Original Article

Preparation & Evaluation Of Oral Boswellic Acid Colon-Specific Microspheres

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Abstract

The aim of this study was to prepare boswellic acid loaded Eudragit microspheres and to evaluate their in vitro characteristics. The microspheres were prepared using solvent evaporation method, and characterized in terms of the morphological examination, particle size distribution, entrapment efficiency, drug loading and invitro release. The surface morphology of the microspheres was smooth, discrete with a regular spherical to near-spherical shape. Size of the microspheres was 4.96±0.76 mm and well-distributed. The zeta potential of microspheres was 29.3 ± 2.1 mv. An average drug loading of 9.3 ± 0.4 % and encapsulation efficiency of 81.1 ± 4.7% was obtained with the optimized preparation parameters. The cumulative release rate of boswellic acid microspheres was followed by a sustained release and fitted for classic higuchi kinetic model, ensuring the efficiency of treatment and improving patient compliance by reducing dosing frequency. It also does not cause any harmful or toxic effect in colon and rectum as evaluated by histopathological studies.

Keywords: Colon targeting, Boswellic acid, Calcium chloride, Eudragit S100,

INTRODUCTION

The extracts from the oleo-gum resin of Boswelliaserrate Roxb. ExColebr. (Family-Burseraceae), also known as SalaiGuggal, find use in traditional Ayurvedic medicine for the treatment of inflammatory diseases, like osteoarthritis and chronic bowel diseases.[1]

The oleo-gumresin is obtained from bark andcontains essential oil (5–9%),mucopolysaccarides (21–22%), and pure resin (65–85%). Amongst tetracyclic and pentacyclictriterpene acids, found in pure resin 11-keto-β-boswellic acid (KBA) and 3-O-acetyl-11-keto-β-boswellic acid (AKBA) are most active, powerful, and promising anti-inflammatory compounds. [2] Fig 1

The extract contains 37.5 to 65 % Boswellic acids. AKBA is a white crystalline powder with a λmax of 250 nm that is soluble inchloroform, methanol, and nearly insoluble in water. The AKBA due to its high lipophilicityand poor absorption have very low bioavaiablity. [3]

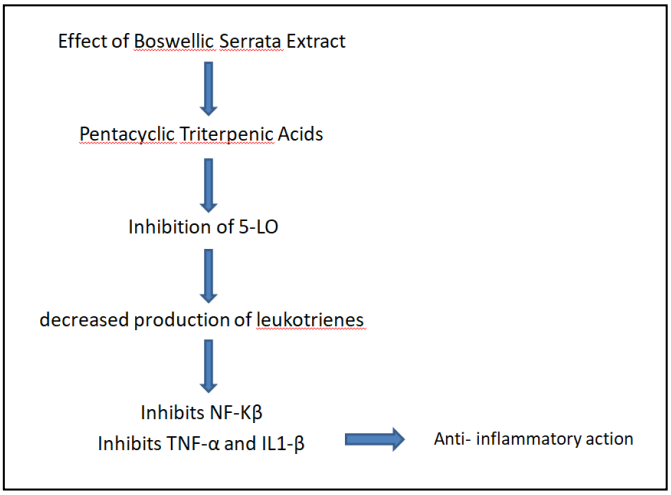

Figure 1: Boswelliaserrata Composition

Oral colon specific drug delivery system (CSDDS) is used to ensure complete drug release in colon. This is very useful approach in treatment of various diseases like colorectal cancer and inflammatory bowel diseases.[4, 5]In CSDDS drug does not get release and degrade either in upper GIT and is released completely only after reaching the colon. [6, 7]
It is seen that unit dosage forms such as tablets and capsules meant for CSDDS have shortcomings such as unpredictable gastric emptying, gastrointestinal (GI) transit variations resulting from inter-subject variability in transit patterns.[8] Inability of polymer coats to dissolve often leads to incomplete drug delivery in colon. Colon specific Multiparticulate drug delivery systems provide answer to such problems by virtue of following advantages it possess:

Less inter and intra-individual variability, more rapid and uniform gastric emptying, more uniform dispersion and reproducible transit through GI tract. [9] Microspheres are form of multiparticulate drug delivery system which is homogeneous, monolithic particles, it improves the outcome of treatment localization of the drug at the site of action and by prolonging the drug release. However it also suffers from the risk of early dissolution and release of the drug before reaching the colon due to its large surface area [10]

The pH-dependent approach to colon specific drug delivery is based on the pH variations of GIT from the stomach (pH 1.5-3.5) and small intestine (pH 5.5-6.8) to the colon (6.4-7.0). Most commonly used polymers used in pH-dependent drug delivery aremethacrylic acid copolymer, anionic polymethacrylate polymers contain methacrylic acid functional groups, which dissociate and render the polymer soluble at the higher pH of the small intestine and colon. Anionic copolymers of methacrylic acid and methyl methacrylate at a ratio of approximately 1:1 (Type A; eg. Eudragit L) and approximately 1:2 (Type B; eg. in Eudragit S) , dissolve at pH 6.0, and 7.0, respectively. These polymers do not dissolve in stomach and intestinal pH due to hydrogen bonding between the hydroxyl groups of the carboxylic moiety and the carbonyl oxygen of ester groups in the polymer molecules. However, they dissolve in the colon because of the ionization of their carboxyl functional groups and release the drug in the colon.[11, 12, 13]
Present work described formation of boswellic acid microspheres using Eudragit polymers to achieve release at colonic pH.

MATERIALS AND METHODS

Materials

Boswellic acid and Eudragit S 100 were received as gift sample from Shivatva Enterprises(Aurangabad, Maharashtra, India) and Evonik Industries (Mumbai, Maharashtra, India) respectively. Methanol, acetone and liquid paraffin were purchased from LOBA Chemie. Pvt. Ltd. (Mumbai, Maharashtra, India). All chemicals and solvents used were of analytical grades.

Preparation of Eudragit Microspheres: *Boswelliaserrata* is a traditional Ayurvedic remedy with anti-inflammatory properties. *Boswelliaserrata* in colonic epithelial cell monolayers exposed to H_2O_2 or INF-γ+TNF-α, and was found to reduce inflammation.[5]

The Eudragit S100 microspheres containing boswellic acid were prepared by the solvent evaporation method. [14] In this procedure, drug and polymer was dissolved ina mixture of acetone and methanol in a ratio of 2:1 v/v. This organic solution (30 mL) was emulsified into liquid paraffin (70 mL) containing the emulsifier Span 80. The system was stirred continuously using a mechanical stirrer at room temperature for 3–4 h to allow complete evaporation of the solvent. The microspheres thus formed were collected and dried in a hot air oven at 50ºC. The influence of drug–polymer ratio, rate of stirring, and concentration of emulsifier on the particle size and size distribution, entrapment efficiency, and Cumulative % drug release from microspheres was studied.

EXPERIMENTAL DESIGN:

Box Behnken design was selected to study 3 factors namely drug and Polymer ratio (Factor A), Stirring rate (Factor B) and concentration of emulsifier (Factor C) at 3 levels. The objective was to optimize and study effect of variables on responses such as drug content (Response 1) and particle diameter (Response 2) Table1&2..[15]

Table 1: Summary of Factors and Levels for the experimental design.

Levels / Factors	Level -1	Level 0	Level +1
Drug: Polymer (A)	1:2	1:3	1:4
Stirring Rate (B)	500	1000	1500
Emulsifier concentration (C)	0.5	0.75	1

Table 2: Formulation Batches F1 to F12 of Boswellic acid microspheres : using Box-Behnken Surface Response Design

Formulation No.	Factor 1 Drug: Polymer %	Factor 2 Stirring Rate Rpm	Factor 3 Emulsifier concentration %
F1	1:3	500	0.5
F2	1:4	500	0.75
F3	1:4	1000	0.5
F4	1:3	1500	0.5
F5	1:2	1000	1
F6	1:4	1000	1
F7	1:3	500	1
F8	1:2	1000	0.5
F9	1:3	1500	1
F10	1:4	1500	0.75
F11	1:2	500	0.75
F12	1:2	1500	0.75

STUDY OF MICROMERITICS OF MICROSPHERES

The micrometric properties of prepared microspheres were measured by properties like angle of repose, bulk density, tapped density, compressibility index, and Hausner's ratio using methods reported in literature. [16]

1. Particle size determination:

Particle size was determined using Malvern Zetasizer ZS 90 (Malvern instruments, Worcestershire, 0U.K), utilizing laser diffraction with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration.

