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Formulation Development, Optimization and Evaluation of Flurbiprofen Microsponge Tablet for the Treatment of Rheumatoid Arthritis (RA) by using Box- Behnken Design

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Abstract

Introduction: Rheumatoid arthritis (RA) is a chronic, autoimmune and inflammatory disease that mostly impacts the joints. Chronotherapeutics refers to a treatment method in which *in-vivo* drug availability is timed to match rhythms of disease in order to optimize therapeutic outcomes and minimize side effects. Flurbiprofen is a non-steroidal anti-inflammatory drug, indicated for the relief of inflammation.

Objectives: The aim of the present study was to develop & optimize the microsponges based of Flurbiprofen tablet for Chronotherapeutics for enhanced therapeutic effect.

Methods: Microsponges were developed by Quasi Emulsion solvent diffusion method. Prepared microsponges were optimized in order to analyze the effects of independent variables like concentration of PVA (X1), Volume of Dichloromethane (X2) & stirring speed (X3) on the Entrapment Efficiency (Y1), Mean particle size (Y2) and Drug release at 8 hr (Y3) using box Behnken design. The optimized formulation was subjected to in vitro study and Comparison with marketed formulation. With release kinetics study.

Result: The optimized formulation Batch (F-18) Show particle size of 49.12µm, entrapment efficiency

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of 87.46%, and drug release at 8 h 70.49%, which is under the acceptance criteria, which is more effective compared with Marketed tablet.
Conclusion: The results showed that, as stirring speed increases, the particle size decreases and entrapment efficiency increases. While volume of dichloromethane increases, particle size decreases. Morphology was found to be porous and spherical. Optimized batch of Flurbiprofen microsponge was further formulated in future for invivo study and clinical trials.

Keywords Flurbiprofen; Microsponge; Box- Behnken design

Introduction

A polymeric system made up of porous microspheres is called the Microsponge Delivery System (MDS). Their small, spherical, sponge-like particles are made up of several interconnected gaps inside a non-collapsible structure that has a large porous surface area that allows the regulated release of the active component. By capturing the less water-soluble medications in its pores, the microsponge system in oral drug delivery accelerates their solubilization. The medicine is effectively reduced to microscopic particles due to the extremely small pores, and the substantial increase in surface area accelerates the solubilization process. Rheumatoid arthritis is a chronic inflammatory systemic disease, mainly characterized by synovitis of small joints, especially of hands and feet. Persistent synovitis leads to pain, joint swelling, stiffness, decreased mobility and joint space narrowing; synovial hyperplasia causes erosions and joint deformities [1,2,3]. The biological and physiological processes of the brain and body depend heavily on circadian rhythms. Chronobiology plays a crucial part in rheumatoid arthritis (RA), where major symptoms including stiffness and pain in the joints are most noticeable in the morning. These symptoms may be mediated by circadian cycles of hormone and cytokine levels. According to chronobiological principles, the best outcomes may come from chronotherapy, which synchronises treatment schedules with each patient's unique circadian rhythm. Studies on patients with RA who received Flurbiprofen or NSAID. chronotherapy indicate that this strategy can enhance results and lessen side effects. One method to solve such problems is to find dosage form capable of releasing the drug such as Chronotherapeutical modified release microsponge tablets [4]. Chronotherapeutics refers to a treatment method in which in vivo drug availability is timed to match rhythms of disease in order to optimize therapeutic outcomes and minimize side effects. Flurbiprofen is a non-steroidal anti-inflammatory drug, indicated for the relief of inflammation [5]. Flurbiprofen is an important analgesic and non-steroidal anti-inflammatory drug (NSAID) also with anti-pyretic properties whose mechanism of action is the inhibition of prostaglandin synthesis. Flurbiprofen is rapidly eliminated from the blood, its plasma elimination half- life is 3-6 hours and in order to maintain therapeutic plasma levels [6].

Objectives

The aim of the research work was to design microsponges for chronotherapeutic drug delivery system to improve efficacy of a drug. Some drugs produce gastric irritation in stomach. Some drugs produce unwanted systemic side effects like increase risk of blood clot, Oedema, heartburn, enzymatic degradation of drug in small intestine. Formulating these drugs as microsponges can address these problems effectively. Microsponges can be designed to work as Chronotherapeutics drug delivery system where drug release can be programmed in synchronization with circadian cycle of the body and disease condition like rheumatoid arthritis. This can effectively make drug available to have better monitoring of drug concentration in the body [7,8].

