Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (5):52-59
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Statistical optimization of mucoadhesive ketoprofen multiparticles to target the small intestine

Sivakumar Ramakrishnan[*a], Rajendran N.[b] and Narayanan N.[c]

[a]*Department of Pharmaceutics, The Grace College of Pharmacy, Palakkad, Kerala*
[b]*Department of Pharmaceutics, Swamy Vivekananda College of Pharmacy, Tiruchengode*
[c]*Jaya College of Pharmacy, Chennai, India*

ABSTRACT

The aim of the study was to apply statistical optimization technique for the development of ketoprofen loaded mucoadhesive multiparticles to target the small intestine. The mucoadhesive multiparticles were prepared by quassi- emulsion solvent diffusion method and filled in enteric coated capsules. All the formulations were characterized for particle size, mucoadhesion, drug loading, entrapment efficiency, in vitro release studies. A 3^2 factorial design was used to optimization. The dependable variables were PVA concentration (X1) and rotational speed of stirring elements(X2). The chosen response variables were particle size (Y1), percentage of drug release for 24 h (Y2), mucoadhesion (Y3) and entrapment efficiency (Y4). The optimized formulation according to the study was mucoadhesive microspheres prepared with 0.5% w/v of PVA and 400 rpm of stirring speed i.e (-1, +1). The results of this optimized formulation showed with mean particle size of 225.5 µm, percentage of drug release for 24 h 97%, 83.43% of mucoadhesion and 88.4% of entrapment efficiency.

Keywords: Ketoprofen, Small intestine, controlled delivery, mucoadhesive multiparticles, statistical optimization.

INTRODUCTION

Mucoadhesion of multiparticles is the successful method of retain drug in the target site for prolonged release and therefore for prolonged action of drug. This dosageforms were improve the absorption and systemic bioavailability the drug that were normally poorly absorbed[1].

Ketoprofen is a Non steroidal anti-inflammatory drug (NSAID) used in rheumatic disorder and used for moderate pain.[2]. It has short half life (4.5 h) required frequency of administration. Also the drug causes gastric irritation to the stomach. The drug has poorly solubility in water and acidic condition and therefore its bioavailability remains problematic in stomach region.[3, 4]. The drug exhibit maximum absorption from small intestine following oral administration[5]. So that small intestine specific ketoprofen loaded mucoadhesive multiparticles is the preferred option to avoid gastritis, improved bioavailability, reduced frequency of administration. Biocompatible polymers are preferred as they are nontoxic and eliminated from the body. Eudragit RS 100, Chitosan and Carbopol 934 P have been used over the years in the development of mucoadhesive multiparticles [6, 7, 8].

Factorial design and response surface methodology is an important statistical tool to study effect of several factors influencing responses by varying them simultaneously by carrying them simultaneously by carrying out limited

number of experiments[9, 10]. Literature search revealed no study carried out to formulate small intestine specific mucoadhesive multiparticles and to demonstrate the influence of formulation variables using a factorial approach. The aim of the present study was to develop optimized formulation of mucoadhesive multiparticles of ketoprofen to target the small intestine using factorial design approach

MATERIALS AND METHODS

Ketoprofen USP was purchased from BEC chemicals Pvt. Ltd, (Mumbai. India). Poly Vinyl Alcohol (PVA) was obtained from Loba Chemie Pvt Ltd (Mumbai, India). Chitosan (MW 150 KDa) was obtained from Central Institute of Fisheries Technology, (Cochin, India). Carbopol 934 P and Eudragit RS100 were purchased from sigma chemicals (Mumbai, India). All other chemicals, reagents and solvents used were of analytical grade.

Experimental design for preliminary Trials

Preliminary trial formulations were designed by quasi-emulsification and solvent diffusion method using various drug: polymer (s) ratio (1: 1, 1: 3 and 1: 6) Table1. Based on the results of preliminary trials best drug to polymer ratio was selected for the optimization.

