JBAAR
Journal of Basic And Applied Research
ISSN 2413-7014

Formulation and Evaluation of Chitosan-Gellan Beads as Controlled Drug Delivery System of Antihypertensive Drug

Rohit R. Bhosale[a*], Riyaz Ali M. Osmani[a], H. V. Gangadharappa[a], D. V. Gowda[a], Afrasim Moin[b].

[a]Department of Pharmaceutics, JSS College of Pharmacy, JSS University, Mysuru- 570015, Karnataka, India.
[b]Department of Pharmaceutics, College of Pharmacy, University of Hail- 2440, Kingdom of Saudi Arabia.

*corresponding author: bhosalerohit707@gmail.com

Received: 29-3-2016
Revised: 12-4-2016
Published: 15-4-2016

Keywords:
Chitosan,
Gellan gum,
Diltiazem HCl,
Beads,
Drug Delivery

Abstract: The use of natural polymers in the design of drug delivery formulation has received much attention due to their excellent biocompatibility and biodegradability. Among them, chitosan and gellan are very promising and have been widely exploited in pharmaceutical industry for controlled drug release. In the present study, an attempt has been made to formulate chitosan-gellan beads of Diltiazem hydrochloride using chitosan and gellan as ionic polysaccharide, and calcium chloride as cross-linking agent. The formulations were prepared by varying the concentration of gellan gum and chitosan. The prepared beads were evaluated for drug polymer interaction by FT-IR, DSC andsurface morphology by SEM. Drug content, swelling studies and *in vitro* drug release were carried out on prepared beads. FT-IR and DSC studies showed no interaction between drug and polymers. SEM showed spherical nature of beads with inward dents. Swelling of beads depends upon pH, concentration of polymers and crosslinking agent. The *in vitro* release of drug from beads was controlled for 12 h. Chitosan-gellan beads as controlled drug delivery were prepared by changing the polymer concentration. Swelling studies showed that with an increase in pH from acidic to basic, a considerable increase in swelling in all the formulations was observed.

INTRODUCTION

High blood pressure, termed "hypertension," is a leading cause of morbidity and mortality. Hypertension is much more than a "cardiovascular disease" because it not only affects heart but also affects other organ systems of the body such as kidney, brain, and eye. The term "hypertension" can apply to elevations in mean arterial pressure, diastolic pressure, or systolic pressure (Chien, 1992). The oral drug delivery has its own importance because of its ease of administration and patient compliance. Though the conventional oral drug delivery systems achieve both local and systemic effects, there is no control over drug release from dosage forms that may lead to local or systemic toxicity. In the past ten years, there has been a rapid development in the field of drug delivery. In particular, a number of drug delivery or release systems have been developed, having influences on drug release control. Recently, greater emphasis has been placed on controlling the rate and/or site of drug release from oral formulations for the purposes of improving patient compliance and treatment efficacy. These systems maintain plasma concentrations within the therapeutic range, which minimizes side effects and also reduces frequency of administration. The low development cost and time required to formulate a novel drug delivery system is also an advantage. Hence this system gains an importance over immediate release systems for their effectiveness in treatment, reduced side effects and also increased patient compliance (Fishburn, 1965).

Microencapsulation has played a vital role in the development of controlled release drug delivery systems. Microcapsules or beads have been of particular interest from the pharmaceutical point of view providing the possibility to achieve controlled drug release (Hejazi and Amiji, 2003). A Bead may be defined as a particle with size varying from 0.9 to 2.1 mm containing a drug dissolved, dispersed or adsorbed on to the surface of the particle. Recently, the use of natural polymers in the design of drug delivery formulation has received much attention due to their excellent biocompatibility and biodegradability. Among them, chitosan and gellan are very promising and have been widely exploited in pharmaceutical industry for controlled drug release (Aminabhavi et al., 2004; Kedzierewicz et al., 1999). The model drug used in the present study is Diltiazem hydrochloride (Diltiazem HCl), calcium channel blocker commonly used as an antihypertensive agent. The dose varies from 30 mg to 60 mg q.i.d depending on the severity in divided doses; it has a half-life of 3 to 5 h.