2. Percentage yield:

The percentage yield of the microspheres was calculated as the ratio of the mass of microspheres collected to the total mass of inner phase solid contents.

Percentage yield = (Total weight of Microspheres/Total weight of internal phase) × 100

3. Surface morphology

The surface morphology of the microspheres was studied byobserving under stereo zoom microscope (ALMICRO)

4. Entrapment efficiency: [17]

Accurately weighed microspheres equivalent to 100 mg of boswelic acid, were dissolved in 10ml methanol and sonicated for 15 mins. The solution was filtered throughWhatman filter paper no.41 . 1 ml of this solution was withdrawn and further diluted to 10 ml with methanol. The absorbance of the resulting solution was measured at 250 nm against methanol as a blank.

Percentage entrapment efficiency = (Actual drug content/Theoretical drug content) x 100

5. *In-Vitro* Drug Release Study Of Microspheres Using Changing Ph Method

he drug release from microspheres was studied using USP Type II Dissolution Test Apparatus (Lab India DS 8000). The test was carried at 100 rpm at 37°C in dissolution medium with changing pH. The microspheres were transferred to the dissolution medium, and aliquotes were withdrawn at selected time intervals, filtered through Whatman filter paper no. 41, and analyzed using a UV spectrophotometer (V-730 Jasco) at 250 nm. Initiallyfor 2 hours, the dissolution medium was 700 mL of 0.1 N HCl (pH 1.2). After this, 200 ml of 0.2M tribasic sodium phosphate buffer solution was added to all the dissolution vessels, and the pH was adjusted to 6.4 for 1 hour, 6.8 for the next 2 hours, and 7.2 till the end of the study using 2 M NaOH.[18]

6. Differential Scanning Calorimetry:

The DSC thermogram for the boswellic acid, Eudragit, and microspheres was recorded using differential scanning calorimeter (Mettler Toledo). 5mg of sample was heated in a sealed, pierced aluminum pan from 30°C - 300°C at a heating rate of 10°C/min under a stream of nitrogen at a flow rate of 40ml/min. DSC study was carried out to determine the drug-polymer interaction studies.[19]

7. Stability studies: The required amount of microspheres was added to capsule and wrapped in aluminum foil these were stored at 40C and 75% RH for 6 months. The microspheres were subjected to drug content and dissolution at equally spaced intervals of one month. The stability protocol designed was based on the ICH guidelines.[20]

RESULTS AND DISCUSSION

UV Spectrophotometry of Boswellic acid

A calibration curve (fig 2) using boswellic acid marker was prepared by UV spectrophotometric approach. The linearity equation was y =0.0095x +0.0042 with an R squared value of R2 = 0.999

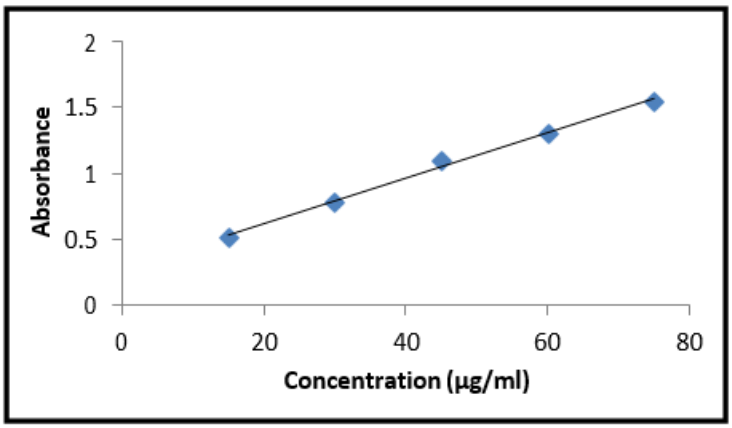

Figure 2. Calibration curve of Boswellic acid in Phosphate buffer pH 7.4.

