DEVELOPMENT & ASSESSMENT OF COLON TARGETTED MICROSPHERES OF BOSWELLIC ACID USING SODIUM ALGINATE & CALCIUM CHLORIDE BY IONIC GELATION TECHNIQUE

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ABSTRACT

Background: This work is based on development of microspheres of boswellic acid for colon targeted drug delivery.

Method: Microspheres were prepared using sodium alginate, calcium chloride by ionic gelation method.The prepared microspheres were evaluated for micromeretic properties, surface morphology etc. Levels of sodium alginate and calcium chloride were varied to obtain optimum composition for smallest possible particle size, maximizing entrapment efficiency and cumulative drug release at 6hr. The selected composition was used to prepare microspheres that were subjected to coating by Eudragit S100 in hydroalcoholic solvent system. Dissolution of these microspheres was studied by pH progression method. Drug release mechanism was found be characterized by swelling and erosion.

Results: The microspheres showed good flow properties and spherical shape. The formulation was optimized and optimized models were found to be valid. It was observed that the microspheres prepared using 1.48 % w/v of sodium alginate and 4.71% w/v of calcium chloride showed optimum drug release of 89% at colonic conditions, 78% entrapment efficiency and 340 microns particle size.

Conclusion: It was concluded that boswellic acid microspheres prepared using ionic gelation method and coated with Eudragit S 100 can be a promising approach for colon targeting.

KEYWORDS: Colon targeting, boswellic acid, sodium alginate, calcium chloride, Eudragit S100, optimization.

INTRODUCTION

Many colonic diseases like Crohn's disease, inflammatory bowel disease, and irritable bowel syndrome etc face create challenges in clinical management due to factors like poor drug absorption from the upper part of the gastro intestinal track leading to poor bioavailability. Enzymatic or acidic degradation also contribute to this. The complications arising from these diseases can include frequent constipation or diarrhea as well as incapacitating inflammatory bowel diseases leading to colon cancer[1-3].

Colon targeted drug delivery system is proposed to be a promising approach to overcome these issues and enhancing the success of the therapy. Delivery of drug directly to the colon can ensure direct treatment at the affected area with lower dose and less systemic side effects. Such formulations can also be used as the threshold entry of the drugs into blood for macromolecules which get degraded or poorly absorbed in upper GIT. The colon targeted dosage forms can also be beneficial for chronotherapy for treatment of various diseases like arthritis, angina and asthma[4-6].

There are certain advantages of using a multiparticulate drug delivery system. The risks of dose dumping, gastric irritation and systemic toxicity are significantly lowered. Since each subunit is separately coated, failure of coating process is a much lesser matter of concern[7-8]. Microspheres of various polymers incorporating the active drug and coated with required release modifying polymer present a promising approach in targeted drug delivery systems especially for colon targeting[9]. Alginic acid derivatives like sodium alginate have been proposed for making such microspheres. Biocompatibility as well as compatibility with many drugs, easier processes, being biodegradable and non toxic favors the choice of alginates. Brown seaweeds like *Laminariadigitata*, *Laminariahyperborea*, *Laminaria japonica*, *Macrocystispyrifera* and *Ascophyllumnodosum* are principle sources of aliginates[10]. Alginates croslinked with calcium and magnesium ions can be used for encapsulating various drugs. Such type of divalent cation cross linking usually produces spherical particles that are better with respect to formulation aspects[11].

MATERIALS AND METHODS

Boswellic acid and Eudragit S 100 were received as gift sample from Shivatva Enterprises and Evonik Industries, Mumbai respectively. Sodium alginate, calcium chloride was purchased from SD Fine Chemicals, Mumbai. Ethanol, hydrochloric acid, disodium hydrogen phosphate and other chemicals were purchased from Loba Chemicals, Mumbai. All the chemicals and reagents used were of AR grade.

Preparation of alginate microspheres

Alginate microspheres were produced by Ionic gelation method using alginate as anionic polymer and calcium chloride as crosslinker[12-14]. Preformulation studies were carried out to select a range of suitable concentrations of the polymer and cross linker. Initially sodium alginate was dissolved in water to obtain different solutions of 1%, 3% and 5% concentration. Boswellic acid was dissolved in small quantity of ethanol. This solution was added to the solution of sodium alginate with constant stirring. Calcium chloride solution was prepared separately with concentration of 3%, 4% and 5%. From a constant height, calcium chloride solution was added dropwise into sodium alginate solution using 22 gauge needles. The system was kept under constant stirring at 400 rpm.After 2 hours of stirring, the solvent was decanted and the product was washed several times with distilled water, dried and stored in a closed container.

