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In vitro evaluation of polymeric beads of riboflavin formulated at different cross-linking time

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Abstract

Sodium alginate beads were evaluated as sustained release vehicles for the delivery of Riboflavin. It has been shown that the structure of the cross-linked calcium alginate network is of primary importance in the retention and/or release of the Riboflavin. The Riboflavin bead formulations were prepared by dispersing Riboflavin together with a mixture of sodium alginate and plasticizer solution and then dripping the dispersion into a solution of 5% (w/v) calcium chloride for different time of cross linking. Prepared beads were evaluated for Particle size, Flow property, Drug entrapment efficiency and in-vitro drug release. The formulations showed the particle size and angle of repose within acceptable range having good flow property but the beads formed by high cross linking time demonstrated nearly same particle size and showed poor flow property as compared with other formulations. Drug entrapment efficacy of all formulations was in the range of 55.76 - 74.21% and entrapment efficacy of beads decreases with increase the cross linking time. Riboflavin in-vitro release from beads was studied. The Beads prepared by maximum 15 minutes cross linking time shows 77% drug release. The values of coefficient correlation were calculated and drug release was found to follow Higuchi matrix order release.

Key words: Sodium alginate, riboflavin, angle of repose and cross linking time.

INTRODUCTION

Ideally, a drug delivery system releases the drug in the right body compartment at the rate required for a specific treatment. Most available drug delivery systems use biodegradable, biocompatible and natural biopolymers and are capable of rate and/or time controlled drug release [1]. Considerable research efforts are being spent on oral sustained drug delivery systems, with the majority of these systems being solid dosage forms [2].

Sodium alginate is a polymer which can be extracted from brown seaweed and kelps. It is one of the structural polymers that help to build the cell walls of these plants. It has some unusual properties and a wide variety of uses [3]. Chemical reaction between sodium alginate and

calcium chloride to form calcium alginate was utilized for beads formation. The gelation of alginate is cause by forming an egg box junction to associate divalent metal ions of alginate polymer chain [4]. Sodium alginate has been used as matrix material to achieve a sustained release of drugs [5,6]control release drug delivery [7], targeting gastric mucosa [8,9], and increasing the bioavailability of drugs [10] because of sodium alginate's ability to form a stable and bioadhesive gel with calcium ions [11].

It has some unusual properties and a wide variety of uses and applications in the biotechnology industry [12]. Alginate has been used successfully for many years in the food [13,14] and beverage industry [15] as a thickening agent [16], gelling agent and colloidal stabilizer and in pharmaceutical industries, such as disintegrate, and tablet binder, thickening stabilizing agents in mixtures and as gelling agents in confectionary.

Riboflavin [7,8-dimethyl-10 (1'-D-ribityl) isoalloxazine, B-complex vitamin, Dolo-neurotrat, flavin, flavine, lactoflavin, riboflavine, vitamin B2, vitamin G], a model of narrow absorption window drug [17,18] was used to demonstrate the impact of cross linking time on particle size, flow property, drug entrapment efficacy and controlled drug release from the calcium alginate beads. This model drug is advantageous because it lacks adverse effects and has no pharmacological effect on gastric motility [19].

Riboflavin is used for the treatment of anemia , dermatitis (skin irritation)[20], Migraine headache prevention, parkinson's [21], ariboflavinosis [22,23] associated with weakness, throat soreness/swelling, tongue swelling (glossitis)[24], angular stomatitis/cheilosis (skin cracking or sores at the corners of the mouth) and malaria, cognitive function, pre-eclampsia [25], some research suggests that riboflavin may lead to slight improvements in motor function, cognitive behaviour, and diarrhoea. This is also used as a tracer of medication compliance in the treatment of patients with alcohol dependence [26], mental disorders, and other conditions. Urinary riboflavin levels [27, 28] may be measured in order to determine level of compliance. It is readily absorbed from the upper GIT being its absorption window, 60% of drug is bound to plasma proteins, its $t^{1/2}$ 66-84 min, 9% of drug is excreted unchanged in urine make it a suitable candidate for floating drug delivery system [29].