Preparation of mucoadhesive multiparticles

Mucoadhesive ketoprofen multiparticles were prepared by quasi-emulsification and solvent diffusion method[11] for preliminary trial. To prepare inner phase, Eudragit RS 100 was dissolved in isopropyl alcohol (5ml) and the drug was added to the solution under constant stirring at 200 rpm at 37° C. The inner phase was added drop wise introduced into water phase (outer phase) containing carbopol 934 P in 200ml of stabilizing agent (polyvinyl alcohol 0.5%) with constant stirring at 200 rpm for 30 min. Chitosan was dissolved in 15 ml of 1 % v/v aqueous acetic acid solution and dropped into the gently agitated solution of outer phase and stirred at 200 rpm for 2 h. The multiparticles were filtered using what man filter paper (No 56) to separate the multiparticles and dried in an oven at 40º C for 12 h. The dried multiparticles were stored in a dessicater.

Preparation of Enteric capsules

The coating solution was prepared by dissolving enteric polymer Eudragit L100 (10% w/w) Caster oil (5%w/w), titanium dioxide(0.5%w/w), methanol (10%w/w) methelene chloride (1%w/w) in 100 ml of isopropyl alcohol. Weight equivalent to 50 mg of drug containing multiparticles were filled into hard gelatin capsules and coated with the enteric coating solution using dipping and drying technique. At each stage the capsules were kept in a hot air oven for15 minutes at 45° C. The capsules were weighed and the weight gain limited to (8 %w/w) indicating completion of enteric coating.

Statistical optimization by 3^2 factorial design:

A 3^2 full factorial design was used for optimization of the formulation variables. Amount of PVA(X1) and stirring speed (X2) were selected as independent variables. Particle size (Y1) In *vitro* release studies (Y2) , Entrapment efficiency (Y3), Percent mucoadhesion (Y4) were selected as dependent / response variables. Data were analyzed using Minitab 2002 – V13. 20 software to generate the study design and the response surface plots. Statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y= b_0 +b_1X_1+b_2X_2+b\ X_2+b_{12}\ X_1X_2+ b_{11}X_{12}+ b_{22}X_{22}$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b1 is the estimated coefficient for the factor X1. The main effects (X1 and X2) represent the average result of changing one of the factors at a time from its low to high value. The interaction term (X_1X_2) show response changes when two factors are simultaneously changed. The polynomial terms (X_{12} and X_{22}) are included to investigate non –linearity.

Characterization of multiparticles

Particles size analysis

Particle size analysis was performed on multiparticles formulations by Malvern Mastersizer (Malvern instruments, Mastersizer 2000, UK). The results are the average of three analyses. The values (d_{50}) were expressed for all formulations as mean size range.

Drug loading and Entrapment efficiency [12]

100 mg of accurately weighed multiparticles were crushed in a glass mortar pestle and the powered multiparticles were suspended in 25 ml of phosphate buffer (6.8) for 12 h at room temperature to release the entrapped drug. After 12 h the solution was filtered using micropore filter and the filtrate was diluted and analyzed for the ketoprofen content using UV spectrophotometer at 256 nm. The amounts of ketoprofen present in the multiparticles were determined using a calibration curve. The drug loading and entrapment efficiency were calculated using Equation (1) and(2), respectively.

A. Drug Loading (%)

$$\text{Drug Loading} = \frac{\text{Amount of ketoprofen in multiparticles}}{\text{Multiparticles weight}} \times 100$$

B. Entrapment efficiency (EE)

$$\text{Entrapment Efficiency} = \frac{\text{Actual weight of ketoprofen in sample}}{\text{Theoretical weight of ketoprofen}} \times 100$$

Test for mucoadhesion

The mucoadhesive property of PMP was evaluated by *In vitro* wash-off test[13]. A 1x1 cm piece of intestinal mucosa of goat was tied onto a glass slide using thread. Mucoadhesive multiparticles were spread (~50) onto the wet rinsed tissue specimen and the prepared slid was hung onto one of the grooves of a USP tablet disintegrating test apparatus. When the disintegrating test apparatus was operated, where by the tissue specimen was given slow, regular up-and-down moment in the beaker of the disintegration apparatus, which contained the phosphate buffer (pH 6.8). At the end of 6 h, 12 h, 18h and 24 hours, the number of multiparticles still adhering onto tissue was counted.