Experimental design

A 3^2 full factorial design was used to prepare the formulations[15-16]. Polymer and cross linker concentration were identified as formulation variables. Levels of sodium alginate were varied from 1% to 5% whereas calcium chloride levels were kept between 3% to 5%. Boswellic acid quantity, stirring speed and all other factors were kept constant. Entrapment efficiency and percent drug release were selected as response variables. Composition of formulations as per the experimental design is shown in Table 1.

Table1: Experimental design for preparation of boswellic acid microspheres

Formulation No.	Concentration of sodium alginate (%w/v)	Concentration of calcium chloride (%w/v)
F1	1	3
F2	1	4
F3	1	5
F4	3	3
F5	3	4
F6	3	5
F7	5	3
F8	5	4
F9	5	5

Evaluation of the microspheres

Flow properties[17]

Bulk and tapped density

Bulk and tapped densities of the formulations were calculated using a bulk density apparatus.

Angle of repose

Angle of repose was calculated by funnel method. A glass funnel was mounted on a stand with tip placed at 2 inch height from the horizontal surface. A fixed quantity of microspheres was added into the funnel. The height and radius of the pile formed was measured and angle of repose was calculated.

Carr's Index

Carr's index was calculated using following formula:

$$\text{Carr's index} = \frac{(\text{tapped density} - \text{bulk density})100}{\text{tapped density}}$$

Hausner's ratio

Hausner's ratio was calculated using following formula

$$\text{Hausner's Ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

Particle size

Particle size was measured using optical microscopy. Initially the eye piece micrometer was calibrated using a stage micrometer. The sample was mounted on a glass slide and observed against the calibrated scale. About 100 particles from each batch were observed and average particle size was calculated.

Yieldof microspheres

The percent yield of the microspheres was calculated considering total mass of the starting materials and mass of the product obtained after drying using following formula:

$$\text{Percent yield} = \frac{\text{total weight of the microspheres x100}}{\text{total weight of the internal phase}}$$

Surface morphology

Surface morphology of the microspheres was studies using stereo zoom optical microscope. Shape, surface texture and formation of aggregates if any were examined[18].

Entrapment efficiency

After the preparation of microspheres, the dispersion was filtered and microspheres were separated by filtration. The washings were added to the filtrate. The amount of drug detected in the filtrate represents un-entrapped drug. From this, drug entrapped into the microspheres was calculated.

Drug release

Microspheres equivalent to 50 mg of drug were accurately weighed. Dissolution studies were carried out in 900 ml of phosphate buffer pH 7.2, maintained at 37 ± 0.5°C at 100 rpm using USP type I dissolution test apparatus under sink conditions[19]. At predetermined time intervals, 5 ml of each sample was withdrawn and replaced by equal volume of fresh medium kept at same temperature. Samples were filtered through Whatmann filter paper no. 41 and analyzed on UV-visible spectrophotometer at 250 nm.

Coating of microspheres

The coating solution was prepared as follows. Initially Eudragit S100 was dispersed into a diluent mixture containing isopropyl alcohol, acetone and water and stirred well for 30 minutes using a magnetic stirrer. Separately, talc and triethyl citrate were added to the diluent mixture of the same composition and stirred for the same duration. This dispersion was slowly added to the Eudragit dispersion with constant stirring for 60 minutes and filtered[20]. Coating solution was freshly prepared for each coating process.

The optimized batch of microspheres was selected for coating. After few preliminary trials coating parameters like spraying rate, pan rotation speed, drying temperature etc were fixed to get optimum coating. These parameters are listed in Table no. 2

Table 2: Boswellic acid microspheres coating parameter

Coating parameter	Specification
Batch Size	5gm
Spray rate	2ml/min
Nozzle Diameter	1mm
Atomizing Air Pressure	0.5 bar
Pan speed	30-40 rpm
Air inlet temperature	40-50°C

In-vitro Drug release study of microspheres using pH progression method

To study the release profile from microspheres pH progression method was used. USP Type I apparatus was used at 50 rpm speed and the temperature was maintained at 37 °C. The samples were collected at chosen time intervals, filtered through Whatmann filter paper no. 41 and analyzed by UV spectrophotometer (V-730 Jasco) at 250 nm.

