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Hydrogel based biodegradable enteric coated alginate beads for colon targeted drug delivery of embelin

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ABSTRACT

Alginate, a natural polysaccharide has been extensively used as a matrixing agent for number of allopathic drugs. In the present investigation attempt is made to develop a sustained release multi particulate dosage form containing herbal molecule. *Embelin*, a well-proven anthelmentic from herbal origin was formulated in to gel beads of sodium alginate. A 2^3 full factorial design has been adopted for optimization of cross-linked sodium alginate beads containing *embelin*. Sodium alginate (10% w/v) was used as a polymer. Based on preliminary studies, the constraints of independent variables X1 (concentration of cross linking agent), X2 (type of hardening agent) and X3 (drying temperature for beads) have been fixed. The dependent variable that has been selected was Y1 (time taken for 80% drug release from beads, T80). The application of factorial design gave systematic approach in optimization on formulation variables. Concentration of cross linking agent (10% and 20% $CaCl_2$) and type of hardening agent (Isopropyl alcohol and Glutaraldehyde) were found to be effective in controlling the gel bead size and T80. The drug release was found to follow Korsmeyer model of diffusion. A USP dissolution guideline, similarity factor f_2 for drug release from gel beads with and without colonic fluid showed dissimilarity in drug release pattern. Further, optimized gel beads were film coated with HPMC phthalate to prevent the early release of *embelin* in upper GI tract and allowing majority of drug to release in lower intestine.

KEY WORDS: Biodegradable, Cross-linked Alginate beads, Embelin

1. INTRODUCTION

In the last decade much attention has been paid to formulate herbal medicines using modern technique. The present work is an attempt made to formulate multi particulate colon targeted drug delivery system containing *Embelin*, one of the well-known anthelmentic from herbal origin. *Embelin* (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) is the active constituent of the fruits of the plant *Vidang* (*Embelia ribes*, Family: Myrsinaceae). The plant is native to India and south-east Asia and distributed throughout India, Srilanka, Malaya, Singapore and South China[1]. Embelin is water insoluble, but forms a water soluble, violet colored complex in alkaline medium. In the Ayurvedic system of medicines it is recommended as an anthelmentic [2].

In recent years, the biomedical and pharmaceutical industries have shown much increased interest in the use of biopolymers, particularly alginates [3]. Alginates are linear unbranched polysaccharides containing varying proportions of -d-mannuronic acid (M) and -l-guluronic acid (G) residues. The M and G monomers are 1?4 linked by glycosidic bonds, forming homopolymeric MM or GG blocks, which are interspersed with heteropolymeric MG or GM blocks [4,5]. The naturally occurring alginate polymer used as binders and disintegrants in tablet manufacturing. Alginate, due to its hydration capacity and forming a gelatinous layer make it suitable for the development of controlled-release systems.

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The cross linking of alginate is a function of both the alginate composition and the length of the molecule. The affinity of the cross linking cation for the alginate is also of great importance in the cross linking reaction. The preparation of alginate hydrogel beads described here is based on the cross linking properties of this polymer with $CaCl_2$. The calcium ion is believed to interact with five different oxygen atoms of two adjacent guluronate units in intra-chain binding. In addition it makes an 'egg-box model' through inter-chain binding of calcium to two or more alginate chain. The enclosed drug is released from the alginate matrix at a controlled rate.

As compared to unit dosage form multi particulate system would cover larger area and hence better localized effect of drug can be achieved [6,7].

Keeping the above facts in focus the study was aimed to formulate colon specific biodegradable multi particulate dosage form contain-

ing *embelin.* In this study, factorial design based response surface method was adopted to optimize the effect of formulation on release characteristic of embelin from calcium alginate (ECA) beads. A 2^3 factorial design was employed to evaluate the combine effect of the selected independent variable (concentration of cross linking agent, type of a hardening agent and drying temperature) on time required for 80% drug release from beads (T80). Drug loaded alginate beads undergo enzymatic degradation in presence of colonic fluid. The drug release profile of ECA beads with and without colonic fluid was compared using USP dissolution specification f_2 value, a similarity factor.

2. MATERIALS AND METHODS

Powder of Vidang (Embelia ribes, F: Myrsinaceae) was purchased for L.Gandhi and Sons, Ahmadabad, India. Sodium alginates, 40 Cps (1%w/v), Isopropyl Alcohol, Glutaraldehyde, HPMC Phthalate were purchased from S. D. Fine Baroda, India) and used as received. All other solvent and chemical are used as received and were of analytical grade. *Embelin* was isolated in large amount from *Vidang* powder, by the method described by Sarin and Ray [8].

