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Formulation and Optimization of Flurbiprofen Loaded Eudragit® S-100 Delayed Release Colon Specific Microspheres using Box-Behnken Experimental Design

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ABSTRACT

One among many technologies to colonic delivery of non-steroidal anti-inflammatory drugs (NSAIDs) to reduce early morning complications associated with arthritis was pH dependent colon specific delayed release system. The current investigation was projected to develop and optimize pH sensitive colon specific delayed release microspheres formulation of flurbiprofen which facilitate release of the drug in colonic region and minimize the premature release in the upper gastrointestinal tract. A Box-behnken design was chosen to optimize process and formulation variables. The selected dependent and independent variables were the amount of flurbiprofen(X_1), Eudragit® S-100(X_2) and stirring speed(X_3); and Particle size (Y_{PS}), entrapment efficacy (Y_{DEE}), % flurbiprofen release at 5th h (Y_5) and 10th h (Y_{10}) respectively. Scanning electron microscopy study revealed that developed colon target pH sensitive microspheres formulation was smooth and spherical. Fourier transform infra-red spectroscopy has revealed that flurbiprofen was dispersed in polymer in its optimized formulation without any measurable interactions. Accelerated stability studies on the optimized formulation revealed that there was no significant difference in Y_{PS}, Y_{DEE}, Y_5 and Y_{10} before and after study. The flurbiprofen loaded microspheres based on pH sensitive approach can be preferred for the better management of early morning complications associated with the rheumatoid arthritis.

Keywords: Microspheres, colonic drug delivery, flurbiprofen, Eudragit® S-100, Box-behnken design.

INTRODUCTION

Colon specific drug delivery technologies (CSDDT) are being enormously improved in current years. The major impediments in drug targeting to the colon are degradation pathways and systemic absorption of the drugs in the upper GI tract. Many research studies are being done in CSDDT to meet the needs of ever-increasing gastrointestinal complications such as Crohn's disease and ulcerative colitis [1-3] amebic colitis, [4,5] to treat colon cancer, [6-9] to deliver the protein and peptide drugs [10] and for the healing of ailments such as nocturnal asthma,

[11,12] early morning arthritis, [13,14] nocturnal angina [15] and hypertension [16] influenced by circadian rhythms.

A range of strategies/systems have been developed/practiced for better colonic drug delivery. Among all other strategies, the pH sensitive drug delivery system is more frequent and acceptable. These systems are designed to reduce/avoid the degradation and absorption of the drugs in the starting portion of the gastrointestinal tract and they undertake pH dependent disintegration in the colon and delivering the therapeutic agents.
Nonsteroidal anti inflammatory drugs (NSAIDs) are extensively used in the management of pain and inflammation associated with osteoarthritis, rheumatoid arthritis. They also preferred for the management of inflammatory bowel disease and large intestine cancers. [17,18] Flurbiprofen, a nonsteroidal anti-inflammatory drug, successfully used for the management of inflammation, pain associated with rheumatoid arthritis, was selected as a model drug. It has plasma half-life 3-6 h. Its high dose frequency is due to its shorter half-life [19, 20].

The objective of this study was to formulate and optimize pH sensitive colon specific microspheres using Eudragit S-100 (insoluble at pH below 5, but soluble at pH >7) as pH sensitive polymer. A 15 run, 3-factor, 3-level, BBD was employed to identify optimum levels of selected variables for the development of pH dependent colon specific microspheres, and to evaluate pharmacokinetics in rats.

MATERIALS AND METHODS

MATERIALS

Flurbiprofen was supplied as a gift sample from FDC, Ltd, Mumbai, India. Poly vinylalcohol (PVA) was procured from HiMedia Laboratories Ltd., Mumbai, India. Eudragit® S-100 was provided by AET Laboratories Pvt. Ltd., Hyderabad, India. All other chemicals used were of analytical grade.

