alginate based beads (GP_1-GP_4). Similar high encapsulation efficiencies were achieved for various model drugs having low solubility (Imai *et al.*, 1993; Torre *et al.*, 1998; Gursoy and Cevik, 2000; Kulkarni *et al.*, 2001). This may be due to the low solubility of glipizide in the calcium chloride solution, which prevents drug partitioning during bead formation. Further, increasing the drug loading from 0.5 to 2 % w/v did not have a significant effect on encapsulation efficiency, suggesting that the amount of sodium alginate used was sufficient enough to entrap the drug at levels used in the present study. Encapsulation efficiency of beads was also unaffected by change in calcium chloride concentration (GP_3, GP_7 and GP_8). A curing time of two hours was used for the beads prepared. In order to study the effect of curing time on encapsulation efficiency and drug release, additional batches containing the composition of batch GP_3 were prepared and cured for 4, 8 and 12 hours. It was observed that only 1.2, 1.8 and 2.6 % of drug

was lost for beads cured for 4, 8 and 12 hours, respectively, suggesting slow diffusion of the encapsulated glipizide to the surrounding medium.

In vitro drug release

The effect of sodium alginate concentration on glipizide release from different batches of microspheres is shown in Figure 2. A significant decrease ($p<0.05$) in the rate and extent of drug release was observed with an increase in sodium alginate concentration.

This could be attributed to the increase in density and in the diffusional path length that the drug molecules have to traverse (Thanoo *et al.*, 1992, Rajinikanth, *et al.*, 2003). Figure 3 shows the effect of varying concentrations of sodium alginate and gellan on glipizide release from beads prepared with both the polymers.

Fig.2.Effect of sodium alginate concentration on in vitro drug release of glipizde beads. Bars represent mean ± SD (n = 3).

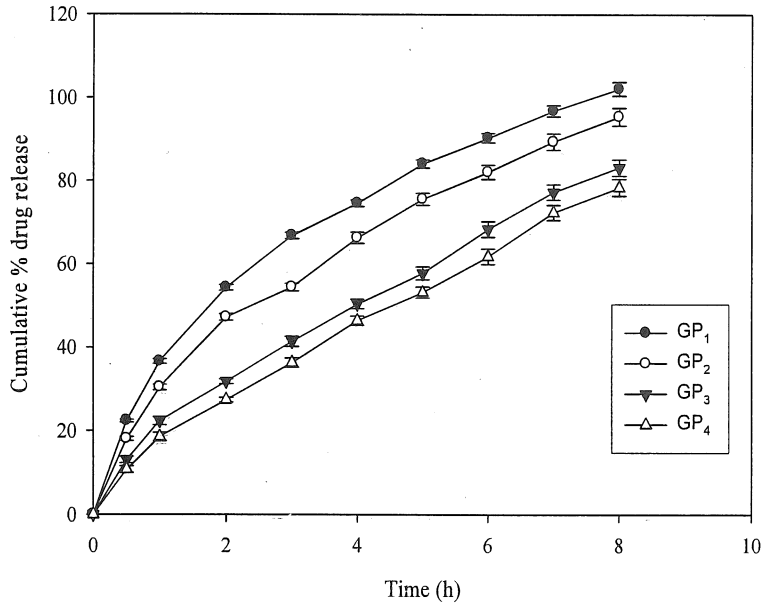
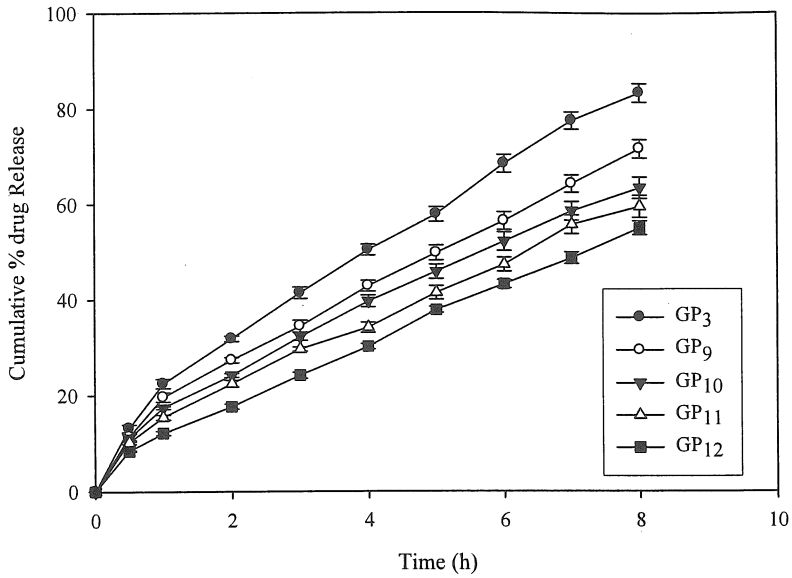


