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Development of alginate gel beads-entrapped liposome for colon specific drug delivery of Prednisolone

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ABSTRACT

Delivery of drugs to the systemic circulation through colonic absorption represents a novel mode of introducing peptide and protein drug molecules and drugs that absorb poorly from the upper gastrointestinal tract (GIT), as the colon lacks various digestive enzymes that are present in the upper GIT. Colon-specific drug delivery systems (CDDSs) can be used to improve the bioavailability of drugs given through the oral route. A novel formulation for oral administration using Eudragit S 100 coated calcium alginate gel beads-entrapped liposome and prednisolone as drug has been investigated for colon-specific drug delivery in vitro. Drug release studies were done in conditions mimicking stomach to colon transit. Result shows that the drug was protected from being released completely in the physiological environment of the stomach and small intestine. The release rate of prednisolone from the coated calcium alginate gel beads-entrapped liposome was dependent on the concentration of calcium and sodium alginate, the amount of prednisolone in the liposome, as well as the coating.. The colonic arrival time of the tablets is normally 4–5 h. The results clearly demonstrated that the coated calcium alginate gel beads entrapped liposome is a potential system for colon-specific drug delivery.

Keywords: Controlled drug Delivery, Colon Targeting, Entrapped Liposome, Site Specific drug delivery, Coated Alginate Beads

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INTRODUCTION

Oral route is not suitable for a number of drugs because of their side effects in stomach and degradation of drugs by the digestive enzymes of the stomach and the small intestine. But the oral route is by far the most popular route for drug administration due to patient compliance and

the manufacturing of formulation for oral route is easy due to the reason no need of sterilization etc. A number of studies have been carried out in attempts to deliver prednisolone effectively into the systemic circulation. Delivery of drugs to the systemic circulation through colonic absorption represents also a novel mode of introducing peptide and protein drug molecules and drugs that absorb poorly from the upper gastrointestinal tract (GIT), as the colon lacks various digestive enzymes that are present in the upper GIT. Colonic drug delivery is also useful for systemic absorption of drugs, especially protein and peptide drugs, because of the less hostile environment prevailing in the colon compared with the stomach and small intestine. The different approaches for targeting orally administered drugs to the colon include coating with pH-dependent polymers, design of timed-release dosage forms and the utilization of carriers that are degraded exclusively by colonic bacteria. [1, 2] The poor site-specificity of pH-dependent systems, because of large variations in the pH of the gastrointestinal tract, has been very well established. [3,4] The site-specificity of timed-release dosage forms is considered poor because of large variations in gastric emptying times [5] and in passage across the ileocaecal junction. [6] Alginate is a natural polyacid, and has a unique property of gel formation in the presence of multivalent cations, such as calcium ions in aqueous media, which takes place mainly at junctions in the G–G sequence rich chain region know as the ‘‘egg box junctions’’. Therefore, alginate is used as an immobilization matrix for cells and enzymes as well as pharmaceutical and food adjuvants. [7-9] when an aqueous solution of sodium alginate is added drop wise to an aqueous solution of calcium chloride, a spherical gel with regular shape and size is obtained. The spherical gel is termed an ‘‘alginate bead’’. Alginate beads have the advantages of being nontoxic orally and having high biocompatibility. [10] Another advantageous property is their ability of reswell. This property is susceptible to the pH of an environment, so acid-sensitive drugs incorporated into the beads would be protected from gastric juice. [11]. Because of this, many results have been reported concerning the use of alginate beads as a drug-controlled release formulation. [12-15] However, the porosity of alginate beads results in a very low efficiency of incorporation with drugs that have a low molecular weight and are water-soluble drugs, and they result in a fast release of incorporated drugs. On the other hand, liposomes also provide protection of the entrapped drugs from enzymatic degradation. [10, 17-18] The aim of this study was to evaluate a new colon-specific drug delivery system (CDDS). Instead of entrapping the drug inside the matrix during fabrication of the calcium alginate gel beads, we propose coating the alginate beads and entrapping a drug-loaded liposome with Eudragit S100. In this experiment, we prepared the calcium alginate gel beads containing liposome-loaded prednisolone and investigated the factors that influenced the release of the model drug, prednisolone.

