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RESEARCH ARTICLE

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Design and Characterization of Mucoadhesive Microspheres of Etodolac

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ABSTRACT

Mucoadhesive microspheres are drug delivery system intended for drug targeting to a specific region. Etodolac is a Non-steroidal anti-inflammatory drug. Sustain released Etodolac loaded mucoadhesive microspheres were prepared to overcome the relatively short residence time of Etodolac in the GIT tract before elimination. Solvent evaporation method was used for preparation of mucoadhesive microspheres with the help of Carbopol 974P, HPMC K100M and HPMC K4M as the polymers. Central composite design was selected for the development of the formulation. The formulations were evaluated for their particle size, surface morphology, degree of swelling, entrapment efficiency, drug content and *in-vitro* drug release study was done. Based on the results obtained from the preliminary formulations three optimized formulations were designed. The percentage mucoadhesion and swelling index of these formulations were obtained in the range of 66-70% and 82.50-83.84% respectively. Optimized formulation releases 90.94% to 92.11% of drug after 10 hours and follows zero order kinetics.

Keywords: Mucoadhesive microsphere, Etodolac, Optimization, Mucoadhesion.

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INTRODUCTION

Microencapsulation is a novel method which is helpful in delaying and modifying the release of the drug. It is a process of coating small particles or droplets inside a shell resulting in the formation of microspheres. [1] Microspheres are multiparticulate drug delivery systems which are made up of protective substances such as natural, semi-synthetic or synthetic polymers. The particle size falls in the range of 10µm-1000µm. [2] Microspheres give numerous advantages especially for sustain release, controlled release and site specific delivery. Microspheres are also helpful in reducing drug toxicity; improve efficacy, stability and better patient compliance. [3]

Mucoadhesive delivery of drugs has gained prominence in recent times as a means of drug administration. The mucoadhesive microspheres adhere more intimately with the mucous membrane. The intimate contact of the mucoadhesive polymer with the mucous surface can result in an increased drug retention time, increasing bioavailability and increasing contact time between drug and mucosa. [4]

Mucoadhesive microspheres enhance the bioavailability and improve the absorption of the drug as they connect with the mucus membrane intimately. [5]

Etodolac (ET) is NSAID (non-steroidal anti-inflammatory) prescribed for the treatment of acute pain, osteoarthritis, and rheumatoid arthritis. ET is the most selective COX-2 inhibitor; it possesses 10-fold COX-2 selectivity over COX-1. [6] Etodolac is BCS class-II drug having half-life 6.4 hours and low and pH-dependent solubility between pH 3 to 7. As many NSAIDs, ET has side effects, as gastro-toxicity, cardiovascular risk. [7-9] Formulation of ET loaded mucoadhesive microspheres may improve the safety and efficacy of the product and help to target the active substance for a better efficacy.

MATERIALS AND METHODS

Materials used

Etodolac, Carbopol 974-P, HPMC K4M and HPMC K100M were obtained from Yarrow Chem products. Dichloroethane, Light liquid paraffin, and Span 80 were obtained from Himedia. All the reagents used in the study were of analytical grade.

Methods

Compatibility studies

Fourier transforms Infrared spectroscopy (FTIR) of drug along with polymers was recorded using Shimadzu FTIR system. The samples were scanned in the range of 4000 to 400 cm^{-1}. [10]

Central composite design (CCD)

In central composite design (CCD) response surface methodology was selected for the development of the formulation. CCD has three groups of design points, two-level factorial or fractional factorial design points, axial points and central points. Effect of Carbopol 974P (A), HPMC K100M (B), and HPMC K4M (C) was selected as independent variables. Higher (+1) and Lower (-1) value of the independent variables were selected. These values were put in the optimization software and get the different formulations. Swelling index, percentage mucoadhesion and percentage *in-vitro* drug release were selected as response.