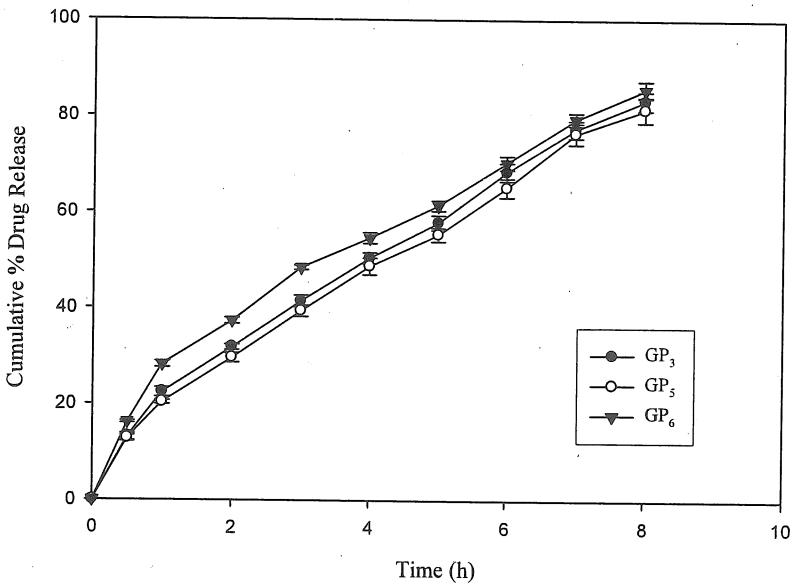
Fig. 3. Effect of gellan concentration on in vitro drug release of glipizide beads. Bars represent mean ± SD (n = 3).



The results indicated that the amount of drug released from these batches decreased significantly ($p<0.05$) when compared to the corresponding batch of bead containing 3.5 % w/v sodium alginate alone. As the gellan concentration increased progressively from 0.25 to 1 % w/v, the drug release decreased significantly. Gellan forms three-dimensional networks due to the formation of coordinates by cross-linking with cations and it has the propensity to form relatively stiffer gels in comparison to sodium alginate when calcium is used as the coordinating cation, as is the case in the present study (Sugawara *et al.*, 1990).

The effect of drug loading on glipizide release from the prepared beads is shown in Figure 4. The results indicated that the drug release was significantly ($p<0.05$) higher during the initial phase of drug release from the batches containing 2 % glipizide loading. However, there was no difference in rate and extent of drug release between the batches containing 1 and 0.5 % of the drug. The initial high release observed in cases of batch containing 2 % of glipizide could be attributed to the presence of glipizide at close proximity to the surface of the beads.

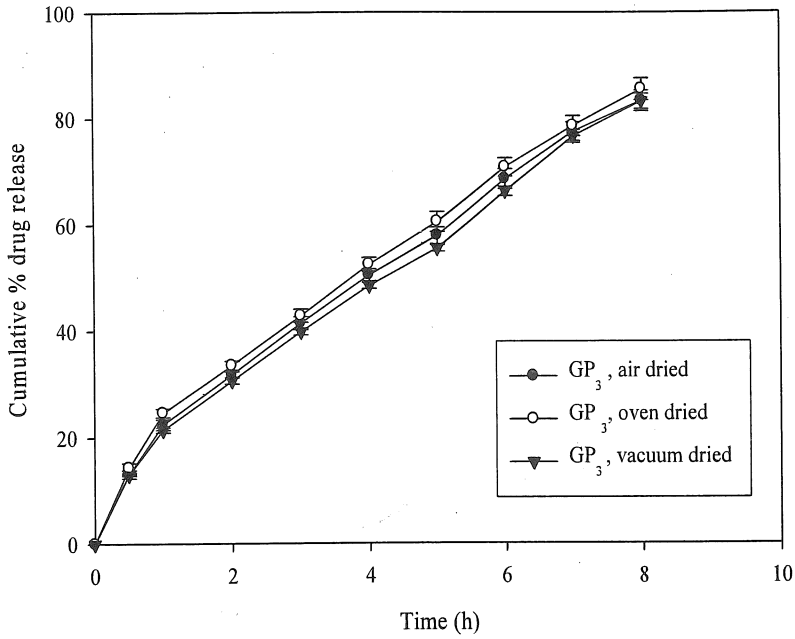
Fig. 4: Effect of Drug Loading on Glipizide release from the prepared beads
Bars represent mean ± SD (n = 3).



