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Formulation and evaluation of deflazacort loaded mucoadhesive microsphere for colon drug delivery system

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ABSTRACT

Irritable bowel disease is very common colon disease. Deflazacort is one of the best drug with clinical activity against Irritable bowel disease. Microsphere system are effectively protect drugs against premature degradation, to localize drug molecules at the target site of action and to control the time and rate of release. Mucoadhesive microspheres enhance the bioavailability of orally given drugs by lengthened contact time of drug with the intestinal mucosa. The main disadvantage of these microspheres is adherence to the substrate by non-specific interaction. To overcome this limitation, microspheres are prepared by emulsification method to treat irritable bowel disease. Chitosan microspheres were prepared by Ionotropic Gelation method. Microspheres were coated with Eudragil S using solvent evaporation method.

Keywords: Deflazacort, Mucoadhesive, Microspheres, Irritable bowel disease

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INTRODUCTION

Colon was considered as a part of body solely responsible for absorption of water, electrolyte and temporary storage of stools, but now it is accepted as an important site for drug delivery. Targeted delivery ensures the direct treatment at the disease site, lower dosing, and minimizing side effects. Drug targeting to colon is highly desirable for local treatment of a variety of bowel diseases. Moreover, dosage forms with mucoadhesive properties may prolong the residence time at the site of drug absorption. Mucoadhesive microparticulates coated with a pH-dependent polymer are proposed to initiate the release of the drug at the putative colonic pH 7–8.[1-3] Deflazacort is a drug of choice in Ulcerative colitis with proven anti inflammatory and immunosuppressive effect. Deflazacort is a corticosteroid that works by acting within cells to prevent the release of certain chemicals that are important in the immune system. These chemicals are involved in producing immune and allergic responses, resulting in inflammation by decreasing the release of these chemicals in a particular area, inflammation is reduced. Deflazacort has a shorter biological half life of 1.1-1.9 hr. Thus by using deflazacort microspheres half life is increased and provide sustained release of drug for longer duration and thus bioavailability problems associated with oral administration is also improved and microspheres with mucoadhesive properties may prolong the residence time at the site of drug absorption.[3-4]

MATERIAL AND METHODS

Material

Deflazacort was collected as a gift sample from Alcon bio sciences PVT Ltd, Mumbai. Eudragit-S 100 obtained from Degussa India Pvt Ltd. Sodium Alginate and chitosan were received from Loba Chemie Pvt. Ltd (Mumbai, India). All other reagents and chemicals used were of analytical grade.

Methods

Preparation of mucoadhesive microsphere of Deflazacort

Chitosan microspheres were prepared by ionotropic gelation method.

Preparation I: Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature.

Preparation II: The drug and sodium alginate was dissolved in 100 ml of water.

Preparation III: 1% Sodium tripolyphosphate solution was prepared.

Preparation IV: Solution of preparation I was slowly added in preparation III with continuous starring on magnetic stirrer.

Preparation II was added in preparation IV through a disposable syringe needle into a gently agitating. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Gel like beads were obtained which was air dried for twenty four hours followed by oven drying for six hours at 40°C. [5-6]

Table 1: Formulations of the mucoadhasive microspheres

Sr. No	Formulation Code	Deflazacort (mg)	Chitosan (mg)	Sod. Alginate (mg)
1.	F1	10	25	50
2.	F2	10	25	75
3.	F3	10	25	100
4.	F4	10	50	50
5.	F5	10	50	75
6.	F6	10	50	100

Coating of mucoadhasive microspheres

Microspheres were coated with Eudragil S (ES) using solvent evaporation method. mucoadhasive microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolution of 500 mg of ES in ethanol:acetone (2:1) to give 5:1 (coat:core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated microsphere were filtered, washed with n-hexane, and dried in desiccator.[7-8]

Evaluation of microspheres

Percentage Yield

The prepared microspheres with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.[5-6]

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Drug Entrapment

The various formulations of the mucoadhasive microspheres were subjected for drug content. 10 mg of mucoadhasive microspheres from all batches were accurately weighed and crushed. The powder of microspheres were dissolved in 10 ml 7.4 pH Phosphate Buffer and centrifuge at 1000 rpm. This supernatant solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.4 pH Phosphate Buffer. The percentage drug entrapment was calculated using calibration curve method.[7-8]