Preparation of microspheres by solvent evaporation methods:

The system used was an organic polymer phase emulsified in immiscible oil, this constituted an O/O type system. The elimination of water significantly reduces the tendency of the drug to partition into the continuous phase, provided that the drug is insoluble in the external oil.[21]Mineral oil was a useful dispersion medium for microencapsulation of hydrophilic active such as Boswelic acid.In such systems the components are initially dissolved in a mixture of acetonitrile/ethanol and optionally water, or only acetone or in a mixture consisting of methanol and ethanol. [12]

Experimental Design:

A 3 factor 3 level full factorial design will need 27 runs hence Box Behnken design which includes middle points was employed to explore main effects as well as interaction. The desired properties such as entrapment, drug release are dependent onsolubility of drug in polymer, solubility of polymer in solvent, ratio of external to internal phase. When the polymer content is high it improves the drug internalization but also increase the particle size due to higher viscosity of internal phase, this in turn will affect drug release. Similarly a higher stirring rate will produce smaller droplets of internal phase and lead to higher surface area allowing loss of drug to external phase, while surfactants will reduce particle size and similarly affect the drug entrapment. [22, 23]

Table 2 : Particle size, entrapment and cumulative drug release values of optimization batches

Formulation No.	Particle size Micron	Entrapment Efficiency %	Cumulative Drug Release %
F1	14.6 ± 0.42	68.2 ± 2.56	98.8 ± 4.18
F2	13.2 ± 0.43	72.3 ± 2.98	98.4 ± 3.42
F3	12.5 ± 0.39	72.1 ± 2.94	99.3 ± 4.11
F4	13.9 ± 0.32	68.4 ± 2.36	90.5 ± 3.14
F5	14.8 ± 0.58	79.3 ± 2.87	93.2 ± 3.42
F6	15.2 ± 0.54	80.5 ± 3.21	96.2 ± 3.85
F7	13.9 ± 0.45	69.8 ± 3.10	99.5 ± 3.93
F8	11.8 ± 0.30	64.3 ± 2.25	98.0 ± 3.76
F9	12.4 ± 0.44	56.7 ± 2.58	92.4 ± 3.45
F10	14.3 ± 0.55	79.9 ± 3.34	90.7 ± 3.27
F11	12.4 ± 0.39	76.5 ± 2.79	99.1 ± 3.26
F12	10.2 ± 0.21	71.2 ± 2.96	99.4 ± 3.72

Particle size ranged between 10.2 to 15.2 microns, entrapment efficiency ranged between 68.2 to 80.2 % and drug release at the end of 8 hours was above 90 % for all the batches. (Table 2).

Analysis of experimental results was done by using the Stat-Ease Design Expert. A 2 factor interaction (2F1) model was suggested to describe effect of main factors and reveal the interactions. The coefficients of the polynomial equations generated for particle size and % EE using Design expert 7.0

Table 3 : Polynomial equation for obtained model.

Particle size	
Final Equation in Terms of Coded Factor	=+13.05-1.59*A -0.60*B -0.0875*C - 0.1750*AB - 0.25*AC -0.025*BC
Drug Content	
Final Equation in Terms of Coded Factor	= +70.40 -7.63*A -2.94*B- 0.1375*C -1.57*B- 0.6750*AC

The negative coefficients for variables indicate an unfavourable effect on the response variables, while the positive coefficients for the interactions between 2 variables indicate a favourable effect while the small coefficients indicate that these terms contribute the least in prediction of particle size and drug content. (Table3)