Table 1: Independent variables (Formulation Factors)

	Symbols	Levels (mg)	
		-1	+1
Carbopol974P	X1	150	225
HPMC K100M	X2	40	75
HPMC K4M	X3	100	150

Preparation of Etodolac mucoadhesive microspheres

Solvent evaporation method was used to prepare Etodolac loaded mucoadhesive microspheres. Ethanol and dichloromethane were used as solvents. Carbopol 974-P, HPMC K4M, HPMC K100M was the polymer used in the preparation. First the polymer was dissolved in the solvents followed by adding of the drug. The final solution was kept in sonicator for 20 mins. 100 ml of liquid paraffin was taken in a container along with 2% span 80 and the solution containing drug and polymers were extruded in it. The solution was stirred at 1800 rpm using a three blade propeller for 5 hours at 50°C so the solvent will completely evaporate. The solution was then filtered and microspheres were collected and washed with petroleum and dried at temperature of 50°C for 2 hours. [11]

Table 2: Composition of mucoadhesive microspheres of ET

Formulation code	Etodolac (mg)	Carbopol 974P (mg)	HPMC K100M (mg)	HPMC K4M (mg)
F1	300	187.5	40.0	125
F2	300	225.0	57.5	125
F3	300	150.0	75.0	100
F4	300	187.5	75.0	125
F5	300	187.5	57.5	125
F6	300	150.0	40.0	100
F7	300	225.0	40.0	150
F8	300	187.5	57.5	150
F9	300	187.5	57.5	100
F10	300	225.0	40.0	100
F11	300	225.0	75.0	100
F12	300	150.0	75.0	150
F13	300	225.0	75.0	150
F14	300	150.0	40.0	150
F15	300	150.0	57.5	125

Characterization of microspheres

Particle Size

Particle size analysis of the samples was done using optical microscope. Microspheres were placed on a glass slide with the help of a thin brush and then it was covered with a cover slip and placed on the stage of the microscope. Then it was observed under 10X magnification. Hundred particles were counted from each batch and average particle diameter was determined. [12]

Micromeritic Study

Angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio was determined for the prepared microspheres. Microspheres were evaluated for their flow. [13]

Swelling study

Swelling index was determined by measuring the extent of swelling of microspheres. 100 mg of microspheres were placed in 6.8 pH phosphate buffer for 24 hours and were allowed to swell. The excess drops on the surface of microspheres were removed by blotting method and the microspheres were weighed. [11]

Percentage Yield

Percentage yield of the microspheres were calculated by following formula

$$\%\ yield = \frac{\text{actual yield}}{\text{theoritical yield}} \times 100$$

The total quantity of the microspheres obtained was divided by the total quantity of the drug and excipients taken for the preparation gives the percentage yield. [5]

Drug Content and Entrapment Efficiency

50 mg equivalent of weighed microspheres were crushed in a glass mortar and the powdered

microspheres were suspended in 15 ml of methanol and kept in magnetic stirrer for 1 hour. The solution was then centrifuged and the supernatant liquid was collected. The liquid was then diluted suitably with 6.8 pH phosphate buffer and analysed for drug content. The drug content was analysed by measuring absorbance in UV spectrophotometer at 200 - 400 nm (UV spectrophotometer-1800, Shimadzu-Japan) using 6.8 pH phosphate buffer as blank. [5, 11]

Surface Morphology

Surface morphology study was done using scanning electron microscopy (JEOL JSM 6380LA, Japan). Small amount of the sample was taken and mounted scotch double adhesive tape. Sample were coated with gold to thickness 100A using Hitachi Vacuum Evaporator (HUS 5GB). Coated samples were analysed in a Scanning Electron Microscope operated at 15Kv and photographed. [12]

Percentage Mucoadhesion Test

Mucoadhesion test was done using egg shell membrane as it matches the properties of animal stomach mucosa having similar composition and thickness. The membrane was extracted from the fresh chicken eggs. The external shell was removed after removing the egg contents, and the membrane was removed. The egg membrane was cut and placed on a glass slide and it was tied. Approximately 10 mg of microspheres were spread on the wet membrane and the prepared slides were hung onto the groves of a USP tablet disintegrating test apparatus so that the glass slide will get a regular up and down movement in to a beaker having 6.8 phosphate buffer. The microspheres adhering to the surface of the membrane were counted after 1, 2, 3, 4, 5 and 6 hours. [11]

In-vitro Drug Release Study

The *in-vitro* dissolution studies were carried out using USP Type-II Dissolution apparatus for up to 10 hours. Microspheres equivalent to 50 mg of Etodolac taken and placed in dissolution apparatus containing 900ml 6.8 pH phosphate buffer using 1% SLS which was maintained at 37±0.2°C and at a speed of 50 rpm. At predetermined time intervals 5 ml of the sample was withdrawn and same volume of fresh medium was replaced into the basket. Aliquot of 5 ml was withdrawn at time intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours. The concentration of drug released was estimated by using UV spectrophotometer at 222 nm. The percent of drug released at various time intervals was calculated and plotted against time. [11]

