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Research Article

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FOMULATION, PHYSICAL CHARECTERISATION AND *In-vitro* RELEASE STUDIES OF PREDNISOLONE ALGINATE BEADS FOR COLON TARGETING BY IONOTROPIC GELATION

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ABSTRACT

This article shall give an overview on drug delivery systems for new therapeutic strategies in the treatment of inflammatory bowel disease. Conventional drug delivery systems are tightly adapted from developments of colonic delivery by oral administration triggered by release mechanism owing to the physiological environment that these systems encounter in the colonic region. The newer developments in this context aim for an increased selectivity of drug delivery by targeting mechanisms which have a closer relation to patho physiological particularities of the disease. The objective of the present study was to microencapsulate the anti inflammatory drug (prednisolone) to provide controlled release and colon targeting. Alginate beads of prednisolone were formulated by ionotropic gelation and further coated with Eudragit S-100 and the variables studied includes concentration of sodium alginate, different cross linking agents were evaluated with respect to particle size, surface characteristics entrapment efficiency and in vitro release behavior. IR spectroscopic study confirmed the absence of any drug interaction. DSC analysis revealed that the drug was uniformly dispersed in the alginate beads. The mean particle size increases with increasing the polymer concentration. The shape of alginate beads has acceptable sphericity and surfaces were rough which were confirmed by SEM photograph. The entrapment efficiency in different formulation varied from 69% to 81%. The in vitro release profiles were also altered significantly by changing various parameters. The kinetic modeling of the release data indicates that prednisolone released from alginate beads followed by Korsemeyer's model. The above observations suggest that prednisolone can be developed as colon targeting drug delivery system with sodium alginate 2.5% using Calcium chloride as cross linking agent and coated with Eudragit S-100.

Keywords: Prednisolone, alginate, beads, colon-specific, Eudragit S-100, scanning electron microscopy.

INTRODUCTION

Inflammatory bowel disease[1] (IBD) encompasses several chronic inflammatory conditions of the gastro intestinal tract, which can impact the small or large bowel. The most known subtypes are Crohn's disease (CD) and Ulcerative Colitis. Conventional drugs for the treatment of IBD include aminosalicylates, corticosteroids, antibiotics and immunosuppressive agents. The colon[2] is an attractive site where poorly absorbed drug molecules may have an improved bioavailability. Additionally, the colon has longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. By this technique, absorption of the drug from the stomach and small intestine can be minimized[3] until the dug reaches the large intestine. Various drug delivery systems have been designed[4] that delivers the drugs quantitatively to the larger bowel and subsequently to trigger the release of active drug. The site-specific delivery[5] of the drugs to the target receptor sites has the response. However, for successful colonic drug delivery, many physiological barriers must be overcome, the major one being absorption or degradation of the active drug in the upper part of gastrointestinal tract. For example, colon specific drug delivery systems protect peptide drugs from hydrolysis and enzymatic degradation in the duodenum and jejunum, and eventually release drugs in the ileum or colon, which leads to greater systemic bioavailability. The specific release in the colon[6] also affects a time delay between administration and onset of action, which can be useful for diseases, such as asthma and arthritis. Various colon specific drug delivery systems are being developed, by taking advantage of the luminal pH in the ileum and microbial enzymes in the colon.

Various strategies[7], currently available to target the release of drugs to colon, include formation of prodrug, coating of pH sensitive polymers, use of colon-specific biodegradable polymers, timed released system, osmotic systems, and pressure controlled drug delivery systems. Among the different approaches to achieve targeted drug release to the colon, the use of polymers especially biodegradable by colonic bacteria holds great promise. Prednisolone[8], a corticosteroid is used in the treatment. Similarly, an approach by using an experimental pH-sensitive polymer Eudragit S-100 allowing further retention of the drug release as the polymer dissolves at pH 7.5. Sodium alginate, which is a polysaccharide

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originally obtained from marine brown algae, it contains two uronic acids arranged in homopolymeric blocks. A remarkable property of alginate[9] is its ability to form a gel with divalent or multivalent metal ions. This property has been used to prepare alginate beads.

MATERIALS AND METHODS

Materials

Prednisolone was received as a gift sample from Wyeth pharmaceutical, Goa, India and Eudragit S-100 was purchased from Bharat Coats, Chennai, India. Sodium alginate AR was procured from Hi media biosciences Ltd, Mumbai, India. Calcium chloride AR and Methanol AR were procured from S D Fine chemicals Ltd, Mumbai, India. Barium chloride AR and Dichloromethane were purchased from Qualigens Fine chemicals Ltd Mumbai, India.