Material and Methods

Material

Flurbiprofen powder was supplied by Vetina Healthcare, Pune, Eudragit polymers (RS-100) powder were obtained from Evonik, Mumbai, polyvinyl alcohol (PVA) from Loba chemie, India. All other materials used in this study were of analytical grade [9].

Method

Flurbiprofen loaded microsponge preparation: Flurbiprofen microsponge preparation: By using

a quasi-emulsion solvent diffusion method, the microsponges containing Flurbiprofen were made. An internal phase was added, consisting of 1% triethylcitrate (TEC) and Eudragit RS-100 dissolved in dichloromethane, to increase the polymer's plasticity. Flurbiprofen 100 mg was then added and dissolved at 35°C using ultrasonication. Following that, the mixture was added to 100 millilitres of polyvinyl alcohol aqueous solution, which was used as the external phase. The combination was stirred for two hours at 500, 1000, and 1500 revolutions per minute. As a result of dichloromethane evaporating out of the system, microsponges were created [10]. To ascertain the manufacturing yield, the microsponges underwent a 12-hour drying process at 40°C after being cleaned with water, filtered, and kept for later study. [11]

Experiment design (Box-Behnken design) & Optimization

Preliminary trial batches were taken for selection of polymers like Eudragit RS 100, Eudragit RL100 and Ethyl cellulose. Eudragit RL100 and ethyl cellulose were not used to manufacture spherical, stiff microsponges. Eudragit RS 100 produced the necessary micrometre-sized microsponges that were spherical and stiff. Eudragit RS 100 was therefore utilised as a polymer for additional research [12].

Tablets, the primary variables influencing Entrapment Efficiency (%), Mean Particle Size (µm) and %Drug Release were PVA (A), DCM (B), and Stirring speed (C). To look into the effects of the polymers and stirring speed, which were chosen for the study's high, medium, and low level based trials as independent variables.Tablet formulations were made and their dependent variables, namely Entrapment Efficiency (%), Mean Particle Size (µm) and %Drug Release were characterised Table- 1. Once we created the final formulation of the dependent and independent variables, we examined the

Table 1: Independent Variables and their levels

Factors	Actual Value			Levels used (Coded Value)		
	Low	Medium	High	Low	Medium	High
Conc. of PVA	200	400	800	-1	0	+1
Volume of DCM	5	10	15	-1	0	+1
Stirring Speed	500	1000	1500	-1	0	+1