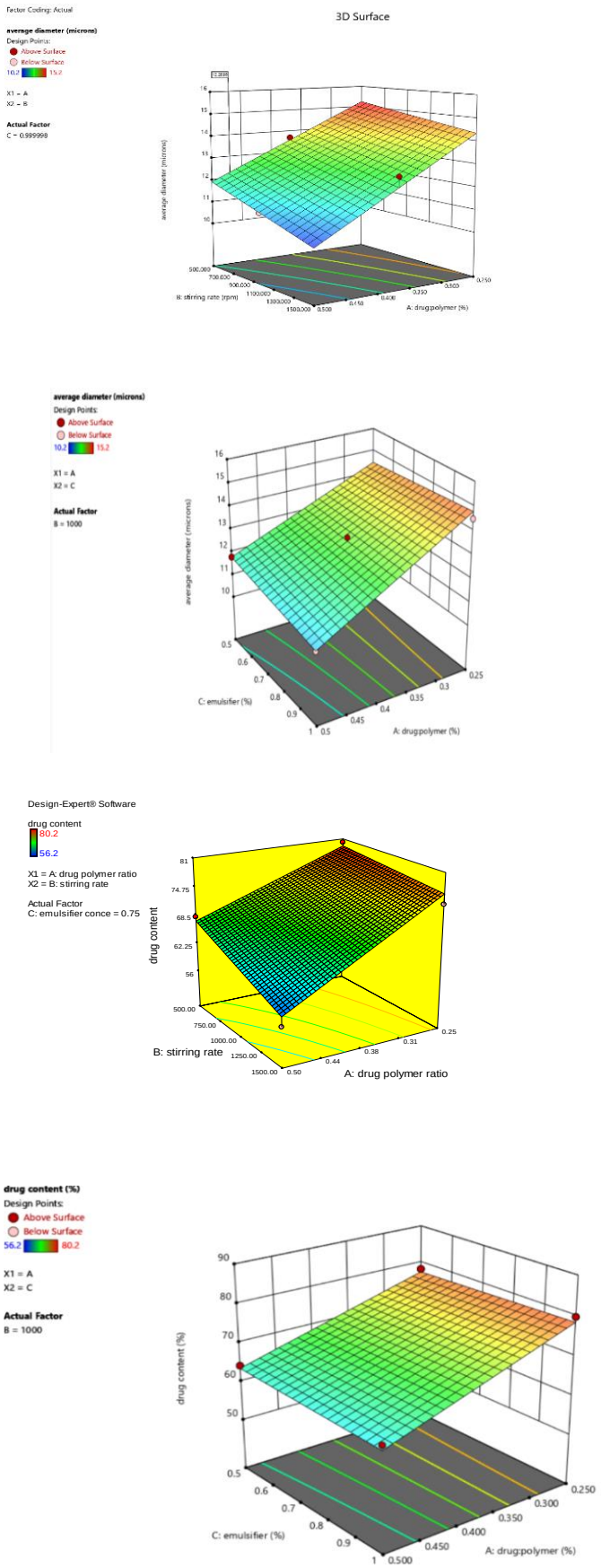

Figure 3: Surface Response diagrams of effect of variables like stirring speed, emulsifier concentration and polymer ratio on particle size and drug content.

Drug polymer ratio was seen to have prominent effect on particle size and drug content as compared to rate of stirring.

Table 4 : Validation of optimization model

Drug: polymer ratio (%w/w)	Stirring rate (rpm)	Emulsifier Concentration (%w/w)	Particle size (micron)		Entrapment efficiency (%)		Cumulative drug release (%)	
			Predicted	Observed	Predicted	Observed	Predicted	Observed
1:3	500	0.25	15.2	16.8	80.2	81.5	96.2	94.6

Physical–chemical characteristics
Micromeritic properties
The flow properties of microspheres evaluated using various parameters were found to be good.

Table 5: The micromeritic properties of prepared microspheres and were indicative of excellent flow properties.

Formulation No.	Bulk density gm/ml	Tapped density gm/ml	Carr's Index	Hausner's Ratio	Angle of Repose
F1	0.43	0.48	11.6	1.12	22.3°±0.23
F2	0.44	0.46	4.6	1.05	36°±0.45
F3	0.49	0.52	6.12	1.06	32.6°±0.34
F4	0.39	0.42	7.7	1.08	25.8°±0.45
F5	0.31	0.32	3.23	1.03	42°±0.57
F6	0.38	0.41	7.89	1.08	25°±0.55
F7	0.35	0.38	8.57	1.09	26.8°±0.26
F8	0.36	0.39	8.33	1.08	22.1°±0.15
F9	0.28	0.29	3.57	1.04	32°±0.45
F10	0.54	0.58	7.41	1.07	41°±0.46
F11	0.34	0.37	8.82	1.09	28°±0.47
F12	0.42	0.48	14.29	1.14	35°±0.41