In-vitro wash off test

The mucoadhesive property of microspheres was evaluated by an *in vitro* adhesion testing method known as the wash-off test. Freshly excised pieces of intestinal mucosa from sheep were mounted onto glass slide. About 100 microspheres were spread onto wet rinsed tissue specimen and immediately thereafter the slides were hung onto the arm of a tablet disintegrating machine. Then the machine was operated. The tissue specimen was given a slow, regular up and down movement in the test fluid at about 37°C contained in a vessel of the machine. At the end of 1, 2, 3, 4, 5, 6, 7,8 hrs the machine was stopped and the number of microspheres still adhering to the tissue was counted. The test was performed at 0.1N hydrochloric acid solution.

$$\% \text{ Mucoadhesion} = (Na\text{-}Nl) / Na \times 100$$

Where, Na = number of microspheres applied; Nl = number of microspheres leached out.[9-10]

Measurement of mean particle size

The mean size of the microspheres was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement. [11]

Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.[9-11]

Shape and Surface Characterization of Microspheres by Scanning Electron Microscopy (SEM)

From the formulated batches of microspheres, formulations (F_3) which showed an appropriate balance between the percentage releases were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 10KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.[10-12]

***In-vitro* Release Studies**

***In vitro* drug release in gastrointestinal fluids of different pH**

The prepared microsphere was evaluated for *in vitro* drug release. The drug release studies were carried out using USP XXII paddle type Dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows:

- *1st hour:* Simulated gastric fluid (SGF) of pH 1.2.
- *2nd and 3rd hour:* Mixture of simulated gastric and Intestinal fluid of pH 4.5.

- *4th to 5th hour:* Simulated intestinal fluid (SIF) of pH 6.8.
- *6th hour and onward:* SIF pH 7.5

A weighed quantity of formulation (100 mg) was spread over the surface of dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by continuous media. The samples withdrawn were assayed spectrophotometrically at 242.0 nm for deflazacort and using UV visible spectrophotometer. The release of deflazacort was calculated with the help of Standard curve of deflazacort.[9-12]

Drug release kinetic data analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).[10-12]

RESULTS AND DISCUSSION

Evaluation of deflazacort microspheres

Percentage Yield

Percentage yield of different formulation was determined by weighing the Microspheres after drying. The percentage yield of different formulation was in range of 82.56 – 93.58%.

Table 2: Percentage Yield for Different Formulation

Formulation	Percentage Yield
F1	82.56±0.45
F2	83.56±0.25
F3	89.98±0.32
F4	85.56±0.45
F5	88.85±0.47
F6	93.58±0.85

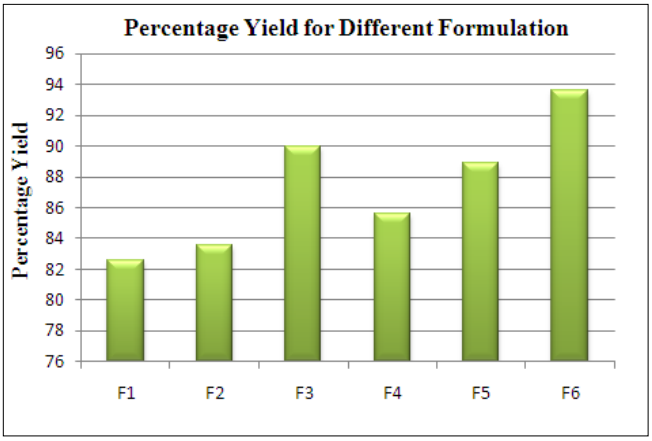

Figure 1: Percentage Yield for different formulation

Drug Entrapment

The drug entrapment of different formulations was in range of 78.05- 83.25% w/w. This is due to the permeation characteristics of HPMC that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of deflazacort microspheres.

