



PREPARATION AND EVALUATIONS OF MULTI-PARTICULATES SYSTEM OF CELECOXIB: OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

The objectives of the present investigation were: (1) to elucidate the effect of formulation variables e.g., amount of Eudragit RS 100, concentration of Tween 80 and agitation speed on in-vitro release profiles of celecoxib from multi-particulates system, formulated with the combination of ethyl cellulose and Eudragit RS 100 by using a novel quasi emulsion solvent diffusion method; (2) to optimize the formulation variables by response surface methodology (RSM) ; and (3) to characterize the products on the basis of FTIR, thermal, particle size, SEM, X-ray analyses and drug release kinetics studies. Experiments were designed and data was collected according to a three levels face centered central composite design. It was found that in-vitro release (Y_1 - Y_3) were decreased significantly ($p < 0.05$) with increase in amount of Eudragit RS 100 but increased significantly ($p < 0.05$) with increase in surfactant concentration and stirring speed. The analysis of dissolution kinetics data showed that it followed Higuchi and zero-order model rather than first order model. It was observed that the drug release data of the selected formulation was close to the predicted release pattern. Therefore this approach suggested that the combination of Eudragit RS 100 and ethyl cellulose microspheres may be useful for the delivery of maximum amount of celecoxib in intact form to the colon.

Keywords: Celecoxib • Eudragit RS100 • Central composite-face centered design • RSM • Multi-particulates system • Delayed release colon targeted Delivery System

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INTRODUCTION

A Colon Targeted Drug Delivery System could be of additional value when a delay in systemic absorption is desirable from a therapeutic point of view, as for diseases that have peak symptoms in the early morning and exhibit circadian rhythm, e.g. rheumatoid arthritis. This can be achieved by a bed time administration of a drug delivery system which, with a delayed start of drug release, can provide adequate protection in the early morning [1-4]. Chronotherapy refers to a therapeutic scheme in which in vivo drug availability is time dependent on the circadian rhythm to produce the maximum health benefit and minimum harm to the patient [1]. Arthritis is mainly two types: osteoarthritis (OA) and rheumatoid arthritis (RA). Many researchers reported the use of non-steroidal anti-inflammatory drugs (NSAIDs) as a treatment option, the toxicities of these agents, and possible solutions, including a new class of anti-inflammatory drugs. Delayed action (at the morning / night time) is needed for arthritic patients. Osteoarthritis tend to have less pain in the morning and more at night; while those with rheumatoid arthritis, have pain that usually peaks in the morning and decreases throughout the day, which is caused just because of C -

reactive protein level increases as per the circadian rhythm, which leading to enhanced pain and inflammation[1,5].

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites [3,6,7].

Eudragit RS is insoluble in aqueous media but they are permeable and have pH-independent release profiles. The permeability of Eudragit RS in aqueous media is due to the presence of quaternary ammonium groups in their structure. Several publications on the formulation and use of drug containing microspheres have utilized the Eudragit (Rohm Pharma) series of polymers as the encapsulating materials [8-10].

The solvent evaporation method is commonly, used to microencapsulate water-insoluble drugs within water-insoluble polymers. The very poor aqueous solubility and wettability drugs, however, give rise to difficulties in the design of pharmaceutical formulations and lead to variable oral bioavailability [11-12].

Celecoxib is an anti-inflammatory drug, that also exhibits analgesic & antipyretic activities and especially used in

arthritis. The mechanism of action of celecoxib is believed to be due to the inhibition of prostaglandin synthesis, primarily via inhibition of Cyclo-Oxygenase-2. COX-2 inhibitors, have an advantage by showing a lower risk for the development of GI bleeding. Celecoxib is thus, associated with a lower incidence of gastro duodenal ulcers than other nonsteroidal anti-inflammatory drugs, which are nonspecific inhibitors of cyclo-oxygenase. Unlike aspirin, celecoxib does not inhibit platelet aggregation and does not increase bleeding time. But, for patients who require chronic use of NSAIDs and are at high risk for NSAID-related gastro-duodenal toxicity, primary therapy with a COX-2 selective inhibitor is a reasonable option. Celecoxib has site specific and saturable absorption kinetics. Studies showed that celecoxib can exhibit chemoprotective effects on tumors of the colon and reduce the risk of colon cancer. Celecoxib exhibits poor water solubility, with biological half life; 6-11 hours, and conventional dosage form is administered twice daily to fulfill the therapeutic level of the patient. only 40% of administered drug is bioavailable through oral route and bioavailability may be greater if it is targeted to the colon [13-15]. Celecoxib was selected as a model drug because it has good indication for colonic delivery.

The aim of this work was to prepare microspheres drug delivery system of celecoxib using combination of ethyl cellulose (EC) and Eudragit RS100. Being a very weakly acidic (pKa 11.1) & BCS class II drug [13-15], the bioavailability of celecoxib can be increased by targeting the drug into the colon by which solubility may be increased in presence of colonic fluids at higher pH. The specific goal of this research was to evaluate the effect of amount of polymer (Eudragit S100), surfactant concentration (Tween 80) and stirring speed on the particle size, percentage encapsulation efficiency and in-vitro release of drug from the formulations. Experiments were designed and data was collected according to a three levels face centered central composite design. The influences of formulation variables on the microsphere properties were examined and the microsphere formulations suitable to achieve our goal were determined and optimized by Response surface Methodology (RSM). In the present study we aimed to delay the drug release by designing multiparticulate systems in the form of microspheres, that would be efficiently released the drug into the colon. Following bedtime administration, microspheres are expected to maintain low drug plasma concentration overnight when the arthritic pain are reported to be the minimum and release the optimal concentrations in the morning between 6-9 hrs; when pain is found to be the maximum.

MATERIAL AND METHODS

Materials

Celecoxib was purchased from Exim-Pharm International, Mumbai, India. Eudragit RS100 was obtained as gift sample from Dr. Reddy's Laboratories, Hyderabad, India; Ethyl Cellulose (EC) in a viscosity grade of 14 cps was procured from Wilson Brothers, Mumbai, India. All the others chemicals used were of analytical grade.

Experimental design

Before application of the design, a number of preliminary trials were conducted to determine the conditions at

which the process resulted to microspheres. The levels of the factors were also determined randomly by evaluating the depended variables (responses) of trial batches. A three levels face centered central composite design was used for optimizing the formulation.

Statistical analysis of the data and validation of the optimization model

The Design Expert software (Version 8.0.4.1 Trial, Stat-Ease Inc, Minneapolis, Minnesota) was used in the current study for the generation and evaluation of statistical experimental design [16-18]. Polynomial models including interaction terms were generated for all the response variables using multiple linear regression analysis. The influence of factors and their interaction, on each of the response are represented graphically.

In order to validate the polynomial equations, one optimum checkpoint (e.g., formulation composition and process) and two random checkpoints were selected by intensive grid search, performed over the entire experimental domain. The criterion for selection of optimum check point i.e. usable amount of polymer used (X_1), concentration of surfactant (X_2) and stirring speed (X_3), was mainly based on the highest possible values of response parameters (Y_1 - Y_5). Formulations corresponding to these three check points were prepared and evaluated for all the five responses (Y_1 - Y_5). The resultant experimental data of response properties were subsequently compared quantitatively with the predicted values.

Preparation of microspheres

The modified version of earlier reported methods [11, 19] was employed to prepare microspheres by using the quasi-emulsion solvent diffusion method of the spherical crystallization technique [11, 12]. Table 1 shows the compositions of various formulations and the studied factors along with their levels and the corresponding responses are summarized in table 2. Celecoxib (0.9 g) was dissolved with ethyl cellulose (0.45 g) & Eudragit RS100 (0.45- 1.05 g) in a mixed solution of acetone (good solvent, 6.0 ml), and chloroform (bridging liquid, 6.0 ml). The dispersion of drug into the polymer solutions was aided by sonication for 20-30 min. Necessary, extra cold water was added to the sonic bath to prevent excessive warming of the drug-polymer solutions. Then, Aerosil (1.0 g) was suspended uniformly in the drug-polymer solutions under vigorous agitation. The resultant drug-polymer-Aerosil suspension was poured into 200 ml distilled water containing 0.5-1.5% (v/v) of Tween 80 under agitations speed (500-1000rpm), with digital mechanical stirrer (Remi Motors, Delhi, India) and thermally controlled at 30-35°C. The suspension was finely dispersed into quasi-emulsion droplets immediately under agitation, and the drug and polymers co-precipitated in the emulsion droplets. After agitating the system for 20 min, again 200 ml of distilled water containing 0.5-1.5% (v/v) of Tween 80 was added slowly to promote the diffusion of the good solvent from emulsion droplets into this solvent resulting in enhancement of the solidification of quasi-emulsion droplets and stirring was continued until evaporation of the organic solvents were completed (typically 2-3 hrs) and till the translucent quasi-emulsion droplets turned into opaque and stable solidified microspheres. The solidified microspheres were recovered by filtration and

washed with 200 ml water. The resultant products were dried overnight in an oven at 37°C.