2.1. Preparation of embelin loaded ECA beads

The biodegradable alginate hydrogel beads containing embelin were prepared by ionotropic gelation method. In the present investigation, effect of calcium chloride concentrations, type of hardening agent and drying temperature were selected as independent variables, whereas T80 was chosen as dependent variable. The formulations and processing conditions in the factorial design are shown in table 1. After carrying out preliminary studies, the two significant variables, concentration of sodium alginate (10 %w/v) and drug to polymer ratio (1:2) were kept constant. All the formulations were prepared according to the experimental design.

Drug loaded calcium alginate beads were prepared by dissolving finely powdered sodium alginate in 100 ml alkaline buffer (pH 7.4) with slow agitation. *Embelin* (100 mesh sieved, 5g) was dispersed in alkaline buffer solution of sodium alginate based on its solubility study [9, 10]. The homogeneous dispersions were added drop wise through nozzle (21 gauge) at a rate of 10 ml/min into a solution of calcium chloride with gentle agitation at room temperature. The resultant ECA beads were allowed to remain in the solution for 2 h and then filtered through thin muslin cloth. After washing twice with distilled water ECA beads were suspended for 10 min in hardening agent for rigidization and then again washed twice with distilled water and filtered through vacuum before drying them under various temperature conditions for 12 h.

22*In-vitro* Characterization of the ECA beads

2.2.1. Determination of Particle size

The Embelin loaded Calcium alginate beads were characterized by optical microscopy. The mean diameter of 50 dried beads was determined. Average of triplicate batch is summarized in Table 1.

2.2.2 Determination of swelling ratio

The swelling ratio of alginate beads (50 mg) was measured in different medium, i.e. water and alkaline phosphate buffer pH 7.4. ECA beads were kept in contact with 100 ml of each of medium for 12 h without stirring. The magnitude of swelling was presented by the ratio of diameter of swollen bead to dried bead. Average of 20 beads was taken for calculating swelling ratio. Results are summarized in Figure 1.

2.2.3 Enzymatic degradation of ECA beads

Naturally occurring alginates are degraded by enzymes present in colonic flora. The effect of rat ceacum content (RCC) on degradation of ECA beads was studied using 4% w/v RCC prepared in 7.4 pH alkaline buffer. The selection of percent RCC solution is based on earlier report of Momin e.al, on natural polysaccharides for colonic drug delivery system. Dried ECA beads (50 mg) were added in two beakers containing 100 ml 7.4 pH alkaline buffer and 100 ml alkaline buffer (pH 7.4) containing 4% w/v RCC. The swelling ratio and appearance of swollen beads with and without addition of RCC after 12 h were observed and the results are summarized in Figure 2.

2.2.4. Determination of entrapment efficiency of Drug

To determine the efficiency of drug entrapment, ECA beads were assayed for drug content. The drug loaded ECA beads (100mg) of each batch were finely powdered in glass mortar and pestle and dissolved in phosphate buffer (pH 7.4) containing 10% ethyl alcohol with stirring at 100 rpm for 5 h. The resultant dispersion was exposed to ultrasonic treatment (Vibronics, Bombay, India) for 20 minutes. This treatment was repeated thrice with a resting period of 30 minutes. *Embelin* content was determined in supernatant using UV (291 nm) spectrophotometric method (Shimatzu UV- 1700) [11].

The percent of drug entrapped and the percent of free drug were calculated by following eqns,

$$\% \text{ free drugs} = \frac{\text{Amount of free drug present in 100 mg formulations}}{\text{Total amount of drug present in 100 mg formulations}} \times 100$$

$$\% \text{ drugs entrapment} = \frac{\text{Amount of drug present in 100 mg drug loaded beads}}{\text{Total amount of drug present in 100 mg beads}} \times 100$$

2.3. *In vitro* drug dissolution study

In-vitro dissolution study was carried out using accurately weighed amount of ECA beads equivalent to 100 mg *embelin*. Drug dissolution kinetics from the ECA beads was studied using USP XXII basket apparatus at 100 rpm at 37°±0.5 C. During dissolution studies physiological condition of stomach to colon was mimicked using different dissolution media. First 900 ml of 0.1 N HCL solution was used as a dissolution medium and the study was continued up to 2 hour. The medium was then discarded carefully and refilled with 10 % v/v ethanolic phosphate buffer of pH 7.4 and the study was continued for additional 4 hour. After total of 6 hour, depending on the design 4 % rat ceacum content (RCC) was added to the dissolution medium and the test was continued for a predetermined time [12]. Control study

(without RCC) was also carried out by continuing the dissolution study in 10 % v/v ethanolic phosphate buffer pH 7.4 for a predetermined time. Samples (10 ml) were withdrawn after regular sampling hour (2 h) and filtered through 0.45 µm membrane filter. The withdrawn amount of sample was replaced by fresh dissolution media. *Embelin* content was determined in the filtrate either directly or after appropriate dilution with dissolution media. An established spectrophotometric method of analysis of embelin was used to determine total *embelin* content (UV at 291 nm). The average value of T80 of each batch is reported in Table 1. All dissolution runs were performed in triplicate.