Table 3: Drug Entrapment for Different Formulation

Formulation	Drug entrapment (% w/w) of prepared microsphere
F1	78.05±0.25
F2	79.98±0.32
F3	82.56±0.14
F4	80.12±0.57
F5	81.14±0.54
F6	83.25±0.45

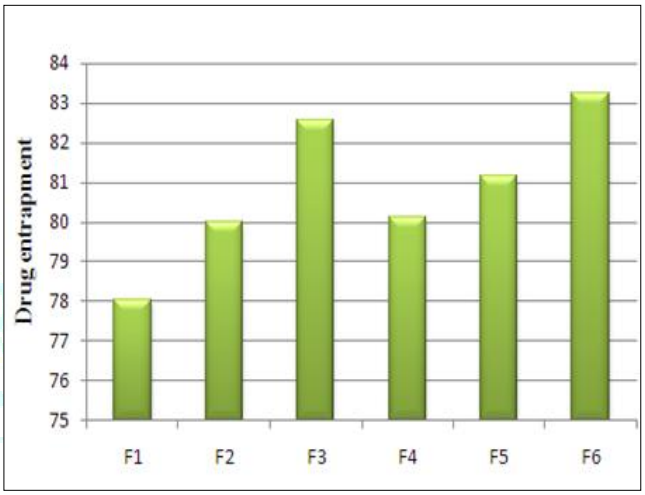

Figure 2: Drug Entrapment for Different Formulation

The maximum Percentage Yield, Drug Entrapment was found to be formulation F3. The optimized formulation of batches subjected to further studies.

Results of *In-vitro wash* off test

Table 4: Results of *In-vitro wash* off test

Formulation code	1hr	2hr	4hr	6hr	8hr
F1	78	74	68	65	61
F2	81	75	69	65	60
F3	92	85	80	78	75
F4	85	78	70	65	60
F5	78	70	65	60	58
F6	75	68	60	55	45

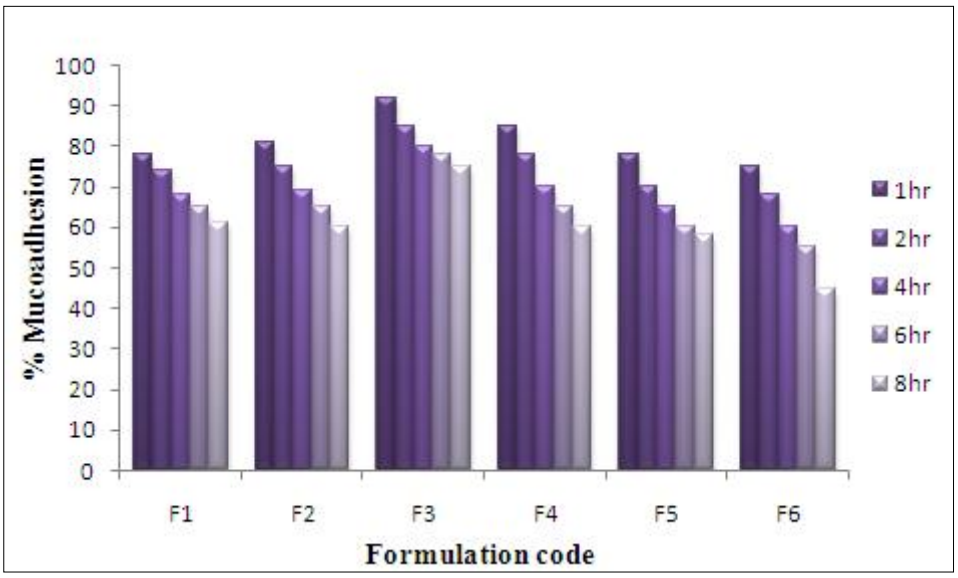

Figure 3: Graph of Results of *In-vitro wash* off test

Particle size analysis

The mean size of the microspheres was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F3 microsphere was found 150.9 nm respectively.