MATERIALS AND METHODS

Alginate was purchased from the Hi-Media (India). Prednisolone was obtained from Kwality Pharmaceutical, India. Eudragit S100 was obtained as gift sample from by the Rohm (Mumbai). All other chemicals used were of analytical grade.

Preparation of liposome containing calcium alginate gel beads

For the preparation of calcium alginate gel beads loaded with prednisolone liposomes, firstly liposome were prepared by shake flake method. 100 mg of phosphatidylcholine was dissolved in

5 ml of chloroform and spread on the inner wall of a round bottom flask as a thick lipid film. The flask was kept in a vacuum to remove the solvent completely. Prednisolon dissolved in phosphate buffer (pH 7.4) was added to disperse the lipid by reverse rotation of flask. The suspension containing liposome was suspended in 10 ml of the sodium alginate solution and mixed completely. Using a 10ml syringe (5 gauge needle), this solution was dropped into about 30 ml calcium chloride solution with mild agitation and stirred slowly for 1 h and kept for 6 hours to cure the calcium alginate gel beads. Eudragit S100 solution (2.0%, w/v) was prepared by dissolving 2.0 g of Eudragit S100 in 100 ml water. The wet calcium alginate beads were transferred into Eudragit S100 solution and kept for 30 minutes under gentle magnetic stirring. The resulting magnetic alginate -Eudragit S100 beads were collected and rinsed with deionized water and dried in air overnight. Then the calcium alginate gel beads were dried in a vacuum for 12 h. The morphology of the gel beads before and after the coating was characterized by scanning electron microscopy (S.E.M.). The coated beads were stored in a sealed polythene bag before use.

Characterization of the calcium alginate gel beads

The amount of prednisolone entrapment was measured by 50 mg of crushed gel beads in 5 ml 5 Mmol/l EDTA/100 mmol/l NaCl buffer (pH 7.4). A 2-ml solution sample was transferred into a microcentrifuge tube and 2 ml of extractant (methanol) was added. The two phases were mixed by vortex for 2 min and the micro centrifuge tube was centrifuged at 15000 rpm for 5 min. The prednisolone content of the methanol phases was determined using UV spectrophotometer, and compared with a standard curve of data obtained by assaying known concentrations of prednisolone solution. The drug encapsulation efficiency, which is the percentage of prednisolone contained within the gel bead in relation to the initial amount employed, was calculated by the equation:

$$\text{Encapsulation efficiency} = \frac{[\text{Prednisolone}]^{\text{in beads}}}{[\text{Prednisolone}]^{\text{initial amount}}}$$

The sizes of the calcium alginate gel beads were observed using motic microscope. The time at which the calcium alginate gel beads reswell is one of the important formation parameters. Dried calcium alginate gel beads were placed in phosphate buffer pH 7.4. During reswelling, a white circle covering around the can be observed.

Scanning electron microscopy

For observation of the surface morphology of the beads and coated beads S.E.M. was used. The samples were mounted onto stubs and sputter coated with gold in a Bio-Rad E-5200 Auto Sputter coater.

Release of Prednisolone

Drug release studies were carried out using a USP Dissolution Rate Test Apparatus (USP Apparatus 1, 50 rpm, 37º±1ºC). As the average gastric emptying time is about 2 h the coated calcium alginate gel beads were tested for drug release for 2 h in 0.1 M HCl (250 ml) to mimic the stomach conditions,. Then the dissolution medium was replaced with pH 6.8 phosphate buffer (250 ml) and tested for drug release for 3 h, as the average small intestinal transit time is

about 3 h. Next, pH 7.4 phosphate buffer (250 ml) was used to test for drug release for 7 h. Samples were taken initially at 30-min intervals and after 4 hr., 60 min interval and after 8 hr. 2 hr. intervals. The amount of prednisolone released is shown in graph.

RESULTS AND DISCUSSION

Effect on the characterization of the calcium alginate gel beads

As shown in Table 1(a, b, c) the results demonstrated that the sodium alginate concentration, calcium chloride concentration and the ratio of liposome/sodium alginate affected the beads' characteristics and the percentage of the release of prednisolone. The results prove that on increasing the sodium alginate concentration used for the beads, the reswelling time and the diameter of the dried gel beads increased for a given calcium chloride concentration. Similarly, on increasing the calcium chloride concentration, the reswelling time and the diameter of the dried gel beads also increased for a given sodium alginate concentration. However, on changing the ratio of liposome/sodium alginate, there were no significant effects on the reswelling time or on the diameter of the dried gel beads.