Box-Behnken Experimental Design

Production and analysis of the statistical experimental design were carried out with the software Design Expert® (Version 7.0.0, Stat-Ease Inc., Minneapolis, MN). The investigated primary variables were amount of flurbiprofen (X_1), Eudragit® S-100 (X_2) and paddle speed (X_3). The secondary variables were particle size (Y_{PS}), entrapment efficacy (Y_{EE}), % flurbiprofen release at 5th h (Y_5) and 10th h (Y_{10}). An optimization design layout containing of 15 trial runs was assembled [13, 21, 22]. An interactive 2nd order polynomial model was used to calculate all the secondary variables:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_1 + b_5X_2^2 + b_6X_3 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3 \ldots. (1)$$

where bi (i = 0-9) is the regression coefficient, X_1, X_2 and X_3 are the primary variables investigated and Y is the measured secondary variable connected with each primary variable level permutation. Table 1 illustrates the primary variables and their low medium and maximum levels and Table 2 illustrates an optimization design layout with the trial runs, permutation of the primary variables for each run and the measured secondary variables for the same. The optimum levels of primary variables were chosen with the constrains of minimum, maximum, target as 70 % and in the range for Y_5, Y_{EE}, Y_{10} and Y_{PS} respectively (Table 1).

Table-1: Variables in Box–Behnken experimental design

Independent variable	**Level**		
	-1	**0**	**1**
X_1: Amount of Flurbiprofen	50	100	150
X_2: Amount of eudragit S-100	100	250	400
X_3: Paddle speed	300	500	700
Dependent variables			**Constrains**
Y_{PS}: Particle size			In the range
$Y_{DEE:}$ Drug Entrapment efficacy			Maximum
Y_5: % cumulative drug release at 5^{th} hr			Minimum
Y_{10}: % cumulative drug release at 10^{th} hr			Target as 70%

Preparation of Microspheres

The flurbiprofen loaded Eudragit® S-100 microspheres were fabricated by quasi emulsion solvent evaporation method using the flurbiprofen (50, 100 and 150 mg), Eudragit S-100 (100, 250 and 400 mg) levels. For the preparation of discontinuous portion, Eudragit S-100 was dissolved in a solvent mixture of 15 ml containing equal portion of ethanol and dicloromethane. Then, flurbiprofen was dissolved in the polymeric solvent mixture. The resulting discontinuous portion was poured into 100 ml of continuous aqueous phase comprising of 1g PVA. Following 3-5h of continuous stirring (300, 500 and 700 rpm), harden polymeric particles were separated by filtration. The resulting microspheres were dried in hot air oven for 8 h at 40 o C and stored in a self-sealing poly bags until for further study [13, 23-25].

Particle Size Analysis of Microspheres

Particle size was analyzed by using a high resolution optical microscope. [13,24,25] Microspheres from each trial run were sprinkled on the surface of the dried microscopic slide separately. Then, the individual particle diameter was measured for 100 particles with pre-calibrated eye piece micrometer. The particle size analysis data were represented as mean particle diameter ± standard deviation for all trial runs [13,24,25].

Micromeritics Properties

The micromeritics of developed flurbiprofen loaded microspheres were analyzed by measuring angle of repose, bulk density, tapped density. The fixed base cone method was adopted for determination of angle repose. Bulk and tapped density was measured using USP tapped density apparatus. All the micromeritics of optimized flurbiprofen loaded delayed release microspheres formulation were calculated using the following equations:

$$\text{Tan}\theta = \frac{h}{r} \quad \text{..... (2)}$$

$$\text{Bulk density}(\sigma b) = \frac{\text{Mass}}{\text{Poured Volume}} \quad \text{..... (3)}$$

$$\text{Tapped density}(\sigma t) = \frac{\text{Mass}}{\text{Tapped Volume}} \quad \text{..... (4)}$$

$$\text{Hausner's ratio} = \frac{\sigma t}{\sigma b} \quad \text{..... ..... (5)}$$

$$\text{Carr's index}\ (\%) = \frac{\sigma t - \sigma b}{\sigma t} x100 \quad \text{..... (6)}$$

Drug Entrapment Efficacy

Flurbiprofen loaded Eudragit® S-100 microspheres (50 mg) were dissolved in 7.4 pH phosphate buffer. Then, solution was kept in orbital shaker and allowed for shaking about 24 h at room temperature. The resulting sample was subjected for centrifugation at 1000 rpm for 8 min to avoid interference of undissolved matter, supernatant was analyzed for flurbiprofen using UV-visible spectrophotometer (Systronics PC Based, 2202, Ahmedabad, India) at 276 nm. The same procedure was repeated for all formulations. Encapsulation efficacy was calculated using the following equation:

$$Encapsulation\ Efficacy = \frac{Measured\ amount\ of\ drug\ in\ microspheres}{Theoritical\ amount\ of\ drug\ in\ microspheres} X\ 100$$