Methods

Formulation of sodium alginate uncoated beads containing Prednisolone: The beads of prednisolone were prepared by ionotropic gelation technique[8]. 100ml of Sodium Alginate (SA) solutions at different concentration were prepared by stirring sodium alginate powder in deionized water[2] for 30 minutes then, an accurately weighed quantity of prednisolone was added to afford homogenous dispersions. The SA-drug dispersion were then added drop wise into a 100ml of cross linking solution (different concentration and type) using a 10ml of hypodermic syringe fitted with a 20 gauge needle and stirred at 500 rpm. The formed alginate beads were cured at different time interval. On expiration of this period the solution of cross linking agent was decanted and the alginate beads were washed repetitively for three times with 50ml de ionized water. The alginate beads were thereafter dried at 60°C for 2hours in a hot air oven. The various formulations are as given in Table **1**.

Table 1. Compositions of different batches of prednisolone sodium alginate uncoated beads

Formulation code	Drug (mg)	Sodium alginate (%w/v)	Cross-linking agent	Cross-linking (%w/v)	Curing time (min)
F1	400	2.5	$Cacl_2$	3	30
F2	400	2.5	$Bacl_2$	3	30
F3	400	3.5	$Cacl_2$	3	30
F4	400	3.5	$Bacl_2$	3	30
F5	400	5.0	$Cacl_2$	3	30
F6	400	5.0	$Bacl_2$	3	30

Coating of prednisolone sodium alginate beads with Eudragit S-100: Optimized alginate beads were coated with Eudragit S-100[9,10] using solvent evaporation method. Beads were dispersed in Eudragit S-100 in dichloromethane and methanol (4:1) ratio to obtain 10% weight gain. And the solvent was evaporated in a rotary evaporator by applying vacuum and rotation rate was 50 rpm, then vacuum dried in desiccators.

Physical Characterization

The particle size distribution analysis was performed by using an optical microscope[11]. The shape and surface characteristics of beads were observed by scanning electron microscopy[12] and are depicted as in figure **1 a-f** for 6 formulations coded as F_1, F_2, F_3, F_4, F_5, F_6 respectively.

Drug Content Analysis

Determination of drug encapsulation efficiency: 50 mg of drug loaded alginate beads from each batch was placed in 100 ml conical flask containing 50 ml of phosphate buffer (pH7.4).The beads were agitated on mechanical shaker for 24 hours, to promote swelling and breakup of the cross - linked structure. Then solutions were filtered and the drug was quantified at 248 nm spectrophotometrically after appropriate dilution with buffer. The encapsulation efficiency[13] (EE) was determined by using the following empirical relationship. Each determination was performed in triplicate manner.

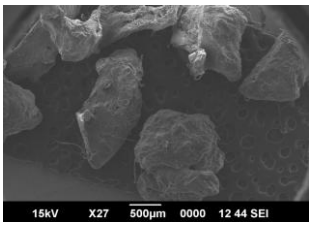
$$Entrapment\ efficiency\ (\%) = \frac{Actual\ drug\ content\ (AC)}{Theoretical\ drug\ content\ (TC)} \times 100$$

AC - Actual quantity of drug present in the beads a

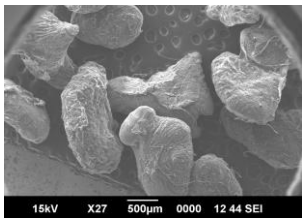
TC - 100% theoretical quantity of drug present in the beads (actual initial dose)

