

Research Article



Formulation and Evaluation of Nifedipine Microbeads Using Guar gum as a Release Modifier

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ABSTRACT

A great deal of attention has been given to the formulation of alginate micro beads which has potential as carrier in controlled drug delivery. The drug Nifedipine has low bioavailability, hence to improve its bioavailability; Nifedipine-loaded mucoadhesive microbeads were prepared by Ionotropic gelation and cross linking technique by using sodium alginate as the hydrophilic carrier in combination with Guar gum as release modifier. Micro beads with different ratio of sodium alginate and guar gum were formulated and evaluated for particle size, swelling ratio, and percentage yield. An optimized batch was selected from the previous study and four different concentrations of Nifedipine were loaded. The drug loaded batches were evaluated for drug entrapment, bio adhesiveness, *in vitro* release, and release kinetics. Particle size distribution of beads was measured by both optical microscopy and SEM. No significant drug-polymer interactions were observed in FT-IR studies. *In-vitro* drug release profile of Nifedipine micro beads in phosphate buffer pH 6.8 exhibited zero order release with kinetics of super case II-transport. The *in vitro* wash-off test using goat intestine revealed that the sodium alginate micro beads of Nifedipine possess good mucoadhesive properties. The drug loaded batches were found to have good drug loading. Hence the formulated Microbeads of Sodium alginate with guar gum as release modifiers could be used as an alternative and cost effective carrier for the development of oral controlled release capsules or tablets the of Nifedipine as once daily formulation.

Keywords: Nifedipine, sodium alginate, micro beads, Ionotropic gelation, Guar gum, release modifier.

INTRODUCTION

Nifedipine is used in the management of various cardio vascular diseases in long term therapy. Still it has various disadvantages such as low bioavailability and frequent dosing through conventional dosage forms. This problem can be attenuated by designing the drug in the form of mucoadhesive beads eventually it would prolong the drug residence time at the site of absorption so as to enhance the bio adhesiveness and thereby the bioavailability^{1, 2}. Oral multiparticulate drug-delivery systems offer biopharmaceutical advantages such as free dispersion and predictable distribution in the intestine.^{3, 4} Beads with lowest shrinkage and highest mechanical strength could be prepared from alginates having high content of a-L-guluronic acid.⁵ By the selection of appropriate type of alginate, cross linking agent, and release modifiers, (coating agents) the alginate beads of various morphology, mechanical strength, distinct porosity, dehydration, and drug loading capacity can be fabricated⁶. This high degree of flexibility can result in delivery of drugs over extended period of time ranging from minutes to months in a sustained manner⁷.

When administrated orally via immediate-release solid dosage forms, absorption of Nifedipine is poor. Therefore, preparation of modified release formulation may be beneficial in reduction of dose and dose related

side effects.⁸ The cross linking property of alginate beads make them attractive as a suitable bio polymer for the encapsulation of wide variety of drugs including proteins, DNA Oligo nucleotides, proteins, cardio vascular drugs like Verapamil, Diltiazem, and Nicardipine, anti-inflammatory drugs like Diclofenac sodium, Aceclofenac, Ibuprofen etc, and even stem cells.^{9, 10}

The aim of the present investigation is to optimize the suitable concentration of guar gum as release modifier for microbeads so as to make a micro bead carrier system ready for the development of controlled release capsules or tablets the for the modified oral delivery of Nifedipine.

MATERIALS AND METHODS

Materials

Nifedipine was a gift sample from Alkem Pharmaceuticals Ltd, Mumbai. All other chemicals like sodium alginate, Guar gum, calcium chloride and glutraldehyde were from Nice chemicals, Bangalore, India. Other reagents used were of analytical grade. Double distilled water was used throughout the study.

Drug-Polymer compatibility study

This study was carried to find out the possible interaction between the selected polymers like sodium alginate, Guar gum with the drug Nifedipine and also to identify the compatibility between the drug and the polymer. Small



amounts of triturated samples were taken into a pellet maker and were compressed at 10kg/cm² using a hydraulic press. The pellet was kept on to the sample holder and scanned from 4000cm⁻¹ to 400 cm⁻¹ in shimadzu FTIR spectrophotometer.¹¹

Formulation of placebo micro beads of sodium alginate using guar gum as release modifier by Ionotropic gelation and cross linking method.