In vitro release and kinetics Study [14, 15, 16]

Each Enteric capsules containing weight equivalent to 50 mg of drug loaded on multiparticles were subjected to the dissolution studies. The studies were carried out using the USP XX111 dissolution test apparatus (apparatus1, 50 rpm, 37ºC ± 0.5ºC) for 2 h in 900 ml of 0.1 N HCl (artificial gastric fluid). Then the dissolution medium was replaced with 900ml of pH 6.8 phosphate buffer (artificial Intestinal fluid) and the experiment was continued. At different time interval samples were withdrawn and replaced with an equal volume of fresh medium to maintain a constant total volume. The aliquots were diluted suitably, filtered and analyzed for the drug content by UV spectrophotometer method at 256 nm.

The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows: zero order kinetic model, first order kinetic model, Higuchi's model, Korsmeyer equation / Peppa's model. This model was used frequently in predicting the relative importance of Fickian (n= < 0.43) or non Fickian (n= >0.43) and case 11 (> 0.85) in anomalous diffusion, and super case 11 transport where n> 1.0. Table 4.

RESULTS AND DISCUSSION

In the present study Eudragit RS, chitosan, Carbopol 934 P were selected for the preparation of multiparticles; While Eudragit can provide this formulation with sustained release characteristics, chitosan and carbopol 934 P offer mucoadhesive properties. The layout and results factorial design are shown in Table 2 – 5 and Fig 1-4. Preliminary trial batches were prepared to study the effect of the drug to polymer ratio on particle size, mucoadhesion, drug release character and entrapment efficiency and characteristics of the multiparticles. On the basis of the preliminary trials a 3^2 factorial design was employed to study the effect of independent variables (PVA concentration X_1 and Stirring speed X_2) on particle size, percentage of drug release, mucoadhesion and entrapment efficiency characteristic of the mucoadhesiv3e multiparticles. Best formulation PF 3 were considered for the optimization based on the characteristics of the multiparticles. The inner phase, Isopropyl alcohol in varying proportion 5 ml, 10ml, and 15 ml were attempted on the formation of multiparticles and it was observed that 5 ml of inner phase

yielded the best results, PVA 0.5 % as a stabilizing agent, stirring speed 400 rpm and stirring time 2 h were selected. The formulation of multiparticles could be described in the following process. The formation of droplets by quassi emulsion solvent diffusion. The rapid diffusion of Isopropyl alcohol (IPA) (solvent for Eudragit RS100 and drug) in aqueous medium might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in water. The instant mixture of the IPA and water at the interface of the droplets at the interface of the droplets induced the precipitation of polymer. Thus formation of shell enclosing the IPA and the dissolved drug counter diffusion of IPA and water through the shell promoted further crystallization of drug in the droplets from the surface in wards. The finely disperse particles of polymer solution of the drug were solidified in the aqueous phase via diffusion of the solvent. The interactions between the cationic chitosan polymer and the anionic carbopol 934 P helped form mucoadhesion layer on the multiparticles surface. The stirring time and speed on the multiparticles formulation were selected based on earlier study. It has been reported that the increased mechanical shear force produced by the increasing the stirring speed divided the suspension of drug and polymer into the small droplets rapidly. In our study the multiparticles prepared with 400 rpm had a smaller particles size 225.47 µm and having homogenous size distribution. The stirring time was extended to 2 h in the study owing to use of more polymers (Eudragit RS 100, Chitosan, Carbopol 934 P).

From the data from the particle size analysis all multiparticles in f1 – f9 were nearly spherical uniform, and free flowing (angle of repose value < 30º) The particle size of multiparticles ranges from 225µm to 280µm and showed good correlation coefficient (0.9633). The results indicate that the effect of X1 is more significant (PVA concentration) is more significant than X2 (i.e. stirring speed). Thus as the PVA concentration increases the particle size decreases. The result of the study indicates that the PVA at higher concentration stabilizes the dispersed droplets by reduces the interfacial forces between the droplets during the process of preparation and so the particle size was deceased as the concentration of PVA increased. Particle size Y1, percentage of drug release during 24th hourY2, mucoadhesion Y3, and drug entrapment efficiency Y4. The entrapment efficiency and percentage of drug release are important variables for assessing the drug loading capacity and the drug release profile that suggest the amount of drug available at site. The drug entrapment efficiency of multiparticles varied from 62.4% to 88.4 % and showed good correlation coefficient 0.9207.The results of equations indicates that the effects of X1 (PVA concentration) more significant than X2 (stirring speed). Thus, as the stabilizing agent (PVA) concentration increases the drug entrapment efficiency increases. However, stirring speed increased the particle size and thus drug entrapment efficiency decreased. The effect of PVA concentration and stirring speed 400 rpm (X1, X2) appears to influence the particle size result an increase the size. Though increasing the stirring speed showed increasing the particle size with reduction in drug entrapment efficiency the net effect of X1 X2 appears to increases the drug entrapment efficiency. The results indicates the stirring speed does not appear to influence either the particle size or the drug entrapment efficiency and changes in particle size and the drug entrapment efficiency are significantly influenced by the concentration of the PVA.