Figure 1. SEM of Formulations F1-F6

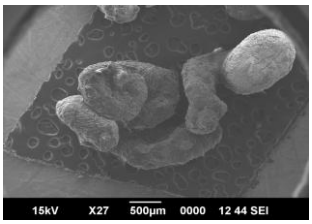
a) SEM of F1



b) SEM of F2

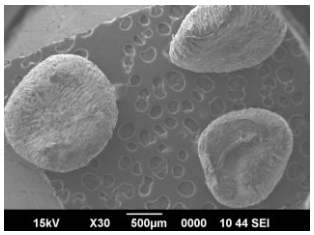


c) SEM of F3



d) SEM of F4

e) SEM of F2



f) SEM of F2

In-vitro drug release studies from core alginate beads

An accurately weighed amount of drug loaded uncoated alginate beads equivalent to 50mg were evaluated for *in-vitro* drug release. The study[14] was carried out in the USP XXIV Type I apparatus using 900ml phosphate buffer solution. A muslin cloth was tied over the basket to prevent the slippage of beads from the basket. The rotating speed of paddle was maintain at 100 rpm at 37±1c for first two hour study in pH 1.2(0.1N hydrochloric acid).samples was withdrawn every 1 hour, and then next two hour study was carried in phosphate buffer pH 5.8. In every one an hour 1ml of sample was withdrawn from pH 5.8 medium, diluted with phosphate buffer and make up to 10ml. At the same time 1ml of phosphate buffer was added to the dissolution medium to maintain the sink condition. The same procedure is repeated for entire 8 hour study with pH 7.4. The absorbance of the sample withdrawn at every one an hour was determined UV-visible spectrometer at 248 nm. The concentrations of dissolved drug in each sample were extrapolated from the calibration curve from its absorbance and are as depicted in figure **2**.

In-vitro drug release studies from coated alginate beads

Coated alginate beads were performed for *in-vitro* drug release study by the method similar to that of core beads. The study was carried out in the USP XXIV Type I apparatus and are as depicted in figure **3**.

Dissolution Kinetics of Drug Release

To study the release kinetics[15], data obtained from in vitro drug release studies were plotted in various kinetic

models: Zero order (cumulative amount of drug released vs. time), First order (log cumulative percentage of drug remaining vs. time), Higuchi's model (cumulative percentage of drug released vs. square root of time), Hixon-Crowell (cube root of amount remained to be absorbed vs. time) and Korsmeyer's (log cumulative percentage of drug released Vs log time).

Figure 2. *In-vitro* dissolution profile of core microbeads

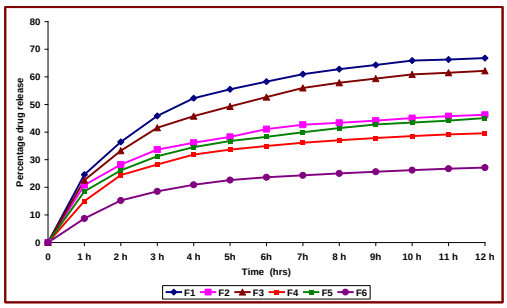
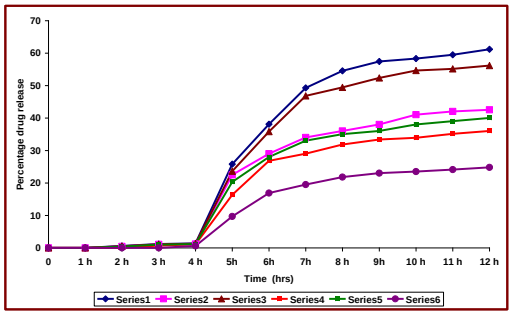


Figure 3. *In-vitro* dissolution profile of coated micro beads



RESULTS AND DISCUSSION

The objective of the present work is to develop different colon-specific formulations containing prednisolone and to study its *in-vitro* dissolution profiles. Alginate beads of prednisolone were formulated by ionotropic gelation and further coated with Eudragit S-100. Six batches of micro beads were prepared using different concentrations of sodium alginate and different cross linking agents. The results observed are mentioned in the following sections.

Physical Characterization

By optical microscopy it was found that the particle size distribution of each formulation was very well within a narrow size range. But the mean particle size was different among the formulations as reported in Table **2**. The effect of concentration of polymer on the size of beads formed were studied and it was found that there was an increase in the average diameter of particles as there was an increase in the concentration of polymer.

Table 2. Average mean diameter of the alginate beads

Formulation Code	Length (µm)	Surface (µm)	Volume (µm)	Volume surface (µm)
F1	987	1019.56	992.38	940.18
F2	1177	1181.06	1185.14	1193.33
F3	1278	1281.99	1286.01	1294.07
F4	1375	1378.66	1382.33	1389.71
F5	1500	1502.93	1505.90	1511.85
F6	1533	1535.22	1537.45	1541.93

In all formulations the alginate beads were more or less spherical in shape and the exterior surfaces were rough and covered with a network of small cracks and fissures, the drug was uniformly dispersed at the molecular level in the alginate beads. The spherical shape of the beads in wet state was usually lost after drying especially for beads prepared with low concentration of SA and cross -linking agent. With the increase of SA concentration the shape of the beads retained considerably.