MATERIAL AND METHOD

Materials used included Riboflavin (Batch no. V227505), and Sodium alginate, food grade, viscosity 45 cps. (Batch no. G521208) were purchased from Loba Chemie PVT LTD, Mumbai (India). Calcium chloride, purchased from S. D. Fine Chemicals, (India). All other compounds were of analytical grade.

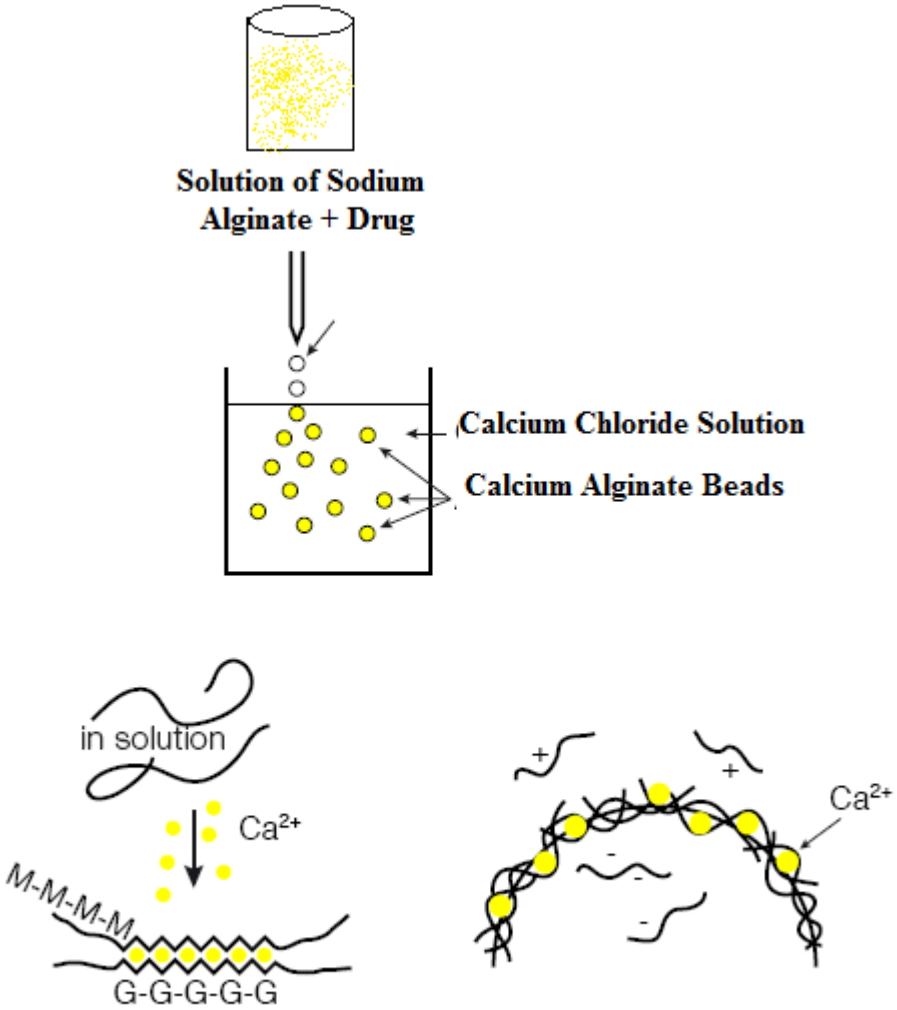
Table 1. Composition of various formulations with different cross linking time