For first 2 hours, to simulate the pH at the gastric environment, 700 ml of 0.1N HCl was used as dissolution media. After 2 hrs, 200 ml of 0.2M tribasic sodium phosphate buffer was added to all the dissolution vessels and pH is adjusted to 6.4, 6.8 and 7.2 by using 2M NaOH or 2M HCl for 1, 2 h and till the end respectively[21].

RESULTS

Flow properties

The flow properties of microspheres evaluated using various parameters were found to be good. A summary of the evaluation is listed in Table 3.

Table3: Flow properties of the microspheres

Formulation No.	Bulk density gm/ml	Tapped density gm/ml	Carr's Index	Hausner's Ratio	Angle of Repose
F1	0.40±0.05	0.43±0.0 8	6.9±0.16	1.08±0.09	26°±0.45
F2	0.45±0.05	0.52±0.0 7	13.4±0.33	1.15±0.06	31.3°±0.23
F3	0.40±0.09	0.45±0.03	11.4±0.19	1.13±0.05	32.6°±0.33
F4	0.38±0.04	0.41±0.05	7.3±0.55	1.08±0.05	25.8°±0.45
F5	0.26±0.08	0.34±0.09	23.5±0.65	1.30±0.06	41°±0.56
F6	0.45±0.04	0.50±0.05	10±0.24	1.11±0.09	30°±0.55
F7	0.35±0.04	0.40±0.04	12.5±0.15	1.12±0.08	32.8°±0.25
F8	0.47±0.06	0.55±0.05	14.5±0.23	1.17±0.06	31.1°±0.15
F9	0.35±0.08	0.40±0.07	12.5±0.36	1.14±0.08	33°±0.45

n=3

Particle size

The particle size of the microspheres was found to vary between 312 to 498 microns. It was found to get increased with increase in polymer content. The outcomes are summarized in Table 4.

Entrapment efficiency

The entrapment efficiency was found to vary between 65 to 94%. More the polymer content more was the drug entrapped into the microspheres as shown in Table 4.

Drug release

The cumulative drug release after 8 hours was found to vary between 67% to 98%. The microspheres with lesser polymer content showed more drug release. Results are summarized in table 4.

Yield

Yield of different batches of microspheresvaried in the range of 95% to 98% as shown in table 4.

Table 4: Evaluation of microspheres

Formulation No.	Particle size Micron	Entrapment Efficiency %	Cumulative Drug Release %	Yield %
F1	312±10	65.10±1.2	98.21±1.3	95.1
F2	320±17	69.35±1.3	95.34±1.4	96.2
F3	333±23	71.40±1.2	92.16±1.5	97.3
F4	370±21	79.14±1.4	78.45±1.2	95.4
F5	388±24	82.22±1.3	75.12±1.3	98.7
F6	401±29	85.45±1.5	72.68±1.4	97.1
F7	460±30	88.17±1.4	71.10±1.1	96.4
F8	472±28	90.63±1.3	69.13±1.2	97.3
F9	498±35	94.05±1.5	67.21±1.1	98.3

n=3

Surface morphology

The microscopic observations revealed that the microspheres had oval to spherical shape with rough surface texture.