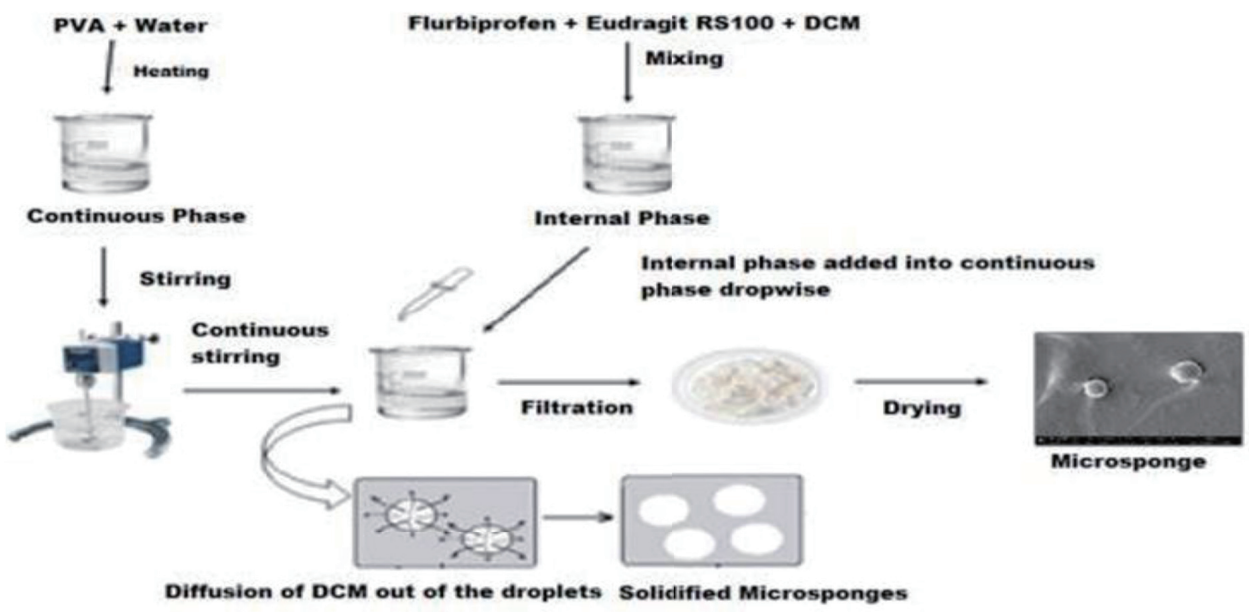


Figure 1: Formulation of microsponge by QESD

specific factors and responses. Table-3 displays the recommended outputs of 17 runs, which align with the DoE investigations [13].

3D Response Surface Plots & Contour plot

The possible correlation between three variables examined by 3D wireframe and surface plots [14].

Desirability Criteria

The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value [15].

Optimization Entrapment Efficiency

Flurbiprofen loaded microsponges theoretically equivalent to 100 mg of Flurbiprofen were weighed crushed and extracted with 5 ml of methanol. After the sample had been appropriately diluted with methanol, it was centrifuged for 10 minutes at 2000 rpm, filtered, and subjected to spectrophotometric analysis at 247 nm. The effectiveness of entrapment was calculated using a formula [16].

Drug Encapsulation efficiency = (Actual Drug Content/ Theoretical Drug Content) X100

Particle size analysis

Optical microscopy was used to measure particle size of microsponge by using digital microscope (Motic CV5-2), calibrated with ocular micrometer (AmScope MR400 Microscope calibration slide). A small amount of the water-dispersed selected microsponge formulation was put on a glass slide in the shape of droplets. Through the use of a digital microscope, the dispersion drop was examined. 300 particle sizes were averaged, and the result was computed [17]

In Vitro Drug Release Study up to 8 hr

Drug release was performed by using the USP-II apparatus. The dissolution test was performed using 900 mL at Phosphate buffer (pH 7.4) at the 37±.5 C and 100 RPM. Aliquots were withdrawn at predetermined interval times for 8 h from the dissolution medium and replace immediately with fresh medium. The amount of drug present in the sample was measured by the absorbance of the solution at L max 247 nm using UV-Visible spectrophotometer. The cumulative percentage of drug release is calculated using an equation obtained from a standard curve. Result are shown in Table No. 7, [18]

Statistical analysis of data, Optimization and validation of experimental design

Statistical Analysis

The effect of independent variables on the dependent variables were estimated by using DoE software (Design Expert Trial Version13., Stat-Ease). Polynomial equations were generated for the dependent variables entrapment efficiency, particle size, drug release at 8 hr. The optimized formulation was selected on the basis of particle size & high entrapment efficiency & drug release at 8 hr and maximum desirability [19].

Preparation of Tablet and optimized batch

Specific weight of selected prepared microsponge's equivalent to 250 mg of Flurbiprofen. 1% magnesium stearate as a lubricant, and, starch, MCC, lactose was mixed well using mortar and pestle for 15 min, then undergo physical evaluation before compression. The powder combinations' compressibility, bulk density, tapped density, and angle of repose were assessed prior to the creation of tablets. [20]

Preparation of coating solution

The Flurbiprofen tablets were further coated with Eudragit S-100 solution. 2.5 % w/v of coating solution of Eudragit S-100 was prepared in a mixture of Isopropyl alcohol: acetone (1:1). The coating of the matrix tablets was performed by immersion in the coating solution followed by dip coating technique [21].

Evaluation of Optimized Batch (F18)

1. Dissolution test

In vitro drug release studies were performed using USP dissolution test apparatus (Type II). The dissolution studies were performed at 100 rpm at 37± 0.5 °C in pH 1.2 pH for first 2 hrs,

6.8 pH for 8 hrs and pH 7.4 for restof studies. Aliquots were withdrawn periodically and replaced with fresh medium & analyzed at 247 nm [22].