Drug-excipients compatibility studies

Compatibility of drug with excipients was studied by Fourier Transform Infra-red spectroscopy (FT-IR). Effect of process of entrapment on crystallinity of the drug was studied by Differential Scanning Calorimetry (DSC) [20].

FT-IR Spectroscopy

The IR spectrum of pure drug, bulk polymers, physical mixtures of drug and polymers, and drug loaded microspheres were obtained in potassium bromide pellet by FTIR Spectrophotometer (Prestige-21, Shimadzu, and Tokyo, Japan) to monitor the interactions of drug with excipients between the ranges of 400 to 4000 cm^{-1} .

Thermal analysis

The thermal analysis of pure celecoxib, bulk ethyl cellulose, bulk Eudragit RS-100, physical mixtures of drug and polymers, and of best selected microsphere formulation, were carried out by differential scanning calorimetry (DSC) equipped with a thermal analysis data system (Perkin Elmer, California, USA). Samples weighing 3-5 mg were heated in flat-bottomed sealed aluminum pans over a temperature range of 40-250°C at a constant rate of 10°C/min under nitrogen purge (50 ml/min).

Evaluations and characterizations of microspheres

Drug Encapsulation Efficiency

Drug encapsulation efficiency [21, 22] of the prepared microspheres was carried out by crushing the microspheres using mortar and pestle. The powdered microspheres equivalent to 100 mg of drug was taken in 100 ml volumetric flask containing 50 ml methanol and 20 ml of phosphate buffer (pH 7.4) and the solution was sonicated to ensure dispersion of the powdered microspheres and it was further shaken for 2 hrs by water bath shaker at 37°C. After volume made up to 100 ml with phosphate buffer (pH 7.4), the samples were filtered through membrane filter (0.45 μm) and after suitable dilution; the drug content was analyzed with the help of UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan) at 250 nm. Each determination was carried out in triplicate and percent entrapment was calculated as: Eq.1.

$$\text{Encapsulation efficiency} = \frac{(\text{Experimental drug content})}{(\text{Theoretical drug content})} \times 100$$

%Yield

The calculation of percentage yield [21, 22] was done by using the following formula:
Eq.2.

$$\text{Percentage practical yield} = \frac{[\text{Practical yield}/\text{Theoretical yield}] \times 100$$

Particle size analysis

Particle size of the microspheres was measured by Malvern instrument (Hydro 2000MU (A), Mastersizer, Malvern, UK). The completely dried particles were dispersed with 400 ml of distilled water and it was placed on the sample tray with an in built vacuum and compressed air system to suspend the particles. The laser obscuration range was maintained between 8% - 12%. All the measurements were carried out in triplicate and 50th percentile diameter (d 0.5) of the cumulative particle size

distribution was considered as mean values and it was expressed for all formulations as mean size range.

Surface topography by Scanning Electron Microscopy (SEM)

The shape and surface morphology of the microspheres was studied by using scanning electron microscope. Morphological examinations of the surface of formulations were carried out using a scanning electron microscope. Scanning electron microphotographs of microspheres were obtained using JEOL instruments (JSM-6100, Tokyo, Japan). The particles were vacuum dried, coated with thin gold-palladium layer by sputter coater unit and observed microscopically at an accelerating voltage of 10 kV.

X-Ray Diffractometry

X-ray diffractometry of different powder samples (pure drug, physical mixtures of drug and polymers and selected formulation) were investigated using Philips XRD Machine set up with generator (PW1830), Goniometry (PW 1820) and diffractometer (PW1710, Eindhoven & Almelo, Netherlands, Europe). Cu K_{α} radiation was used (30 kV, 50mA with an α_1/α_2 ratio of 0.5). The XRD patterns were recorded at diffraction angles (2θ) with 4°/min scanning speed, and 5°-45° 2θ range.

In vitro release of celecoxib-loaded microspheres in simulated GI conditions

Type II dissolution apparatus for delayed release products, specified in USP [23], was followed to study the in vitro drug release of the prepared microspheres. Dissolution studies were carried out using USP XXIV paddle type apparatus (Campbell Electronic, Mumbai, India) using 0.1N hydrochloric acid (pH 1.2) and phosphate buffer (PB) solutions (pH 6.8 & 7.4) with 0.5 % (w/v) sodium lauryl sulphate (SLS) as dissolution mediums.

The microspheres were tested for the in vitro drug release in simulated GI fluids [24-25]. An accurately weighed amount of microspheres, equivalent to 25 mg of celecoxib, introduced in a muslin cloth which was fitted with paddles and 900 ml of dissolution medium was added. The drug release from microspheres was processed at 100 rpm and temperature was maintained at 37±0.5°C. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of gastro intestinal (GI) pH variations was accomplished by modifying the pH of the dissolution medium at various time intervals. For each dissolution run, a mean of three determinations was recorded in three different medium (one after another).

The pH of the dissolution medium was kept at 1.2 for 2 hrs with 0.1 N HCl solutions. Then, dissolution medium was replaced with phosphate buffers (pH 6.8) with 0.5 % SLS. The released rate analysis was run for another 3 hrs. After that, the pH of dissolution medium was further adjusted to get simulated to colonic fluid i.e. phosphate buffers (pH 7.4) and release rate analysis was run for another 4 hrs. A sample volume of 5 ml was withdrawn from the medium at various time intervals and replaced with fresh dissolution medium. The samples were then filtered through Whatmann filter paper and diluted suitably. The amount of drug present in the various dissolution media was analyzed at 252 nm, using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan).

The effects of depended factors (X_1 - X_3) on in vitro drug release of microspheres were also evaluated by employing RSM. 0.5 % SLS was used to maintain the sink condition,

because solubility of celecoxib is very poor in different medium and more over good correlation between bioavailability and in vitro dissolution rate of poorly soluble drug was observed when surfactant was used in vitro dissolution study. Although intestinal fluids contain bile salts, to simulate the intestinal fluids 0.5 % SLS solutions was added in phosphate buffer solutions (pH 6.8 & 7.4).

Stability studies

Stability testing has become an integral part of formulation development, it generates information on which proposal for self-life [26] of drug or dosage forms and their recommended storage conditions are based. Best selected microspheres (RCF2 & RCF14) were tested for stability. All the preparations were divided into 3 sets and were stored at 4°C (refrigerator), room temperature and 40°C with 75±5 % RH. After 15, 30, 60 and 90 days drug contents of all the formulations was determined by the method discussed previously in entrapment efficiency section. In vitro release study was also carried out on same formulation.

Statistical analysis of Response surface methodology

A second order polynomial model [27-29] was employed to fit the data individually for the responses; Y_1 - Y_5 , by the general model:

Eq.3.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Y= dependent variable;

b_0 = arithmetic mean response of sixteen runs;

b_i = estimated coefficient for the factor X_i ;

$X_1, X_2, \& X_3$ = average result of changing one factor at a time

Table1: Compositions of various formulations of microspheres

Sl. No.	Ingredients	Amount
1	Celecoxib	0.9 g
2	Ethyl Cellulose	0.45g
3	Eudragit RS 100 (g)	0.45(-1), 0.75(0), 1.05(+1)
4	Aerosil	1 g
5	Acetone	6 ml
6	Chloroform	6 ml
7	Tween 80 (% w/v)	0.45 (-1), 0.75(0), 1.05(+1)
8	Purified water	400 ml

The quasi-emulsion solvent diffusion method of spherical crystallization technique has been accepted as a useful technique for particle design for pharmaceuticals. It could provide remarkable advantages over conventional microspheres preparation methods. However, further application of quasi-emulsion solvent diffusion method to produce solvent deposition system & to improve the dissolution rate of poorly water soluble drug has been reported by some researchers [11-13,19]. When the drug, polymers and aerosil dispersed system of organic solvent was poured into aqueous phase with stirring, the finely dispersed gel like emulsion droplets were formed instantly. Generally both the drug and polymers have excellent attraction to the organic solvents (mixtures of acetone and chloroform), that's why the emulsion droplets could not be diffused into aqueous phase at once. But as agitations were going on and organic solvents were evaporated gradually, stable and solidified droplets were

from its low to high value;

$X_1X_2, X_1X_3, \& X_2X_3$ = shows how the response changes when two factors are changed simultaneously.

Face centered central composite design was eight factorial points, six axial points and two identical central points with three factors and each factor coded to be in the range of -1, 0, +1. The coded points for the experimental design and responses models for the regression analysis are given in table 2 & 3. Models were evaluated in the terms of significant coefficient, F (ANOVA) & p, standard error (SE), R-squared and lack-of fit values.

RESULTS AND DISCUSSION

Preparation of microspheres

The present study was taken to formulate and evaluate controlled release with delayed action microspheres of celecoxib, by quasi-emulsion solvent diffusion method. Compositions of experimental formulations are enlisted in table 1 and factors along with their levels and the responses are summarized in table 2.