Formulations code	Con. Of drug (mg)	Polymer (% w/v)	Plasticizer (% w/v)	Cal. Chloride solution (% w/v)	Cross linking time (min.)
F1	0.1	2 %	0.1%	5	15
F2	0.1	2 %	0.1%	5	20
F3	0.1	2 %	0.1%	5	25
F4	0.1	2 %	0.1%	5	30
F5	0.1	2 %	0.1%	5	35
F6	0.1	2 %	0.1%	5	40

F7	0.1	2 %	0.1%	5	45
F8	0.1	2 %	0.1%	5	50
F9	0.1	2 %	0.1%	5	55
F10	0.1	2%	0.1%	5	60

Formulation of Riboflavin containing alginate beads

Various formulations (F1 to F10) of riboflavin containing alginate beads were prepared by gel formation (Fig. 1) through the interaction between calcium ions and sodium alginate at different cross linking time as shown Table1. A solution was prepared by dissolving 0.1 g Riboflavin in 5 ml distilled water. The solution was dispersed in 20 ml alginate solution (2 %, w/v) containing 0.1% plasticizer. The dispersion was added drop wise via 26 gauge hypodermic needle fitted with a 10ml syringe into 50ml 5% w/v of cross linking agent (Calcium chloride) solution, being stirred at 200 rpm for 10min . The formed beads were further allowed to stir in the solution of cross-linking agent for an additional of 1hour. So the gelatinous precipitate is formed by chemical reaction between sodium alginate and calcium chloride. The fully formed beads were collected and washed with three times 50ml volume of deionized water. The beads were there after dried at 60 ^{o}C for 2.5 hrs in a hot air oven.



Mechanism of gel formation through the interaction between calcium ions and sodium alginate.

Fig. 1

Evaluation of Riboflavin containing alginate beads

Size Analysis [30]

The average diameter of twenty dry beads was determined randomly using a caliper (Aerospace, China) in triplicate.

Flow Property [31, 32]

Angle of repose method was employed to assess the flowability. Beads were allowed to fall freely through the funnel fixed at 2 cm above the horizontal flat surface until the apex of conical pile just touched the tip of the funnel. The angle of repose (θ) was determined by formula.

$$\theta = \tan\text{-}1\ (h/r)$$

Where, h- Cone height of beads, r- Radius of circular base formed by the beads on the ground.

Drug entrapment efficacy [33]

The prepared beads were evaluated for drug entrapment efficacy. An accurately weighed sample of beads (10 mg) suspended in 100ml of water and kept for 24hrs. Next day it was stirred for 5min and filtered. About 20 ml of methanol was added to precipitate sodium alginate which was removed by centrifuging for 5min at a rotational speed of 1000 rpm. After suitable dissolution with 0.1 N HCL (pH 1.2) Riboflavin content in the supernatent was analysed spectrophotometrically at 444 nm using Shimadzu UV spectrophotometer. Finally, drug encapsulation efficacy is calculated by-

$$\text{Drug Entrapment Efficacy} = \frac{\text{Actual drug content x 100}}{\text{Theoretical drug content}}$$

In-vitro drug release

The in vitro dissolution study was carried out using six station USP paddle dissolution rate test apparatus at 50rpm. The dissolution medium consisted of 900 ml of 0.1 N HCl dissolution media (pH 1.2) 37 ± 2^0C temperature. Aliquots of 5ml were withdrawn every one-hour and an equivalent amount of fresh dissolution fluid equilibrated at the same temperature was replaced. The samples were analyzed spectrophotometrically at 444 nm after suitable dilution.

Stability Study

Stability study was carried out on the all formulations. The formulations was wrapped in aluminium foil and then placed in an amber coloured bottle. Stability study was performed at 2-8°C and at ambient temperature for three months. Results obtained were compared with the data obtained for zero time at room temperature and humidity (Temperature 28 ± 2^OC and 42% ± 2% relative humidity).

RESULT AND DISCUSSION

Size analysis

The size of dry riboflavin containing alginate beads are determined by counting methods, such as microscopy or single particle counting (calipers), provide quantitative results, since data was collected from individual beads Table 2.