2. Kinetic data analysis

Examine the data on in vitro release. The release kinetics were described using a variety of kinetic models. Systems in which the drug release rate is independent of concentration are described by the zero order. The release from a system where the release rate depends on concentration is described in the first order [23,24,25]. Based on Fickian diffusion, Higuchi defined the release of pharmaceuticals from an insoluble matrix as the square root of a time-dependent process. The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs.

time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model) and log cumulative % drug release vs. log time (Korsmeyer model) shows in Table no.-2.[26]

Results & Discussion

Experimental Design

Displays the results of the various evaluation tests performed on design batches Confirmed that the model was significant and independent variables of the model had significant effect on repose variables. The impact of independent factors on response variables, response surface plots were created. For a range of response variables, the contour plot 3-D response surface plot are given in Fig 2,3,4. The equation for all the responses is mentioned below the interaction plots.

% Entrapment Efficiency

The % entrapment efficiency of designed batches was between 75.25 and 89.78%. Batch B7 had the highest efficiency of 89.78 % whereas batch B1 had the lowest efficiency of 75.25%. Among all independent variables, amount of PVA and stirring speed had a significant effect on the drug entrapment efficiency. Regression analysis results indicated that stirring speed had a favourable impact on microsponges' entrapment

Table 2: Equation of Kinetics Model

Sr. no	Model	Equation
1	Zero order	$Q = Q^0 + K^0t$
2	First order	Log Ct = Log C0 – kt / 2.303
3	Higuchi diffusion model	Q = A [D (2C - Cs) Cst] 1/2
4	Hixson-Crowell	Q^0 1/3 – Qt 1/3 = KHCt
5	Korsmeyer - Peppas equation	Mt / Ma = Ktn

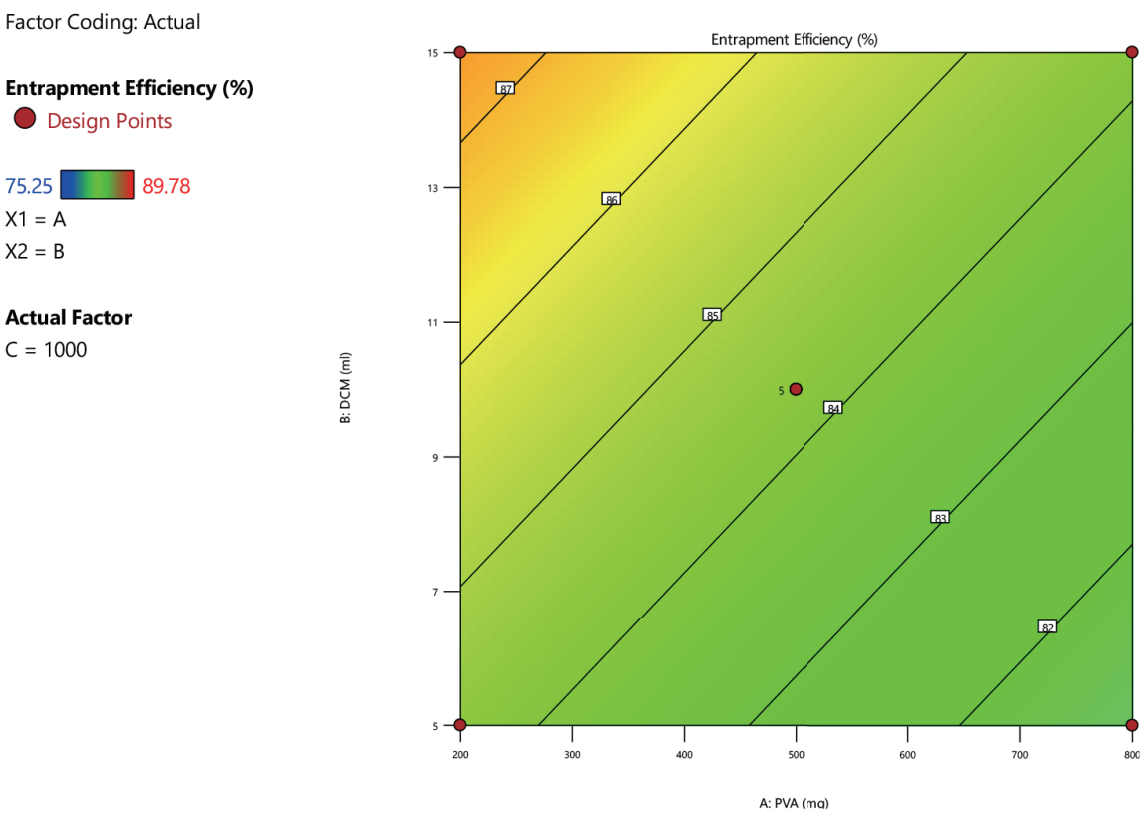


Figure 2. Contour plot depicting the effect of Dichloromethane and PVA concentration on %Entrapment efficiency

