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Formulation and Optimization of Ketoprofen Microspheres using Response Surface Methodology

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Abstract: In the present study, a central composite design based on response surface method was employed to prepare experimental trials using different ratio of drug and polymer at varying r/min. to optimize the microsphere formulations. Formulations were prepared by emulsion- solvent evaporation method using a mixture of dichloromethane: chloroform (2:3) as solvent system for the drug and polymer. The solution was added to 0.5%w/v sodium carboxy methyl cellulose solution and stirred at varying r/min. until all the organic solvent was evaporated completely resulting in the formation of microspheres. The prepared ketoprofen microspheres were discrete and free flowing and indicated that the concentration of polymer, stirring rate significantly influenced the formation of microspheres and ketoprofen entrapment while concentration of the polymer have a significant positive impact on ketoprofen release over a period of 12 hours and the stirring rate have minimal effect on the drug release. The results demonstrated a good relationship between the predicted and experimental values, confirming the validity of the model. Drug release mechanism indicated a best fit model of zero order release. The optimized final formulation KEC1 showed better analgesic and anti-inflammatory activity as compared with standard drug ketoprofen. The optimized formulation was found to be stable when subjected to accelerated and long term stability studies as per ICH guidelines. The results obtained indicated that response surface methodology can be successfully used to analyze the effect of formulation variables and develop an optimized formulation thereby reducing the number of trials, time and cost of formulation development.

Key words: Microspheres, Ketoprofen, Response surface methodology.

INTRODUCTION:

Microencapsulation is one of the novel methods for retarding drug release from dosage forms and minimizing the adverse effects thereby increasing the patient compliance. Emulsion-solvent evaporation is one such microencapsulation method that can be used to coat a water insoluble drug with a water insoluble polymer for sustaining the drug release[1,2]. Ketoprofen is a nonsteroidal anti-inflammatory drug with well established analgesic and antiarthritic effect[3] by inhibiting the prostaglandin and leukotriene synthesis. It is widely used for the treatment of rheumatic disorders[4]. But it requires frequent administration to maintain the plasma drug concentration as the half life of the drug is less and when administered orally Ketoprofen shows adverse effects on gastric mucosa[5]. Oral sustained release formulations need to improve the therapeutic plasma drug concentration level by at least for a period of 12 hours there by decreasing the frequency of dosing[6]. The response surface method has been applied to dosage form design of various drugs[7, 8]. In this study a central composite design based on response surface method was applied to optimize the microsphere formulations. The study analyzes the effect of formulation variables on the properties such as drug encapsulation and drug release in the

preparation of ketoprofen loaded ethyl cellulose microspheres.

MATERIALS AND METHODS:

Ketoprofen drug was purchased from BEC Chemicals, Mumbai, India. Ethyl cellulose and Sodium carboxy methyl cellulose was purchased from BDH Chemicals, Mumbai, India. Chloroform, Methanol and Dichloromethane were purchased from Merck Ltd. All other chemical used were of AR grade.

Experimental design of Ketoprofen loaded Ethyl cellulose microspheres:

Experiments were carried out systematically to analyze the effect of factors such as stirring rate revolutions per minute(r/min.) and drug-polymer ratio using a response surface methodology and to develop an optimized formulation. A central composite design was employed to prepare experimental trials using different ratio of drug and polymer at varying r/min. The formulation and processing conditions were shown in table -1.

The two independent formulation variables evaluated were

X_1: Ratio of drug: polymer and X_2: Rate of stirring.

The dependent variables investigated were Y_1: Drug encapsulation and Y_2: *In vitro* drug release. Formulations were prepared according to the central composite design by emulsion- solvent evaporation method[9]. Ketoprofen and ethyl cellulose were dissolved in a mixture of dichloromethane: chloroform (2:3). The resulting solution was added as a thin stream in to 200ml of 0.5%w/v sodium carboxy methyl cellulose solution and stirred at varying r/min. Stirring was continued until all the organic solvent was evaporated completely resulting in microspheres. The product was collected by vacuum filtration and washed thrice with water and air dried to obtain free flowing microspheres. Total of 9 batches of microspheres were prepared by maintaining other parameters such as volume of dispersion medium and volume of polymer solution constant.

Evaluation of Microspheres

Surface morphology: The microspheres were coated with gold vacuum at high voltage (800-1500V) using ion coater. Samples were examined with scanning electron microscope (LEICA S 440 i).