In this formulation, EC and Eudragit RS100 were used as a bond and retarding agent in order to bind the aerosil into microspheres and control the release rate. To produce a colon specificity delivery in a controlled manner with delayed action, Eudragit RS100 was used to prevent drug release from microspheres in the stomach and small intestine, until they reach the terminal ileum; where EC ensures to control the release of celecoxib, following degradation by the abundant colonic micro flora and in presence of colonic fluids. Aerosil, an inert solid dispersing carrier, was introduced in this formulation to improve the dissolution rate of poorly water-soluble drug [19].

concurrently observed. Most commonly, tween 80 used as a surfactant and most suitable emulsifying agent of the o/w type emulsion; in this present study it was employed to enhanced the stability of emulsion droplets and to retard the formation of agglomerations and lumps. The aerosil was introduced in this microspheres formulation as an inert solid dispersing carrier to improve the dissolution rate of drug. Due to its large surface area, high porosity, and unique adsorption properties, aerosil has been successfully used as a dispersing agent to increase the dissolution rate of sparingly soluble drugs [19]. Simultaneously, being an effective anti-adhering agent, aerosil could speed up the solidification of emulsion droplets promptly to form spherical microspheres. These suggested that higher rate of yield value of microspheres could be obtained comparing with other conventional methods of microspheres.

Table 2: Variables of face centered central Composite Design: factors & responses.

Independent variables (Factors)	Levels used		
	Low(1)	Middle(0)	High(1)
X ₁ = Amount of Eudragit RS100 (mg)	450	750	1050
X ₂ = Concentration of Tween 80 (v/v %)	0.5	1.0	1.5
X ₃ = Agitation speed (rpm)	500	750	1000
Dependent variables (Responses)	Constraints		
	Low	High	Goal
Y ₁ = Cumulative % Drug released in 2 hrs in 0.1 (N) HCl	Minimize		
Y ₂ = Cumulative % Drug released in 5 hrs in PB pH 6.8	Minimize		
Y ₃ = Cumulative % Drug released in 9 hrs in PB pH 7.4	70	90	80
Y ₄ = % Entrapment Efficiency	70	90	80
Y ₅ = Particle Size (µm)	In the range		

Drug-excipients compatibility studies

FT-IR Analysis

The FT-IR spectra of pure celecoxib shown characteristic bands at 1229.04 & 1274.06 cm⁻¹ (-CF₃), 1446.35 cm⁻¹ (-N-N-), 3417.24 cm⁻¹ (-SO₂NH₂). Broad bands were shown at 3300-3500 cm⁻¹ due to -NH₂ stretching and 1550-1600 due to -N-H stretching. The FT-IR spectra of blank ethyl cellulose were shown peaks at 1112.46, 1414.73, 2362.12, 1561.35 & 1642.31, & 3451.61 cm⁻¹. Eudragit RS 100 shown 1244.13, 1583.61, 1645.33, 2883.68, 3119.00 cm⁻¹ and a most intensified peak at 1726.17 cm⁻¹ due to C=O stretching of acrylate. Physical mixtures and microspheres showed all characteristic bands of drug and bands around at 1726 cm⁻¹ (C=O stretching band) which attributes the presence of eudragit RS100 and it has also been reported earlier in the literature; due to for acrylate polymer [9-10]. All the characteristic peaks of celecoxib were observed in

the spectra of all the microspheres, thus indicating that no chemical interaction or changes took place during the preparation of the formulations and that the drug was stable in all the formulations. A strong characteristic band for ethyl cellulose at 1112 cm⁻¹ was also observed. A very slight shift in the bands was observed in combination of substances formulation which may be due to the reduction in purity of substances.

Differential Scanning Calorimetry studies

Celecoxib (fig. 1C) showed a single sharp endothermic peak corresponding to the melting of the drug at 165.21°C (T_{onset}=161.15°C, T_{end}=167.73°C, area=1066.29 mJ, ΔH_f=0.1066 KJ/mol and ΔH=106.63 J/g). The DSC curves for bulk eudragit RS100 and bulk ethyl cellulose (fig. 1F & 1D) showed endothermic peak at 60.56 °C and 185 °C respectively, for the melting of polymers.

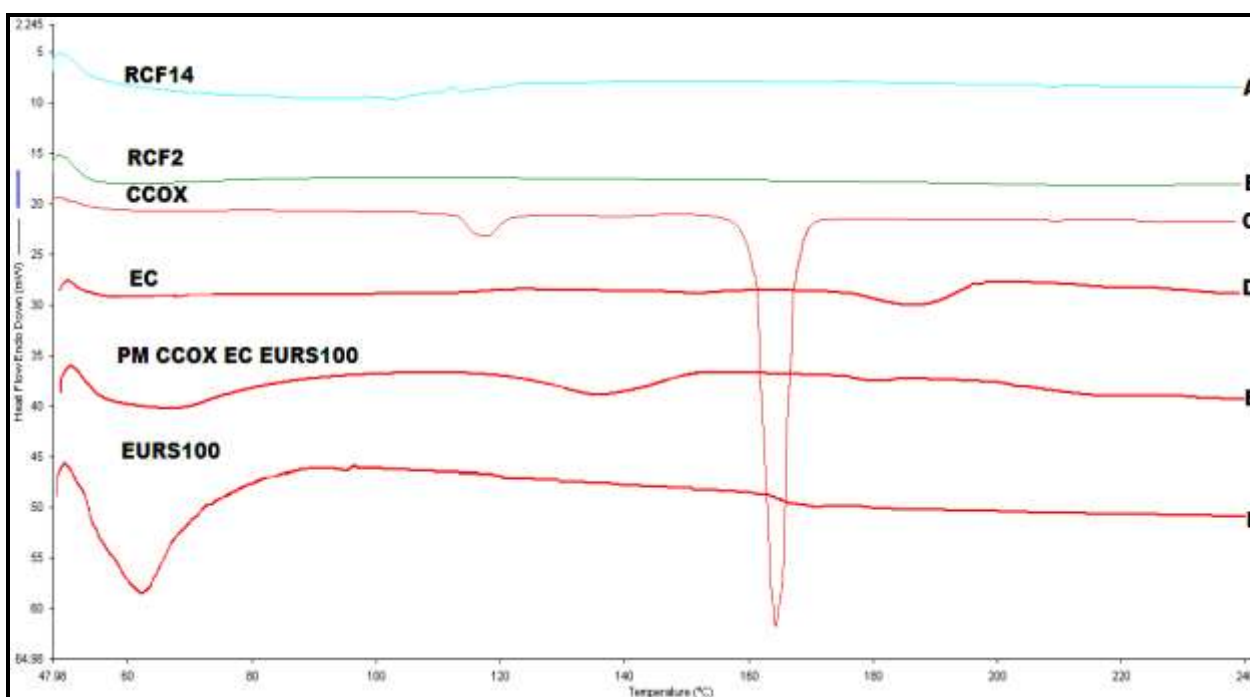


Figure 1: DSC thermogram of (A)RCF14, (B)RCF2, (C) Celecoxib, (D) Ethyl cellulose, (E) Physical mixtures of drug, Eudragit RS 100 & EC (2:1:1) & (F)Eudragit RS100.

The physical mixtures of drug with eudragit RS100 and ethyl cellulose in ratio of 2:1:1 showed endothermic peaks (fig. 1E) at 64.45, 134.24 and 183.26 °C respectively, demonstrating a marked shift in the endothermic peaks from original endothermic peaks of drug and polymers. Also, a considerable decrease in peaks height of the thermogram was observed, which may be due to the reduction in purity of substances. DSC thermogram of RCF2 (fig. 1B) microspheres was showed no endothermic peak & RCF14 (fig. 1A) was showed peaks at 103.10 &

113.94°C and disappearing of the original endothermic peaks of drug & polymers verified that celecoxib could be dispersed homogenously in the amorphous state. The variation of crystalline state was further confirmed by XRD analysis.

Evaluations and characterizations of microspheres

The encapsulation efficiency of the prepared microspheres was in the range 70.57 ±1.04 to 97.52 ±2.40 % (table 3). The production yield of celecoxib microspheres was varied from 70.90±1.05 % to 84.10 ±1.24%.

Table 3: Face centered central Composite Design: randomized runs and the response.