Table 1(a): - The effects of concentration of sodium alginate on the characteristics of gel beads

Sr. no	Concentration of sod. Alginate	Factors				
		Appearance	Diameter (in mm)	Encapsulation efficiency	Reswelling time (in min.)	Percentage Release (in 8 hr.)
1	1% , w/v	Non Uniform	0.61±0.01	32.11±0.10	4~5	94.21±2.45
2	2% , w/v	Spherical	1.23±0.07	54.23±0.21	7~9	88.46±1.57
3	3% , w/v	Spherical	1.74±0.04	74.82±0.17	14~16	74.89±1.93
4	4% , w/v	Spherical	2.32±0.05	91.05±0.62	19~21	62.52±0.93

Table 1(b): -2.0% (w/v) of sodium alginate liposome: sodium alginate :: 1:2

Sr. no	Concentration of calcium chloride(mmol/l)	Factors				
		Appearance	Diameter (in mm)	Encapsulation efficiency	Reswelling time (in min.)	Percentage Release (in 8 hr.)
1	50	Spherical	0.65±0.02	41.23±0.10	5~6	92.14±1.95
2	100	Spherical	0.91±0.05	51.21±0.21	7~9	90.68±2.51
3	150	Spherical	1.37±0.03	77.65±0.17	11~14	87.39±1.74
4	200	Spherical	1.72±0.02	93.17±0.62	15~19	92.42±1.33

Table 1(c): -200 mmol/l of calcium chloride 2.0% (w/v) of sodium alginate

Sr. no	Liposome/sodium alginate	Factors				
		Appearance	Diameter (in mm)	Encapsulation efficiency	Reswelling time (in min.)	Percentage Release (in 8 hr.)
1	1:1	Spherical	1.28±0.01	63.21±0.42	10~12	91.84±2.13
2	1:2	Spherical	1.37±0.03	88.32±0.33	10~12	86.49±1.72
3	1:3	Spherical	1.52±0.06	91.15±0.49	10~13	61.42±0.98

Data are mean ±SD and n = 25 for measurements of diameter and the reswelling time of dried gel beads; n = 3 for measurements of encapsulation efficiency. Diameter given is of dried beads.

On the other hand, the use of concentrated sodium alginate solutions significantly reduced the release percentage of prednisolone from beads for 12 h. So, regardless of the calcium chloride

concentration, the use of 4.0% w/v sodium alginate solutions resulted in a prednisolone release of around 60%. However, the release percentage of prednisolone from beads for 12 h was not significantly affected by variations in calcium chloride solutions from 0.05 to 0.2 M. As shown in Table 1, the ratio of the liposome/alginate affected the release of the prednisolone. It was observed that the higher the amount of alginate, the lower was the release percentage of prednisolone from beads for 12 h. The results showed that the higher the concentration of sodium alginate and calcium chloride solution, the higher the drug encapsulation efficiency was. On the other hand, the drug encapsulation efficiency increased as the ratio of liposome/sodium alginate decreased. However, when the ratio of liposome/sodium alginate was lower than 1:2, no significant influence on the drug encapsulation efficiency was observed. All these results can be explained by the gel formation process, which is assumed to be an almost instantaneous and irreversible process that is governed by the diffusion of the two components involved in it: sodium alginate and Ca2 + ions. In this respect, the metallic cation is smaller than the polymer molecules. So, it is mainly the cation that diffuses between the alginate chains, binding to unoccupied binding sites on the polymer. Thus, on increasing the number of biopolymer molecules per unit solution volume, the number of binding sites for Ca2 + ions also increases. As a result, a more densely crosslinked gel structure will probably form. This would explain the results that the percentage of drug release decreases with increasing alginate concentration, for a given calcium chloride concentration. In the experiment, we observed qualitatively that the beads obtained from 1.0% w/v sodium alginate solutions had not gelled well and sticky surfaces and were very fragile and difficult to handle. This means that a more porous gel bead was formed on decreasing the number of biopolymer molecules per unit solution volume for a given calcium chloride concentration. On the other hand, on increasing the calcium chloride concentration, the cross-linked gel density increases for a given sodium alginate concentration. This kind of phenomena caused the increase of the drug encapsulation efficiency due to an increase in the diffusional resistance that liposomes suffer as they flow through an environment with a lower porosity for gel structures. In addition, a decrease in bead size was mediated by lower alginate concentration or lower calcium concentration, as shown in Table 1.