Micromeritic properties: The average particle size of the microspheres was determined by using optical microscope. The flow properties and packing properties were investigated by measuring the angle of repose, tapped density and bulk density.

Drug entrapment: Accurately weighed microspheres equivalent to 100mg of drug was dissolved in 25ml of 75% methanol and sonicated for 3 min. The solution was then filtered, diluted suitably and analyzed for drug content spectrophotometrically at 260.5nm. The percentage drug entrapment was calculated as:

% Drug Entrapment = Practical drug loading/ Theoretical drug loading X 100

Dissolution studies: Dissolution test was performed in USP XXIII dissolution test apparatus by paddle method. The dissolution media used was 900ml of phosphate buffer pH 7.4 maintained at 37±0.5^{o}C and rotated at 100 r/min. Aliquots samples were withdrawn at specified time intervals and replaced with same volume of fresh media, filtered and analyzed spectrophotometrically (Shimadzu 1600) at 260nm for cumulative drug release.

Statistical analysis: The effect of formulation variables on the response variables were statically evaluated by applying one-way ANOVA using Design-Expert® 6.05 (Stat Ease, USA).

Acute Toxicity studies: The animal experiments were carried out with prior permission from the Institutional Animal ethics Committee approval (IAEC NO: MSRCP/P-07/2008). The acute toxicity studies were carried out as per OECD guidelines for testing of Chemicals – No. 423. Female Albino rats of Wistar strain weighing 170-190g were taken for the study. The selected rats were housed in acrylic cages under laboratory conditions. They were fasted overnight but had access to water. The test procedure was started with a dose of 5 mg/kg administered orally and the animals were observed for 7 days. Based on the mortality rate, the dose was subsequently increased up to 300mg/kg and the animals were observed for any toxic symptoms. On the 8th day, the animals were sacrificed, blood samples were withdrawn and analyzed for haematological and biochemical parameters. The tissues were subjected to histopathological study.

Anti- inflammatory activity: The study was carried out by Carrageenan induced rat paw edema method. The animals were divided into 3 groups, each group containing 6 animals. Albino rats Wistar strain of either sex weighing 170-190g were taken for the study. To the first group normal saline was injected. To the second group 9mg/kg of standard Ketoprofen was

administered orally and to the third group the optimized final microsphere formulation was administered orally. After 30minutes, 0.05ml of 1%w/v carrageenan was injected in the sub plantar region of the left paw to both control and treated groups. The paw volumes of both control and treated groups were measured at the intervals of 15, 30, 60, 120, 180, 240, 300 and 360min. after carrageenan administration. Percentage difference in the paw volumes of each animal of control and treated groups were calculated.

Analgesic activity: The study was carried out by Writhing stimulus method. The animals were divided into 3 groups, each group containing 6 animals. Swiss albino mice of either sex were taken for the study. Animals are injected intraperitoneally with 1ml/100g of 0.6% acetic acid solution. Animals that do not exhibit writhing within 30 seconds are discarded from the test. The formulation is administered subcutaneously 15 to 20 min prior to administering acetic acid solution. Animals showing no response are analgesic positive. The percentage protection at each dose level is calculated for each group. The values were statistically analyzed by one way ANOVA using Graph Pad Instat. P value < 0.5 was considered significant.

Stability Studies: The stability protocol was designed based on the ICH 'Q1AR2' guidelines. The microspheres formulations chosen were stored at 30 ± 2^0 C and 65 ± 5% RH for a period of 12 months and at 40 ± 2^0 C and 75 ± 5% RH for a period of 6 months. The stored samples were tested for their drug content and for any physical change. The testing was carried out at 0, 2, 4 & 6 months for accelerated storage condition and at 3-month intervals for a period of 12 months for long-term storage condition[10].

Table – 1: Experimental design batches for ketoprofen micropshere formulations with variable levels and response parameters

Batch Code	Variables		Response parameters	
	Drug-polymer ratio (g) (X1)	r/min. (X2)	% Drug Entrapment (Y1)	% Drug Release in 12 hours (Y2)
F1	1: 4.62	1250.00	81.0	63.0
F2	1:2.50	1250.00	71.5	92.0
F3	1:2.50	189.34	55.0	90.0
F4	1:1.00	2000.00	53.1	94.0
F5	1:2.50	2310.66	77.0	91.0
F6	1:4.00	2000.00	82.0	64.0
F7	1:0.38	1250.00	43.0	101.0
F8	1:1.00	500.00	34.0	98.0
F9	1:4.00	500.00	62.0	60.0