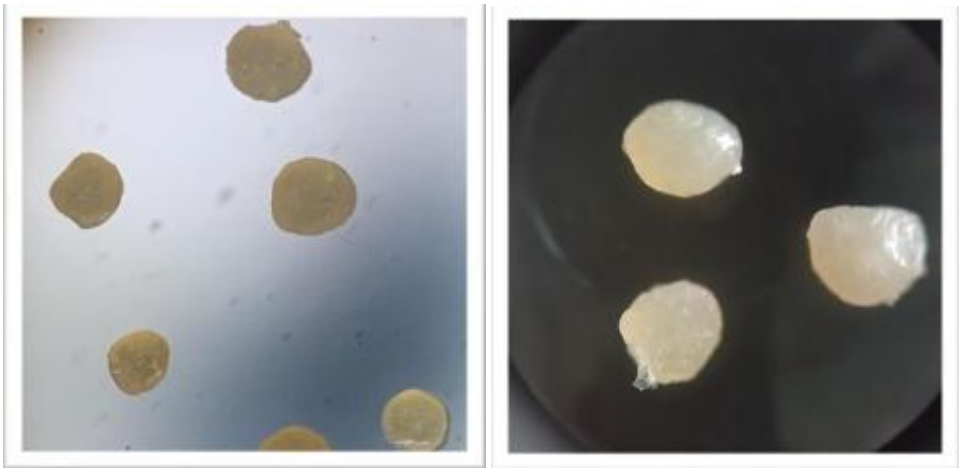

Figure 1: Microspheres image under stereo zoom microscope

Search for composition of optimum formulations

After evaluation of the microspheres for response variables i.e. particle size, entrapment efficiency and drug release with respect to the composition of the batches, the results were processed through Design Expert software. ANOVA report for the same analysis is shown in Table 5. The software was asked to predict the composition of the optimum formulation considering minimizing the particle size, maximizing the drug entrapment and drug release. The software suggested that the optimum composition of microspheres contains 1.48 %w/v of sodium alginate and 4.71 %w/v of calcium chloride.

Table 5: ANOVA for the experimental design

Model	**R^2**	**Adeq. Precision**	**Model F value**	**P value**
Particle Size	0.9992	70.643	743.91	<0.0001
Entrapment efficiency	0.9989	63.226	536.65	0.0001
Cumulative drug release	0.9957	29.566	139.47	0.0009
Particle size = $+384.78+77.50A+15.00B+4.25AB+12.83A^2+2.83B^2$				
Entrapment efficiency = $+82.39+11.17A+3.08B-0.10AB-2.49A^2-0.18B^2$				
Cumulative drug release = $=76.01-13.55A-2.62B+0.54AB+7.27A^2-0.90B^2$				
Where A = sodium alginate and B = calcium chloride				

Response surface plots

Response surface plots were generated to study the effect of formulation factors on response variables[22]. These images are depicted in Figure 2,3 and 4.