Invitro Drug Release Test under GI Simulation Conditions

The release behavior of flurbiprofen from Eudragit® S-100 microspheres was studied in simulated enzyme free gastrointestinal fluid using United States Pharmacopeia apparatus-II (paddle type). The speed of the paddle, temperature, volume of dissolution study medium was 50 rpm, 37^{o} C and 900 ml respectively. Samples (5 ml) were collected at predetermined time points and analyzed for flurbiprofen using UV-visible spectrophotometer (Systronics PC Based, 2202, Ahmedabad, India) at 276 nm. The simulation of gastrointestinal passage conditions was attained by using the different release medium. The initial 2 h was carried out in enzyme free SGF (simulated gastric fluid) and next 3 h in enzyme free mixed gastrointestinal fluid (pH 4.5). The remaining time up to complete flurbiprofen release carried out in enzyme free simulated intestinal fluid (SIF; pH 7.4).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of flurbiprofen, Eudragit S-100 and flurbiprofen loaded microspheres were recorded using a FTIR spectrophotometer (Bruker Alpha-T-1020, Ettlingen, Germany). The samples were processed by preparing KBr disks at 10-ton pressure using KBr press.

Scanning Electron Microscopy (SEM)

The surface appearance of the developed flurbiprofen microspheres was investigated by SEM. The particles were sprinkled on adhesive surface and coated with gold to achieve a thickness of ~300 Å using a sputter coater. Then observed under scanning electron microscope (S-3700, Hitachi, Japan) and microphotographs were recorded.

Stability analysis

Stability analysis was carried out according to the ICH guidelines by storing the developed flurbiprofen loaded microspheres at 40^{o}C /75 ± 5% RH for six months and evaluated for particle size, encapsulation efficacy, amount of drug release at 5th and 10th h [27,28]

RESULTS AND DISCUSSION

Box–Behnken experimental design was adopted in this work to optimize the pH dependent colon specific delayed release system with constraints on the entrapment efficacy, particle size, % cumulative drug release at 5th and 10th h. The constraints applied were to maximize drug entrapment efficacy, to minimize % cumulative drug release at 5th h, and at 10th h was target as 70% and particle size was within the range.

Table-2: Observed responses in Box-Behnken design for flurbiprofen microspheres

Formula Code	Independent variables			Dependent variables (Mean ± SD)			
	X_1 (mg)	X_2 (mg)	X_3 (rpm)	Y_{PS} (µm)	Y_{DEE} (%)	Y_5 (%)	Y_{10} (%)
F1	-1	-1	0	90.25± 2.00	61.98±2.71	29.23±1.16	98.61±1.94
F2	1	-1	0	105.64± 5.25	58.17±0.15	35.58±1.33	99.39±1.26
F3	-1	1	0	120.01± 3.10	78.16±1.21	10.40±1.91	60.37±1.81
F4	1	1	0	125.24± 8.65	64.75±0.82	25.45±1.61	72.17±4.61
F5	-1	0	-1	180.65± 5.00	71.84±1.08	14.09±1.81	65.61±4.63
F6	1	0	-1	185.05± 9.54	64.45±0.24	27.47±0.36	78.94±1.85
F7	-1	0	1	36.20± 4.27	67.43±1.35	15.78±1.29	66.21±3.28
F8	1	0	1	40.10± 3.69	59.00±0.24	17.43±4.21	68.18±3.77
F9	0	-1	-1	170.30± 4.81	59.97±0.72	31.05±1.23	99.25±3.46
F10	0	1	-1	188.25±10.87	66.09±1.12	16.98±2.27	67.31±5.16
F11	0	-1	1	38.05±8.34	57.08±0.45	32.6±5.60	98.55±5.20
F12	0	1	1	43.90±4.80	65.61±0.90	18.68±2.25	70.69±2.84
***F13**	0	0	0	110.22±7.00	63.25±0.15	30.85±1.70	100.91±3.19
***F14**	0	0	0	112.21±7.30	62.24±0.31	31.47±3.17	97.79±1.65
***F15**	0	0	0	113.81±7.75	64.03±0.31	28.89±4.08	98.80±2.52

The observed responses for the 15 runs are presented in Table 2. The in-vitro drug release profile for the 15 runs is represented in Figure 1. To prepare pH dependent colon specific delayed release microspheres formulations and drug entrapment efficacy and particle size measurement was performed as previously reported [13,23-25].