The drug release profile of the multiparticles indicate neither the PVA concentration nor the stirring speed did influence the *in vitro* drug release and the correlation coefficient was 0.2975.The percentage drug release of all formulation f1 – f2 ranged from 83.10 % to 97 %. The formulation f7 showed highest percent drug release (97%) as compared to other formulation. However statistical analysis indicates no significant difference in the release rate between the formulation suggest that the release rate of drug from the multiparticles is independent of the PVA concentration and stirring speed used in the present study. However all formulations showed slow release profile of the drug during 24^{th} h possible due to combined chitosan and carbopol and Eudragit RS100.

The *invitro* release data of all formulations were fit into best model for analyzing the release kinetics and mechanism of release. It was observed that r value of zero order plots were in the range of 0. 91 – 0.98 and first order plot were in the range of 0.81 – 0.89. Based on maximum r values it can be conclude that the formulations f1 – f9 follow zero order kinetics. When the slope n values were from the range of 0.8- 1.05. (>0.45) indicating the drug release by non Fickian diffusion mechanism. Non- Fickian is anamolous transport, in the process polymer chain relaxation / erosion or both involved

The *in vitro* wash off test for the percent mucoadhesion after 24 h varied from 64.26 % to 83.43%. However showed poor correlation coefficient 0.5933 (Y3).The results indicate that the PVA concentration increases (X1) the percent mucoadhesion increases. Whereas the stirring speed increases i.e. X2, the percentage of mucoadhesion decreases. However both the effect are insignificant from the statistical analysis. Though all the formulation f1 – f9 showed good mucoadhesion for 24 h. The PVA concentration as well as the stirring speed did not affect significantly the

mucoadhesion of the multiparticles. Further this study included chitosan and carbopol in the fixed ratio in all the multiparticles and show the significant difference in the mucoadhesion was not observed between the formulations Due to the effect of PVA concentration (X1) and stirring speed (X2). Based on the above results f7 was found to be the best formulation. Table 4.

TABLE 1: COMPOSITION OF MULTIPARTICLES FOR PRILIMINARY TRIALS

Ingredients (mg)	Code: PF1	Code:PF2	Code:PF3
Ketoprofen	100	100	100
Eudragit RS100	50	150	300
Chitosan	25	75	150
Carbopol 934 P	25	75	150

TABLE 2: LAY OUT OF 3^2 FULL FACTORIAL DESIGN

Code	Variable levels		Y1	Y2	Y3	Y4
	X1	X2	%	%	%	%
f 1	-1	- 1	269.2 ± 7.2	95.5 ±4.2	77.33 ±7.5	68.6±4.2
f 2	-1	0	275.7 ± 6.9	89.2±3.9	64.26 ±4.5	65.3±4.7
f3	-1	1	280.2 ± 8.3	87.2 ±5.2	68.00 ±4.8	62.4±2.9
f4	0	-1	243.2 ± 9.8)	83.1 ±3.7	71.08 ±5.2	75.6±3.6
f5	0	0	262.3 ± 7.1	91.5 ±4.7	75.66 ±6.3	78.3±3.6
f6	0	1	266.1 ± 7.7	93.0 ±3.5	70.33 ±5.7	80.1±4.1
f7	1	-1	225.5 ± 8.3	97.0 ±2.9	83.43 ±8.3	88.4±3.1
f8	1	0	233.8 ± 8.0	95.3 ±3.3	75.23 ±6.2	85.2±2.6
f9	1	1	237.5 ± 5.9	95.0 ±4.2	73.10 ±5.3	82.3±4.8

Translation of coded level in actual units

Independent variables	-1	0	+1
X1= PVA (% w/v)	0.25	0.5	0.75
X2 = Stirring speed (rpm)	200	400	600

Response Variables: Y1= Particle Size (μm) Y2= Percentage of drug release for 24h (%) Y3= Mucoadhesion (%) Y4= Entrapment efficiency (%). All the values are average of three such determinations.