Table – 2: Summary of ANOVA results for Surface linear model of drug entrapment and Quadratic model of drug release for the formulation batches

Response	Model	Sum of Squares	F Value	Prob > F
Drug Entrapment	Surface Linear	2508.75	40.55	< 0.0001
Drug Release	Quadratic	2211.51	23.67	< 0.0003

Table – 3: Comparison chart of predicted and observed values for optimized ketoprofen formulation (KEC1)

Observation	Drug-polymer ratio	r/min.	% drug entrapment*	95% drug release in 12 hrs*
Predicted Values	1:2.13	1485.17	70.00	94.99
Observed values	1:2.1 (Rounded off)	1500.00 (Rounded off)	75.00	96.55

* Average of three determinations

Table – 4: Micromeritic properties of optimized ketoprofen formulation (KEC1)

Parameters	Observed values*
Particle size	136.1±0.36 µm
Angle of repose	21.3±0.45 ^{0}
Tapped density	0.589±0.02 g/cm3
Bulk density	0.531±0.01 g/cm3
Carr's Index	16.1± 0.25%

*-Average of three determinations

Table -5a: Anti inflammatory of the optimized ketoprofen formulation (KEC 1)

Treatment groups (n=6)	Dose (mg/kg)	Paw volume in ml after 6 hr (mean±SEM)	% inhibition of paw oedema after 6 hr
Control (Normal saline)	10ml/kg	0.57±0.02	--
Standard Ketoprofen	9.0	0.14±0.02*	75.4
KEC 1	9.0	0.19±0.02*	66.6

$p < 0.01$ compared to control

Table -5b: Analgesic activity optimized ketoprofen formulation (KEC 1)

Treatment groups (n=6)	Dose (mg/kg)	No. of writhings (mean±SEM)	% inhibition of writhing
Control (Normal saline)	10ml/kg	17.1±0.30	--
Standard Ketoprofen	9.0	7.33±0.33*	58.0
KEC 1	9.0	10.1±0.36*	40.5

* $p < 0.001$ compared to control

Table -6: Regression co-efficients and rate constants for release of optimized ketoprofen formulation

Formulation code	Zero order		First order		Higuchi matrix		Peppas koresmeyer		Hixon Crowell	
	r	K1	r	K2	r	k3	r	k4	r	k5
KEC 1	0.961	7.62	0.893	2.02	0.933	5.93	0.959	1.20	0.924	4.61

Figure 1: SEM Photomicrograph of the optimized formulation KEC1

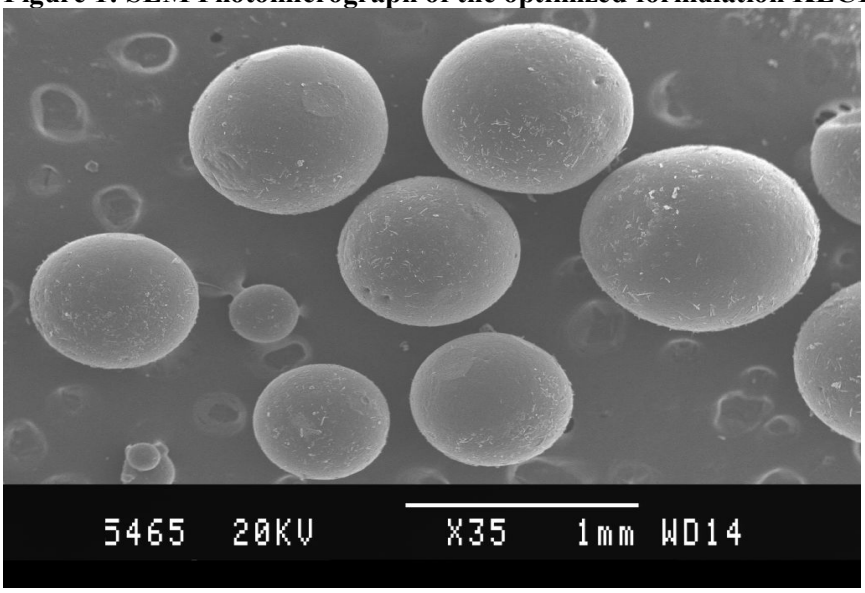


Figure 2: Response surface plot showing the effect of amount of Polymer (X_1) and r/min (X_2) on the response Drug entrapment (Y_1).