Based on the experimental design produced, the independent variables permutations resulted in different responses. These responses revealed that all these formulations yielded acceptable particle size for the developed microspheres formulations and the observed range was 36.20 ± 4.27 to 188.25 ± 10.87 µm. Similarly, it can be subject from equation 3 and 4 that selected two among three independent variables, namely amount of flurbiprofen and amount of Eudragit® S-100 have an intense effect on the drug release at 5th and 10th h. The % cumulative drug release at 5th and 10 h ranged from 10.40 ± 1.91 to 35.58 ± 1.33 % and 60.37 ± 1.81 to 100.91 ± 3.19 %, respectively. Based on the experimental design, the factor combinations provided different drug entrapment efficacy. The range of the drug entrapment efficacy was 57.08 ± 0.45 to 78.16 ± 1.21 %.

To attain a formulation having increased, lower and about 70 % of drug entrapment efficacy, % cumulative drug release at 5th and 10 h, respectively, response surface method optimization was utilized to find out the levels of these factors. The summary of results of regression analysis for responses Y_{PS}, Y_{DEE}, Y_5 and Y_{10} was shown in Table 3. The polynomial equations concerning the responses were represented in the equations 7, 8, 9 and 10.

$$Y_{PS} = 110.66+3.61\ X_1+9.14\ X_2-70.75\ X_3 \ldots\ldots \quad (7)$$

$$Y_{DEE} = 62.36-4.28\ X_1+4.46\ X_2-1.74\ X_3-2.66\ X_1\ X_2+3.36\ X_1^2 \ldots\ldots\ldots\ldots \quad (8)$$

$$Y_5=30.4+4.55X_1-7.12X_2-2.93X_1X_3-5.69X_1^2+6.02X_3^2 \ldots\ldots (9)$$

$$Y_{10}= 99.17+3.48X_1-15.66X_2-15.37\ X_1^2-14.06X_3^2 \ldots \quad (10)$$

In the above polynomial equations, a +ve value shows direct relationship; while a –ve value shows an inverse relationship between the primary and secondary factors. The effect of X_1 on Y_{PS}, Y_5 and Y_{10} was positive, whereas negative on Y_{DEE}. The influence of X_2 on Y_{PS} and Y_{DEE}. was positive, whereas negative on Y_5 and Y_{10}. The effect of X_3 on Y_{PS} and Y_{DEE} was negative, whereas it was insignificant effect on Y_5 and Y_{10}. However, the influence of X_1 on Y_{PS} three folds than X_2, around ten folds less than X_3. The influence of X_1 and X_2 on Y_{DEE} was almost all equal while the influence of X_3 was 2.5-fold more than X_2 on Y_{DEE}. The influence of X_2 on Y_5 was 1.6-fold than the X_1, and it was 4.5-fold than X_1 on Y_{10}. The influence of interactive terms on the selected dependent variables also observed from the above polynomial equations. The influence of X_1X_1 on Y_{DEE} was positive, whereas negative on Y_5 and Y_{10}. The influence of X_3X_3 was positive on Y_5 and negative on Y_{10}.

2D contour and 3D response surface plots were shown in Figure 2 which are valuable to study the interaction effects of the selected independent factors on the dependent factors. In all the represented figures, the third independent factor was kept at a constant level. The relationships among all the three variables are non-linear.

The optimum formulation was chosen based on the condition of achieving the particle size within the observed range, maximum drug entrapment efficacy and minimum % cumulative drug release at 5^{th} and applying constrains on Y_{10} (70%). The composition of the selected microspheres formulation and overall desirability were represented in Table 4.