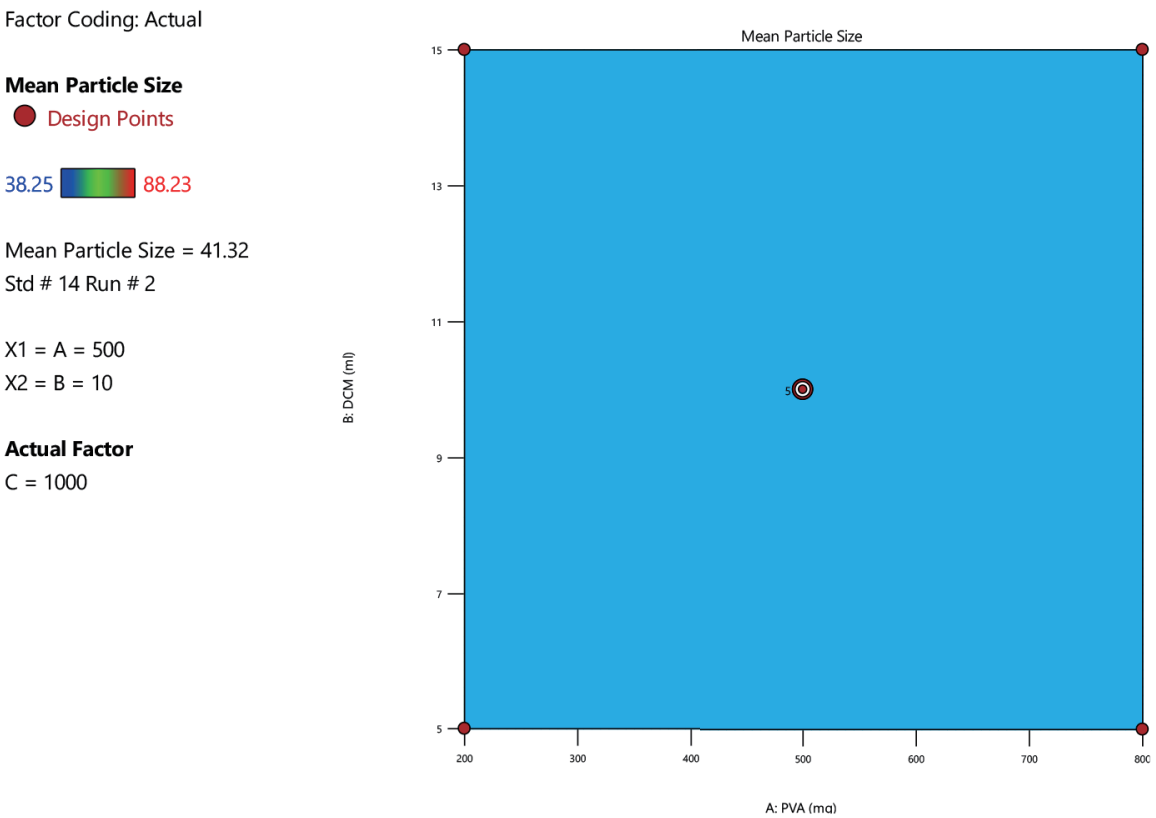


Figure 3. Contour plot depicting the effect of Concentration of Dichloromethane and PVA concentration on Mean Particle size.