In the present study, an attempt has been made to formulate Chitosan-Gellan beads of Diltiazem HCl using chitosan and gellan as ionic polysaccharide, and calcium chloride as cross-linking agent.

MATERIALS AND METHODS

The materials required for the present work were procured from diverse sources. Diltiazem hydrochloride (Diltiazem HCl) was procured from FDC Ltd., Mumbai, India; while chitosan was provided as gift sample by Evonik Pharma, Mumbai, India. Gellan gum and calcium chloride were procured from Central Drug House Pvt. Ltd., Mumbai, India. All the other ingredients used were of analytical grade, and were used as procured.

Methods

Formulation of chitosan-gellan beads

Seven formulations of beads were prepared to study the effect of different formulation variables. The formulation chart is given in Table 1. The formulations were prepared by varying the concentration of gellan gum (1.5%-2.5%) and chitosan (1.0%-1.5%). Formulation F1-F6 contains 5% HPMCP whereas F7 does not contain the enteric polymer. Keeping other parameters like drug loading, gellan gum pH, and concentration of cross-linking agent constant (Kedzierewicz et al., 1999; Fujji et al., 2005; Patil et al., 2006).

Characterization of beads

The prepared beads were characterized by Fourier transform infrared spectroscopic analysis, Differential scanning calorimetric analysis and surface morphology by Scanning electron microscopy (Agnihotri et al., 2006).

Fourier transform infrared spectroscopy (FT-IR)

The sample powder was dispersed in KBr powder and pellets were made by applying 6000 Kg/cm^2 pressure. FT-IR spectra were obtained by powder diffuse reflectance on a FT-IR spectrophotometer type 8400S Shimadzu.

Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out on DuPont 9900 thermal analyzer with 910 DSC module. Calorimetric measurements were made with empty cell (high purity alpha alumina discs of Dupont Company) as the reference. The dynamic scan, were taken in Nitrogen atmosphere at the heating rate of 10°C min^{-1}.

Scanning electron microscopy (SEM)

SEM photographs were taken for the prepared beads with a scanning electron microscope, Joel-LV-5600, USA, at the required magnification in room temperature. The photographs were observed for morphological characteristics and to confirm the spherical nature of beads.

Evaluation of beads

Drug content

The prepared beads were soaked in 7.4 pH buffer for overnight. The final solution was filtered using Whatmann filter paper and estimated spectrophotometrically for drug content (Agnihotri et al., 2006).

Swelling studies

The swelling properties of the beads were carried out using pH 7.4 buffer. The beads of known weight were placed in 50 ml of the buffer solution for 24 h. At regular time intervals the beads were removed and excess surface liquid was removed by blotting paper and their weight was recorded. The percentage swelling (S) was determined by the following equation (Pasparakis and Bouropoulos, 2006).

$$S = \frac{\text{Weight of swollen beads} - \text{Weight of dry beads}}{\text{Weight of dry beads}} \times 100 \quad (1)$$

Particle size analysis

The particle size was carried out by using optical microscope. They were dispersed in liquid paraffin and observed under optical microscope. Stage micrometer was used to calculate calibration factor. Averages of 50 particles were counted and size was determined (Patil et al., 2006; Pasparakis and Bouropoulos, 2006).

% Yield

Determining whether the preparation procedure chosen for incorporating a drug into the polymers is efficient is of prime importance. The raw materials, amount of active compound, and other process parameters are deciding factors for the yield of the product during the preparation of beads (Akbuga and Sezer, 1995). The yield was determined by weighing the beads and then finding out the percentage yield with respect to the weight of the input materials, i.e., weight of drug and polymers used. The formula for calculation of % yield is as follows:

$$\% \text{ Yield} = \frac{(\text{Wt. of drug} + \text{Wt. of polymers})}{\text{Wt. of beads}} \times 100 \quad (2)$$

Table 1: Formulation chart

Formulation code	Drug added (%)	Dropping solution		Receiving solution		
		Gellan (%)	pH	Chitosan (%)	$CaCl_2$(%)	HPMCP (%)
F1	40	1.5	12	1.0	3.0	5.0
F2	40	2.0	12	1.0	3.0	5.0
F3	40	2.5	12	1.0	3.0	5.0
F4	40	1.5	12	1.5	3.0	5.0
F5	40	2.0	12	1.5	3.0	5.0
F6	40	2.5	12	1.5	3.0	5.0
F7	40	2.5	12	1.0	3.0	-

Drug loading and encapsulation efficiency

Drug loading is important with regard to release characteristics. Generally, increased drug loading leads to an acceleration of the drug release. Drug entrapment efficiency represents the proportion of the initial amount of drug, which has been incorporated into the beads (Agnihotri et al., 2006; Akbuga and Sezer, 1995).