To confirm the validity design, a fresh batch with the selected formula was prepared and evaluated for Y_{PS}, Y_{DEE}, and Y_5 and Y_{10}. The data from the observed and predicted response for the selected formulation was shown in Table 4. The closeness between predicted and observed values was observed. Thus, it can be concluded that the produced polynomial equations were valid for calculating the Y_{PS}, Y_{DEE}, Y_5 and Y_{10}.

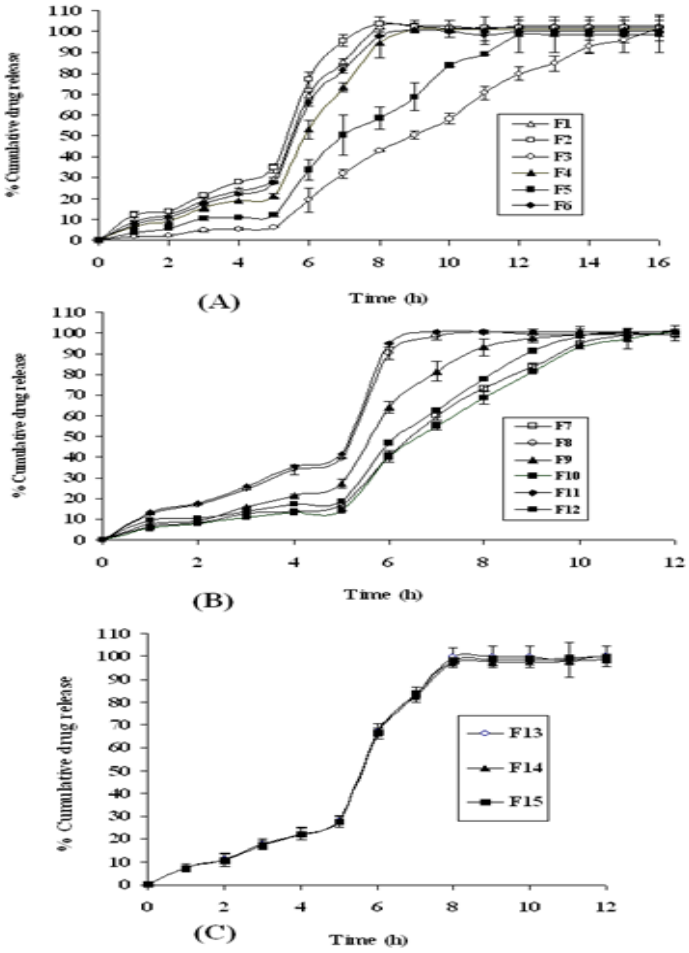

Figure-1: Dissolution profiles of flurbiprofen loaded colon specific delayed release microspheres according to the Box-Behnken design (A) F1–F6, (B) F7–F12and (C) F13–F15.

Table-3: Summary of results of regression analysis for responses Y_{PS}, Y_{DEE}, Y_5 and Y_{10}

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV	Remarks
Response (Y_{PS})						
Linear	0.9952	0.9939	0.9900	4.24	3.83	Suggested
Second order	0.9967	0.9942	0.9837	4.11	3.72	-
Quadratic	0.9969	0.9913	0.9524	5.05	4.56	-
Response (Y_{DEE})						
Linear	0.7938	0.7376	0.5791	2.79	4.36	-
Second order	0.8645	0.7629	0.3651	2.65	4.14	-
Quadratic	0.9674	0.9088	0.4996	1.65	2.57	Suggested
Response (Y_5)						
Linear	0.6420	0.5443	0.3574	5.40	22.12	-
Second order	0.7015	0.4777	-0.0673	5.78	23.69	-
Quadratic	0.9714	0.9198	0.5974	2.26	9.28	Suggested
Response (Y_{10})						
Linear	0.5652	0.4466	0.2872	12.02	14.51	-
Second order	0.5834	0.2710	-0.2998	13.79	16.65	-
Quadratic	0.9916	0.9766	0.8851	2.47	2.99	Suggested

Table-4: The observed and predicted response values for the optimized formulation

Independent variable	Optimized level	
X_1	50.00	
X_2	326.0	
X_3	470.0	
Overall desirability	0.863	
Dependent variables	Expected	Observed
Y_{PS}	122.40	120.3
Y_{DEE}	73.77	70.97
Y_5	15.08	16.25
Y_{10}	70.00	73.08

The scanning electron microscopy study revealed that the resulting microspheres were spherical as represented in the Figure 3. The study also revealed that there were no visual crystalline flurbiprofen particles on the surface of the microspheres. The micromeritics, such as angle of repose (30.46 ± 1.25º), Hausner's ratio (1.121 ± 0.005) and Caar's index (10.22 ± 2.41) reveled that microspheres from selected formulation was free flowing.