In-vitro drug release kinetics

The dissolution profile of all the batches was evaluated for Zero order, First order and Higuchi to ascertain the kinetic modelling of the drug release. The results obtained from *in-vitro* release studies were plotted in four kinetics models of data treatment as follows:

Cumulative percentage drug release Vs. Time (zero order rate kinetics),

Log cumulative percentage drug retained Vs. Time (first order rate kinetics),

Cumulative percentage drug release vs. √T (Higuchi's classical diffusion equation),

Log of cumulative percentage drug release vs. log Time (Peppa's exponential equation). [12]

Stability studies

The stability study of the mucoadhesive microspheres was done and determined by drug content and *in-vitro* drug release study. The selected batch was packed in an aluminium foil and was kept in a petridish at accelerated temperature (40±2°C/75±5% RH) for a period of 90 days. [14]

RESULTS AND DISCUSSION

Compatibility studies

Compatibility studies were performed using FTIR spectrophotometer. The FTIR of pure drug and drug along with the polymers were done by making KBr disc. The peaks of both the mixtures were compared and correlated to find any changes. The FTIR of pure drug is characterized by N-H stretch at 3344.41 cm^{-1} , C-O stretch at 1144.22 cm^{-1}, C=C stretch at 1616.91 cm^{-1} and C-H stretch at 3055.05 cm^{-1}. All the characteristic FTIR peaks related to ET also appeared in the FTIR spectrum of mixture of ET with Polymer, so there was no chemical incompatibility between ET and polymer. Functional groups and their IR range of Etodolac and the physical mixture are showed in figure 1 and 2.