The dried ECA beads swell and forms hydrogel when comes in contact with physiologic fluid of GI tract. *Embelin* present on the outermost surface of the bead get released from swollen hydrogel beads giving premature release of *embelin* in stomach and upper intestine. Hence, optimized ECA hydrogel beads were film coated with HPMC phthalate to prevent the early release of *Embelin* in upper GI tract and allowing majority of drug to release in lower intestine.

2.4 Statistical Analysis

The results from factorial design were evaluated using Sigma plot software (Systat Software Inc, version 3.0 Richmone, CA software). Step-wise backward linear regression was used to develop polynomial equation for dependent variable T80.

$$Y = B0+B1X1+B2X2+B3X3+B11X12+B22X22+B33X32+B12X1X2+B13X1X3+B23X2X3+B123X1X2X3$$

Where, Y is dependent variable, B0 is arithmetic mean response of eight batches, and B1, B2, B3, are estimated co-efficient for factor X1, X2 and X3 respectively. The main effects (X1, X2 and X3) represents average result of changing one factor at a time from its low and high value. The interaction terms (X1X2, X2X3, X1X3) shows how the response changes when three factors are simultaneously changed [13]. The present investigation is aimed to target the natural polymer based drug delivery system to release the drug in colon. Natural polysaccharides get degraded by colonic microbial flora. Hence, it will be very informative to carry out drug release study which mimics the enzymatic degradation of drug delivery system. The formulated ECA beads were subjected to enzymatic degradation test using rat ceacum content. The drug release profile of ECA beads (n=3) in the dissolution medium with and without rat ceacal fluid was compared using USP dissolution specification f2 value, a similarity factor [14]. A value greater than 30 was considered significant value indicating dissimilarity profiles. Drug release kinetics study for model fitting was done for optimized formulation.

3. RESULTS AND DISCUSSION

The present study was aimed at developing calcium alginate based biodegradable hydrogel beads containing Embelin for colon targeted drug delivery system. The present formulation is a multi particulate device, which has benefit of being biodegradable as well as enhanced localized effect. These dual advantages make the formulation more suitable for drugs that have localized effect in large intestine and colon.

Alkaline buffered solution of sodium alginate containing embelin was dropped into calcium chloride solutions and gelled spheres were formed instantaneously due to ionotropic gelation mechanism. The ECA beads were easily manufactured without any sophisticated equipment. The preliminary studies revealed that the sodium alginate concentration below 10% did not obtained good sphericity of the gel beads. The lower viscosity of polymer solution led to oblong, tailed beads, while higher than 20 % concentration of polymer was difficult to handle. Hence, alginate concentration was finalized at 10 %w/v. Further, when the drug polymer ratio was kept 1:1 and 1:1.5, no significant effect was observed on entrapment efficiency of the drug in beads compared to 1:2 drug polymer ratio and hence, this ratio was selected for experimental design.

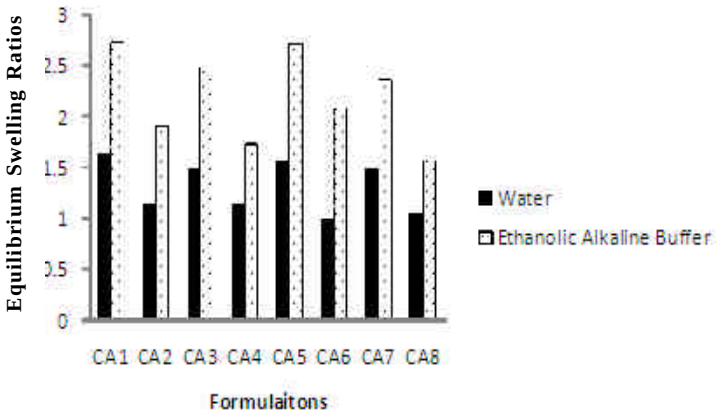

Fig. 1. The equilibrium swelling ratios of ECA beads in water and pH 7.4 ethanolic buffer