TABLE 3: SUMMARY RESULTS OF REGRESSION ANALYSIS

Coefficient	b_0	b_1	b_2	b_{12}	R_2
Y1	254.87	- 21.390	7.660	0.260	0.965
Y2	91.890	02.583	-0.045	1.602	0.297
Y3	73.149	03.695	-3.380	- 0.250	0.595
Y4	76.244	09.333	- 1.301	0.025	0.920

TABLE 4 : OPTIMUM VALUES FOR PRODUCTION OF KETOPROFEN MUCOADHESIVE MULTIPARTICLES

Specification	Optimum values
Drug to polymer(s) ratio	1:6
Amount of drug	0.1g
Stabilizing agent	PVA 70,000
Concentration of stabilizing agent	0.5%
Inner phase solvent	Isopropyl alcohol
Amount of water in outer phase	200 ml
Temperature of inner phase	37º C
Stirring type	magnetic stirrer
Stirring rate (rpm)	400 rpm
Stirring time (min)	60

TABLE 5: DRUG RELEASE KINETIC DATA FOR OPTIMIZED BATCH (f7)

Batch Code	Zero r^2	First r^2	Higuchi r^2	Korsmeyer - peppas r^2	n	Mechanism
f7	0.099	0.894	0.969	0. 969	1.01	Case 11 transport