100 mg of beads were weighed and transferred to 100 ml volumetric flask containing pH 7.4 phosphate buffer. From this, 1 ml of solution was transferred to 10ml volumetric flask and diluted up to the mark. Further 1 ml of this solution is diluted to 10 ml and absorbance was measured at 236 nm. The drug content was calculated by using the formula-

$$\text{Amount of drug} = \frac{\text{Conc. from standard graph} \times \text{Dilution factor}}{1000} \quad (3)$$

Percentage encapsulation efficiency is found out by calculating the amount of drug present in 100 mg of beads. It is further calculated by using formula-

$$\% \text{ Encapsulation efficiency} = \frac{\text{Drug content}}{\text{Total amt. of drug added}} \times 100 \quad (4)$$

***In vitro* drug release**

The *in vitro* release of drug from the beads was carried out in basket type dissolution tester USP XXIII, TDT-08L, with auto sampler containing 900 ml of pH 1.2 buffer for the first 2 h and in 7.4 pH phosphate buffer for the next 10 h. The volume of the dissolution media was maintained at 900 ml while constant stirring (50 rpm) and temperature of bath was maintained at 37 ± 0.5°C. Aliquots (5 ml) of dissolution media were sampled at specified time points and replaced with fresh media immediately after sampling. And the samples are analyzed for drug content by UV visible spectroscopy (Shimadzu UV 1601). The release data obtained were fitted into various mathematical models to know which mathematical model is best fitting for the obtained release profile (Bajpai and Tankhiwale, 2006; Anal and Stevens, 2005).

Mathematical model fitting

The *in vitro* release studies data was fitted into various mathematical models to determine the best-fit model. The various parameters like n-time exponent, k-release constant and R-regression coefficient were calculated (Liu et al., 2008; Neau et al., 2002; Torrado et al., 2004).

Stability study

Stability is defined as the ability of a particular drug or a dosage form in a specific container to remain with its physical, chemical, therapeutic and toxicological specifications. Optimized formulation of the Diltiazem beads was selected for stability studies. Formulations were packed in a screw-capped bottle and studies were carried out for 90 days by keeping at 25°C and 60 ± 5% RH, 30°C and 65 ± 5% RH, and 40°C and 75 ± 5% RH**.** Samples were withdrawn on 0th, 15th, 45th and 90th day and were analyzed for drug content (Torrado et al., 2004).

RESULTS

Characterization of beads

Fourier transform infrared spectroscopy (FT-IR)

Pure Diltiazem and their formulations were subjected to FT-IR analysis. The obtained spectra are given in Figure 1. The characteristic peaks of

pure drug were compared with the peaks obtained for their respective formulations.

Differential scanning calorimetry (DSC)

In order to study any possible interactions between the drug and polymers, DSC studies were carried out. The DSC thermograms obtained are reported in Figure 2. From the thermograms it was observed that, Diltiazem HCl displayed a single sharp endothermic peak at 220.09°C corresponding to its melting point, and a peak at the same temperature was observed in the formulation. Hence, it can be observed that there was no significant interaction between the drug and the polymers used.

Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out to observe the surface morphology, texture and porosity of the beads. The SEM photographs are given in Figure 3a and 3b. From the photographs it was observed that the beads were spherical in nature (mean size of around 1.7 mm), having a smooth surface with inward dents and shrinkage due to the collapse of the wall of the beads during dehydration. The fibrous network was found on the surface.

Evaluation of beads

Drug Content

The test for drug content was carried out to ascertain whether the drug is uniformly distributed in the formulation. From the results it can be inferred that there was a proper distribution of Diltiazem HCl in the beads and the deviation is within the acceptable limits.