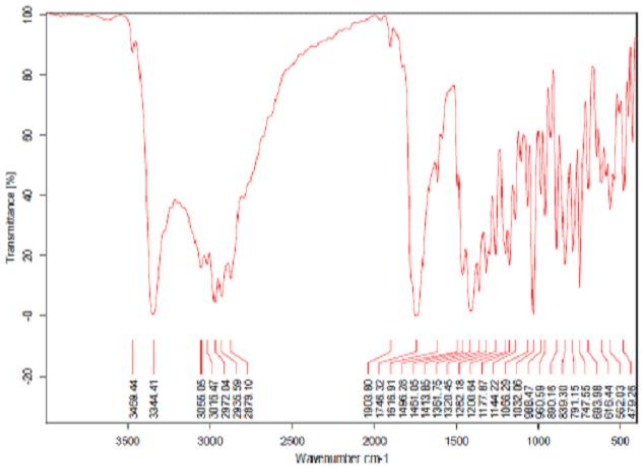

Fig. 1: FTIR spectra of Etodolac

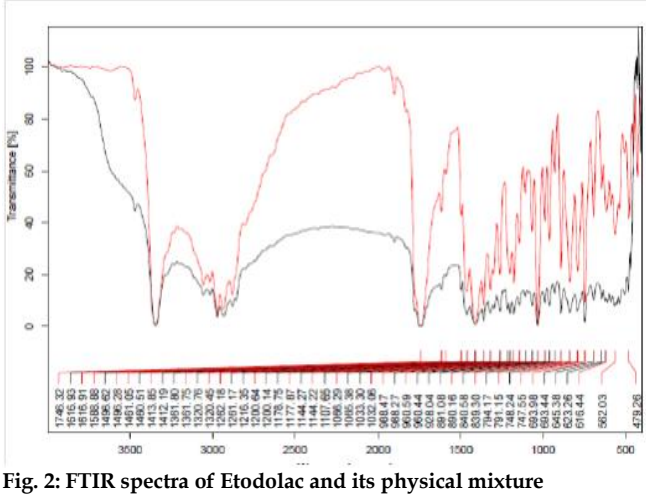

Fig. 2: FTIR spectra of Etodolac and its physical mixture

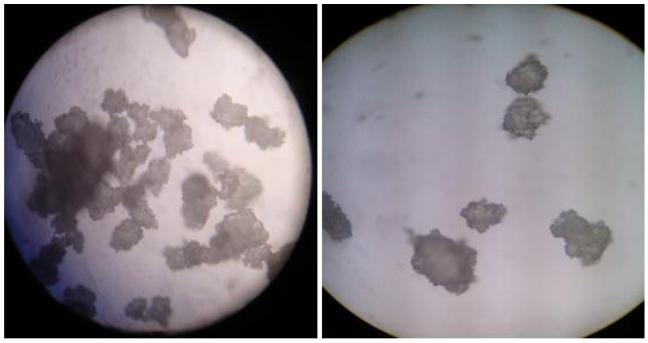

Fig. 3: Microscopic view of ET mucoadhesive microspheres

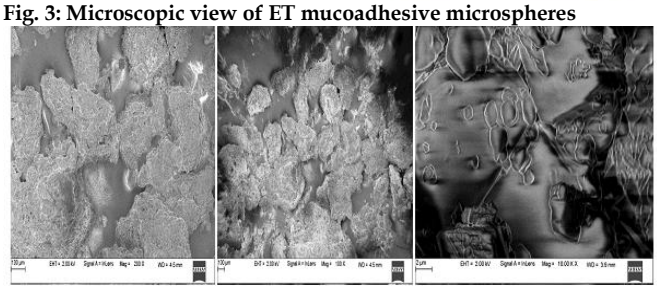

Fig. 4: SEM of ET mucoadhesive microspheres

Shape and surface morphology

Particle size

Particle size of the mucoadhesive microspheres ranges from 63.67µm to 161.85µm (Table 4). Mean particle size increases if drug and polymer ratio increases this may be due to the increase in the viscosity leading to increased particle size.

Micromeritic study

The flow property of the prepared microspheres was studied from the angle of repose and Carr's index value. The obtained data's are shown in Table 3. The angle of repose value ranges from 21°.70′±0.89 to 37°.00′±0.12 which are passable. Carr's index value was obtained in the ranged of 20°.65′±0.45 to 28°.95′±0.35. From this result it could be concluded that the mucoadhesive microspheres exhibited good flow property.

Drug content and entrapment efficiency

The drug content was found in the range of 30.50% to 43.40% and Entrapment efficiency in the range of 62.90 to 87.00%. Entrapment efficiency increases with increase in polymer concentration due to increase in viscosity and leading to larger particle size.

Shape and surface morphology

The shape and surface morphology of the prepared microspheres were observed by scanning electron microscopy. SEM photographs of microspheres were spherical, discrete with smooth surface.

Percentage degree of swelling

The swelling index study data is shown in table 4. Swelling index of the formulations was found to be in the range of 59.50% to 85.50%. Increase in the amount of Carbopol 974P in the formulation, increases the swelling index.

Percentage mucoadhesion test

Percentage of mucoadhesion was determined by *in-vitro* wash off test done with the egg cell membrane. The % Mucoadhesion of the formulations after 6 hours was found in the range of 52% to 76%. Mucoadhesivity increased with increase in polymer concentration. A result of *in-vitro* mucoadhesion is shown in table 4.

In-vitro drug release

In-vitro drug release data for the mucoadhesive microspheres of ET are represented in Figure 6. The % cumulative drug release of the formulation ranged from 71.41% to 90.00%. The increase in proportion of polymer in the formulation, sustains the release of the drug.

Design and summary of response

Response 1: Swelling index

ANOVA analysis of response 1 i.e. % swelling index showed that the linear model was found to be significant with F-value of 18.46. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F '' less than 0.0500 indicate model terms are significant. In this case A are significant model terms.

Final equation in terms of coded factors: Swelling Index = +72.70+8.60*A+1.75*B+ 1.05*C

Final equation in terms of actual factors: +18.70000+0.22933* Carbopol974P +0.10000* HPMC K100M+0.042000* HPMC K4M

Response 2: % Mucoadhesion

ANOVA analysis of response 2 i.e. % Mucoadhesion showed that the linear model was found to be significant with F-value of 18.17. There is only a 0.01 % chance that a "Model F-value" this large could occur due to noise. Values of "Prob >F" less than 0.05 indicate model terms are significant. In this case A are significant model terms.