As the dissolution medium enters the bulk of the bead, sodium alginate being a hydrogel starts swelling. The further intrusion of the dissolution medium and the resultant swelling triggers the loosening of the polymeric matrix, resulting in changes in the microstructure of the bulk via the formation of pores accounting for rapid release of drug near the outer surface.

In order to optimize the drying conditions, the prepared beads were (a) air dried (b) dried at 40°C in an oven and (c) vacuum dried all were evaluated for *in vitro* drug release. Figure 5 shows the effect of the drying conditions on glipizide release from the prepared beads. The results indicate that there was not much difference in the release pattern of the drug from the beads dried under different condition of drying. However, the drug release from the batch dried at 40°C, showed marginally, a higher release when compared to the beads that were either air dried or dried under vacuum. This could be attributed to the migration of drug molecules to the periphery along with the water molecules during the evaporation, which occurs much faster when dried at 40°C in comparison to the other two drying conditions. Hence it was decided to air dry the beads.

Fig. 5. Effect of drying conditions on glipizide release from prepared beads
Bars represent mean ± SD (n = 3).



The effect of calcium chloride concentration on drug release is shown in Figure 6. The cross-linking/ionotropic gelation of sodium alginate matrix with calcium chloride is well established and documented. Sodium alginate is a linear copolymer consisting of β-(1-4)-D-mannuronic acid (M) and α-(1-4)-L-guluronic acid (G) residues, arranged in homopolymeric blocks of type MM, GG and in heteropolymeric block of MG. The principle of cross-linking or gelation of sodium alginate with calcium chloride is based on the formation of tight junctions between the GG residues. In the present study calcium chloride at 3 different concentrations, 2.5, 5 and 7.5 % w/v were used. The results indicate that as the concentration of the cross-linking agent increased there was a significant decrease ($p<0.05$) in the drug release. Based on the results a calcium chloride concentration of 5 % was selected for studying the effect of curing time. The beads were allowed to cure for 4, 8, and 12 hours. The effect of curing time on glipizide release is shown in Figure 7.

Fig. 6. Effect of calcium chloride concentration on glipizide release from prepared beads Bars represent mean ± SD (n = 3).

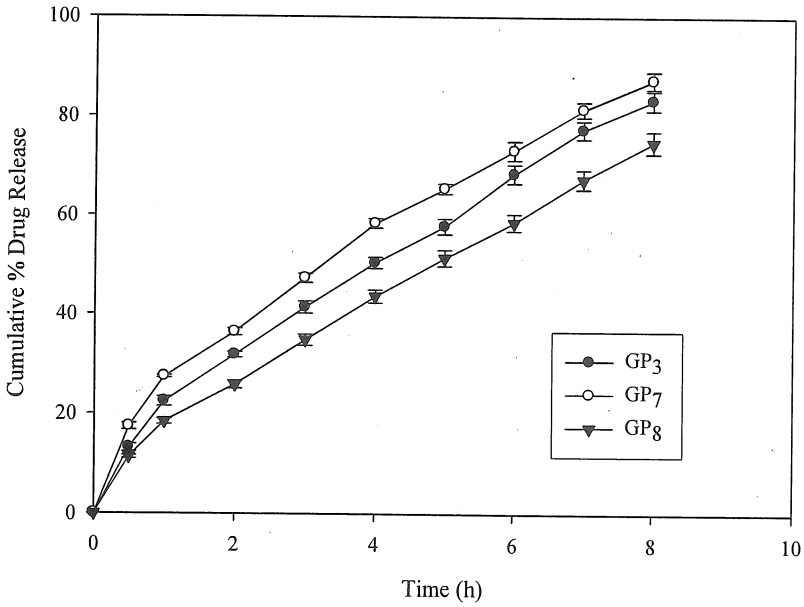
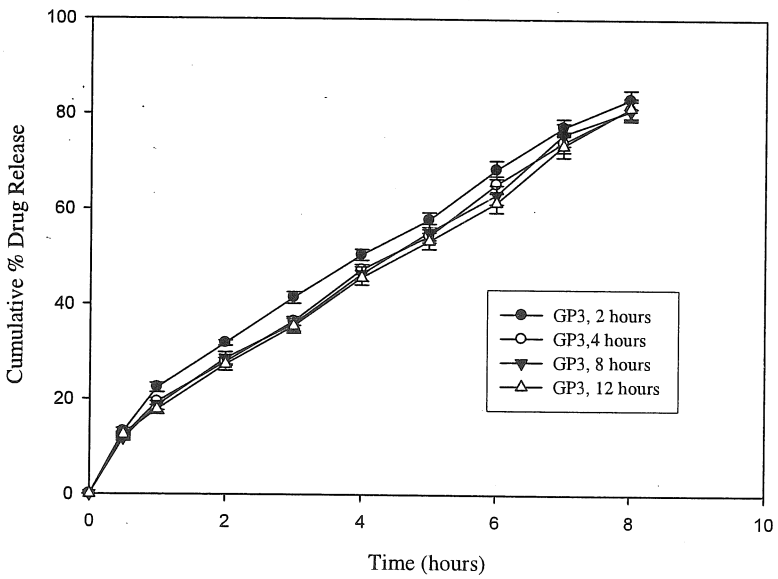