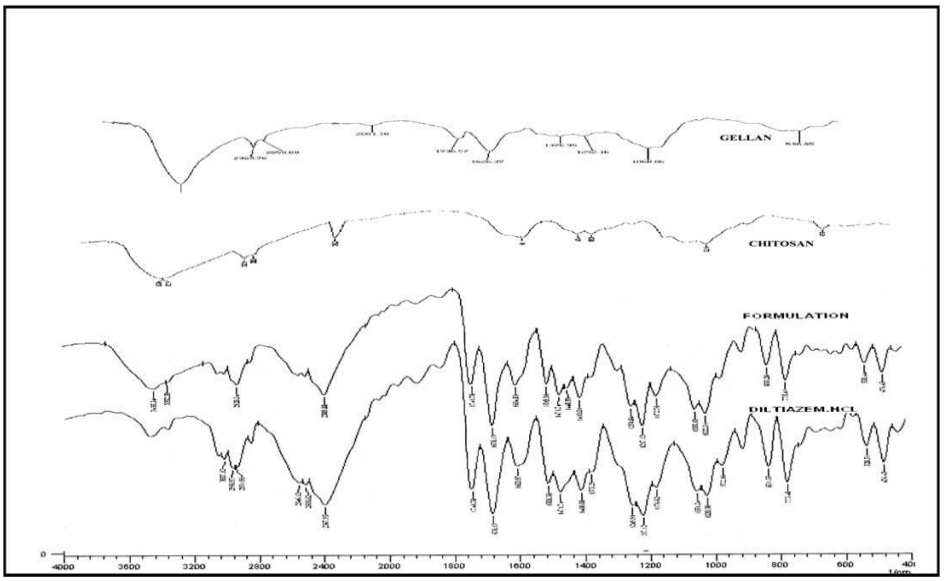

Figure 1: FT-IR comparison of pure diltiazem HCl and formulation F3

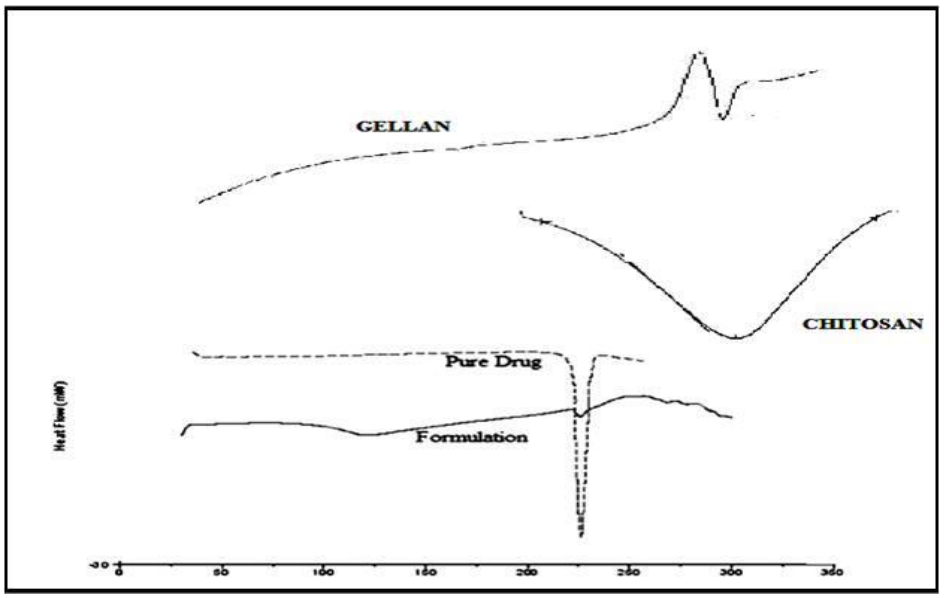

Figure 2: DSC comparison of pure diltiazem HCl and formulation F3

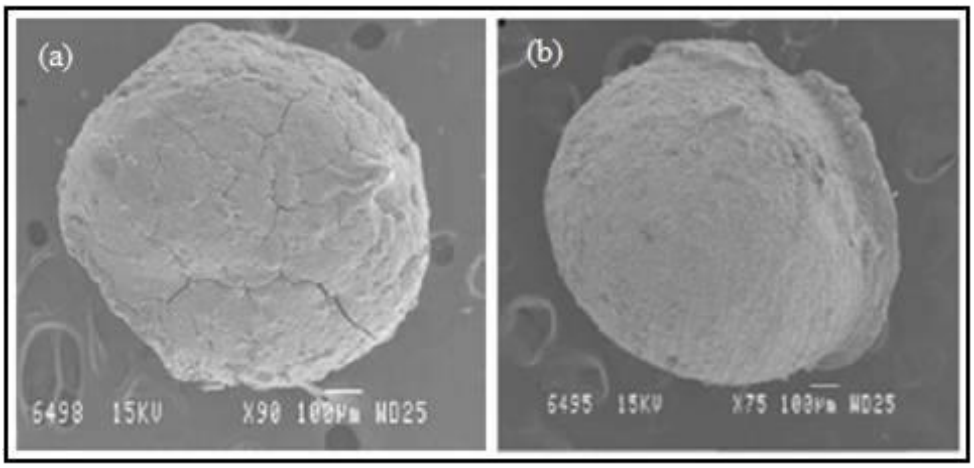

Figure 3: (a) SEM of bead in pH 1.2 PBS (b) SEM of bead in pH 7.4 PBS

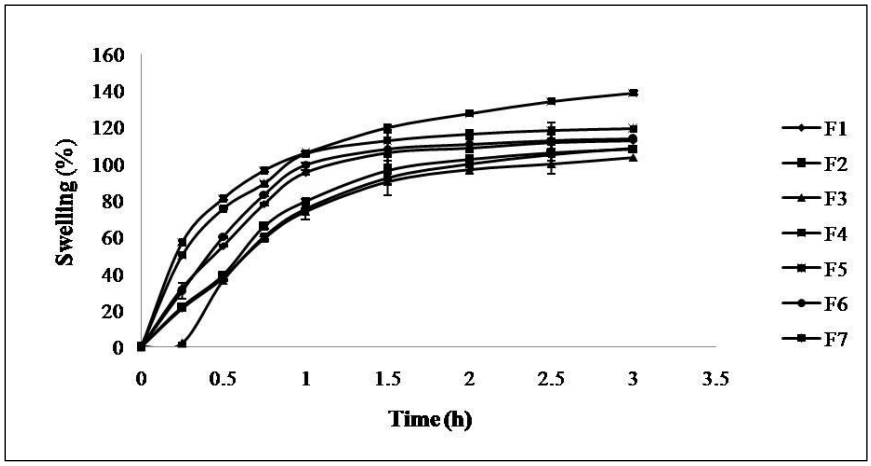

Figure 4: % Swelling of beads in pH 7.4 phosphate buffer

Swelling studies

The swelling studies for the beads (without drug) were carried out in both pH 1.2 buffer and pH 7.4 phosphate buffer to check their pH sensitivity (Figure 4). The beads tend to shrink when exposed to the acidic environment of SGF. At low pH, the carboxylate groups of gellan are protonated and the electrostatic interaction of carboxyl groups of gellan with the protonated amine groups of chitosan is strengthened, thus resulting in a dense structure with minimum water uptake and shrinkage is favored. This explains the non-swelling of beads in gastric pH.

During the process of microencapsulation, the mechanical variables cause loss of final product and hence process yield may not be 100%. Beads were weighed after drying and the percentage yield was calculated. The obtained data is shown in the Table 2.