Final equation in terms of coded factors: % Mucoadhesion= +63.60+8.20*A+0.40*B-1.40* C

Final equation in terms of actual factors: % Mucoadhesion= +28.28571+0.21867*Carbopol

Fig. 5: Photograph of the egg cell membrane containing mucoadhesive microspheres during *in-vitro* wash off test

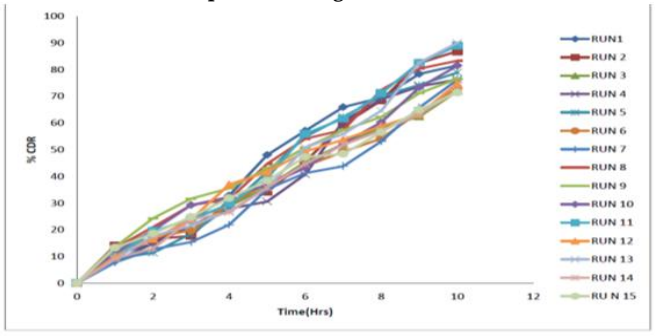

Fig. 6: *In-vitro* release profile of ET mucoadhesive microspheres

Table 3: Micromeritic properties of Etodolac loaded mucoadhesive microspheres

Formulation code	Angle of Repose (θ ± SD)*	Bulk Density (gm/ml ± SD)*	Tapped density (gm/ml ± SD)*	Carr's Index (% ± SD)*	Hausner's Ratio*
F1	37.00 ± 0 .12	0.46 ± 0.11	0.61 ± 0.14	24.31 ± 0.67	1.22 ± 0.105
F2	27.91 ± 0.89	0.51 ± 0.13	0.73 ± 0.17	28.15 ± 0.35	1.35 ± 0.050
F3	30.91 ± 0.46	0.53 ± 0.13	0.71 ± 0.17	24.50 ± 0.50	1.28 ± 0.045
F4	21.70 ± 0.10	0.61 ± 0.14	0.77± 0.18	20.65 ± 0.45	1.24 ± 0.020
F5	24.60 ± 0.22	0.47 ± 0.12	0.62 ± 0.15	23.35 ± 0.45	1.29 ± 0.015
F6	28.13 ± 0.68	0.53 ± 0.13	0.71 ± 0.17	25.50 ± 0.50	1.25 ± 0.050
F7	28.07 ± 0.73	0.57 ± 0.14	0.77 ± 0.19	24.50 ± 0.50	1.25 ± 0.150
F8	30.20 ± 0.34	0.36 ± 0.09	0.51 ± 0.13	28.15 ± 0.35	1.35 ± 0.050
F9	35.50 ± 0.50	0.41 ± 0.10	0.53 ± 0.13	21.95 ± 0.25	1.24 ± 0.040
F10	30.30 ± 0.42	0.64 ± 0.15	0.90 ± 0.23	21.50 ± 0.50	1.30 ± 0.015
F11	32.05 ± 0.55	0.69 ± 0.17	0.97 ± 0.23	26.90 ± 0.70	1.35 ± 0.030
F12	32.50 ± 5.00	0.35 ± 0.08	0.46 ± 0.11	22.10 ± 0.40	1.26 ± 0.025
F13	30.50 ± 0.40	0.62 ± 0.15	0.77 ± 0.19	22.50 ± 0.50	1.20 ± 0.030
F14	26.79 ± 0.67	0.60 ± 0.15	0.86 ± 0.21	28.95 ± 0.35	1.38 ± 0.020
F15	23.70 ± 0.51	0.43 ± 0.10	0.58 ± 0.14	25.00 ± 0.60	1.31 ± 0.030