Formulation Code	Factors ^a			Responses ^{a,b}				
	X ₁	X ₂	X ₃	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
RCF1	-1	1	1	17.60±1.21	75.95 ±2.28	98.26 ±4.20	88.65 ± 1.24	271.22±1.16
RCF2	0	1	0	11.75 ±1.24	51.55±2.15	81.10 ±2.60	74.13 ± 1.04	106.35±1.98
RCF3	1	-1	1	5.60 ±0.60	32.08 ±1.94	61.80 ±2.12	75.06 ±0.09	103.23±1.86
RCF4	-1	1	-1	17.20 ±1.45	75.05 ±1.26	97.25 ±2.15	89.76 ±1.02	421.57±1.24
RCF5	1	1	1	6.70 ±0.46	35.30 ±1.86	64.50 ±2.05	70.57 ±1.04	93.42±1.66
RCF6	1	-1	-1	5.15 ±0.28	32.00 ±1.44	61.15 ±1.24	76.34 ±1.04	102.26±1.86
RCF7	1	1	-1	6.60 ±0.46	34.60 ±1.28	64.05 ±1.22	71.85 ±1.05	71.30±1.63
RCF8	-1	0	0	15.25±1.02	69.60 ±1.48	92.08 ±2.16	93.0 ±2.02	273.67±1.28
RCF9	0	0	0	9.85 ±1.04	46.50 ±1.04	72.60 ±1.24	77.71 ±1.08	269.84±1.24
RCF10	0	0	-1	8.20 ±0.46	45.10 ±1.06	70.75 ±1.26	80.70 ± 1.90	348.92±2.02
RCF11	0	0	0	9.80 ±0.48	46.70 ±1.20	72.30 ±2.85	77.12 ±1.60	270.03±1.28
RCF12	-1	-1	-1	14.25 ±1.24	67.60 ±1.28	90.30 ±1.24	97.52 ±2.40	653.66±2.02
RCF13	0	-1	0	7.65 ±1.20	44.85 ±1.24	71.70 ±1.04	81.3 ±1.24	262.95±1.44
RCF14	-1	-1	1	13.75 ±1.28	64.10 ±1.06	85.95±2.24	95.34 ±1.15	578.30±1.64
RCF15	1	0	0	6.20 ±0.86	33.75 ±0.88	62.80 ±1.06	73.14 ±1.08	77.12±1.78
RCF16	0	0	1	10.20±1.92	48.40 ±1.26	73.95 ±1.08	75.92 ±1.04	178.20±1.98

^a Refer to table 1 for factors and responses, ^{a,b} Mean ±SD (n=3)

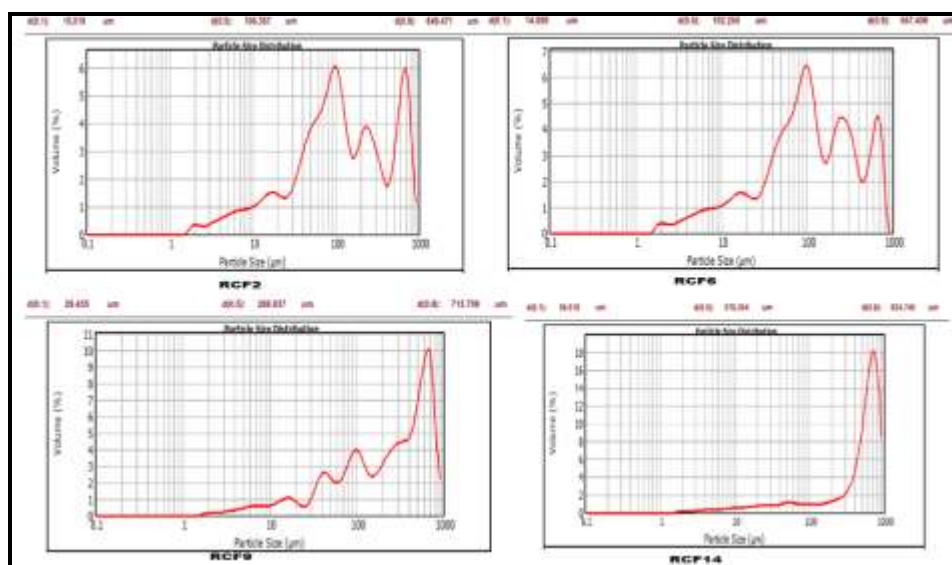


Figure 2: Particle size distribution curves of various celecoxib microspheres of RCF2, RCF6, RCF9 & RCF14.

The mean particle size ($d_{0.5}$) of the microspheres significantly decreased ($p < 0.05$) with increasing polymer concentration. Particle size ($d_{0.5}$) and ($d_{0.9}$) was in the range of 71.30 ± 0.63 to $653.66 \pm 2.02 \mu\text{m}$ and 330.57 ± 1.85 to $877.55 \pm 2.78 \mu\text{m}$ respectively and Particle size distribution curves were shown in fig. 2 & data was represented in table 3.

The SEM images (fig.3) indicated the spherical & non-aggregated nature of the microspheres as well as the presence of pores on their surface. Their spherical nature contributed considerably to their very good flow properties while their hollow nature would mean lower microsphere density.

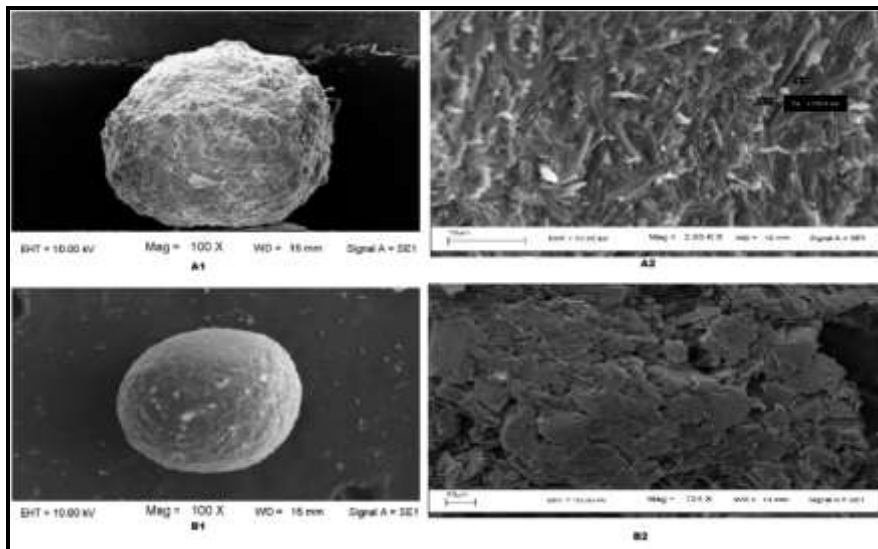


Figure 3: SEM photographs of various celecoxib microspheres of (A1 & A2); RCF2, (B1 & B2); RCF14

The surface of the microspheres prepared with low level of eudragit RS 100 was smoother and crystalline nature of encapsulated drug which was present in the surface of microspheres in higher amount, than of higher level

polymer loaded microspheres. The study of drug loaded microspheres showed the presence of drug particles on the surface, might be responsible for the initial burst release of drug from the entire formulated microspheres.

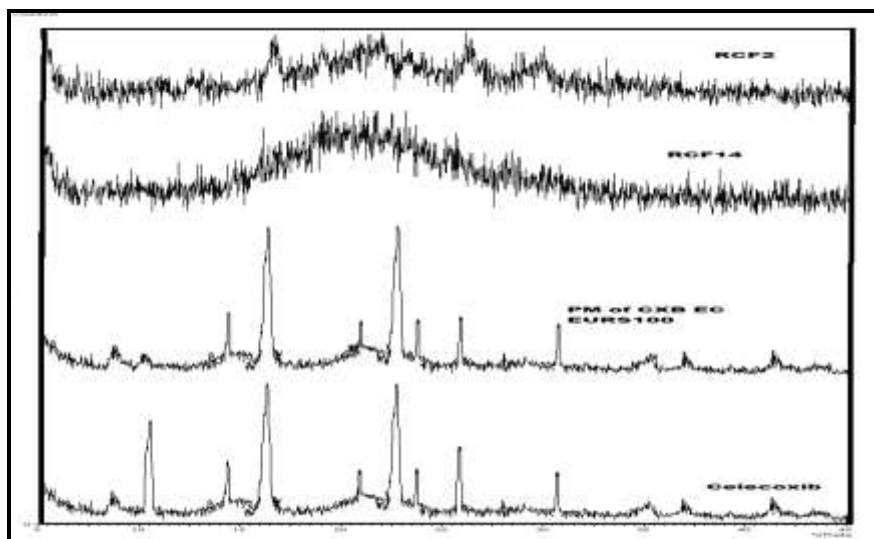


Figure 4: X-ray diffraction spectra of pure drug (Celecoxib), Physical mixtures of pure drug, Eudragit RS100 & EC and microspheres formulations (RCF2 & RCF 14)

X-ray diffraction analysis can be used to evaluate any changes in crystallinity of the drug to formulated microspheres. Figure 4 shows the diffractograms of Celecoxib, physical mixture of drug and polymers and formulated microspheres (RCF2 & RCF14). Celecoxib is a crystalline drug and it gives characteristic peaks at 14.7 , 16.0 , 21.6 , 22.2 , 23.5 & 25.5° and disappeared in RCF2 & RCF14. The X-ray diffraction patterns of drug revealed high crystallinity of the drug with major sharp diffraction peaks of high intensities and while physical mixture having

suppressed peaks with lower intensities. Thus, XRD could be used to study any changes in crystallinity of the drug in an amorphous form, which could be one of the mechanisms responsible for improved dissolution [19]. A significant difference in the crystallinity was observed between formulations (RCF2 & RCF14), physical mixture and drug. This reduction of crystallinity may explain the higher drug release profile by the RCF2 & RCF14 formulations.