Drug Content Analysis

The entrapment efficiency in different formulation varied from 69% to 81% as reported in Table **3**. As drug to polymer ratio was increased, entrapment efficiency was also increased.

Table 3. Effect of concentration of polymer on entrapment efficiency

S.No	Formulation code	Concentration of sodium alginate (%w/v)	Entrapment Efficiency (%)
01	F1	2.5	70
02	F2	2.5	69
03	F3	3.5	76
04	F4	3.5	74
05	F5	5.0	81
06	F6	5.0	79

In-vitro Drug Release Studies

In-vitro drug release of coated and uncoated beads was performed in different pH medium (1.2, 5.8, 7.4) at 37^{0}C ± 0.5˚C. As compared to core alginate beads, coated beads do not show drug release at pH of 1.2 & 5.8 and it show only on pH 7.4. So it protects the release of dug from the upper part of GIT and minimizes the side effects. Above pH 7.0; Eudragit S 100 coating stared to dissolve and exposed the alginate beads for drug release. Among these trials, batch F1 showed good results in *in-vitro* drug release study. Therefore, we can conclude that if the Eudragit S-100 coated beads protect the drug from stomach and small intestine and start drug release upon arrival to colon and gives local action. It may provide site-specific release and reduce systemic side effects.

Dissolution Kinetics of Drug Release

In-vitro data obtained for Eudragit coated prednisolone alginate beads were used to determine the dissolution kinetics. (Table **4**, **5**)

Table 4. *In-vitro* dissolution profile of core microbeads

Time	Percentage release of prednisolone from uncoated sodium alginate beads.					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1 h	24.6	20.8	22.6	15	18.5	8.7
2 h	36.43	28.22	33.23	24.42	26.12	15.21
3 h	45.84	33.63	41.54	28.23	31.23	18.52
4 h	52.25	36.14	45.75	31.83	34.54	20.92
5h	55.46	38.24	49.25	33.64	36.74	22.63
6h	58.26	41.05	52.66	34.93	38.25	23.62
7h	60.96	42.64	55.96	36.13	39.94	24.33
8 h	62.77	43.35	57.86	37.04	41.44	25.03
9h	64.27	44.14	59.36	37.85	42.75	25.63
10 h	65.88	45.05	60.87	38.54	43.45	26.22
11 h	66.27	45.75	61.46	39.14	44.14	26.73
12 h	66.77	46.25	62.17	39.55	45.05	27.13

Table 5. *In-vitro* dissolution profile of coated microbeads

Time	Percentage release of prednisolone from Eudragit coated sodium alginate beads.					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1 h	0	0	0	0	0	0
2 h	0.6	0.45	0.5	0	0.40	0
3 h	1.2	1.10	1.10	0.70	1.0	0
4 h	1.4	1.20	1.30	1.10	1.20	0.60
5h	25.8	22.51	23.61	16.34	20.31	9.70
6h	38.12	29.02	35.82	26.82	28.02	16.91
7h	49.3	34.03	46.83	29.03	33.03	19.52
8 h	54.56	36.04	49.45	31.83	35.04	21.82
9h	57.43	38.04	52.37	33.4	36.03	23.03
10 h	58.32	41.05	54.65	33.93	38.04	23.52
11 h	59.5	42.04	55.16	35.14	39.05	24.13
12 h	61.2	42.55	56.18	36.04	40.04	24.82

These data indicated that the drug release followed the Korsmeyer-Peppa's equation. Diffusion coefficient (n) is

less than 0.45 for all the six formulations, indicating that the release mechanism is diffusion.

CONCLUSION

The proper selections of formulation are important to achieve high encapsulation efficiency and to sustain the release from alginate beads. Ionotropic gelation technique can be successfully used for preparation of prednisolone alginate beads. Prednisolone release from the beads was influenced by varying sodium alginate concentration. By increasing the polymer concentration loading efficiency increased and drug release exhibited more sustained effect. The addition of different cross linking agents was observed to alter the drug encapsulation and release characteristics. The above observations suggest that prednisolone can be developed as colon targeting drug delivery system with sodium alginate 2.5% using Calcium chloride as cross linking agent and coated with Eudragit S-100.

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