Table 4: Results of various characterization studies

Formula	% Yield (± SD)*	Particle size (µm ± SD)*	Drug content (mg ± SD)*	Entrapment efficiency (%±SD)*	Swelling index (%±SD)*	% Mucoadhesivity
F1	81.00 ± 2.00	111.00 ± 1.00	35.30 ± 0.40	70.50 ± 0.50	68.00 ± 1.00	60
F2	74.93 ± 0.70	151.52 ± 0.52	37.10 ± 0.43	74.50 ± 0.50	78.50 ± 0.50	64
F3	70.50 ± 0.50	68.50 ± 0.50	32.50 ± 0.50	65.00 ± 1.00	61.50 ± 0.50	56
F4	70.69 ± 0.48	135.00 ± 1.00	41.50 ± 0.50	83.50 ± 0.50	70.50 ± 0.50	66
F5	80.50 ± 0.50	103.50 ± 0.50	39.87 ± 0.87	80.80 ± 0.68	72.50 ± 0.50	66
F6	88.00 ± 1.00	96.96 ± 0.96	30.50 ± 0.50	62.90 ± 0.90	66.00 ± 1.00	58
F7	73.50 ± 0.50	98.91 ± 0.91	42.50 ± 0.50	85.50 ± 0.50	79.50 ± 0.50	72
F8	71.67 ± 0.47	161.85 ± 0.85	41.25 ± 0.25	82.50 ± 0.50	76.00 ± 0.40	60
F9	85.82 ± 0.26	144.80 ± 0.80	35.68 ± 0.68	71.50 ± 0.50	73.50 ± 0.50	68
F10	75.50 ± 0.50	133.10 ± 1.10	41.02 ± 0.02	81.50 ± 0.50	81.50 ± 0.50	74
F11	92.03 ± 1.03	128.50 ± 0.50	39.48 ± 0.48	78.96 ± 0.96	83.00 ± 1.00	72
F12	83.00 ± 1.00	136.64 ± 0.64	31.80 ± 0.08	64.50 ± 0.50	73.50 ± 0.50	52
F13	71.50 ± 0.50	117.65 ± 0.65	43.50 ± 0.50	87.00 ± 1.00	85.50 ± 0.50	76
F14	81.75 ± 0.75	141.50 ± 0.50	32.55 ± 0.55	65.65 ± 0.65	61.50 ± 0.50	54
F15	89.00 ± 1.00	63.67 ± 0.67	35.61 ± 0.61	71.72 ± 0.72	59.50 ± 0.50	56

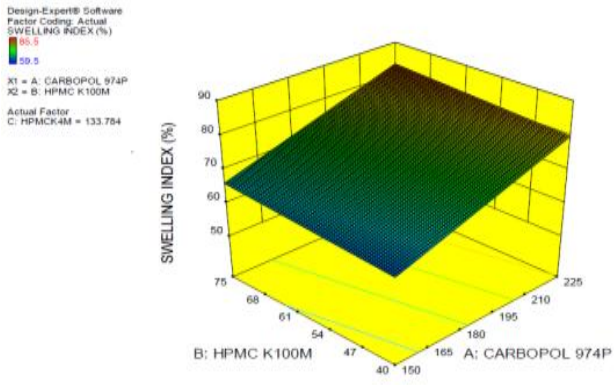

Fig. 7: 3D surface plot of swelling index (%) against amount of Carbopol 974P and HPMCK100M

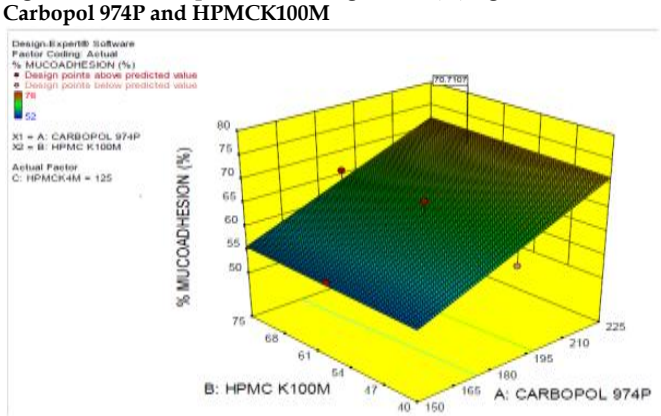

Fig. 8: 3D surface plot of % Mucoadhesion against amount of Carbopol 974P and HPMCK100M

Optimized Formulations

Using the polynomial equations, the optimized formulations were obtained for the response parameters. In the trail runs the optimized formulations were arrived using numerical optimization in design expert 10.0.3 Version. The data for the formulation variables, the response parameters and the constraints placed on them are as follows.

Preparation of the optimized formulation

The optimized formulations were prepared by the method previously mentioned in methodology.

Evaluation of optimized formulations

The data of the optimized formulations are shown in Table 1. The Percentage yield and particle size of an optimized formulation was found in the range of 78.28% to 85.88% and 104.50% to 133.10%, respectively. The drug content was found to be in the range of 38.52% to 41.02%. The Entrapment efficiency was found in the range of 77.10% to 81.98%. The entrapment efficiency was found to increase with increase in polymer concentration due to increase in viscosity of the preparation. Swelling index of the formulations was found to be in the range of 82.50% to 83.84%. Increase in the amount of Carbopol 974P in the formulation, increases the swelling index.