Four different batches of placebo microbeads were prepared by using sodium alginate and guar gum as release modifier. To 25 ml of deionized water, 1% sodium alginate was added and stirred with magnetic stirrer, and then 1% guar gum solution was added to form uniform dispersion. The resulting dispersion was dropped through syringe with a needle slowly into a beaker containing 50 ml of 2% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer. Allowed the beads to be formed by running the stirrer for 15 min. checked the bead formation under microscope. The beads were rigidized by adding 1ml of 25% solution of glutaraldehyde. The stirring was done for further 1 hour at 100 rpm. After stirring the solution was filtered and the beads were collected and dried in an oven for 2 h at 50°C and it was coded as G₁. By following above mentioned procedure three other batches were prepared where the ratio of sodium alginate and Guar gum were different such as 1.25 :0.75, 1.5:0.5, and 1.75: 0.25 they were coded as G₂, G₃ and G₄ respectively. All other variables were kept constant¹².

Evaluation of placebo micro beads and selection of best batches

All the four batches of placebo micro beads were subjected to evaluation study such as Percentage yield, Average particle size, rate of drying and swelling index. The best batches were selected based on the evaluation of physical characteristics.

Yield of production

The yield of production of micro beads of various batches were calculated using the weight of final product after drying with respect to the initial weight of the drug and polymer used for the preparation using the formula given below.¹³

Percentage yield= (Practical yield/ Theoretical yield) × 100

Determination of particle size distribution by optical microscopy

The particle size and particle size distribution of microbeads were evaluated using optical microscope. The dried microbeads were spreaded on a clean and dried glass slide and examined on an optical microscope, and size of the microbeads was measured by using the pre-calibrated ocular micrometer and stage micro meter. About 50 particles of each formulation were observed and counted. Mean diameter of particle size of microbeads was determined by using following formula.

Mean size= ($\Sigma n x / \Sigma n \times$ magnification value)

$\Sigma n x$ =total number of beads × mid-point of the size range

Σn = total number of beads.

Study on drying rate of the beads

A weighed amount of the beads was placed in open glass bottles and kept in an incubator (WTB Binder, Germany) maintained at 37°C. Initially, the beads were removed at short intervals of time (5, 10, and 15, up to 100 min) and later, at longer time intervals (200, 300, up to 550 min). These measurements were continued until attainment of constant mass indicating the complete equilibration. All the masses were measured using an electronic Mettler microbalance (Model AE 240, Switzerland)¹⁴.

Study on swelling behavior of micro beads

Swelling behavior was studied by measuring the percentage water uptake by the beads. About 50 mg of beads were accurately weighed and placed in 100 ml of phosphate buffer (pH 6.8 and 0.1 N HCl (pH 1.2). Beads were removed from their respective swelling media after 8 h and weighed after drying the surface water using filter paper. The water uptake was calculated as the ratio of the increase in weight of beads after swelling to the dry weight.¹⁵

Formulation of Nifedipine loaded micro beads

Preparation of calibration curve

Accurately weighed 100 mg of Nifedipine and was dissolved in minimum quantity of methanol and phosphate buffer mixture (2:1 ratio) in 100ml volumetric flask and made up the volume to 100 ml with phosphate buffer pH 6.8 so as get the concentration of 1mg/ml.(stock I). This stock solution was further diluted to get a concentration of 100µg/ml (stock II). Stock II solution was further diluted to get a series of concentration 1-10 µg/ml. Absorbance of these solution measured at 235nm in UV spectrophotometer by taking 6.8 pH phosphate buffer as blank. Graph was plotted concentration Vs absorbance to obtain the standard calibration curve¹⁶.

Preparation of Nifedipine loaded micro beads containing Guar gum as release modifier

One batch of microbeads using Guar gum as release modifier was selected based on certain evaluation criteria from four batches of micro beads. The drug Nifedipine was loaded into micro beads in various concentrations by following the procedure given below.

Accurately weighed quantity of Nifedipine 5mg was added to the selected batch of guar gum microbeads such as G₄. The resulting dispersion was dropped through syringe with needle slowly into a beaker containing 50 ml of 2% aqueous solution of calcium chloride which is kept under continuous agitation at a slow speed using a magnetic stirrer. Beads were allowed to be formed by running the stirrer for 15 min. The beads were checked under microscope and rigidized by adding 1ml of 25% solution

of glutraldehyde. The stirring was done for further 1 hour at 100 rpm. The solution was filtered and the beads were collected and dried in an oven and it was coded as F1. Similarly three other batches were prepared where, only the quantity of Nifedipine added was different such as 10mg, 20mg, and 30mg. The concentration of polymer was kept constant in all the batches such as 1.75% of sodium alginate and 0.25% of Gelatin and they were coded as F2, F3 and F4 respectively.