% Yield

Table 2: % Yield for diltiazem HCl loaded beads

Formulation	% Yield ± SD*
F1	82.82 ± 2.16
F2	83.29 ±1.92
F3	87.25 ±1.74
F4	84.04 ±1.24
F5	85.41 ±1.53
F6	86.92 ±1.64
F7	84.12 ±1.57

*** Standard deviation, n = 3**

Table 3: Drug loading and encapsulation efficiency

Formulation	Drug loading (mg) Mean ± SD*	Encapsulation efficiency (%) Mean ± SD*
F1	32.3 ± 1.1	50.3 ± 0.7
F2	36.5 ± 0.9	54.6 ± 0.5
F3	40.7 ± 0.5	57.7 ± 0.9
F4	30.3 ± 0.8	48.7 ± 1.2
F5	32.5 ± 1.2	51.3 ± 0.8
F6	35.4 ± 1.0	53.6 ± 1.1
F7	39.3 ± 1.1	56.2 ± 0.8

*** Standard deviation, n = 3**

Drug loading and encapsulation efficiency

The percent of drug loading in the formulations was found to be in the range of 30-40%. The percentage encapsulation efficiency was found to be 48-57%. The results obtained are given in Table 3.

***In vitro* drug release**

Dissolution studies were carried out in pH 1.2 HCl buffer for 2 h followed by 10 h in pH 7.4 phosphate buffer using USP XXIII dissolution apparatus type II. Release studies were carried out for formulations F1-F7. The Dissolution rate-time data obtained are reported in Figure 5.

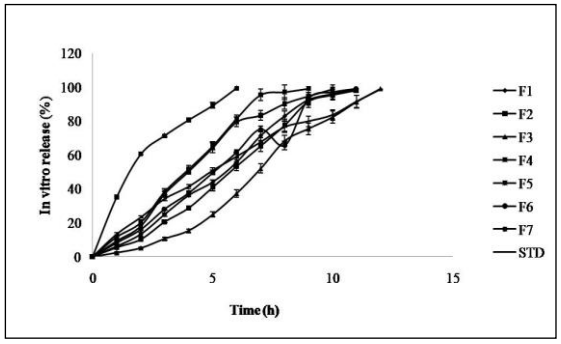

Figure 5: % Drug release profile of diltiazem HCl beads

From the data obtained, it was observed that the release in gastric pH was controlled in formulations F1-F6 whereas it was found to be more (>50%) for the formulation F7. It may be attributed to the presence of HPMCP, enteric release polymer that controlled the drug release of drug in gastric pH. Thus in pH1.2 the release was extended to 5-20% for the formulations F1-F6, which was mainly due to the migration of the drug to the surface during drying of the bead.

Higuchi plot

The amount of drug released versus square root of time was plotted (Figure 6). The plots were linear and the results inferred that drug release from the beads was by diffusion.

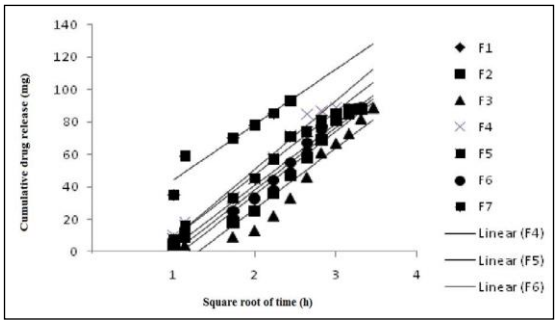

Figure 6: Amount of drug released Vs square root of time

Mathematical model fitting

The *in vitro* release studies data was fitted in to various mathematical models to determine the best-fit model. The results indicated that, the best-fit model was found to be Peppas models. The data obtained from *in vitro* release studies was fit into Peppas model.

Koresmeyer-Peppas equation:

$$M_t / M_\infty = 1 - A (\exp^{-Kt}) \quad (5)$$

$$\log (1 - M_t / M_\infty) = \log A - kt/2.303 \quad (6)$$

Where,

M_t = Amount of drugs released at time t

M_∞ = Total amount of drug loaded

K = Diffusion constant/Release rate constant

R = Regression co-efficient

A = Time exponent

The value of 'A' determined from Korsmeyer-Peppas equation if found to be below 0.45, it indicates that the drug release from the formulation follows Fickian diffusion, if 'A' value is between 0.5-0.85, indicates Non-Fickian diffusion or anomalous mechanism (relaxation controlled) and if 'A' value is above 0.89, indicates Super case II transport. The data obtained from *in vitro* release studies was fitted into Peppas model. The various parameters the intercept A, the release constant K and regression coefficient R^2 were obtained. The data is shown in Table 4.