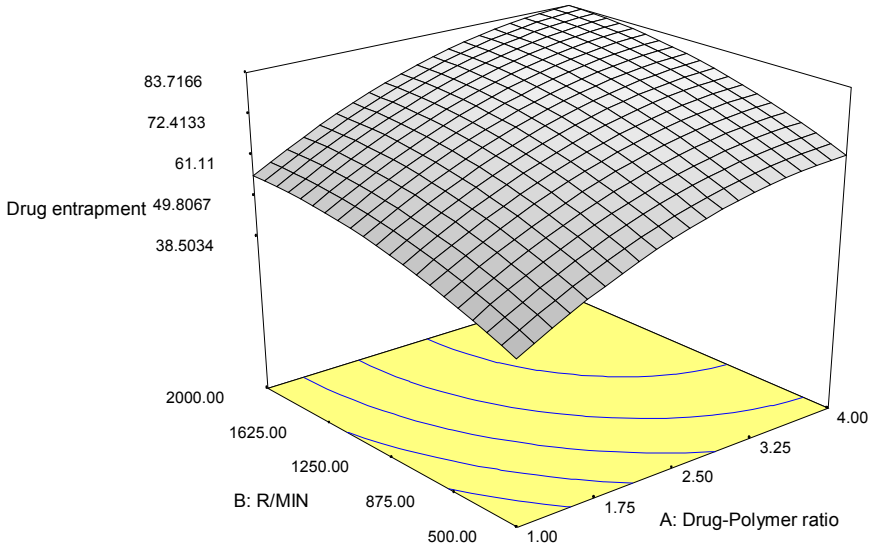


Figure 3: Response surface plot showing the effect of amount of Polymer (X_1) and r/min (X_2) on the response % Drug release in 12 hours (Y_1).

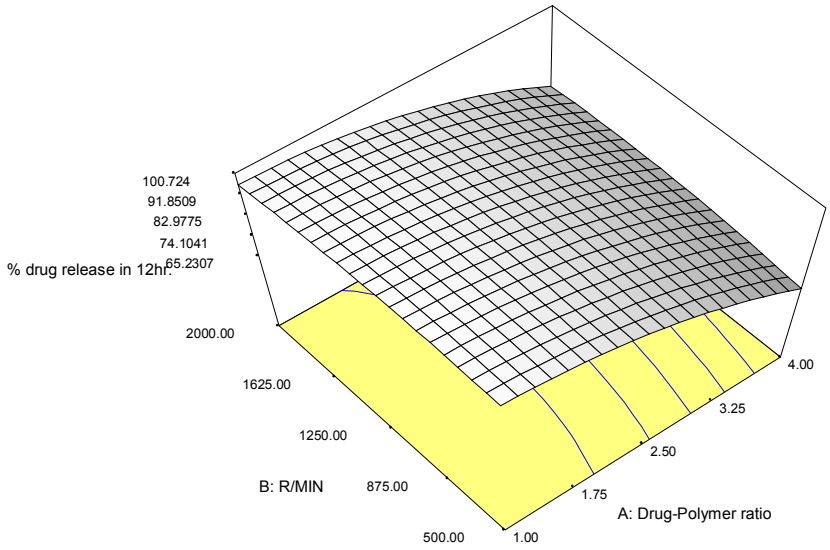
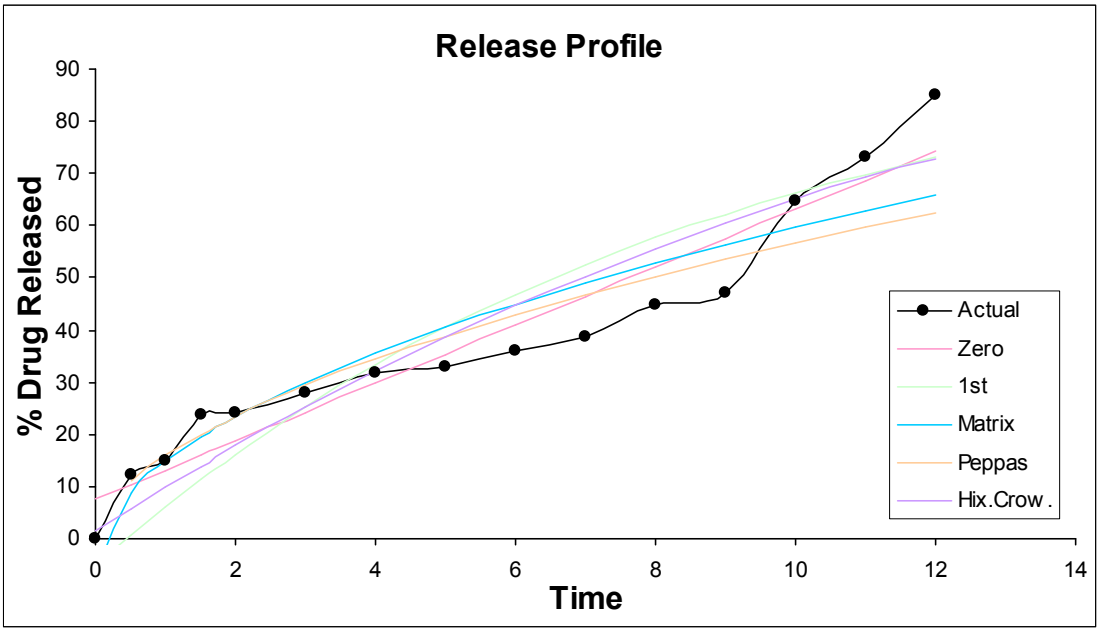