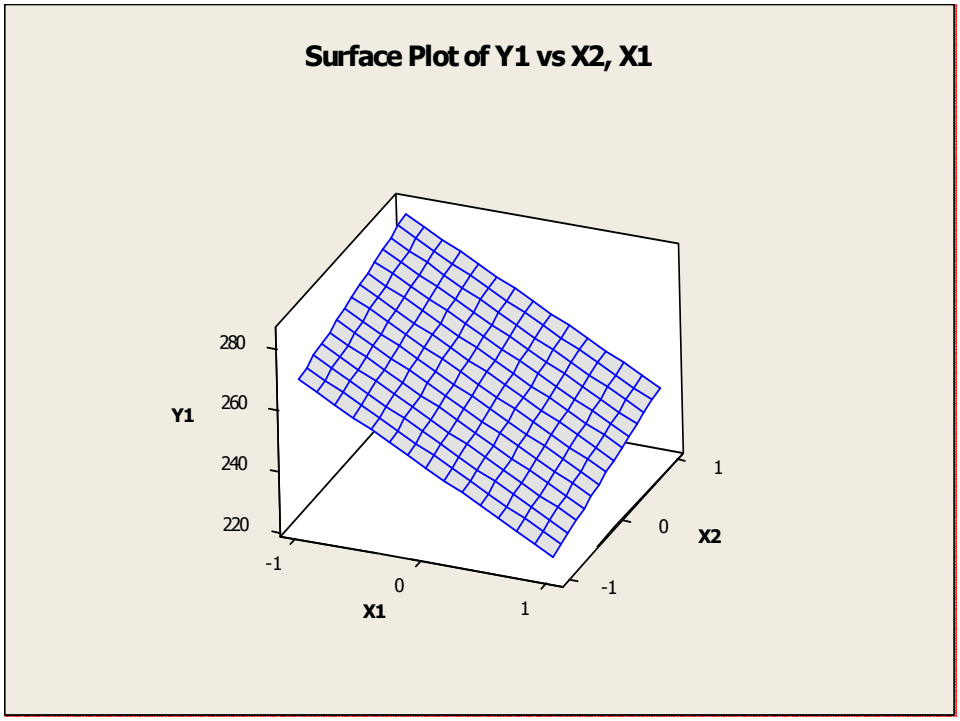

Fig. 1: Effect of dependent variables on particle size

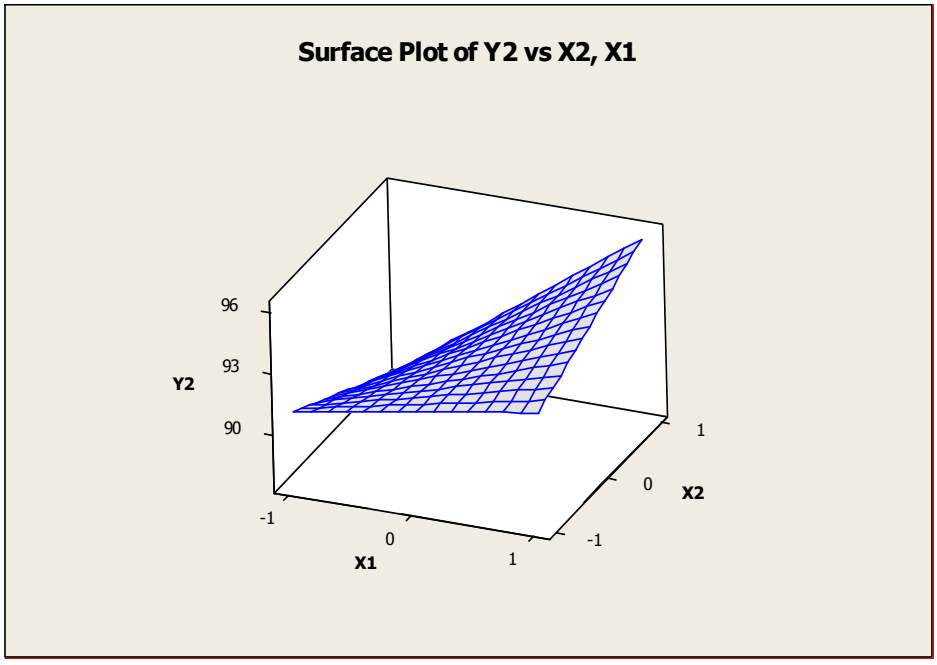

Fig.2: Effect of dependent variables on entrapment efficiency

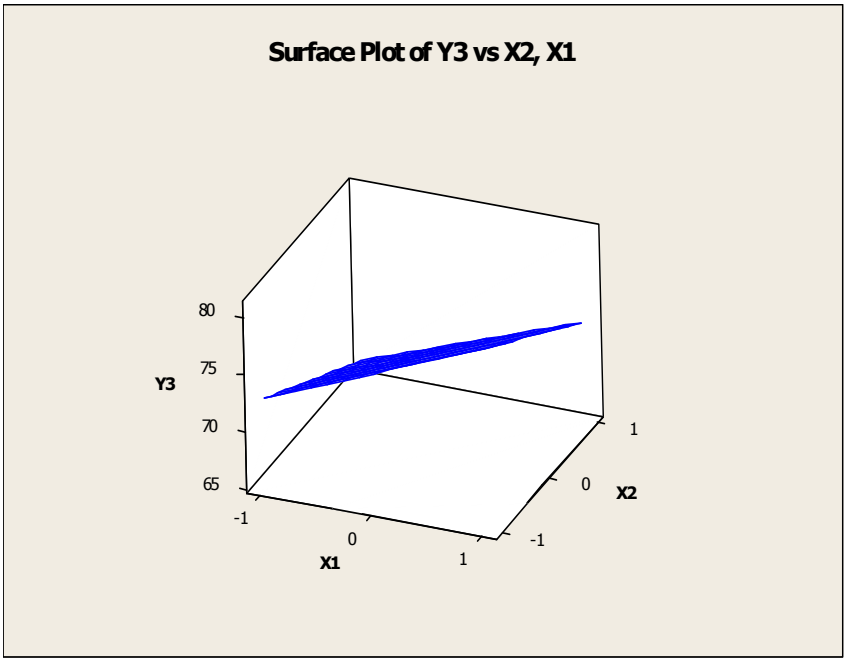

Fig.3. Effect of Dependent variables on mucoadhesion property

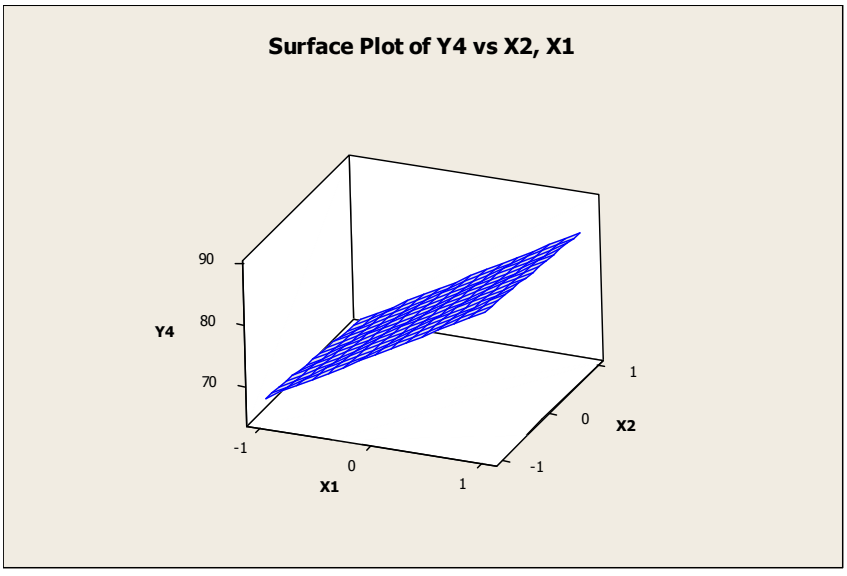

Fig.4: Effect of Dependent variables on Entrapment efficiency

CONCLUSION

In the current work a mucoadhesive multiparticles incorporating ketoprofen is described. A systematic study using a central composite design revealed the most suitable concentration of stabilizing agent PVA and stirring speed of rotational elements. The optimized formulations fulfilled all the requirements of the target set and exhibited suitable values of particle size, mucoadhesion, and dissolution period and entrapment efficiency. The present study clearly indicates the applicability of statistical optimization technique to predict the composition of a formulation and speed of rotational elements that gives optimum product parameters.

Acknowledgements
The authors would like to thank Dr. M. Karunanidhi, Vivekananda Groups of Educational Institution, Tiruchengode (India), for extending required laboratory facilities for the present work.

REFERENCES

[1] Myung -Kwan Chun, Hongkee Sah, Hoo-Kyun Choi. *Int J Pharm*., **2005,** 297, 172 – 179.