Particle size [24]

The particle size of the microspheres was found to vary between 10 to 15 microns. Particle size increased with the increase in polymer concentration. This higher particle size may have been due to higher viscosity at high polymer concentrations, leading to larger emulsion droplets and finally in greater microsphere size. For agitation, as speed increased, the size of microspheres was reduced, but higher agitation speeds again resulted in irregularly shaped microspheres. The concentration of emulsifier had significant effect on particle size distribution of the microspheres. As agitation speed increased, the size of microspheres was reduced, but higher agitation speeds resulted in irregularly shaped microspheres. The particle size was optimum with improved entrapment efficiency atF5, F6 and F12.
Surface Morphology using Stereo zoom microscopy: The stereo zoom images of microspheres provided an understanding of its shape and surface topography. The surface of microspheres was spherical and its surface was smooth which indicated the drug incorporation in polymer matrix. Fig 4

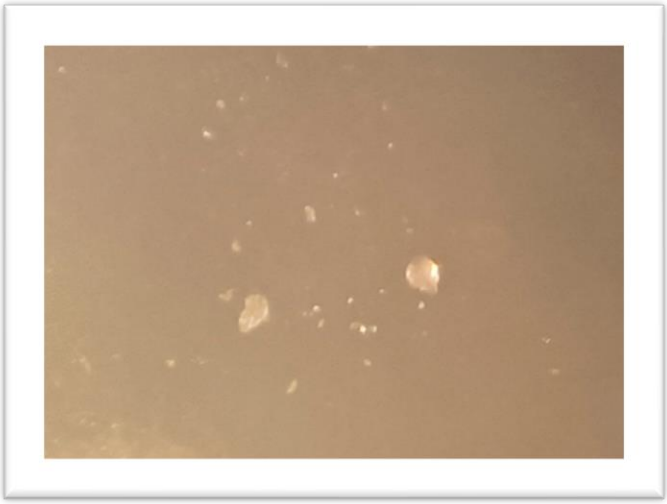

Fig. 4: Image of Eudragit S100 microsphere under stereo zoom microscope.

Entrapment efficiency

The entrapment efficiency was found to vary between 56% to 80%. Entrapment of drug increased with increasedpolymer concentration and particle size. There was no significant effect of stirring rate and emulsifier concentration ondrug loading capacity.The entrapment efficiency was maximum at polymer ratio 1:4.

In vitro drug release:

The cumulative drug release of boswelic acid after 8 hours was found to vary between 67% to 98%. There was an inverserelation between drug release and polymer concentration. The microspheres were subjected to drug release test bychanging pH method. It was observed that the drug release for first 2 h (simulated gastric condition) was almost negligible. As the pH was raised slowly, the microspheres started to release the drug. [25] Complete drug release was observed at the end of 8 hrs. The high polymer concentration in the microspheres, results in increased coat thickness surrounding the drug particles thereby decreasing the drug release. Fig 5