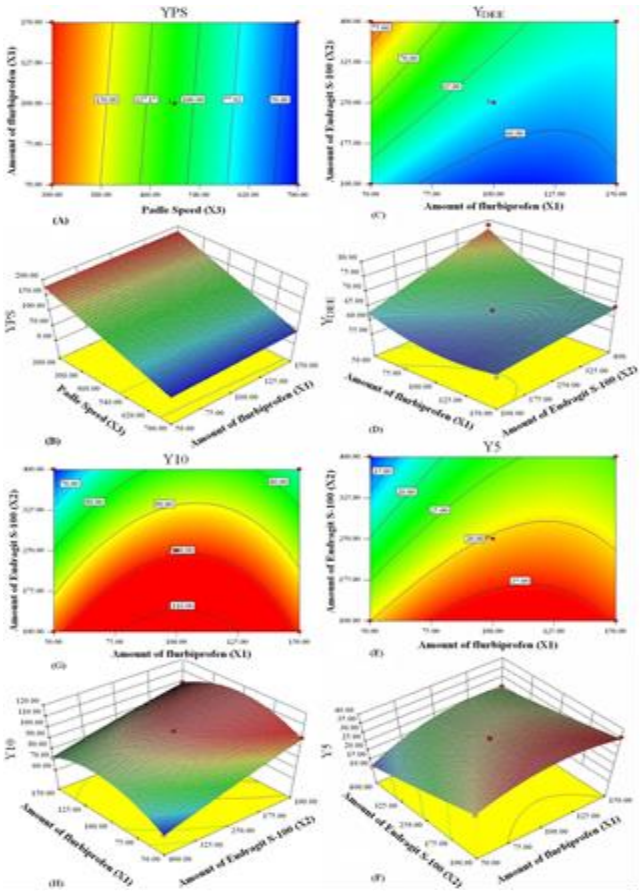

Figure-2: Contour plots (A, C, E and G), Response surface plot (B, D, F and H) showing effect of (X1), (X2) and (X3) on response YPS, YDEE, Y5 and Y10.

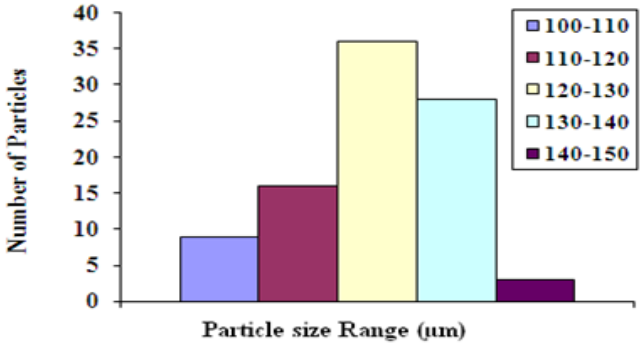

Figure-3: Particle size analysis of optimized flurbiprofen loaded colon specific delayed release microspheres formulation by optical microscopic method

Table-5: Characteristic peaks of pure drug, polymer, and optimized formulation in FTIR spectra.

S. No	Compound	Group	Type of Vibration	Peaks (cm^{-1})
1	**Pure drug**	C-F $-CH_3$ -COOH -COOH Aromatic Hydrocarbons	C-F Stretching C-H Stretching C=O Stretching O-H Stretching Ar-H Stretching C=C Stretching	1075.70;1128.55 1216.92;1324.77 2980.35 1701.59 2522.50;2627.61 3040.69 1461.60;1483.70 1514.16
2	**Polymer**	$-CH_2-CH_3$ $CH_3-C=O$	C-H Stretching C-O Stretching	2853.53;2951.97 1724.31
3	**Optimized formulation**	C-F $-CH_2-CH_3$ $CH_3-C=O$ and -COOH -COOH Aromatic Hydrocarbons	C-F Stretching C-H Stretching C=O Stretching O-H Stretching Ar-H Stretching C=C Stretching	1075.62;1151.00 1200.60;1394.08 2890.66;2980.00 1715.66;1723.06 2720.00 3030.67 1453.11;1500.12