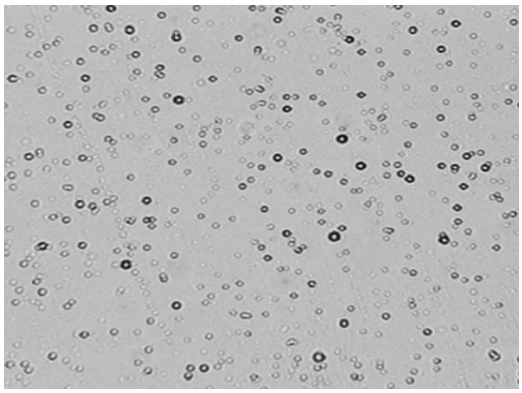

Fig. 1:- Motic image of liposome

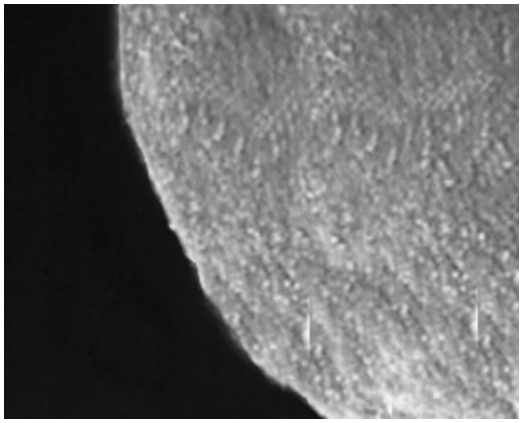

Fig. 2:- Surface morphology of uncoated alginate beads.

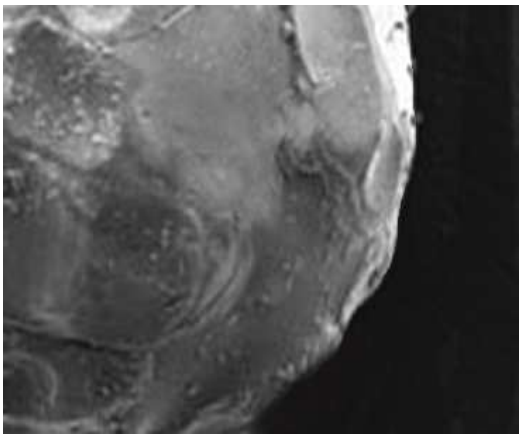

Fig. 3:- Surface morphology of Eudrgit S 100 coated alginate beds.

Scanning electron microscopy

Motic image of liposomes is shown in fig. 1. Scanning electron microscopy (S.E.M.) was used to observe the surface structure of the calcium alginate gel bead before and after coating. The size of spherical beads ranged from 0.91 to 1.7 mm. The appearance of a bead after drying is shown in fig 2. It is obvious that the surface of the bead shrank and a densely cross-linked gel structure was formed. The smooth surface of beads after coating is shown in fig 3.

Release of Prednisolone

Release of prednisolone from coated gel beads up to 12 hr is shown in fig 4 at pH 1.2 followed by a prednisolone -triggered release at pH 6.8 and pH 7.4. Initially release of prednisolone from the coated gel beads was low. A Little amount of prednisolone could be measured in the pH 1.2 medium for 2 h. Only 8.32±0.52% was released after 3 h and 22.32±0.86% after 4 h. After 8 h about 81% and after 12 hrs 93% had been released. Targeting of drugs to the large bowel can be achieved in several ways. Enteric coating has traditionally been used to prevent drug release in the upper gastrointestinal tract. The results of the present study prove that the prednisolone drug was protected completely from acid and enzymes in gastric juice by a membrane coating of

Eudragit S100. At pH 6.8, the membrane coating dissolved, and the gel beads were exposed. Following the polymer matrix swelling and erosion, the prednisolone loaded liposomes was released.