All the formulations prepared in accordance with factorial design yielded spherical beads. As shown in Table 1, the mean diameter of ECA beads ranges between 1.536 and 1.854 mm. Isopropyl alcohol and Glutaraldehyde were selected based on initial trial studies on several non-solvents. The hardening agent promoted the formation of cross-links between sodium alginate molecules. It is thought that the hardening agent cooperates to build a dense surface on the beads. Glutaraldehyde was found to give better hardening of beads compared to isopropyl alcohol. The effect of drying temperature (37° C and 50°C) on diameter of ECA beads was also studied. The diameter of beads was not affected significantly due to variable drying temperature. The extent of swelling of the ECA beads upon rehydration during release was affected by the dissolution medium. Alcohol present in the ethanolic phosphate buffer allows the gel beads to swell more than in water due to greater solvent penetration into the calcium alginate network (Figure 1). The equilibrium swelling ratios depicted in Figure 1 shows that there is an influence of hardening agent on the swelling of the beads in water and ethanolic phosphate buffer. The swollen beads were examined under the microscope to observe the gelling characteristic. In all cases the gels remained intact, although in the ethanolic phosphate buffer they appeared slightly softer. In the enzymatic degradation study, it was observed that the surface of the bead was slightly eroded. The beads were softer and porous in nature.

Table I Formulations, formulation variable and its characteristics.

Formulation	Variables			Parameters		
	X1	X2	X3	Mean Diameter (mm ± S.D.)	T_{80} (Without RCC) (n= 3)	T_{80} (With RCC) (n=3)
ECA1	10	I	37°c	1.691 ± 0.42	14.5	7.20
ECA2	20	I	37°c	1.854 ± 0.025	19.4	9.8
ECA3	10	G	37°c	1.652 ± 0.041	17.2	8.1
ECA4	20	G	37°c	1.797 ± 0.036	21.8	11.5
ECA5	10	I	50°c	1.617 ± 0.041	15.6	7.4
ECA6	20	I	50°c	1.691 ± 0.029	22.6	12.5
ECA7	10	G	50°c	1.609 ± 0.034	19.8	9.1
ECA8	20	G	50°c	1.536 ± 0.017	24.6	24.6

X1 = Concentration of cross linking agent, X2 = Hardening agent, X3 = Drying temperature °C, I = Isopropyl Alcohol, G = Gluteraldehyde, S.D.= value calculated for 50 beads, T_{80} = Time (hrs) required to release 80% of drug from Beads (Without RCC and with RCC)

From the data of experimental design and parameters (Table1) for ECA beads formulations

ECA1 to ECA8, a fitted polynomial equation relating the response (T80) to the transformed factors is shown below,

$$T80 = 4.818 + 0.33X1 - 0.009X2 + 2.85X3 + 0.008X1X2 - 0.125X1X3 + 0.042X2X3 \ldots\ldots \text{Eq 2} \ (R^2 = 0.997, DF = 7)$$

The T80 value of all eight batched show a wide variation ranging from 14.5 h to 24.6 h. In Eq 2, negative sign for co-efficient of factor X2 indicates that T80 of ECA beads increases when drying temperature is low. At higher temperature ECA beads forms hard surface which in turn do not hydrate easily and hence drug release was delayed. The positive sign of X2 and X3 factors indicates that dependent variable value increases as X2 and X3 increases. The negative sign of interaction factors, X1 and X3 indicates that drug release from beads is also affected by concentration of cross-linking agent and type of hardening agent. Hence, it can be concluded that the selected independent variables have statistically significant effect on selected dependent variable.

3.1. Drug release Kinetics studies:

Release studies were carried out to examine the suitability of the calcium alginate gel beads for targeted and prolonged drug release. Different formulation variables such as concentration of calcium chloride, type of hardening agent and the drying temperature were investigated and their effects on the release of embelin are shown in Fig. 2 (response surface plot). This figure shows the effect of the concentration of $CaCl_2$, presence of isopropyl alcohol/gluteraldyhyde and drying temperature on drug release from ECA beads. From all the formulations as per factorial design, batch ECA8 showed best optimized characteristics.

During dissolution studies physiological condition of stomach to colon was mimicked using different dissolution media. All the formulations were subjected to dissolution study with and without rat ceacal content. Time required to release 80% drug was compared for all the formulation in presence and absence of RCC (Figure 2). The drug release profile of all the batches were compared using USP dissolution specification, f_2 value, a similarity factor. All the formulations showed f_2 value greater than 30 indicates dissimilarity in dissolution profile in presence and absence of RCC.