The release profiles obtained for the microspheres formulations are presented in figure 5. Within the first two hours, microspheres showed 5.15 ± 0.28 to 17.60 ± 1.21 % cumulative amount of the drug released in presence of 0.1 N HCl solutions. Cumulative drug released at 5 hrs in phosphate buffer (pH 6.8) dissolution medium was 32.0 ± 1.44 to 75.95 ± 2.28 % and cumulative release of drug after 9 hrs ranged from 61.15 ± 1.24 to 98.26 ± 4.20 %. The influence of different processing condition was evaluated on *in-vitro* drug release. A biphasic *in-vitro* drug released

profiles was observed with initial burst effect for all the formulations prepared. The initial burst release is due to the presence of drug on the surface prepared microspheres. The initial burst release can be attributed as desired effect, which ensures the quick initial plasma therapeutic concentration of drug. All the formulated microspheres retain their shape and size even after dissolution which indicates the release of drug diffusion through the polymer wall of microspheres.

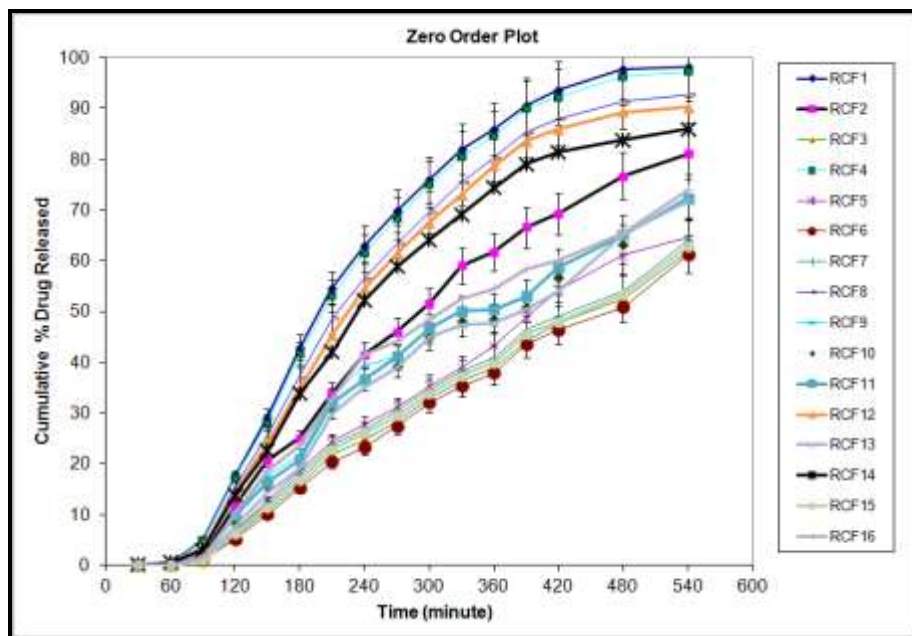


Figure 5: In-vitro zero orders drug release profiles various formulations of RCF1 to RCF16.

Data based on the Higuchi model [] usually provided a clue to the diffusion mechanism of drug release from matrix systems of the microspheres developed in this work. R^2 values of RCF2 and RCF14 on the Higuchi model was 0.993 & 0.971 respectively. As these values were close to 1.0, the drug release mechanism of the developed microspheres can be said to be Higuchian and, therefore, matrix diffusion-controlled.

The results obtained in the stability test showed that the content and release rate of celecoxib from best selected microspheres stored at 4°C (refrigerator), room temperature and 40°C with 75±5 % RH was unchanged during 3 months. The results revealed that the selected formulations (RCF2 & RCF14) were stable, probably due to the fact that the stable excipients such as ethyl cellulose,

eudragit RS 100 and aerosil were employed in the preparation of microspheres.

Optimization of Formulations

Effect of process variables on microsphere properties

Mathematical relationships [16-18] were generated between the factors and responses for determining the levels of factors, which yield optimum responses. A first and second order polynomial regression equations (tab. 4) represents the quantitative effect of factors (X_1 , X_2 and X_3) upon each of the responses; Y_1 - Y_5 . Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. A positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors.

Table 4: Regression equations of fitted models of various variables

Regression Equations	Fitted Model
$Y_1=10.36-4.78X_1+1.35X_2+0.245X_3$	Linear
$Y_2=46.97-18.46X_1+3.18X_2+0.15X_3-1.69X_1X_2+0.43X_1X_3+0.63X_2X_3+4.52X_1^2+1.04X_2^2-0.40X_3^2$	Quadratic
$Y_3=73.38-14.95X_1+3.42X_2+0.10X_3-1.71X_1X_2+0.55X_1X_3+0.65X_2X_3+3.59X_1^2+2.55X_2^2-1.50X_3^2$	Quadratic
$Y_4=77.73-9.73X_1-3.06X_2-1.06X_3+0.68X_1X_2+0.09X_1X_3+0.13X_2X_3+5.18X_1^2-0.16X_2^2+0.43X_3^2$	Quadratic
$Y_5=255.13-175.11X_1-73.65X_2-37.33X_3$	Linear

The amount of celecoxib and ethyl-cellulose at 2:1 ratio was kept constant and the eudragit RS100 was varied. In all five models Y_1 - Y_5 , X_1 had a negative impact (tab. 4) on the all responses. It is considered that the higher drug to polymer ratio in the microspheres, result in increase in polymers thickness surrounding the drug particles thereby increasing the distance travelled by the drug through the dispersed matrix channel of polymers. Increase in the content of eudragit RS 100 would increase polymer matrix density and thus result in increased diffusional path length, leading to a decrease in drug release from the microsphere and the same results reported by some researchers [13, 19]. The reduction in microspheres size with the increase in the amount of Eudragit RS 100 might be due to increase in viscosity of the internal phase, which converted to the formations of tightly bonded smaller particles. As shown in figures 6,7 and 9,10; three-dimensional response surface and two dimensional contour plots also indicated the negative effect of amount of polymer (X_1) on rate of drug released at 5 and 9 hrs in phosphate buffers pH 6.8 (Y_2) and pH7.4 (Y_3) respectively. In all three models Y_1 - Y_3 , X_2 had a positive impact (tab. 4), indicating that as the concentration of surfactants

increased the rate of drug release also increase, but negative impact (tab. 4) of X_2 on Y_4 & Y_5 implied drug entrapment and particle sizes reduced with increasing the value of X_2 . This might be attributed to the fact that average size of microspheres decreased as the concentration of surfactant increased thereby free drug on microspheres surface is available for dissolution. With increasing the concentration of surfactants, particles size of microspheres were decreased, may be due to the formation of smaller stable emulsion droplets and the lower entrapment efficiency may be due to higher amount of drug present into the external phase & greater solubility of drug into the water may loss the drug to entrap into the internal phase and the same results reported by earlier researchers [19]. As shown in figures 6,8 and 9,11; three-dimensional response surface and two dimensional contour plots also depicted the positive effect of surfactant concentration (X_2) on rate of drug released at 5 and 9 hrs in phosphate buffers pH 6.8 (Y_2) and pH7.4 (Y_3) respectively. The mean particle size and percentage entrapment efficiency decreased significantly ($P < 0.05$) with the increased surfactant concentration.

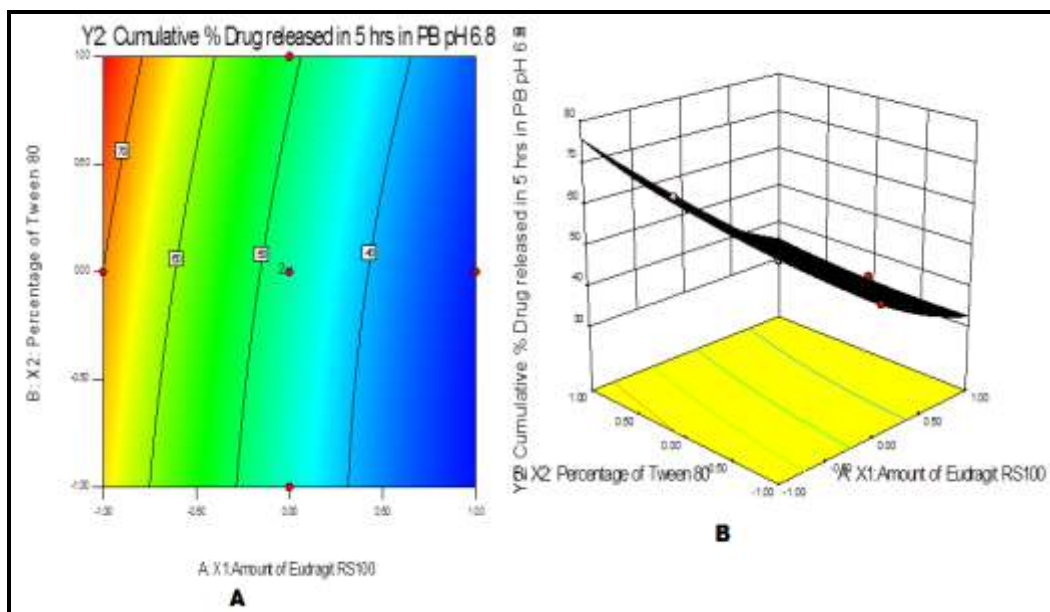


Figure 6: Contour plot (A) and 3D response surface plot (B) showing the influence of amount of Eudragit RS 100 (X_1) & concentration of Tween 80 (X_2) on the response Y_2 .