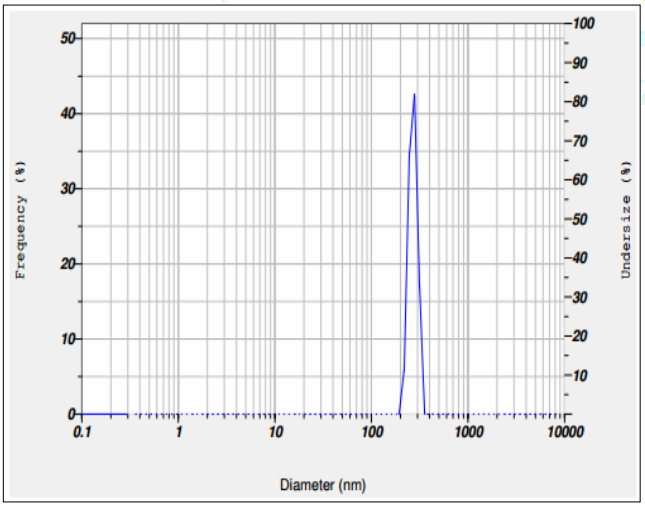

Figure 4: Particle size data of mucoadhesive microsphere

Zeta Potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. Results of zeta potential of optimized formulation F3 microsphere was found -10.7 mV respectively.

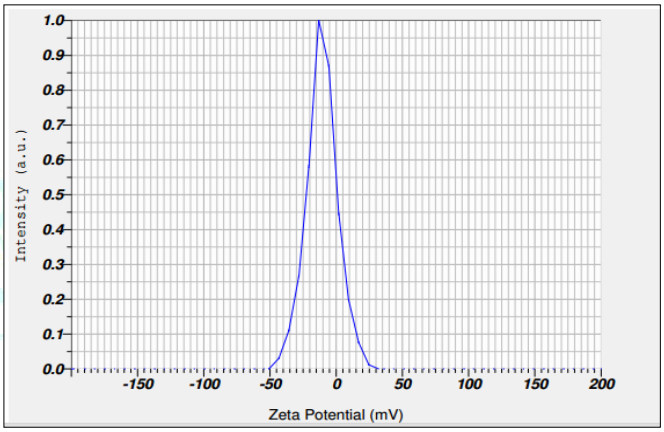

Figure 5: Zeta potential data of mucoadhesive microsphere

Scanning Electronic Microscopy

Shape and surface characteristic of Deflazacort microspheres exam in by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnification 55X which illustrate the smooth surface of mucoadhesive Microspheres.

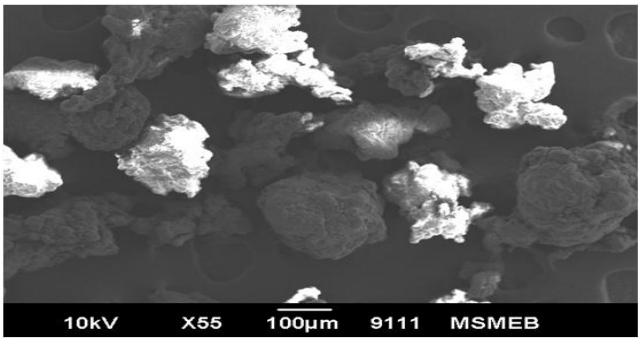

Figure 6: Scanning Electronic Microscopy image of optimized formulation F-3

In Vitro drug release

Table 5: Cumulative % drug release of Deflazacort from plain and coated microsphere at different pH

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release	
			Microsphere	Coated microsphere
1	SGF (pH 1.2)	1	4.8	0.3
2		2	7.2	0.7
3	SGF+SIF(pH 4.5)	3	13.0	1.0
4		4	18.9	4.2
5		5	25.7	9.7
6	SIF (pH 6.8)	6	37.0	16.2
7		7	58.4	34.3
8	SIF (pH 7.5)	8	66.7	45.5
9		9	73.1	53.1
10		10	82.3	62.0
11		12	88.6	67.8