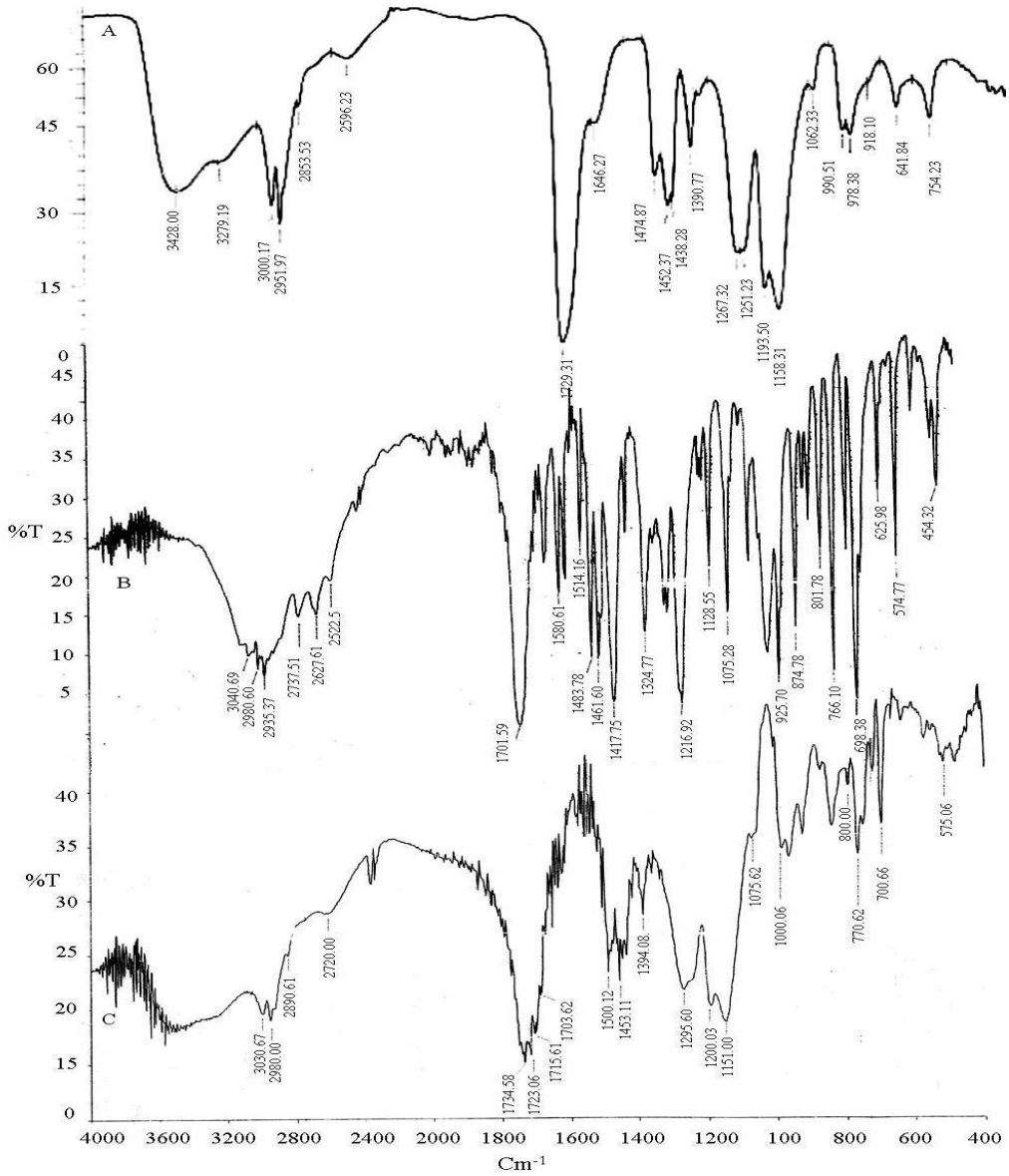

Figure-4: FTIR spectra of A) flurbiprofen, B) Eudragit® S-100 and C) Optimized formulation.