Micromeritic study of optimized formulations

The flow property of the optimized formula was studied from the angle of repose and Carr's index

value. The obtained data's are shown in table 2. The angle of repose value ranges from 290.09'+0.64 to 300.89'+ 0.33 which are good. Carr's index value ranged from 22°.02' ± 0.2 to 25°.4' ± 0.3. From this result it could be concluded that the optimized formula exhibited good flow property.

Table 5: constraints for optimization

Name	Goal	Lower Limit	Upper Limit
A: Carbopol974P	is in range	150	225
B: HPMCK100M	is in range	40	100
C: HPMCK4M	minimize	100	200
Swelling Index (%)	is in range	60	85
% Mucoadhesion	minimize	60	85
% *In-vitro* release	maximize	80	95

Table 6: Optimized formulae obtained and their desirability

Number	Carbopol K100M	HPMC K100M	HPMC K4M	% Swelling Index	% Muco-adhesion	% *In-vitro* drug release	Desirability
OF1	216.550	100.00	158.046	85.00	69.073	93.719	0.625
OF2	216.403	100.00	158.838	85.00	68.997	93.912	0.625
OF3	216.466	100.00	158.501	85.00	69.029	93.830	0.625

Table 7: Evaluation of optimized formulations

No.	Percentage Yield (% ± SD)*	Particle size (µm ± SD)*	Drug content (mg ± SD)*	Entrapment Efficiency (% ± SD)	% Degree of Swelling*
OF1	81.97 ± 0.19	122.42 ± 0.49	40.76 ± 0.44	80.10 ± 0.54	83.84 ± 0.39
OF2	78.28 ± 0.61	133.10 ± 1.10	41.02 ± 0.35	81.98 ± 0.75	83.06 ± 0.72
OF3	85.88 ± 0.43	104.50 ± 0.50	38.52 ± 0.41	77.10 ± 0.76	82.50 ± 0.50

*All Values are expressed as mean ± SD, n=3

Table 8: Results of micromeritic study of optimized formulations

No.	Angle of Repose (θ ± SD)*	Bulk Density (gm/ml ± SD)*	Tapped density (gm/ml ± SD)*	Carr's Index (% ± SD)*	Hausner Ratio* (± SD)*
OF1	30.89 ± 0.33	0.30 ± 0.005	0.40 ± 0.005	24.1 ± 0.2	1.26 ± 0.005
OF2	29.78 ± 0.33	0.34 ± 0.005	0.44 ± 0.010	22.0 ± 0.2	1.26 ± 0.02
OF3	29.09 ± 0.64	0.25 ± 0.010	0.34 ± 0.005	25.4 ± 0.3	1.28 ± 0.06

*All Values are expressed as mean ± SD, n=3

Table 9: Results of % mucoadhesivity of optimized formulations

Formulation code	% Mucoadhesivity					
	1 h	2 h	3 h	4 h	5 h	6 h
OF1	89	82	78	72	64	66
OF2	87	84	82	79	74	70
OF3	90	85	81	77	72	68

Percentage mucoadhesivity of optimized formulations

Percentage of mucoadhesion was determined by *in-vitro* wash off test done with the egg cell membrane. Table 4 shows the *in-vitro* mucoadhesion data. The % Mucoadhesion of the Optimized formulations after 6 hours was found to be in the range of 66% to 70%. Mucoadhesivity increases with increase in polymer concentration.

In-vitro drug release study of optimized formulations

The results obtained in the *in-vitro* drug release studies for the optimized formulations are shown in figure 6. Optimized formulations OF1, OF2 and OF3 show 92.11%, 91.54% and 90.94% release of drug at the end of 10 hours respectively. Values obtained are near to the predicted values.