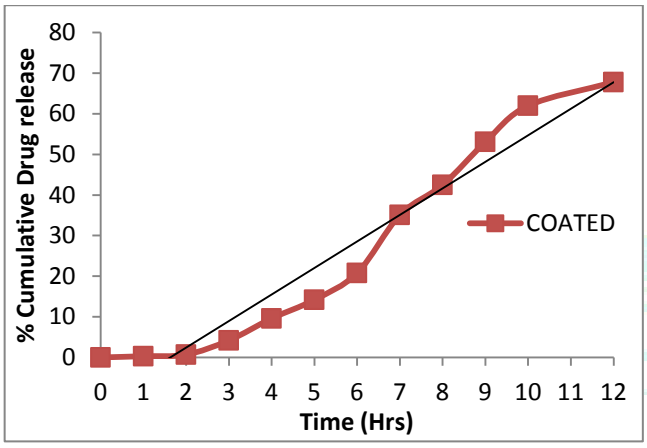

Figure 7: Cumulative Percent Drug Released Vs Time (Zero Order Plots)

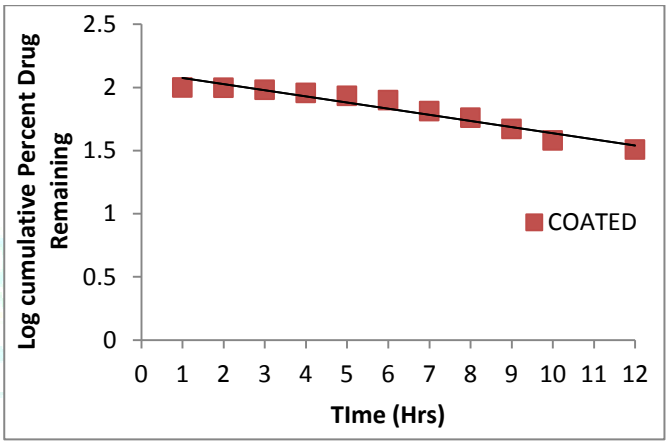

Figure 8: Log Cumulative Percent Drug Remaining Vs Time (First Order Plots)

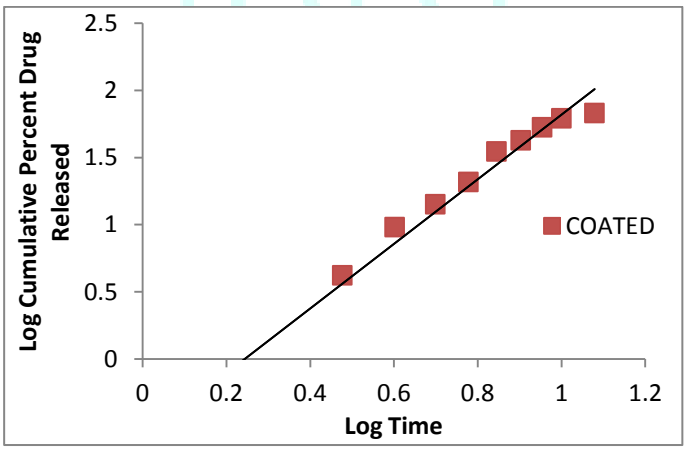

Figure 9: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots)

Table 6 Regression Analysis Data of mucoadhasive microspheres Formulation

Formulation	Zero order	First order	Pappas plot
F3	$y = 6.540x - 10.65$ $R^2 = 0.944$	$y = -0.048x + 2.123$ $R^2 = 0.932$	$y = 2.406x - 0.587$ $R^2 = 0.975$

Stability studies

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer. The particle size of the microsphere was found to increase at RT, which may be attributed to the aggregation of microsphere at higher temperature. At 45±2°C the microsphere aggregate i.e. these microsphere were unstable at higher temperature like 45±2°C. Percent efficiency of mucoadhesive Microspheres also decrease at higher temperature Like 45±2°C.

CONCLUSION

From the result of present study, it can be concluded that chitosan based deflazacort mucoadhesive microspheres offer a high degree of protection from premature drug release in simulated upper GIT conditions and deliver most of the drug load in the colon and allow drug release to occur at the desired site. Based on the results of the physicochemical characterization and *in vitro* drug release studies, it possessed all the required physicochemical characters and with drug releases up to 12 h where it released 67.8 % of the deflazacort. Thus, chitosan based microspheres are a potential system for colon delivery of deflazacort for Ulcerative colitis.

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