Fig. 7. Effect of curing time on in vitro glipizide release from preapred beads Bars represent mean ± SD (n = 3).



The results indicate that there was not much difference between the profiles of the batches cured for 2 (GP_3), 4, 8 and 12 hours. The marginal decrease in the drug

release as observed may have been due to the loss of drug, as observed in the encapsulation studies due to slow diffusion of the encapsulated glipizide to the surrounding calcium chloride solution.

The drug release from the prepared beads was biphasic, characterized by an initial phase of high release followed by a second phase of moderate release. In order to investigate the mechanism of drug release the data were fitted to models representing zero-order, first order and Higuchi's square root of time. The examination of the coefficient of determination (r^2) indicated that drug release followed diffusion controlled mechanism from the prepared beads, as the r^2 values for Higuchi's square root of time (ranged between 0.961 to 0.998) was always higher in comparison to zero (ranged between 0.904 to 0.948) as well as first order (ranged between 0.876 to 0.894). Since the release from the prepared beads followed a biphasic profile, it was decided to use a more stringent test in order to distinguish between the mechanisms of drug release. The release data were fitted to Peppas's exponential model (Korsmeyer *et al.*, 1983) $Mt/M\infty = Kt^n$, where $Mt/M\infty$ is fraction of drug released after time 't' and 'K' is kinetic constant and and 'n' is release exponent which characterizes the drug transport mechanism. When n approaches 0.5, a Fickian diffusion-controlled release is implied, where $0.5<n<1.0$ non-Fickian transport and $n=1$ for zero-order (case II transport). The values 'n' were in the range of 0.7128-0. 0.915, indicating that all the prepared formulations followed non-Fickian diffusion controlled mechanism of drug release.

Gelling rate

In order to understand the effect of gellan addition and the concentration of calcium chloride used for cross-linking, the extent of ionotropic gelation was indirectly assessed through weight variation corresponding to water loss during matrix formation. Determination of the gelling rate allows for the evaluation of speed and degree of polymer reaction on the formation of cross-linked structure. Figure 8 shows the gelling rate beads prepared from sodium alginate alone (GP_3); beads crosslinked with 7.5 % calcium chloride (GP_8) and bead containing 1 % gellan and 2.5 % sodium alginate (GP_{12}). The results showed that the reaction between the polymers and calcium chloride was fairly rapid, being completed in approximately 45 minutes. This was in accordance with results reported earlier (Al-Musa *et al.*, 1999). The gelling rate was reduced significantly with an increase in calcium chloride concentration and with inclusion of gellan in the matrix. These findings correlate with the *in vitro* release studies, where a decrease in drug release was observed with an increase in calcium chloride concentration and with an increase in the proportion of gellan in the beads.

Swelling rate

The swelling behaviour of representative batches of the prepared beads is shown in Figure 9. The swelling behaviour indicates the speed and easiness of a liquid to penetrate the alginate matrix, which is a necessary step for drug release since dissolution from alginate based beads, occur via diffusion through the swollen matrix. The results indicate that the beads when immersed in pH 6.8 phosphate

buffer, recovered their initial production spherical shape. The rate of swelling was higher for GP_3, which contained only alginate.