In all three models Y_1 - Y_3 , X_3 had a positive impact (tab. 4), indicating that as the stirring speed increased the rate of drug release also increase, but negative impact (tab. 4) of X_3 on Y_4 & Y_5 implied drug entrapment and particle sizes reduced with increasing the value of X_3 . Release curves (fig. 5) indicated that with increasing of stirring speed an increased in drug release significantly ($P < 0.05$). This can be attributed to the fact that the drug migration is to be high for low stirrer speed and more amounts of drug remain in the microspheres surface but when stirring speed is increased drug migration is less due to collision of emulsion droplets [10, 19]. The percentage encapsulation efficiency decreased significantly ($P < 0.05$) with increasing the agitations speed. Increase in high shear results the

lower entrapment efficiency may be due to higher amount of drug present into the external phase & greater solubility of drug into the water may loss the drug to entrap into the internal phase. The mean particle size decreased (table 3) significantly ($P < 0.05$) with increasing the stirring speed (500,750 and 1000 rpm) of stirrer. It might be due to increase in high shear results in decrease in the size of micro-droplets of the emulsion, resulting formation of smaller size of microspheres [9-10]. As shown in figures 7,8 and 10,11; three-dimensional response surface and two dimensional contour plots also depicted the positive effect of stirring speed (X_3) on rate of drug released at 5 and 9 hrs in phosphate buffers pH 6.8 (Y_2) and pH7.4 (Y_3) respectively.

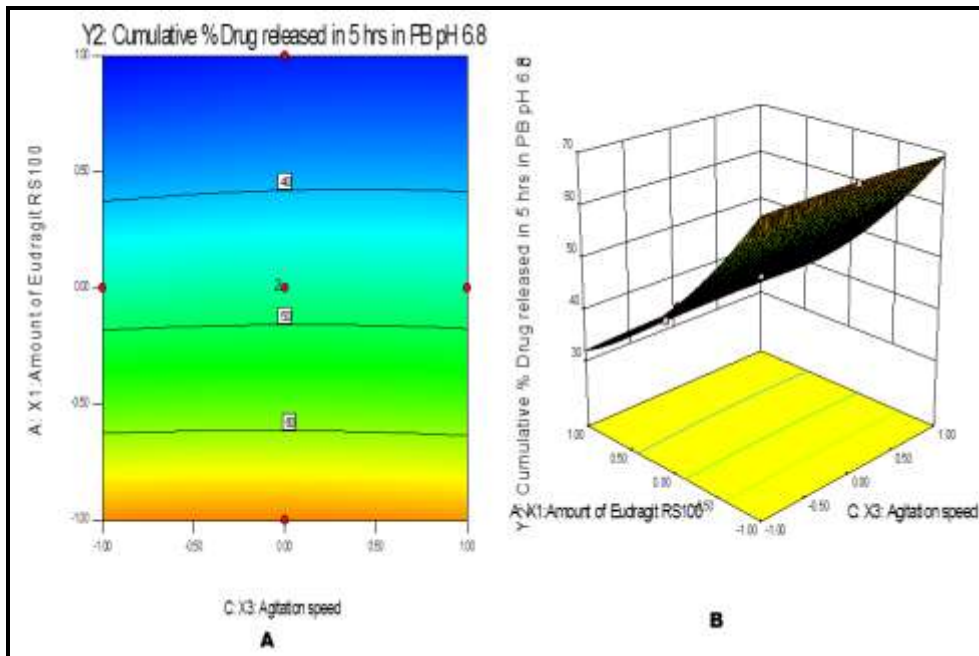


Figure 7: Contour plot (A) and 3D response surface plot (B) showing the influence of amount of Eudragit RS 100 (X_1) & agitation speed (X_3) on the response Y_2 .

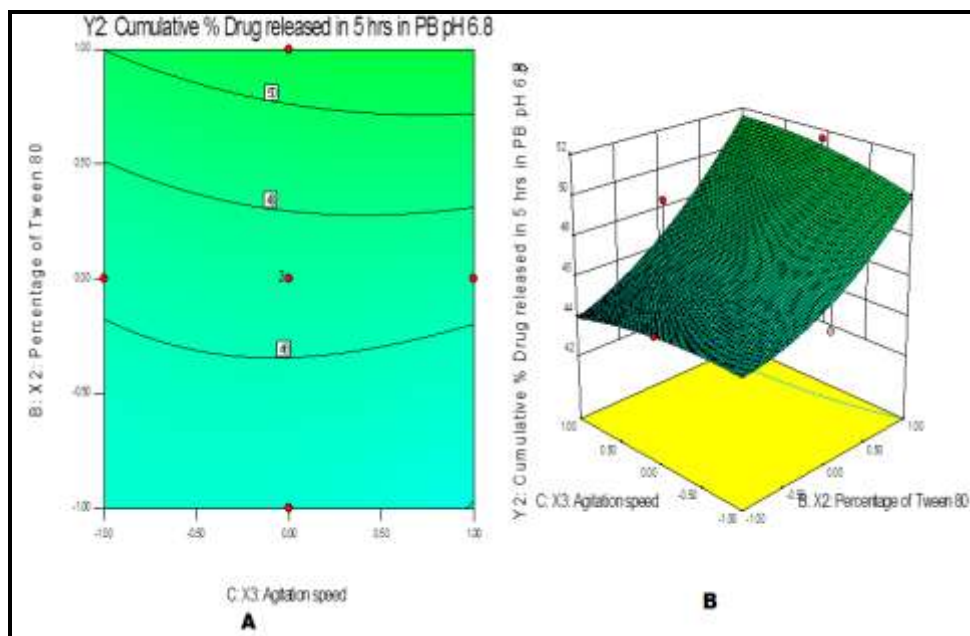


Figure 8: Contour plot (A) and 3D response surface plot (B) showing the influence of concentration of Tween 80 (X_2) & agitation speed (X_3) on the response Y_2 .

Figures 6-11 shows the three-dimensional response surface and two dimensional contour plots, which was resulted from tabulated regression equation (response vs. factors) in tab. 4. It is quite remarkable that the effects are nearly linear and the curvatures appeared were due to the non-linear nature of factors. As shown in figures 5, 6 & 8, 9, the effect of amount of polymer (X_1) on cumulative drug release at 5 and 9 hrs is more pronounced than the concentration of surfactant (X_2) and agitation speed (X_3). In contrary, for the cumulative percent of drug release at 5 and 9 hrs in phosphate buffer solutions pH 6.8 (Y_2) and pH7.4 (Y_3), amount of eudragit RS100 polymer is more important factor than agitation speed and surfactant concentration. Figures 6,7 and 8, indicated that cumulative

% drug released at 5 hrs was in the range of 32.0 to 75.95% and at the same time figure 8,9,10 indicated that Y_3 response varied between 61.15 to 98.26 % respect to all independent variables (X_1 - X_3).

Effect of Response variables on microsphere properties

Analysis of variance (ANOVA) was applied for estimating the significance of the model, at 5% significance level. A model was considered significant if the p-value is less than 0.05 [16-18]. The results of multiple regression analysis and ANOVA are summarized in table 4 & 5. The regression equations and coefficients of fitted model, indicating good fit and it was concluded that model adequately approximated the true surface.

Table 5: Analysis of Variance (ANOVA) for Dependent Variables from Face centered central Composite Design of various celecoxib microspheres.

Depended Variables	Source	Sum of Squares	df ^a	Mean Square	F ^b Value	Significant P ^c -value
Y ₁	Model	247.17	3	82.39	84.17	< 0.0001
	Residual	11.74	12	0.98	-	-
	Total	258.92	15	-	-	-
Y ₂	Model	3625.38	9	402.82	277.18	< 0.0001
	Residual	8.72	6	1.45	-	-
	Total	3634.10	15	-	-	-
Y ₃	Model	2463.79	9	273.75	96.48	< 0.0001
	Residual	17.02	6	2.84	-	-
	Total	2480.0	15	-	-	-
Y ₄	Model	1162.02	9	129.11	132.22	< 0.0001
	Residual	5.86	6	0.98	-	-
	Total	1167.87	15	-	-	-
Y ₅	Model	374819.0	3	124939.7	16.27	< 0.0002
	Residual	92174.72	12	7681.23	-	-
	Total	466993.7	15	-	-	-

^a df-degree of freedom, ^b F-ANOVA test value, ^c p-Probability

For the cumulative % drug released in 2 hrs in 0.1 (N) HCl (Y₁), the F value of linear model was 84.17, implied that the model was good fit and significant. There is only a 0.01% chance that "Model F-Value" could occur due to noise. Values of "Prob > F" (tab. 5) less than 0.05 indicated model terms are significant. In this case, p value for X₁ and X₂ was <0.0001 and 0.001 respectively; indicating significant model terms, but p value of X₃ was 0.449; which was greater than 0.10 indicated that the model terms are not significant. The "Lack of Fit F-value" was 854.21 implied the Lack of Fit is significant relative to the pure error; which having value of 0.0013. There is a 2.67% chance that a "Lack of Fit F-value" this large could occur due to noise, since non-significant lack of fit is good in terms of the model to fit. The "Predicted R-Squared" of Y₁ was 0.92 and which was in reasonable agreement with the

"Adjusted R-Squared" of 0.943. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 25.75 indicated an adequate signal and this model can be used to navigate the design space. In case of cumulative % drug released in 5 hrs in PB pH 6.8 (Y₂), the F value of quadratic model was 277.18, implied that the model was good fit and significant. There is an 8.12% chance that a "Lack of Fit F-value" this large could occur due to noise, since non-significant lack of fit is good in terms of the model to fit. The "Predicted R-Squared" of Y₁ was 0.973 and which was in reasonable agreement with the "Adjusted R-Squared" of 0.994. "Adequate Precision" measures the signal to noise ratio. The ratio of 45.84 indicated an adequate signal and this model can be used to navigate the design space.