Table II Results of model fitting of batch ECA8 (with rat ceacal content)

Model fitting parameters	Zero Order	First Order	Higuchi	Hixon Crowell	Korsmeyer and Peppas	Weibull
F-value	13.445	26.674	3.254	20.312	1.181	3.854
R-square	0.854	0.872	0.986	0.693	0.995.	0.979
Diffusion exponent (n)			1.42			
Mechanism of Drug release			Anomalous diffusion			

Note: Three best models were selected for calculation of F
Minimum F = 1.181 Medium F= 3.254 Maximum= 3.854
Critical value of F (5%), DF (7,7) = 3.76
F (Medium /minimum) = 2.75 The difference is insignificant
F (Maximum /minimum) = 3.26 The difference is insignificant

The dissolution data of gel beads ECA8, a potential candidate for targeted and prolonged drug release, was further analyzed to ascertain the mechanism for drug release [15]. The release profile of ECA8 fitted best to Korsmeyer and Peppas equation (F = 1.181) giving the least residual sum of square as compared to Higuchi equation model with R square value 0.995. It showed anomalous diffusion along with erosion of the system. (Table 2).

Formulation ECA8 almost released 9% of Embelin in physiological environment of stomach and jejunum; this could be further retarded by giving an enteric coat with 10% HPMC Phthalate to the ECA beads (Figure 3)

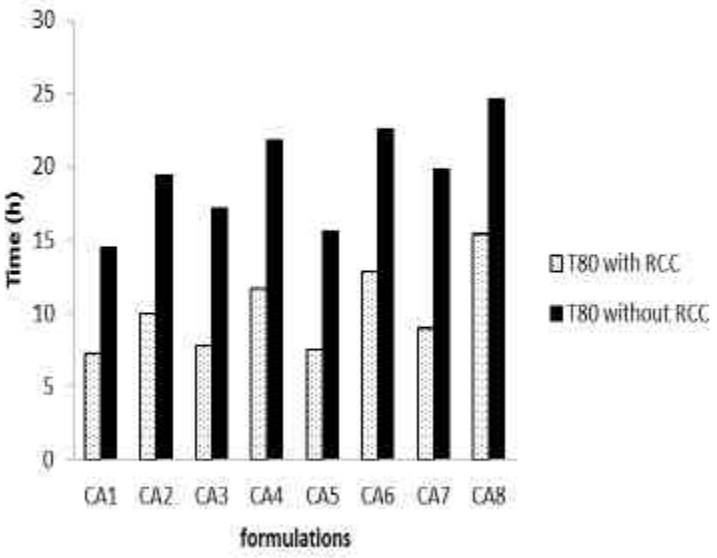

Fig. 2. The effect of presence of Rat Cecal Content medium on T80.

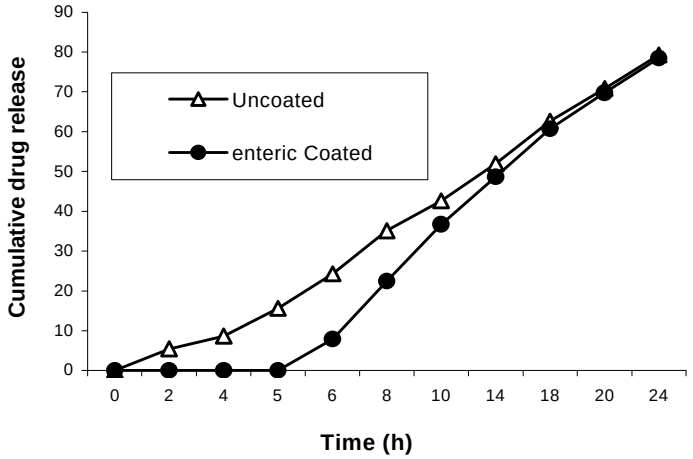

Fig. 3. Comparison of drug release profile of uncoated ECA8 beads with enteric coated formulation

In conclusion, the ECA beads were successfully prepared using ionic gelation technique to provide a controlled release delivery system. Spherical beads with narrow size distributions could be prepared with high yields and good entrapment efficiencies. All investigated factors have significant effect on percentage of drug entrapment, the size of beads and the release of embelin from ECA beads. The mechanism of drug release from ECA beads followed the diffusion controlled model for an inert porous matrix. The natural polymer like alginate based multiparticulate dosage form provided an intermediate erosion pattern for the colonic delivery of *Embelin* beads. Hence, it can be concluded that the drugs like, Embelin can be delivered to colon without releasing in stomach and upper intestine for the better treatment of helminthiasis using natural polysaccharides as a polymer.

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