Estimation of drug encapsulation efficiency

Drug entrapment efficiency of Nifedipine microbeads was performed by accurately weighing 50mg of microbeads and suspended in 100 ml of phosphate buffer pH6.8 and it was kept aside for 24 hours. Then, it was stirred for 15 minutes and filtered. After suitable dilution, Nifedipine content in the filtrate was analyzed spectrophotometrically at 235 nm using shimadzu 1201 UV. Spectrophotometer. The absorbance obtained was plotted on the standard curve to get the exact concentration of the entrapped drug. Percentage of actual drug encapsulated in microbeads was determined using following relationship¹⁷.

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Particle size determination of microbeads by scanning electron microscopy (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan) using gold sputter technique at Cochin University of Science and Technology, (STIC). The particles were vacuum dried, coated to 200 Å thicknesses with gold palladium prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.

In vitro release studies of drug loaded micro beads

In vitro release studies of prepared microbeads were carried out using phosphate buffer (pH 6.8) using USP-basket type apparatus. The quantity of microbeads equivalent to 10 mg of Nifedipine was accurately weighed and put into the basket rotated at a speed of 100 rpm with the temperature maintained at 37±5°C. 900ml of the dissolution medium i.e. phosphate buffer PH6.8 was taken in the inner bath.

The samples were withdrawn at 0.5h, 1h, 2h, 4h, 6h, 8h, 10h, 11h, 12h, 14h, 18h, and 24h. At each time interval 5 ml of sample was withdrawn, at the same time 5 ml of fresh dissolution media was added to maintain sink condition. The samples withdrawn were suitably diluted and measured the absorbance at 235 nm spectrophotometrically. The cumulative percentage drug release was calculated at regular time intervals.

In vitro release Kinetics study of selected batches of Nifedipine micro beads

Determination of order of release of drug loaded batches by graphical method: To determine the order of release of drug from micro beads by graphical method from the dissolution data, a graph was plotted using % drug remaining Vs time. The slope of the curve was calculated to find out the release rate constant. Regression coefficient of the curve was determined to confirm the correlation between X and Y axis. In the second stage, from the same dissolution data, a graph was plotted through log % remaining Vs time. The slope and regression coefficient of the graph were also determined.

Study on mechanism of drug release: In order to predict and correlate the release behavior of drug from the polymeric matrix, it is necessary to fit the *in vitro* release data in to a suitable model. Hence the dissolution data were fitted according to the well-known exponential equation, which is often used to describe the drug release behavior from a polymer system. The equation which is used to describe drug release mechanism is: $M_t / M_\infty = kt^n$

Where, M_t / M_∞ are the fraction release of the drug with respect to the release time. 'k' is the constant, which indicate the properties of the macromolecular polymeric system, and 'n' is the release exponent indicative of the mechanism of release. The 'n' value was used for the analysis of drug release mechanism from drug loaded micro beads. The 'n' values were determined for all batches of drug loaded micro beads.

In vitro Wash off mucoadhesion test of Nifedipine micro beads

The mucoadhesive properties of various formulations of Nifedipine-loaded sodium alginate beads were evaluated by the *in vitro* wash-off method by using goat intestinal mucosa.

Pretreatment of goat intestine: Fresh intestinal mucosa was obtained from a local slaughter house. Intestinal contents were removed and washed with distilled water and then with phosphate buffer pH 6.8.

In vitro wash-off method: Freshly excised pieces of goat intestinal mucosa were cut into (1 cm × 1 cm) it was mounted on a glass slide (7.5 cm×2.5 cm) using thread. About 50 beads from each batch were spread out on each piece of mucosa and then hung from the arm of the tablet disintegration test apparatus. The tissue specimen was given a regular up and down movement in a vessel containing 900 ml of phosphate buffer (pH 6.8) maintained at 37±0.5°C. The adherence of beads was regularly observed. The beads that remained adhered to the mucosa were counted at regular intervals for up to 6h¹⁸.

$$\% \text{ Mucoadhesion} = \frac{\text{Weight of adhered microbeads}}{\text{Weight of applied microbeads}} \times 100$$

RESULTS AND DISCUSSION

Drug-polymer Compatibility study

From the FTIR graphs it was observed that the presence of characteristic functional peaks of Nifedipine, sodium alginate, and guar gum in the drug polymer physical mixture. It has also been evident that there was no major shifting and appearance of new characteristic peaks. Hence it was concluded that there was no interaction between the selected drug and polymers.