Table 2. Average size and angle of repose of the prepared beads.

Formulation code	Average size (mm)±S.D	Angle of repose [a] (θ)	Formulation code	Average size (mm)±S.D	Angle of repose [a] (θ)
F1	1.21±0.03	19^0	F6	1.28±0.04	21^0
F2	1.31±0.04	20^0	F7	1.39±0.03	22^0
F3	1.28±0.06	19^0	F8	1.31±0.06	21^0
F4	1.30±0.03	21^0	F9	1.46±0.07	22^0
F5	1.11±0.06	20^0	F10	1.47±0.03	23^0

± S.D- Standard deviation for (n=3), [a] Mean of three readings

Flow property

The formulation prepared showed the angle of repose within acceptable range having a good flow Property Table 2. But when we see the individual formulation the individual formulation increases the angle of repose because if we increase the time of cross linking the surface of beads erode or rough F10, in formulation F1 angle of repose is very low 19^0, this means if the cross linking time is short the surface of beads will be smooth. Higher hardening time resulted in smaller beads with irregular surface because of shrinkage and showed an increased angle (from 19^0 to 23^0)

Table.3 Drug entrapment Efficacy and *In-Vitro* drug released of the prepared beads

Formulation code	Drug entrapment Efficacy* (%)	*In-Vitro* drug released (%) Mean ± (S.D.)
F1	74.21	77 ±3.45
F2	73.65	75±2.67
F3	72.18	74±2.10
F4	68.91	73±3.75
F5	67.23	71±2.36
F6	64.10	69±3.12
F7	60.13	68±2.09
F8	58.01	65±2.21
F9	57.90	65±2.45
F10	55.76	64±2.90

± S.D- Standard deviation for (n=3), * Mean of three readings

Drug entrapment efficacy

The drug entrapment efficacy of all the formulation was in the range of 77- 64 % Table 3. The plot of drug entrapment efficacy v/s time plotted for all formulations are shown in Figure 2. The drug entrapment efficacy of beads decreases with increases the time of cross linking because if we increase the cross linking time some amount of drug releases in the cross linking solution, so the formulation F10 entrap low concentration of drug and formulation F1 entrap high concentration of drug .

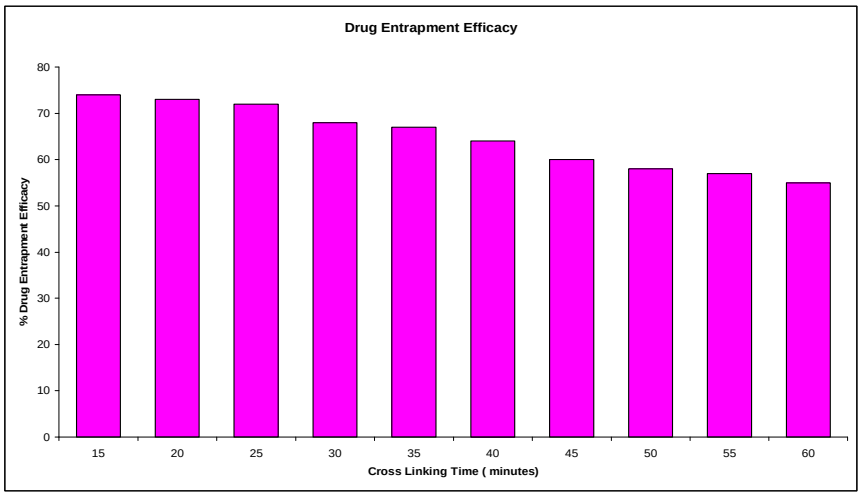


Fig. 2 Percentage of the Riboflavin drug entrapment efficacy as a function of beads formation at different cross linking (Release media, 0.1 N HCl, pH 1.2)