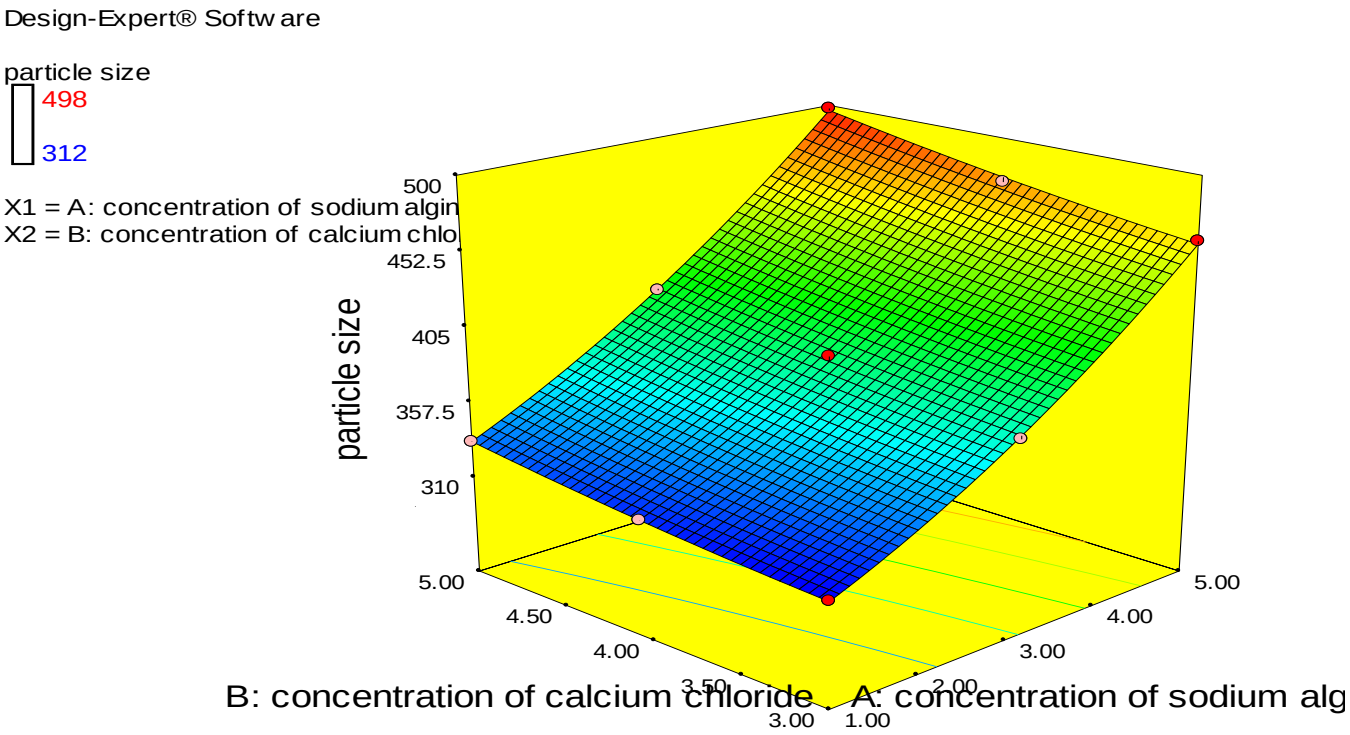

Figure 2: Response surface plot showing effect of sodium alginate and calcium chloride on particle size.

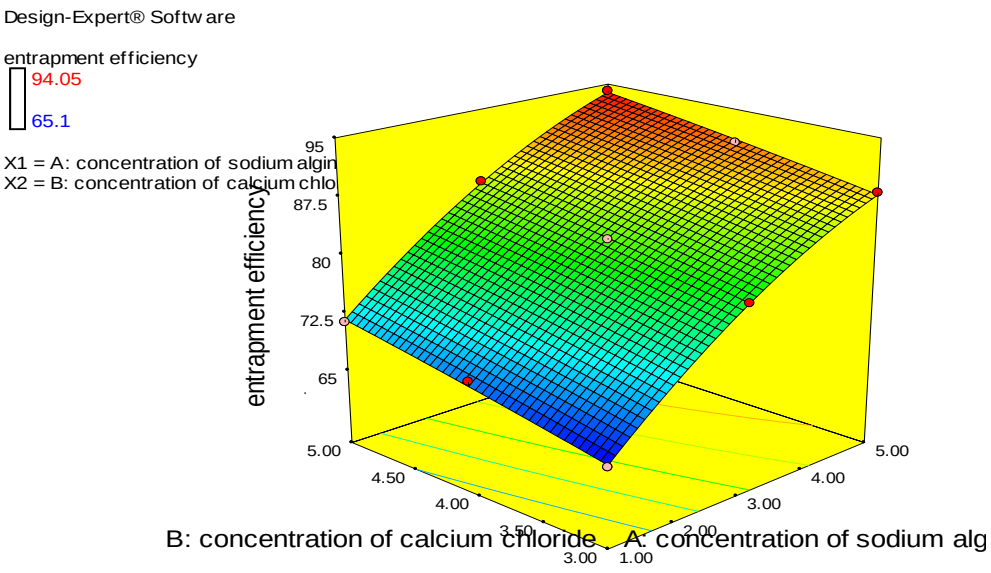

Figure 3: Response surface plot showing effect of sodium alginate and calcium chloride on entrapment efficiency.