Formulation, evaluation and selection of best batches of placebo micro beads

From the table -1 it is evident that all the selected ratio of sodium alginate and guar gum were effective in producing micro beads of various sizes. But among various batches of micro beads made with guar gum as release modifier, the batch G4 was relatively better than other batches in terms of their physical characteristics.

This batch was found to be the best based on its discrete nature, (fig-1) higher yield, lower particle size, lower drying rate and higher swelling ratio. Hence this batch was subjected to subsequent studies using the drug.

Estimation of drug loading efficiency of drug loaded microbeads

From the above table it is evident that all the four batches of micro beads show a satisfactory drug loading capacity. The percentage of drug loading ranges from 58 to 73%. But the drug loading capacity of F2 batch was better at a drug concentration of 10mg /500mg of polymers. The Batch F3 and F4 and F1 showed relatively lower percentage loading than the F2 batch. As there was no much increase in drug loading after a concentration of 10mg/500mg of polymer the batch F2 was considered as the best batch. But all the drug loaded batches were subjected to *in vitro* analysis and *in vitro* wash off study.

Table 1: Physical characters of placebo micro beads with guar gum release modifiers

Ratio of Sodium alginate: guar gum	Formulation code	Av. Size of beads ($\mu\text{m} \pm \text{S.D}$)	Yield ($\% \pm \text{S.D}$)	Rate of drying (h at 50°C)	Swelling Ratio ($\pm \text{S.D}$)
1:1	G1	1208.2 $\pm$ 0.8	78 $\pm$ 1	2.5 $\pm$ 0.2	1210 $\pm$ 1.1
1.25:0.75	G2	1184.02 $\pm$ 1.2	83 $\pm$ 2	2.4 $\pm$ 0.11	1230 $\pm$ 1.2
1.5:0.5	G3	1261.10 $\pm$ 1.5	84 $\pm$ 1	1.5 $\pm$ 0.1	1220 $\pm$ 1.3
1.75: 0.25	G4	1125 $\pm$ 1.5	86 $\pm$ 1	1.2 $\pm$ 0.12	1420 $\pm$ 1.5

Table 2: Drug loading efficiency of microbeads of Nifedipine

Selected batch of Micro beads	Formulation code	Quantity of drug added mg / 500mg of polymer	Drug loading ($\% \pm \text{S.D}$)
G4	F1	5mg	58 $\pm$ 0.5
	F2	10mg	72 $\pm$ 0.8
	F3	15mg	71 $\pm$ 0.2
	F4	20mg	68 $\pm$ 0.6

Particle size determination of microbeads by scanning electron microscopy (SEM)

The morphological evaluation of the batch F2 was done by scanning electron microscopy. The study revealed that the microbeads were almost spherical in shape with rough outer surface, which subsequently enhances the drug release by channel formation.

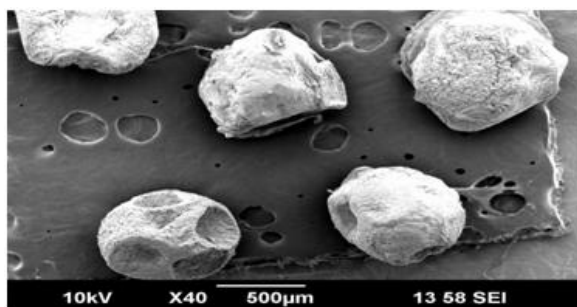


Figure 1: Scanning Electron microscopy of Microbeads (F2)

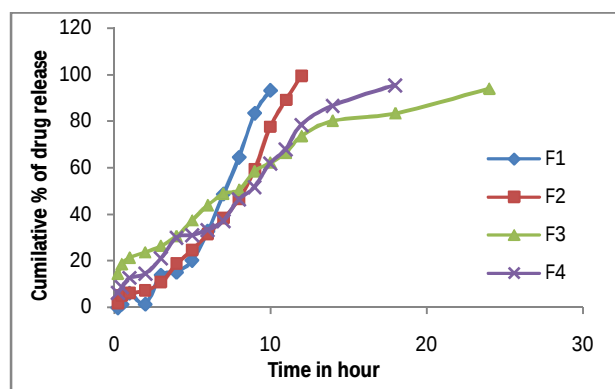


Figure 2: *In vitro* release studies of drug loaded micro beads (Batch F1-F4)

Fig-2 depicts the *in vitro* release data of Nifedipine loaded micro beads. It can be understood that disregard of drug loading, all the drug loaded batches from F1- F4 shows a cumulative percentage release of 92-100% with a sustained pattern for about of 24 hours. It is also clear

that the selected release retardant is effective in retarding the release rate of drug at all selected concentrations. But among the batches the batch F1 and

F2 are not as effective as other batches in sustaining the release rate of drug whereas the F3 and F4 are more consistent in showing a sustained release.