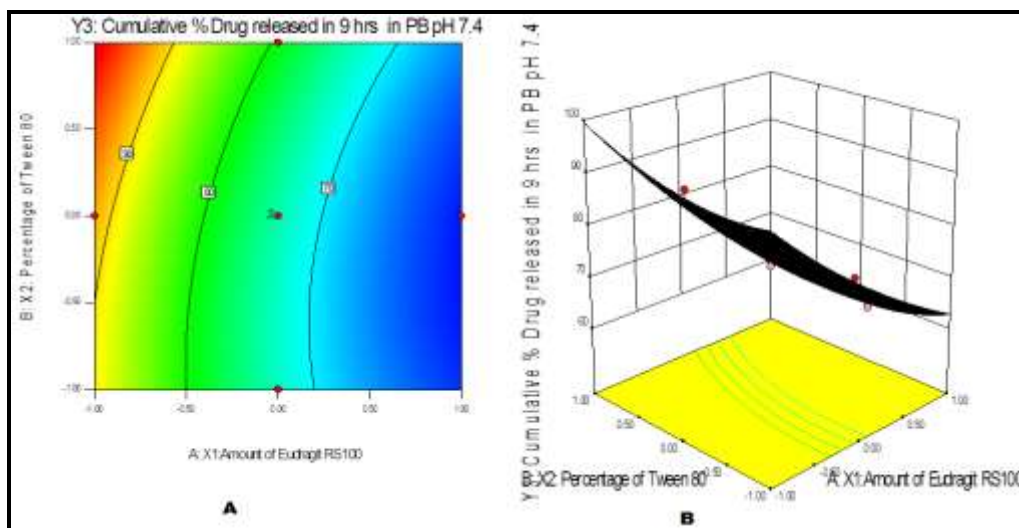


Figure 9: Contour plot (A) and 3D response surface plot (B) showing the influence of amount of Eudragit RS 100 (X₁) & concentration of Tween 80 (X₂) on the response Y₃.

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In case of cumulative % drug released at 9 hrs in PB pH 7.4 (Y_3), the F value of quadratic model was 96.40, implied that the model was good fit and significant. The "Predicted R-Squared" of Y_1 was 0.924 and which was in reasonable agreement with the "Adjusted R-Squared" of 0.983. "Adequate Precision" measures the signal to noise ratio. The ratio of 27.75 indicated an adequate signal and this model can be used to navigate the design space.

For Y_4 , the F value of quadratic model was 132.22, implied that the model was good fit and significant. There is only a 0.01% chance that "Model F-Value" could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. For quadratic model p value for X_1 , X_2 , X_3 & X_1^2 was <0.0001, <0.0001, 0.015 & 0.0001 respectively; indicating a significant model terms, but p value of X_1X_2 , X_1X_3 , X_2X_3 , X_2^2 & X_3^2 was 0.98, 0.80, 0.72, 0.79

& 0.51 respectively; indicated that the model terms are not significant and insignificant model terms may be reduced to improve the model. The "Lack of Fit F-value" was 6.53 implied the Lack of Fit is not significant relative to the pure error; which having value of 0.958. There is a 28.83% chance that a "Lack of Fit F-value" this large could occur due to noise, since non-significant lack of fit is good in terms of the model to fit. The "Predicted R-Squared" of Y_1 was 0.958 and which was in reasonable agreement with the "Adjusted R-Squared" of 0.988. "Adequate Precision" measures the signal to noise ratio. The ratio of 35.47 indicated an adequate signal and this model can be used to navigate the design space.

For Y_5 , the F value of linear model was 16.27, implied that the model was good fit and significant.

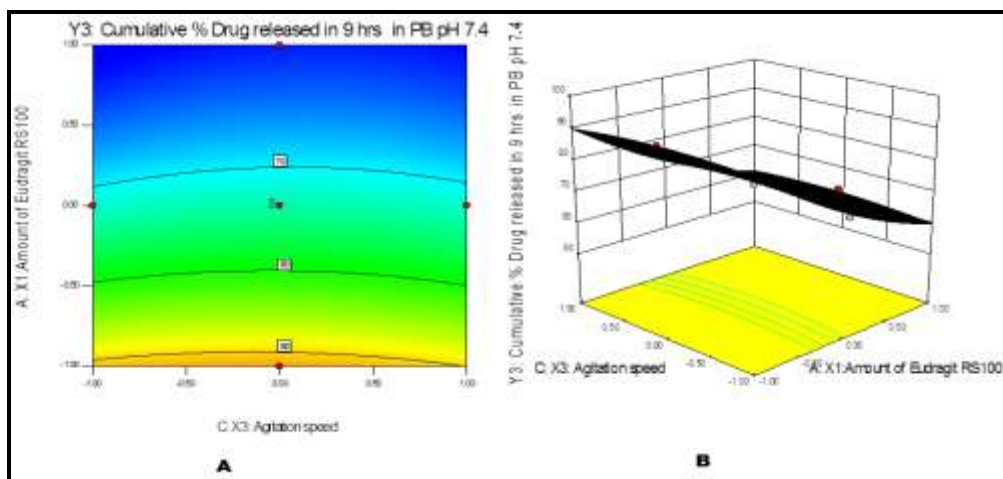


Figure 10: Contour plot (A) and 3D response surface plot (B) showing the influence of amount of Eudragit RS 100 (X_1) & agitation speed (X_3) on the response Y_3 .

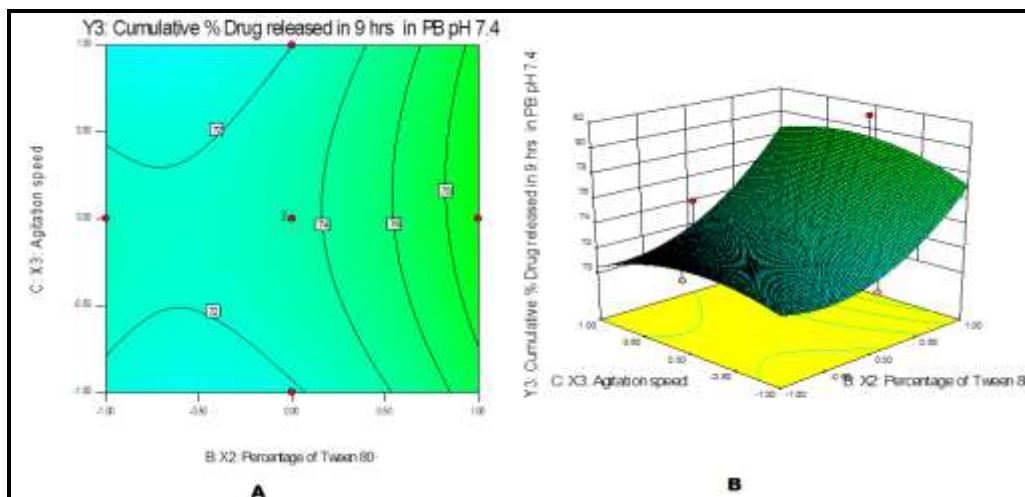


Figure 11: Contour plot (A) and 3D response surface plot (B) showing the influence of concentration of Tween 80 (X_2) & agitation speed (X_3) on the response Y_3 .

As shown in figure 12, the predicted values of Y_1 , Y_2 & Y_3 were compared with corresponding actual values and a combined graph for all three responses were plotted by using Microsoft excel 2003 edition. Furthermore a good agreement between observed and predicted values of Y_1 , Y_2 & Y_3 (fig. 12) and low % bias for all batches showed a good fit of models. Since all the observed values for dissolution were within 95 % confidence level of predicted

values. Figure 12 also shows the regression coefficients values that was 0.955, 0.998 and 0.993 for Y_1 , Y_2 & Y_3 respectively, from that it can be concluded that the optimal surface was chosen correctly and that the model has satisfactory predictive power. In the same manners for Y_4 & Y_5 responses the predicted values were compared with the actual values and the difference in between the two

values were very less and it was well accepted to fit the model significantly.