The analyzed particle size distribution data for the optimized formulation was represented in Figure 3. The FTIR of the flurbiprofen, Eudragit® S-100 and optimized formulation was done. The characteristic peaks due to the flurbiprofen in the optimized formulation, a strong absorption bands at 1715.66 cm^{-1} and 1723.06 cm^{-1} for C=O group. Four peaks were observed at 1075.62 cm^{-1}, 1200.60 cm^{-1}, 1394.08 cm^{-1} and 1151.00 cm^{-1} for the C-F group. Absorption peak was observed at 2720.00 cm^{-1} for O-H stretching in –COOH group. Absorption band was seen at 3030.67 cm^{-1} for C-H stretching in aromatic ring. Two absorption peaks were observed at 1453.11 cm^{-1} and 1500.12 cm^{-1} for C=C Stretching in aromatic ring. Two peaks were seen at 2890.66 cm^{-1} and 2980.00 cm^{-1} for C-H Stretching in $-CH_2$ and $-CH_3$ groups (Table 5 and Figure 4). It can be concluded that from FTIR studies that the drug flurbiprofen was entrapped into the microspheres and there was no chemical interaction, because there is no major shifting of the functional group peaks between the Flurbiprofen, Eudragit S-100 and optimized formulation.

Optimized flurbiprofen loaded microspheres formulation was subjected to the stability study of six months. The stability testing samples were analyzed for Y_{PS}, Y_{DEE}, Y_5 and Y_{10}. The data obtained from stability study shown in Table 7. The data revealed that there was no significant ($p>0.05$) difference between the data before and after study.

The calculated f_2 value of the dissolution release was 84.11 (>50), indicates the similarity between the release data before and after study.

Table-6: Results of stability study of optimized flurbiprofen loaded microspheres formulation

Time (h)	% Cumulative drug Release[a]	
	Initial	After storage at 40°C/75 ± 5 % RH for 180 days
0	0.00 ± 0.00	0 ±0.00
1	3.36 ± 0.47	4.21 ± 0.21
2	4.72 ± 0.16	5.14 ± 0.27
3	9.08 ± 0.56	9.25 ± 0.53
4	9.58 ± 0.31	10.14 ± 2.24
5 [b]	16.25 ± 2.55	17.46 ± 2.36
6	31.86 ± 5.42	28.55 ± 4.11
7	46.76 ± 6.57	44.73 ± 3.42
8	51.21 ± 12.37	54.33 ± 2.87
9	65.20 ± 7.71	63.22 ± 2.58
10 [b]	73.08 ± 2.31	76.88 ± 4.32
11	86.32 ± 0.23	88.32 ± 3.55
12	93.54 ± 5.90	91.32 ± 5.43
14	99.15 ± 5.53	92.24 ± 3.65
16	103.96 ± 6.28	94.22 ± 4.44
Y_{PS} [c]	120.30 ± 10.42	128 ± 8.32
Y_{DEE} [c]	70.97 ± 0.82	68.48 ± 2.43

[a] f_2= >50 (84.11); [b] Y_{Q5}: % cumulative drug release at 5th h and Y_{Q10}: at 10th h.
[c] *p>0.05*

CONCLUSION

In conclusion, the flurbiprofen loaded colon specific delayed release microspheres from Eudragit® S-100 with free flowing; non-aggregating, finely spherical formulations were prepared and optimized using 3-level, 3-factorial Box–Behnken experimental design for the better management of early morning complications associated with rheumatoid arthritis. The quantitative effect of selected primary variables at different levels on the amount of drug release at 5th and 10th h, particle size and encapsulation efficacy could be predicted by using polynomial equations. Closeness observed between the actual and predicted values of the response variables suggested the prognostic ability of the response surface method design. The quadratic response surface methodology studied for particle size, drug entrapment efficacy and the amount of drug release helped in understanding the interaction effects between the selected primary variables. FTIR studies of the optimized formulation proved that the drug and polymer compatibility of the developed microspheres formulation. Thus, the high degree of prediction obtained using response surface method is quite efficient in optimizing colon specific drug delivery systems that exhibit non-linearity in responses.

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