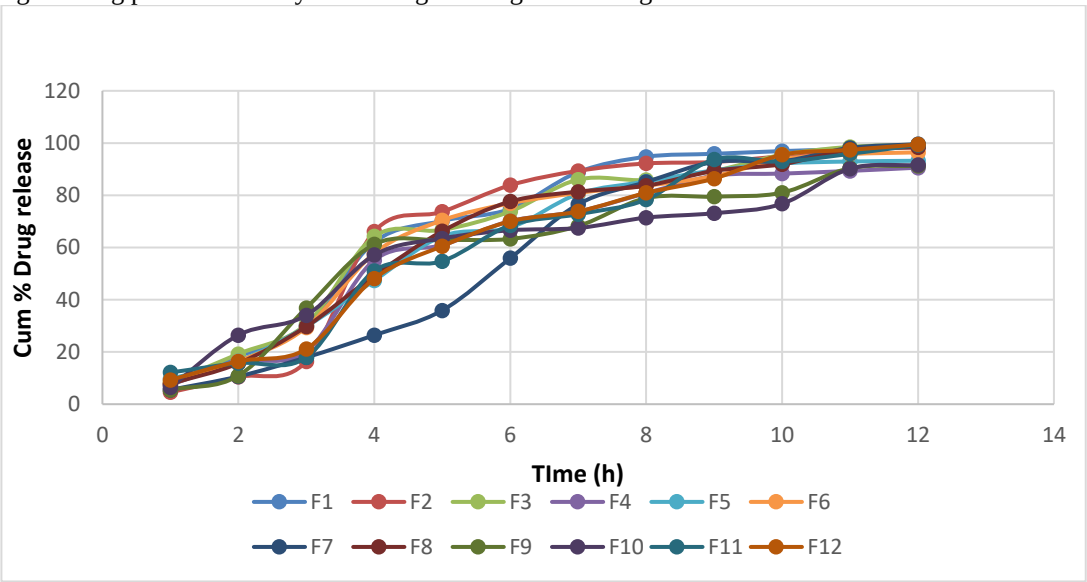

Figure 5: Drug Release profile of Eudragit S100 microspheres.

PERCENTAGE YIELD

The Percentage Yield of different batches of microspheres varied in the range of 95% to 98%.

Differential Scanning Calorimetry:

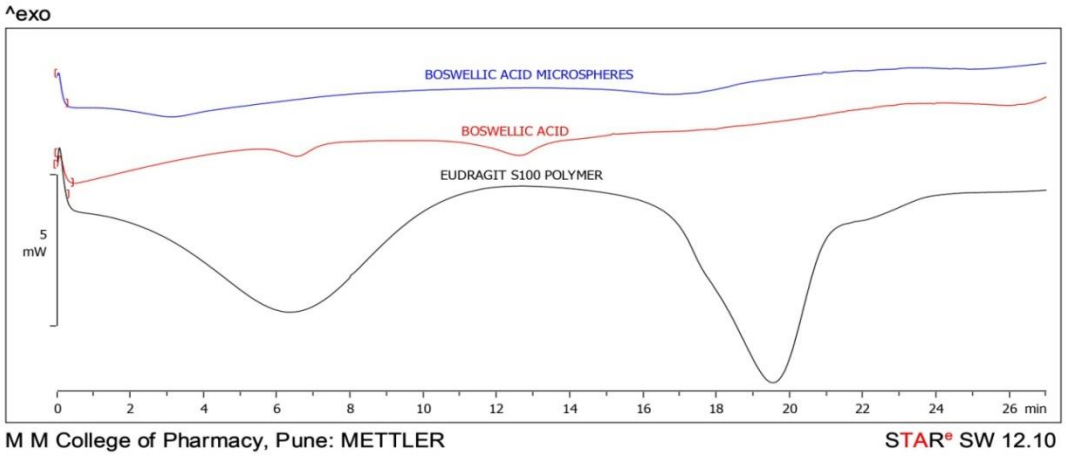

Figure 6: Cumulative DSC Thermogram of A) Boswellic acid B) Eudragit S100 and C)Boswellic acid andEudragit S100 microspheres.

The thermogram of Eudragit S 100 and that of microspheres was similar, there was no evidence of thermal events displayed by boswellic acid in either of these thermograms, this is an evidence of drug entrapment in the polymer matrix. Stability studies: There was observed no significant change in the drug release profile of the microspheres evenafter months. Table 5

Table 6: Table of Stability studies of the ES 100 microspheres.

Parameter/Month	0	1	2	3	4	5	6
Drug release at 2 h	19.98	19.97	19.65	19.24	18.54	18.32	17.98
Drug release at 12 h	96.52	96.21	95.89	95.75	95.21	94.99	94.50
Drug content (%)	80.2	80.15	79.94	79.92	79.64	79.32	78.66

CONCLUSION

Eudragit S -100 microspheres can be prepared by o/o method which is quick and scalable, the drug content, micromeritic and release properties were good. The optimized batch showed no significant change upon storage at accelerated conditions mandated by ICH.

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