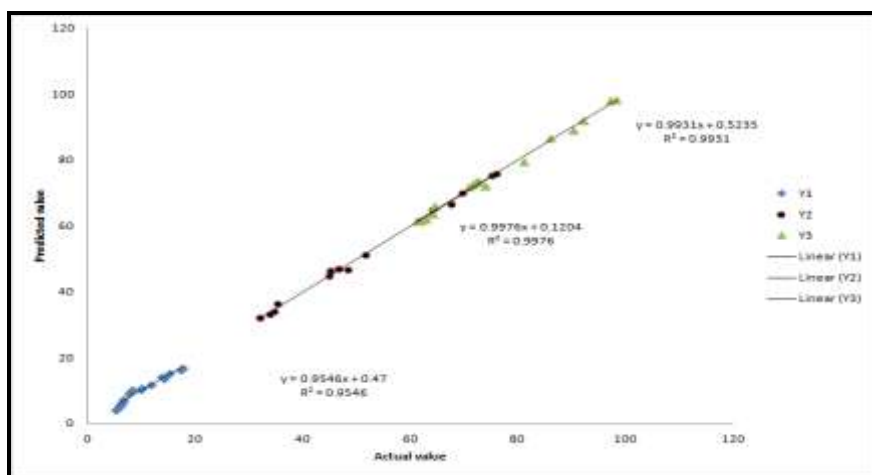


Figure 12: Observed and predicted values of Y_1 , Y_2 and Y_3 for the all sixteen sets of formulations prepared using experimental design

Table 6: Confirmation point for expected optimized formulation and predicted values of various variables with a fixed constraints set.

Independent variables (Factors)	Goal	Level			
A:X1:Amount of Eudragit RS100	is in range	-0.283			
B:X2: Percentage of Tween 80	is in range	0.368			
C:X3: Agitation speed	is in range	-0.280			
Dependent variables (Responses)	Goal	Prediction	Std Dev ^a	SE ^b (n=1)	
Y1: Cumulative % Drug released in 2 hrs in 0.1 (N) HCl	minimize	12.14	0.989	0.300	
Y2: Cumulative % Drug released in 5 hrs in PB pH 6.8	minimize	53.944	1.205	0.554	
Y3: Cumulative % Drug released in 9 hrs in PB pH 7.4	target = 80	79.525	1.684	0.774	
Y4:% Entrapment Efficiency	target = 80	79.999	0.988	0.454	
Y5:Particle Size	in range	288.017	87.642	26.585	

^a Std Dev-Standard Deviation, ^b SE-Standard Error

Numerical optimization techniques using Design Expert software for desirability approach was employed to develop new formulations with the desired responses. Upon comprehensive elucidation of the feasibility search and subsequently exhaustive grid searches, the levels of formulation variables are represented in table 6, as for one example and which may fulfill the maximum requirements of an optimum formulation. To develop an optimum formulation and deliver the drug to the specific target site (colon), a better regulation of drug release rate may fulfill the desired target. To optimize its constraints set for Y_1 and Y_2 was kept minimum value (tab. 2), because the aimed of this work was to develop a delayed release system; and as minimum as possible drug should be released in stomach and intestinal fluids within in the time period of first 5 hrs. Drug would be released maximum 80% at 9 hrs was considered to deliver the drug successfully to the specific target site (colon) and to develop a cost effective products maximum 80% entrapment efficiency was considered, but particle size was kept in the range. On the basis of optimization parameters we did not formulate further to get an optimized formulation but in this study RCF2 &

RCF14 were selected as a best multi-particulates system and which may fulfill the desired target.

Finally, the results of Response Surface Linear Model were evaluated by Design Matrix analysis techniques. Aliases were calculated based on the response selected for taking into an account of missing data points and it was needed to estimate the aliases among the factors or terms (X_1 - X_3). No aliases were found for Linear Model. Degree of freedom (df) was evaluated for model, residuals, lack of fit, pure error and correlation total and it was found 9, 6, 5, 1 & 15 respectively. To ensure a valid lack of fit test minimum of 3 lack of fit df and 4 df for pure error was recommended by RSM, because fewer df will lead to a test may not detect the lack of fit. Power at 5 % alpha level was considered to detect signal/noise ratios of Standard errors, variance inflation factor (VIF), Ri-Squared respect to all the terms [31]. Standard errors should be similar within type of coefficient and smaller is better. Ideal VIF is 1.0 and above 10 causes for alarm and which indicating coefficients are poorly estimated due to multi-collinearity [31]. Ideal Ri-squared is 0.0. High Ri-squared means terms are correlated with each other, possibly leading to poor

models. For all the three terms Standard errors, variance inflation factor (VIFs), Ri-Squared was 0.316, 1 & 0.0 respectively and all the data were within the accepted limit. If the design has multi-linear constraints multi-

collinearity will exist to a greater degree, thus increasing the VIFs and the Ri-squareds, rendering these statistics useless and then power is an inappropriate tool to evaluate response surface designs.

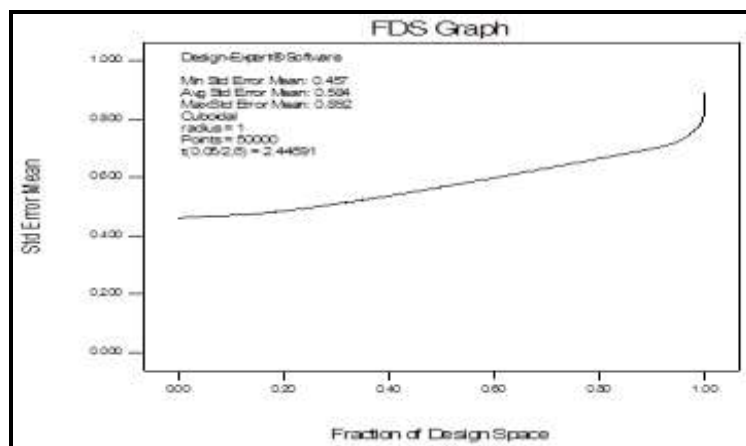


Figure 13: FDS graph for the all sixteen sets of formulations prepared using experimental design.

So, to use precision-based metrics provided in this program via fraction of design space (FDS) statistics. FDS graph (fig. 13) was also considered to further check of the linearity of model and it was found all most linear with slight cubical shape and average standard error mean was 0.584.

Site-specificity release of microspheres

Site-specificity release of microspheres was evaluated on the basis of $t_{50\%}$ & cumulative amount drug release in various dissolution mediums. The complete release profiles of celecoxib microspheres were shown in figure 6. Only 5.15 ± 0.28 to 17.60 ± 1.21 % of the drug was released at 120 min in 0.1N hydrochloric acid which indicated the significant gastric acid resistance of the microspheres. While celecoxib released in simulated intestinal fluids i.e. phosphate buffer solutions pH 6.8 was found between 32.0 ± 1.44 to 75.95 ± 2.28 % of the loaded amount, but cumulative release of drug after 9 hrs ranged from 61.15 ± 1.24 to $98.26 \pm 4.20\%$ in simulated to colonic fluids i.e. phosphate buffer solutions pH 7.4. Drug release from the formulations decreased with increasing the amount of polymer in the microspheres and it was increased with increase the concentration of surfactant and stirring speed. $t_{50\%}$ of RCF2 and RCF14 formulations was showed at 290min and 236min respectively, that indicating that microspheres formulated with EC and eudragit RS10 may be suitable to control the celecoxib release after a certain delayed period of time. Eudragit RS 100 is a copolymer of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups [15]. The ammonium groups present as salts promotes permeability and act as a channeling agent for the entrance of the liquid medium through the microsphere wall, causing it to swell. This facilitates the diffusion of the dissolved drug out of the microsphere into the dissolution medium. Thus, by varying the ratio of EC and eudragit RS 100 to the formulated microspheres, the rate of release of celecoxib can be controlled in a better way, rather than changes of concentration of surfactant and agitation speed; which did not affect greatly on drug release pattern.

Screening of formulations variables was performed on the basis of graphical as well as mathematical models. In particular, graphical analysis of the effects enabled identification of examined variables which are active on the selected responses. The mathematical model for each of the response developed using multiple regression analysis and quantitatively describes the influence of the selected variables on responses under this study. From the significance of main effects and their interactions found in this work, it was possible to predict the influence of the factors within the defined experimental domain.

CONCLUSION

Celecoxib microspheres were prepared successfully by emulsion solvent evaporation method using the combination of ethyl-cellulose and Eudragit RS 100 polymers in various ratios. It was found that the prepared microspheres were spherical, free flowing, high percentage entrapment efficiency and high percentage yielding capacity. It can be concluded from this study that a potential controlled-release drug delivery system may be achieved with a delayed action, using efficient carriers like ethyl cellulose and Eudragit RS 100 polymers and that may be successfully delivered to the colon. The in vitro controlled release of various prepared microspheres formulations have been established in this study. However, the in vitro release characteristics of drug from the microspheres are to be further subjected to in vivo pharmacokinetics, clinical & γ - Scintographic studies to prove the bioavailability and site specificity release. This present study holds promise to improve patient compliance especially for arthritic patients.

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Competing Interests

The authors state no conflict of interest and no financial support have been received from anywhere else for the conduct of the research and preparation of this manuscript.

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