Fig. 8. Gelling rate of prepared beads ,Bars represent mean ± SD (n = 3).

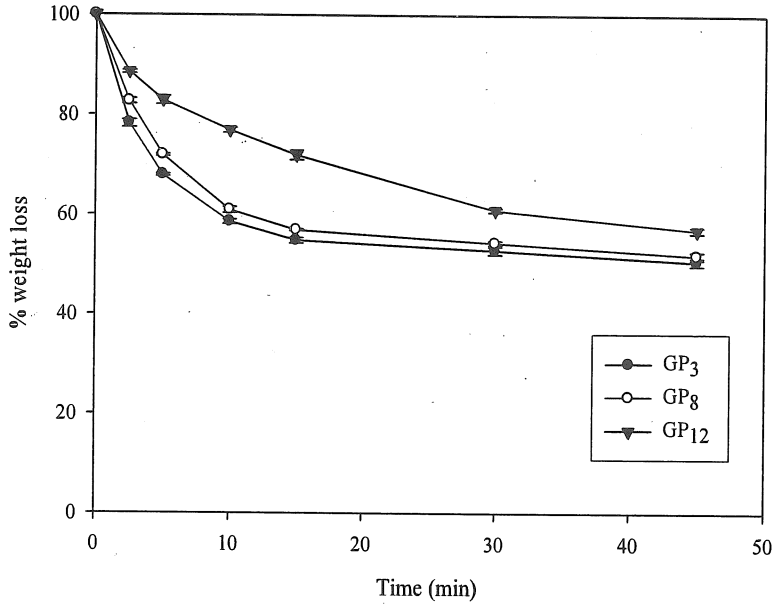
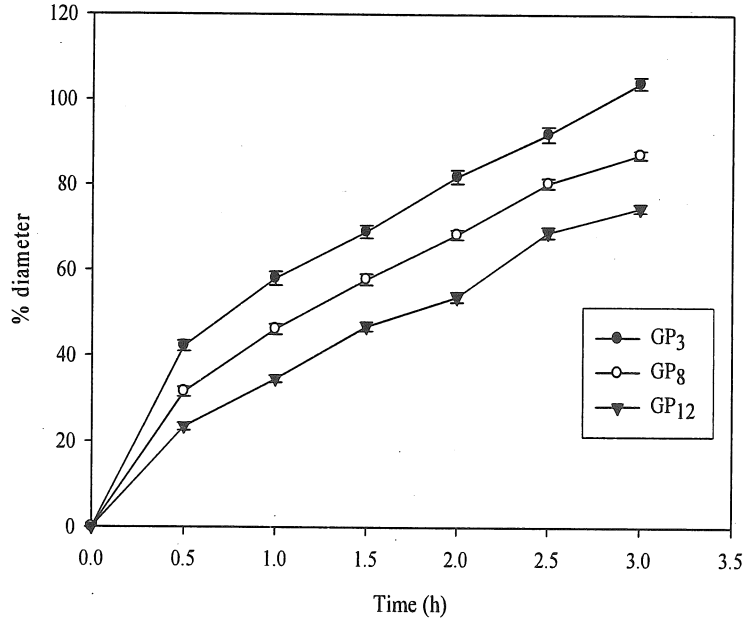


Fig. 9. Swelling rate of prepared glipizide beads in phosphate buffer pH 6.8. Bars represent mean ± SD (n = 3).



The swelling of the beads decreased considerably from batches cross-linked with 7.5 % calcium chloride (GP_8) and the batch containing 1 % gellan (GP_{12}). This correlated well with the results of the *in vitro* studies, wherein a significant decrease in the drug release was observed with GP_8 and GP_{12} with respect to GP_3.

Conclusion

The prepared beads exhibited sustained drug delivery over an 8 hour period, providing an initial phase of high release followed by a phase of moderate release. Incorporation of gellan helped in increasing the incorporation efficiency of glipizide as well as in sustaining the drug release. The results have indicated that the beads prepared with 2.5 % w/v of sodium alginate and 1 % w/v of gellan appear to have a potential for development as a sustained release beads formulation of glipizide. However, further studies wherein combinations with different grades of sodium alginate (based on MM/GG ratio) and gellan (based on degree of deacetylation) needs to be evaluated and a pharmacokinetic profiling in appropriate animal model is essential, which we are currently exploring.

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