***In-vitro* drug release**

Riboflavin *in-vitro* release from alginate beads was studied in 0.1 N HCl dissolution media (pH 1.2) 37 ± 2^0C temperature for the period of 9 hrs. The release pattern of drug from beads was slow and spread over extended period of time. The plot of cumulative % drug released Vs time plotted for all formulations are shown in Figure 3. The beads having more cross linking time (F10, F9, F8 etc.) release the drug very slowly (64%, 65%, 65% respectively) as compare to less cross linking time formulations (F1 F2 F3 etc.) due to high cross linking of polymer Table 3. The values of co-efficient correlation (r) were calculated. The release rate follows Higuchi matrix order release equation and indicate matrix release kinetics.

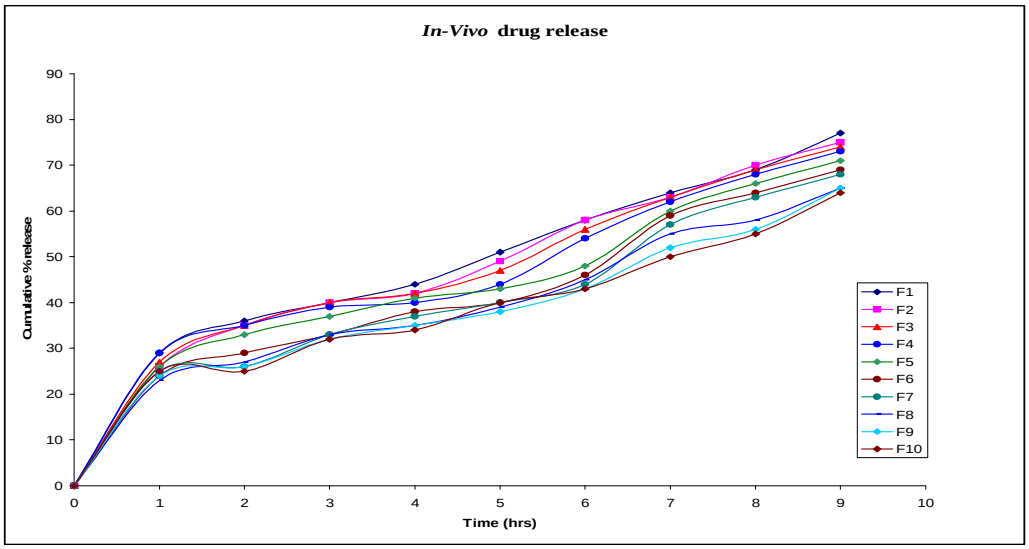


Fig. 3 *In vitro* drug release study of Riboflavin beads (Cumulative % release) (Release media, 0.1 N HCl, (pH 1.2) 37 ± 2^0C)

Stability study

Stability study was performed at different temperature and also at different conditions. But it showed that there is no significant reduction in the percentage drug retained in the formulation and also there was no significant difference in drug release profile for the sample storage at 2-8˚C and at ambient temperature. Also, no significant difference was observed in sample stored in

dark, Riboflavin is a light sensitive drug, so it was suggested that, formulation should be protected from light.

CONCLUSION

The time of cross linking in beads formation is expected to be of primary importance as concerns the entrapment and release properties of the beads. The drug loading efficiency as a function of hydrogel beads formation time (time of contact with the $CaCl_2$ solution) is reported in Figure 2. It clearly appears that, the loading rate of Riboflavin decreases as the time of cross linking increases. This can be explained by the fact that the release of the drug starts to occur during the period of the beads formation. The time of beads formation should also influence the cohesion of the beads. If the structure of the beads is too loose, the polymer network is eroded. Consequently the drug and release outside the beads. The released amount is more important when the time of beads formation is short suggesting that the structure of the beads is strongly dependent on the contact time between Ca^{2+} and alginate. In our conditions, curing time at 15 minutes has been chosen to be the optimum curing time for maximum drug loading efficiency.

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