Table 4: The data obtained for Peppas model fitting

Formulation	Parameters		
	K	A	R^2
F1	-0.189	0.760	0.9958
F2	-0.550	0.900	0.9911
F3	-0.424	0.852	0.9988
F4	-0.189	0.762	0.9976
F5	0.026	0.688	0.9955
F6	-0.324	0.840	0.9953

Table 5: Stability study for drug content of formulation F3

Sampling Interval	% Drug content		
	25 ºC/60% RH	**30 ºC/65% RH**	**40 ºC/75% RH**
0 Days	99.46 ± 1.10	99.42 ± 0.98	99.41 ± 0.82
15 Days	99.43 ± 1.20	99.40 ± 1.25	99.38 ± 1.14
45 Days	99.10 ± 1.12	99.05 ± 1.18	98.91 ± 1.46
90 Days	98.89 ± 1.60	98.81 ± 1.29	98.75 ± 1.81

*** Standard deviation, n=3**

Stability study

The objective of stability studies was to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized formulation (F3) was subjected to stability studies according to ICH guidelines by storing at 25 ºC/60% RH, 30 ºC/65% RH and 40 ºC/75% RH for 90 days. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The obtained data is presented in Table 5.

DISCUSSION

From the FT-IR spectra it was observed that the peaks of pure drug and formulations were found to be similar, indicating that there was no significant interaction between drug and polymer used. DSC thermograms did not show any change in the melting point indicating stability of the formulation. The SEM images indicated that beads in 1.2 pH showed shrinkage and cracks (Figure 3a) and in 7.4 pH showed swelling behavior (Figure 3b). The beads prepared in pH 5 have a porous structure, while those prepared in pH 12 media have the smooth and rigid surfaces. Thus, increasing the gellan gum pH to 12 increases the encapsulation efficiency.

The results indicate that with a change in pH from acidic to basic medium considerable increase in swelling was observed for all the formulations. The obtained swelling profile is shown in Figure 4. The protonated amino groups of chitosan get deprotonated and at the same time carboxylic groups of gellan ionize to give negatively charged – COO^- groups. This ultimately weakens the electrostatic interactions among the two polymer chains, thus making the bead structure rather loose. In addition to this, the ion-exchange between Ca^{2+} ions present in the beads and Na^+ ions of external buffer solution also reduces the extent of cross-linking, thereby increasing the osmotic pressure inside the beads that induces a faster uptake of the aqueous medium resulting in increased swelling. Swelling mainly depends on the extent of cross-linking. At lower cross-linking density, the network is loose with a greater hydrodynamic free volume, so that the chains can accommodate more of the solvent molecules resulting in higher swelling.

A higher concentration of the polymers and pH values corresponding to their ionized state result in an increased charge density of both the polymers and will lead to intense cross-linking with small micropores. This might be the cause of the minimized loss of drug from the PIC beads and higher percent entrapment values at higher polymer concentrations. Hence, as the polymer concentration increases, drug encapsulation increases.

The release in pH 7.4 was extended upto 90% for all the formulations at the end of 10^{th} h except formulation F4 and F7. Formulation F3 was found to give more controlled release upto 12 h. Initial drug release from beads at intestinal environment was characterized by biphasic manner and associated with an initial burst release then slow and sustained release. Increasing chitosan concentration showed fast drug release. This could be attributed to the formation of a more porous structure in the presence of chitosan. The rate and extent of drug release significantly decreased with an increase in the gellan concentration. This could be attributed to the increase of gellan matrix density and the diffusion path length, thereby increasing the distance traveled by the drug molecule through the chitosan-gellan matrix for the release.

In all the cases the value of intercept A were found to be more than 0.5. This indicates that the release of drug from all the formulations was found to be by non-fickian and super case II transport. This kind of diffusion corresponds to a more predictable type of swelling-controlled system. From the data, it was observed that the formulation did not undergo any chemical changes/interaction during the study period.