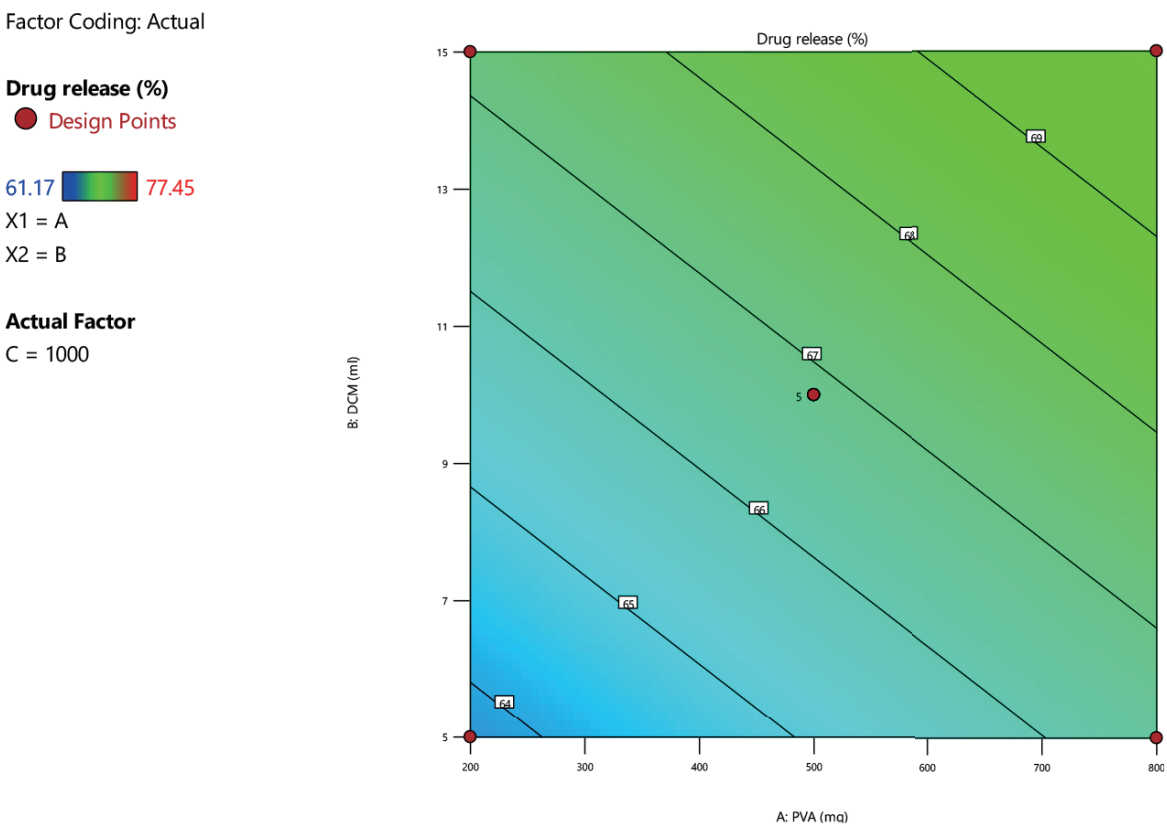


Figure 4. Contour plot depicting the effect of Dichloromethane and PVA concentration on Drug release at 8 hr

efficiency, whereas PVA concentration had a negative effect. High the amount of PVA in the external phase increased the solubility of the drug in water so possibly more amount of drug was able to diffuse from the microsponges to the aqueous phase and thus decreased the entrapment efficiency of microsponges. Shown in Fig 2.

Mean Particle size

The particle size was found to be inversely proportional to quantity of internal phase (DCM). The negative relationship between internal phase volume and mean particle size showed that microsponges' particle sizes reduced as internal phase volume increased. Particle sizes of microsponges can be directly attributed to apparent viscosity of internal phase.particle size are between 38.25 and 88.23%. Batch B14 had the highest efficiency of 88.23 % The globules of the produced emulsion could readily split into smaller droplets when the internal phase with lower viscosity was poured into the continuous phase, resulting in a decrease in mean particle size. The particle size was found to be inversely proportional to the stirring rate. At higher stirring rate, a vigorous, uniform, increased mechanical shear was imposed and this resulted in a rapid division of the formed droplets, which might have less chance of coalescing into bigger droplets as shown in fig. 3. This led to decrease in particle size with increasing stirring rate.

Drug release at 8 hr

The particle size was found to be inversely proportional to quantity of internal phase (DCM). The negative influence of internal phase volume on mean particle size indicated that increasing the internal phase volume decreased the particle size of microsponges. Particle sizes of microsponges can be directly attributed to apparent viscosity of internal phase. Drug release between 61.17 to 77.45%. Batch B5 had the highest efficiency of 77.45 % When the internal phase with lower viscosity was poured into continuous phase, the globules of the formed emulsion could easily divide into smaller droplets and mean particle size decreases. The particle size was found to be inversely proportional to the stirring rate. At higher stirring rate, a vigorous, uniform, increased mechanical