Figure 4: *In-vitro* drug release studies for the optimized formulation (KEC1) with best fit model



RESULTS AND DISCUSSION:

A numerical optimization technique based on the desirability approach was used to generate the optimum settings for the most effective formulation with maximum drug entrapment and drug release. The surface linear model generated for drug entrapment was found to be significant and indicated a significant relation between the variables drug-polymer ratio, stirring rate and the response entrapment of drug. On the other hand the quadratic model generated for the drug release characteristics was found to be significant and indicated that the concentration of polymer have significantly influenced the drug release over a period of time whereas the stirring rate had minimal effect on the drug release. The data of pure error and lack of fit are summarized in ANOVA Table 2. The formulation

variable drug-polymer ratio exerted a significant influence on the drug encapsulation and drug release whereas the other variable stirring rate does not have any impact on the response parameters. A new microsphere formulation with the desired responses was formulated based on the desirability approach. The optimized formulation was evaluated for the response parameters and the experimental values obtained were compared with those predicted by the mathematical models. The experiments were carried out in triplicate and the results obtained were included in Table 3. The optimized formulation KEC1 was assessed for parameters angle of repose, bulk density, Carr's index and the values are indicated in Table 4. The particle size of the microspheres was found to be 136.1 µm and the size of the microspheres was found to increase with increased polymer loads which may be due to increase in viscosity of polymer solutions at higher concentration. The SEM photomicrographs indicated that the microspheres were discrete, spherical and uniform in shape (Fig.1). The results of the optimized formulation KEC1 showed that as the concentration of polymer increases the drug entrapment increases significantly with the drug release sustained over a period of 12 hours. The Response surface plot of KEC1 showing the effect of drug- polymer ratio and r/min. on the response drug entrapment and drug release was shown in Fig 2 and Fig 3. The results demonstrated a good relationship between the predicted and experimental values, confirming the validity of the model.

The acute toxicity studies were carried and it was observed that the animals were stable up to a dose of 50mg/kg and above this dose mortality was observed in the animals. The haematological and biochemical values of the formulation do not deviate much from the control samples. This confirms that the drug or polymer do not have any interaction in the biological fluid. The optimized formulation showed good analgesic and anti inflammatory activity when compared to the standard drug. The results of activities studied were indicated in Table 5a & 5b. The optimized formulation KEC1 showed minor changes in particle size only under long term stability study with no appreciable change in drug content proving good stability of the product conducted both in accelerated and long term stability studies. The drug release kinetics studied using PCP Dissolv software is indicated in Fig.4.Table 6 showed that the R^2 value is 0.9617 that confirms the best fit model was found to be zero order release and the drug release may be by diffusion mechanism.

CONCLUSION:

An emulsion solvent evaporation technique has been successfully employed to produce ketoprofen loaded ethyl cellulose microspheres with maximum drug encapsulation and desirable release profile. The formulation variable drug-polymer ratio exerted a significant influence on the drug encapsulation and drug release whereas the other variable stirring rate, did not had any impact on the response parameters. The results obtained indicated that response surface methodology can be successfully used to analyze the effect of formulation variables and develop an optimized formulation thereby reducing the number of trials, time and cost of formulation development.

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