[2] Tansel Comoglu, Nuruin Gonul, tamer Baykara. I1 *Farmaco*., **2003,** 58, 101-106.
[3] Melike Uner., Umit Gonullu., Gulgun Yene.r, Turan Altinkurt. *IL Farmaco* **2005**, 60, 27-31.
[4] Miao Miao Xi, San qi Zhang, Xin Yi Wang, Kun Quan Fang, Yi Gu. *Int. J. Pharm* **2005**, 298, 91- 97.
[5] 1.Igor E. Shhohin, Julia I. Kulinich, Galina V. Ramenskaya, Bertil et al. *J Pharm Sci* **2012**, 101, 3593-3603.
[6] Jennifer J, Sheng Nehal A Kasim, Ramachandran Chandrasekharan, Gordon L Amidon. *Eur J Pharm* **2009**, 29, 306-314.
[7] Park H, Robinson JR. *Pharm Res*. **1987**, 4, 457 - 464.
[8] Hejazi R, Amiji. *J Control Rel* **2003**, 89, 151 -165.
[9] Kincl M, Turk S, Vrecer F. *Int J Pharm* **2005**, 291, 39-49.
[10] Cochran WG, COX, G.M, Experimental designs, 2nd ed. John Wiley and sons. New York, **1992,** 335-375.
[11] Mine Orlu, Erdal Cevher, Ahmet Araman. *Int J Pharm*, **2006**, 318, 103-107.
[12] Chambin O, Dupuis, G, Champion D, Voilley, A et al. *Int J Pharm* **2001**, 321, 86-93.
[13] Lehr CM, Bouwstra JASchacht EH Junginger HE. *Int J Pharm* **1992,** 78, 43-48.
[14] The United States Pharmacopoeial Convention XXV1 In: The United states pharmacopoeia, Rockville, M.D; The united states pharmacopoeial convention. **2003**, 2528
[15] Ritger P, Peppas NA. *J Control Rel* **1987,** 5, 37-42.
[16] Korsmeyer RW, Gurny R, Peppas. *Int J Pharm* **1983,** 25-35.