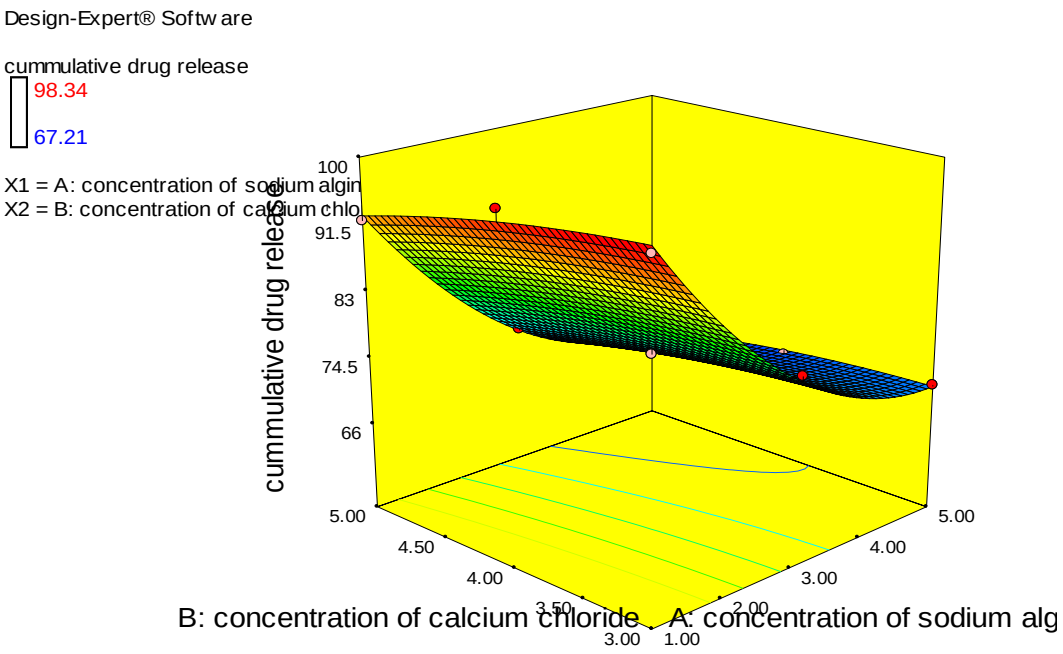

Figure 4: Response surface plot showing effect of sodium alginate and calcium chloride on cumulative drug release.

Validation of the optimization model

The optimum formulation as suggested by the software was prepared and responses were compared for predicted and practical values. It was observed that these values were close enough to conclude that the generated models for response variables were valid. This comparison is shown in Table 6.

Table 6: Validation of the optimization model

Sodium Alginate (%w/v)	Calcium Chloride (%w/v)	Particle size (micron)		Entrapment Efficiency (%)		Cumulative drug Release (%)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed
1.48	4.71	342.8	340	77.61	78	87.92	89

Drug release from the coated microspheres

The optimized formulation was coated with Eudragit S100 and was subjected to drug release by changing pH method. It was observed that the drug release for first 2 hrs i.e. simulated gastric condition was almost negligible. As the pH was raised slowly, the microspheres started to release the drug. Complete drug release was observed at the end of 8 hrs. The drug release profile is shown in Figure 4

DISCUSSIONS

The present work was aimed towards development of colon targeted microspheres of boswellic acid. Sodium alginate with rigidizing agent calcium chloride was used to prepare the microspheres. Sodium alginate is a water soluble polymer. It undergoes cross linking in presence of divalent calcium ions. These ions interfere the stability of the polymeric nets in alginate forming reversible or irreversible inter chain associations. As a result calcium alginate is formed which is gelatinous substance. It can be spheronized by keeping the mixture stirring

while reaction is going on. This technique can be used in encapsulation of many drugs to form a multiparticulate system.

It was observed that the prepared microspheres exhibited good flow properties which are essential to enclose them into dosage form like capsule i.e. unit dosage form. The spherical or near spherical shape of the microspheres contributes to good flow. It was observed that as the polymer content increased, there was increase in the size of the microspheres. Same was the observation with entrapment efficiency. More polymer content builds the particle size as well as encapsulates more amount of drug. More entrapment is essential with respect to viability of commercial manufacturing processes. The effect of polymer content on drug release was opposite. More polymer content tends to hold the drug for longer time, hence the batches with lesser polymer content showed more drug release. It was observed that the content of sodium alginate as well as that of calcium chloride combined together affected these three response parameters. Therefore there was a need to find the best suitable combination of these two factors for optimum performance of the microspheres. A 3^2 full factorial design combines all levels of both factors with minimum experimental runs and search for optimum values throughout the entire design space. It was observed that for all the three responses the models generated as well as the model terms were significant. There was good signal to noise ratio as shown by the adequate precision. High values of R^2 indicated good agreement between the formulation and response variables. Practical validation of this optimization model suggested that the model can be used to find the values of the response variables with selected values of formulation factors within the design space[15, 22-23].

Eudragit S100 is a polymer used for colon targeted release. Chemically it is methacrylic acid-methyl methacrylate copolymer (1:2). It is soluble at pH above 7 which makes it favourable choice for colon targeting. The microspheres coated with Eudragit S 100 were found to hold the drug in acidic pH and release in colonic conditions.

CONCLUSION

It was concluded that microspheres of boswellic acid prepared by ionic gelation method and coated with Eudragit S100 can be a promising approach for colon targeting.

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