CONCLUSION

Chitosan-gellan beads as controlled drug delivery system using Diltiazem HCl were prepared. FT-IR studies indicated no interaction between the

polymers and the drugs as in the formulation; the principal peaks of the drugs are not altered. From the DSC thermograms, it was evident that the decomposition temperatures of both the drugs and their formulations are closer; hence no significant interactions exist between the drug and polymers. From the SEM studies it was observed that beads were found to be smooth (mean size of around 1.7 mm) with inward dents and showed the presence of minute pores, which was minimized by increasing the gellan gum pH to 12. Changing gellan gum pH, reducing the stirring time, and adding drug into external medium have increased encapsulation efficiency upto 45-57%. It was found that increasing the polymer concentration increases the encapsulation efficiency. From the results of swelling studies, it was observed that with an increase in pH from acidic to basic, a considerable increase in swelling was observed in all the formulations, confirming the pH sensitivity of the polymer combination used. At lower crosslinking density, the network is loose with a greater hydrodynamic free volume, so that the chains can accommodate more of the solvent molecules resulting in higher swelling. The *in vitro* drug release was found to be sustained upto 12 h for the formulation F3. As the polymer concentration increases the drug release decreases. The drug release from the beads followed non- Fickian diffusion and super case II transport.

ACKNOWLEDGMENTS

The authors would like to acknowledge JSS University, Mysuru, India, for valuable support during this research. Authors would like to express their sincere gratitude towards Mysuru University, Mysuru for providing the facilities for carrying out particle size, zeta potential, XRD, SEM and polydispersity index analysis.

REFERENCES

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. *Eur J Pharm Biopharm*, *63*, 249-61.

Akbuga, J., Sezer, A. D. (1995). Controlled release of piroxicam from chitosan beads. *Int J Pharm*, *121*, 113-6.

Aminabhavi, T. M., Agnihotri, S. A., Mallikarjuna, N. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J Control Release*, *100*, 5-28.

Anal, A. K., Stevens, W. F. (2005). Chitosan-alginate multilayer beads for controlled release of ampicillin. *Int J Pharm*, *290*, 45-54.

Bajpai, S. K., Tankhiwale, R. (2006). Investigation of water uptake behavior and stability of calcium alginate/chitosan bi-polymeric beads: Part 1. *Reactive Funct Polymers*, *66*, 645-58.

Chien, Y. W. (2nd Eds.). (1992). *Parentral drug delivery and delivery systems*. New York: Marcel dekker.

Fishburn, A. G. (1965). *An introduction to pharmaceutical formulation*. New York: Pergamon Press.

Fujji, T., Ogiwara, D., Ohkawa, K., Yamamoto, H. (2005). Alkaline phosphatase encapsulated in gellan-chitosan hybrid capsules. *Macromol Biosci*, *5*, 394-400.

Hejazi, R., Amiji, M. (2003). Chitosan-based gastrointestinal delivery systems. *J Control Release*, *89*, 151-165.

Kedzierewicz, F., Lombry, C., Rios, R., Hoffmann, M., Maincent, P. (1999). Effect of the formulation on the *in vitro* release of propanolol from gellan beads. *Int J Pharm*, *178*(1), 129-36.

Liu, M., Chen, S., Jin, S., Wang, B. (2008). Preparation of ionic-crosslinked chitosan-based gel beads and effect of reaction conditions on drug release behaviors. *Int J Pharm*, *349*, 180-7.

Neau, S. H., Alsarra, I. A., Betigeri, S. S., Zhang, H., Evans, B. A. (2002). Molecular weight and degree of deacetylation effects on lipase-loaded chitosan bead characteristics. *Biomaterials*, *23*, 3637-44.

Pasparakis, G., Bouropoulos, N. (2006). Swelling studies and *in vitro* release of verapamil from calcium alginate-chitosan beads. *Int J Pharm*, *323*, 34-42.

Patil, S., Sharma, S., Nimbalkar, A., Pawar, A. (2006). Study of formulation variables on properties of drug-gellan beads by factorial design. *Drug Dev Ind Pharm*, *32*, 315-326.

Torrado, S., Prada, P., Torre, P. M., Torrado, S. (2004). Chitosan-poly (acrylic) acid polyionic complex: *in vivo* study to demonstrate prolonged gastric retention. *Biomaterials*, *25*, 917-23.