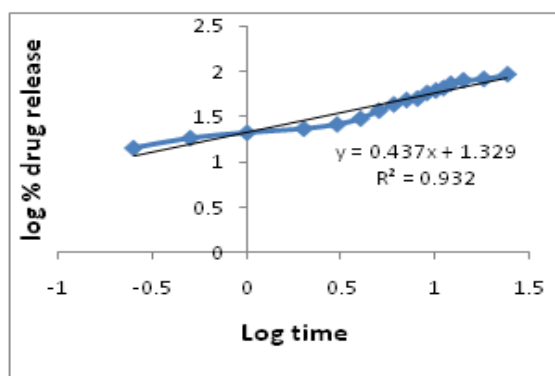


Figure 3: mechanism of drug release of batch F3

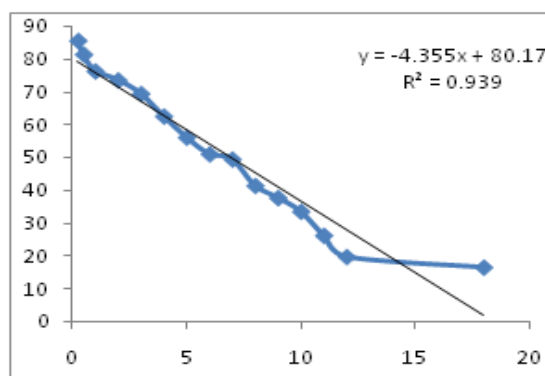


Figure 4: Zero order release from batch F3

Table 3: *In vitro* releases kinetic parameters of micro beads loaded with nifedipine

Formulation code	Zero order (R^2)	Rate constant (K)	Higuchi matrix (R^2)	Kosmeyer's Pappas (R^2)	'n' values
F1	0.909	10.02	0.9752	0.912	1.49
F2	0.910	23.04	0.9179	0.925	2.05
F3	0.939	10.02	0.9752	0.932	1.43
F4	0.913	9.324	0.7656	0.825	1.48

***In vitro* release Kinetics of selected batches of Nifedipine micro beads**

The dissolution data, obtained (table-3) from all the drug loaded batches, a graph was plotted with % drug release Vs square root of time to fit the data to Higuchi's model. Graphs of log % of release Vs log time was plotted and the data was fit into Kosmeyer's- Pappas's model. Based on the 'R-value and 'n' ($n > 1.0$) value of the table it is evident that the selected batches of microbeads (F1, F2, F3 and F4) were found to exhibit a zero order release followed by super case -II transport mechanism.

***In vitro* Wash off mucoadhesion test of Nifedipine micro beads**

The percentage of muco adhesion of micro beads is shown in table 4. The results of the ex-vivo mucoadhesion study reveal that all the four batches of micro beads have good mucoadhesive character which could be due to the combined effect of both polymers. The increased viscosity of the polymer helps to increase adhesion with intestinal mucosa. Therefore, it could be assumed that the prepared micro beads will adhere to the intestinal mucosa for a prolonged period where they release the drug in a sustained manner before being eroded off.

Table 4: Percentage mucoadhesion of selected batches

Formulation code	Mucoadhesion (% $\pm$ S.D)
F1	92 $\pm$ 0.8
F2	90 $\pm$ 1.2
F3	89 $\pm$ 0.6
F4	91 $\pm$ 0.5

CONCLUSION

Sodium alginate microbeads of Nifedipine prepared by Ionotropic gelation method with guar gum as release modifier were found to be a good carrier for the formulation of sustained release capsules or tablets in terms of good drug polymer compatibility, drug loading capacity, swelling behavior, percentage yield, *In vitro* release characteristics, and mucoadhesive strength. Eventually it may improve the bioavailability of the selected drug with concurrent reduction in dose.

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