Table 3: Result of data obtained from experiment

Run	Factor 1	Factor-2	Factor 3	Response 1	Response 2	Response 3
	A:PVA (Mg)	B:DCM (ml)	C:Stirring speed (RPM)	Entrapment Efficiency (%)	Mean Particle Size (µm)	%Drug Release at 8hr
1	1	0	-1	75.25	51.31	66.28
2	0	0	0	86.65	41.32	64.64
3	1	1	0	85.74	45.58	69.12
4	-1	1	0	89.78	41.56	72.46
5	-1	0	-1	81.82	51.62	61.17
6	0	1	-1	86.66	41.5	64.98
7	-1	0	1	89.29	42.97	63.58
8	0	0	0	82.91	46.32	64.12
9	0	1	1	82.24	57.12	70.31
10	0	-1	-1	86.62	44.42	68.56
11	0	-1	1	86.21	43.25	64.81
12	0	0	0	86.62	41.36	66.12
13	1	-1	0	80.65	42.55	62.36
14	0	0	0	87.23	88.23	64.23
15	1	0	1	85.3	38.25	77.45
16	0	0	0	81.23	40.21	68.83
17	-1	-1	0	78.8	45.06	67.11

shear was imposed and this resulted in a rapid division of the formed droplets, which might have less chance of coalescing into bigger droplets as shown in fig. 4. This led to decrease in particle size with increasing stirring rate. As particle size reduced drug release rate increases due to available surface area.

Desirability Function Index

Multiple responses optimization to given experimental factors applying RSM is often unsatisfactory, because what is optimal for one response may not be optimal for other responses. There are several methods to find the best compromise among multiple responses. Derringer's desirability function is the most popular methodology, which searches for a combination of factor levels that simultaneously satisfies the requirements for each response in the design and there result are shown in Table 3 and 4.

Solutions

By DoE 100 Solutions found and out of 100 the highest desirability factor are 0.821 Shown in Table No. 5, and then Optimized Batch-18 evaluated and found result which are shown in Table No. 6

Table 4: Constraints by DoE

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:PVA	Is in range	200	800	1	1	3
B:DCM	Is in range	5	15	1	1	3
C:Stirring speed	Is in range	500	1500	1	1	3
Entrapment Efficiency	None	75.25	89.78	1	1	3
Mean Particle Size	None	38.25	88.23	1	1	3
Drug release	None	61.17	77.45	1	1	3

Table 5: Predicted composition of Optimized Batch formula by DoE

Number	PVA	DCM	Stirring speed	Entrapment Efficiency	Mean Particle Size	Drug release	Desirability	
1	394.476	14.649	964.075	86.152	47.214	67.847	0.821	Selected

Table 6: Results of optimized formulation

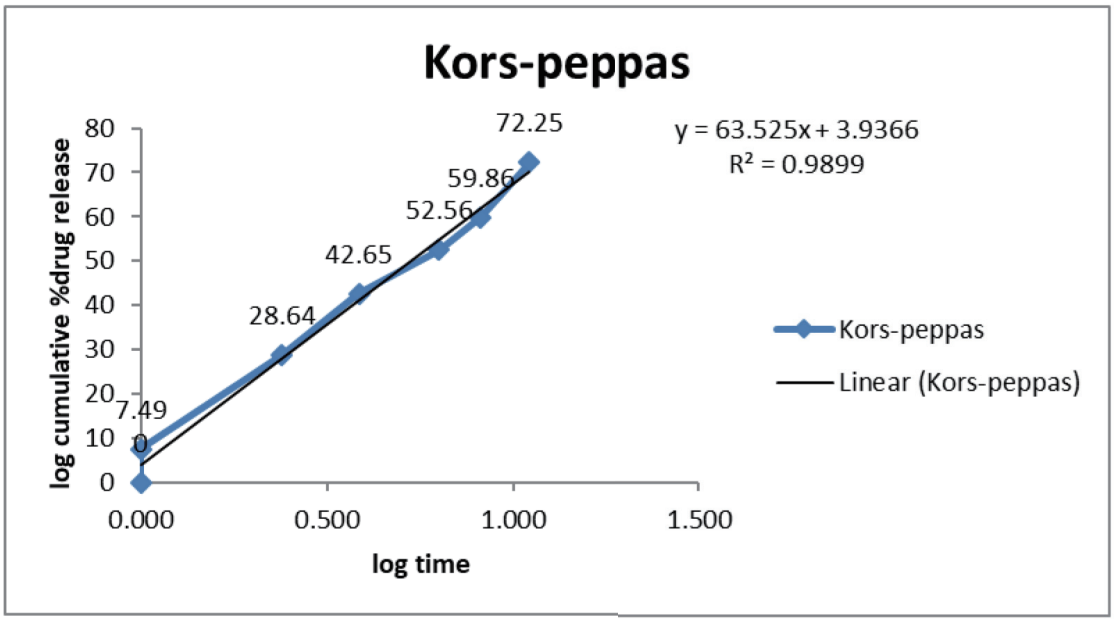
Variables	Predicted response	Observed response up to 10hr	% Predicted error (% PE)	Acceptance criteria for % PE
Entrapment Efficiency (%)	86.152	87.46	1.49%	Less than 5.0 %
Mean Particle Size (μm)	47.214	49.12	3.88%	Less than 5.0 %
Drug release (%)	67.847	70.49	3.74%	Less than 5.0 %