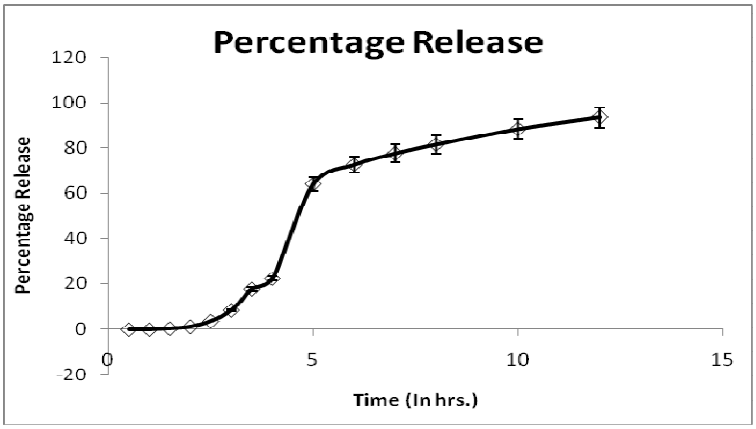

Fig 4: - Percentage release profile of prednisolone from beads
Report: - Mean ±SD, n=3

CONCLUSION

Prednisolone is used for ulcerative colitis and IBD. The release of liposome entrapped prednisolone is strongly influenced by the swelling and erosion of the alginate gel matrix. The characterization of calcium alginate gel beads has a significant dependence on the sodium alginate concentration, calcium chloride concentration and the ratio of liposome/sodium alginate. In addition, the drug release also depends on the coating membrane. Gamma scintigraphy can be used to prove the present study that coated calcium alginate gel beads are a potential system for colonic drug delivery.

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REFERENCES

[1] G. Vanden Mooter, R. Kinget, *Drug Delivery.* **1995**, 2, 81–93.
[2] Y.V. Rama Prasad, Y.S.R. Krishnaiah, S. Satyanarayana, *Indian Drugs*, **1996**, 33, 1–10.
[3] D. F. Evans, G. Pye, R. Bramely, A. G. Clark, T. J. Dysoa, *Gut*, **1988**, 29, 1035–1041.
[4] M. Ashford, J. T. Fell, D. Attwood, H. L. Sharma, P. J. Woodhead, *Int. J. Pharm*, **1993**, 95, 193–199.
[5] S. S. Davis, J. G. Hardy, M. J. Taylor, A. Stockwell, D. R. Whalley, C.G. Wilson, *J. Pharm. Pharmacol*, **1984**, 36, 740–742.

[6] M. Marvola, H. Aito, P. Ponto, A. Kanniksoki, S. Nykanen, P. Kokkonen, *Drug Dev. Ind. Pharm*, **1987**, 13, 1593–1609.
[7] R. P. Lanza, D. Ecker, W. M. Huhtreiber, J. E. Staruk, J. Marsh, W. L. Chick, *Transplantation*, **1995**, 59, 1485–1487.
[8] J. Zimmerberg, F. S. Cohen, A. Finkelstein, *Science*, **1980**, 210, 906–908.
[9] X. Liu, D. Chen, L. Xie, R. Zhang, *Journal of control release*, **2003**, 93, 293-300.
[10] T. Yotsuyanagi, T. Ohkubo, T. Ohhashi, K. Ikeda, *Chem. Pharm. Bull*, **1987**, 35, 1555-1563.
[11] Patent: W. J. Connick, *US Patent* **1983**, 4, 401-456.
[12] C. K. Kim, E. Lee, J. *Int. J. Pharm.*, **1992**, 79, 11–19.
[13] S. Shiraishi, T. Imai, M. Otagiri, *Biol. Pharm. Bull*, **1993**, 16, 1164–1168.
[14] K. Tateshita, S. Sugawarw, T. Imai, M. Otagiri, *Biol. Pharm. Bull*, **1993**, 16, 420–424.
[15] Y. Kato, T. Hosokawa, K. Ito, *Biol. Pharm. Bull*, **1993**, 16, 457– 461.
[16] P. K. Nimase, G. Vidyasagar, *Der Pharmacia Sinica*, 2010, 1(1), 29-35.
[17] K. Muramatsu, Y. Maitani, K. Takayama, T. Nagai, *Drug Dev. Ind. Pharm.*, **1999**, 25, 1099– 1105.