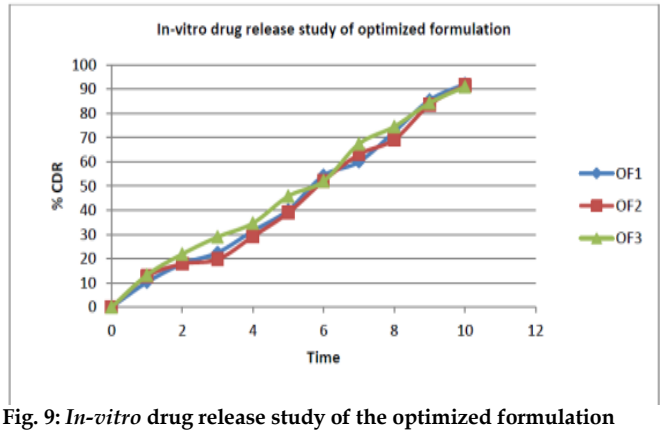

Fig. 9: *In-vitro* drug release study of the optimized formulation

Table 10: Comparison of predicted and actual experimental values

Optimized formulation		OF1	OF2	OF3
% Swelling Index	Predicted	85.00	85.00	85.00
	Actual	81.97	78.28	84.45
	% Error	1.05	-1.4	1.48
% Mucoadhesion	Predicted	66	70	68
	Actual	69.07	68.99	69.02
	% Error	1.05	-1.4	1.48
% *In-vitro* drug release	Predicted	93.71	93.91	93.83
	Actual	92.11	91.54	90.94
	% Error	1.05	-1.4	1.48

Validation of the RSM results

Three check point formulations were selected, for which the results of all the dependent variables were found to be within the limits. Table 10 lists the obtained and predicted values of the check point formulations along with the % prediction error. Linearity correlation chart between the observed experimental values and the predicted values clearly showed there is no much deviation between predicted and experimental values; all are within the range given in the suggested model.

In-vitro drug release kinetics

In order to find out the exact mechanism of drug release from mucoadhesive microspheres of ET, drug release data were fit into various mathematical models, zero order, first order, Higuchi matrix and Peppas. The *in-vitro* release profile of drug from formulations OF1, OF2 and OF3 could be expressed by zero order equation, as the plots shows high linearity (r^2 = 0.9849-0.9946) in comparison to first order (r^2 = 0.8210-0.8941) and Higuchi's release (r^2 = 0.6278-0.8226). So, it was understood that zero order release pattern was followed by all formulations. All mucoadhesive microsphere formulations followed Supercase-II release mechanism as their 'n' values are higher than 0.89 i.e. 0.9244-1.080.

Stability Studies of the optimized formulations

Stability studies were done for 3 months at 40ºC/75% RH. The optimized formulations OF1, OF2 and OF3 were selected for stability studies in order to study the effect temperature and humidity on prepared formulations. The mucoadhesive microsphere were analysed for drug content and *in-vitro* release studies. Formulation OF1 showed drug content of 40.76, 39.78 and 38.21 mg in first, second and third month respectively. Drug release studies conducted on OF1 showed that there was no significant change as it released 92.11%, 91.92% and 89.12% at the end of 10 hours in first month, second and third month respectively. Formulation OF2 showed drug content of 41.02, 40.97 and 39.92 mg in first, second and third month respectively. Drug release studies conducted on OF2 showed that there was no significant change as it released 91.54%, 90.04% and 89.01% at the end of 10 hours in first month, second and third month respectively. Whereas formulation OF3 showed drug content of 38.52, 37.21 and 36.78 mg in first month , second and third month respectively. Drug release studies conducted on OF3 showed that there was no significant change as it released 90.94%, 89.51% and 88.78 % at the end of 10 hours in first month, second and third month respectively. No significant changes in drug content and *in-vitro* release profile were observed in ET mucoadhesive microsphere during study period, thus it can be concluded that prepared formulations were physiochemically stable.

The mucoadhesive microsphere of Etodolac was prepared by non-aqueous solvent evaporation method using Carbopol 974P, HPMCK4M and HPMCK100M as the polymers. Central composite design was selected for the development of the formulation. Micromeritic studies like angle of repose, Carr's index, Hausner's ratio revealed that the prepared microspheres exhibited passable flow property. Microspheres obtained were spherical in shape and entrapment efficacy increases with increase in concentration of polymers. The microspheres of the optimized batches exhibited mucoadhesion in the range of 68.99% to 69.073% after 6 hours, and swelling index of 85.0%. The optimized formulation OF1, OF2 and OF3 could sustain the release of the drug for more than 10 hours and followed zero order release kinetics.

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