Table 7: *In-vitro* drug release of marketed formulation Vs optimized Batch

Formulation	Time (hr)							
	1	2	3	4	5	6	8	10
Marketed Tablet	11.18± 1.32	19.32± 2.10	27.42± 1.83	41.43± 1.54	61.96± 2.15	76.37± 1.71	82.16± 2.37	92.16± 1.45
Optimized	12.12± 1.73	18.56± 1.40	28.42± 1.46	36.71± 2.52	45.82± 2.03	57.10± 2.19	66.70± 1.42	79.28± 1.46

Drug Kinetic release study

The drug release data of optimized Batch were fitted into different kinetic models which show linearity R^2=0.9899 and follow zero order that the drug release from tablet formulations follows



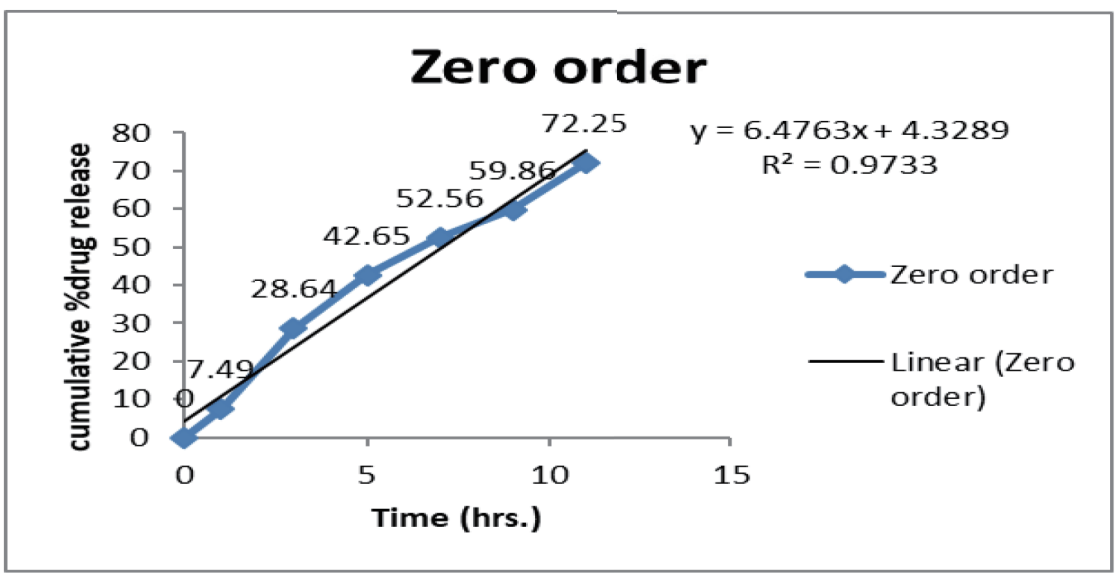


Figure: A) Korsmeyer - Peppas release, B) Zero order

Conclusion

It could be concluded that application of experimental design is helpful tool for the development of microsponges of Flurbiprofen by emulsion solvent diffusion technique using RS 100 as a polymer for enhancement of solubility ,flow properties & and compression characteristics and controlling the release rate up to 10 hrs 79.28±1.46. The optimized batch Microsponge formulation was then formulated into tablet to get controlled release of drug. Drug release kinetics of this formulation correspond best to Korsmeyer & Peppas release model and drug release mechanism as per linearity of Korsmeyer & Peppas was found to be linearity R^2=0.9899 indicates Non- Fickian zero-order release which say that the formulation are sustained release.

Conflict of interest

There are no conflicting interests, as the authors have stated.

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