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Formulation, Characterization & Evaluation of Mucoadhesive Microspheres of Flurbiprofen

Prashant Patil[1]*, Santosh Singh[1], Janardhan Sarvanan[2]
[1]Department of Pharmaceutics, School of Pharmacy Suresh Gyan Vihar University Jaipur, Rajesthan.
[2]Department of Pharmaceutical Chemistry, PES College of Pharmacy Bengaluru, Karnataka.
Email Id : pprash.patil5@gmail.com, pprashant32@yahoo.com

ABSTRACT
The objective of this work is to localize drugs at a certain site (colon) in the body and increasing the residence time of the drug at the absorption site can enhance extent of drug absorption i.e controlled release formulation and The target for interactions of most of the bioadhesive polymers is mucus, the main component of mucus secretion is the glycoprotein fraction, which is responsible for its gel like characteristics. Multiparticulate formulations have advantage over conventional tablet or capsule formulations, since it increases the surface area exposed to the absorption site and thus increasing the absorption of the drug and decreasing the dosing frequency of the drug. This main aim is to formulation and evaluation of mucoadhesive microspheres of Flurbiprofen with various polymers as like HPMC K4M, sodium alginate & microcrystalline cellulose. Flurbiprofen is a non-steroidal anti-inflammatory agent which is prescribed widely in various colon diseases ulcerative colitis, Crohn's disease, carcinomas and infections, Flurbiprofen shows maximum absorption in the lower gastrointestinal tract regions, also shows half life 4-5hours, it shows low bioavailability orally. The mucoadhesive microsphere formulations were characterized for its production yields, actual drug content and encapsulation efficiency. The formulations were prepared by 3^2 factorial design with various ratios of HPMC K4M and sodium alginate and the optimization is done by statistical optimization technique. Three dependent variables considered are; Time required to release 50% of drug ($T_{50\%}$), Time required to release 90% of drug ($T_{90\%}$) and percent drug release at 8h,. The release profile data was subjected to curve fitting analysis for describing the release mechanism of Flurbiprofen from mucoadhesive microspheres of Flurbiprofen. The decrease in Flurbiprofen release was observed by increasing the amount of HPMC K4M and sodium alginate. The evaluation of Mucoadhesive microspheres were done by release study is done by in vitro release analysis and also in vitro and in vivo study for mucoadhesive strength determination.
Key Words: *Microspheres, Mucoadhesive, Ionic gelation, Factorial design.*

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INTRODUCTION

Microspheres formulations have beneficial than conventional drug delivery systems, it increases surface area exposed to absorption area & thus enhances the absorption of the drug and frequency of the drug doses decreases. The colon, as a site for drug delivery, offers distinct advantages on account of a near neutral pH, a much longer transit time, relatively low proteolytic enzyme activity, and a much greater responsiveness to absorption enhancers. Various diseases of colon such as ulcerative colitis, Crohn's disease, carcinoma and infections require local therapy. So, the development of locally acting colon targeted drug delivery systems may revolutionize the treatment of colonic diseases. The biological surface can either be a epithelial tissue or it can be the mucus coat on the surface of a tissue. If adhesive attachment is to a mucous coat, the phenomenon is referred to as 'Mucoadhesion' [1-5].

MATERIALS AND METHODS

Flurbiprofen was gifted from Teva Pharma (Pvt.) Ltd while Hydroxypropyl methyl cellulose (Methocel K4M) (Colorcon Ltd., UK). Different software was used i.e. central composite design was successfully applied from Design Expert software, version 7.0.0, State-Ease, Inc., Minneapolis. Microsoft Excel, DD solver and SPSS 17.0 (SPSS Inc) were used for the assessment of drug release data. Micropsheres formulations were evaluated for release study, Percentage yield, actual drug content & encapsulation efficiency.

Preparation of Microspheres:

Ionic gelation:

Sodium alginate was added to mucoadhesive polymer & dissolved in purified water forms homogenous polymer solution. Drug adds to polymer alginate mixture stirred to form clear solution resulted solution was then added drop wise into 5% calcium chloride solution by syringe. Added droplets were retained in calcium chloride solution for 25mins to complete reaction produce spherical & rigid microspheres. Product washes with water & dried 45^0c for 12 h. A 3^2 factorial design was implanted for optimization of oral controlled release microspheres. According to the model it contains two independent variables at three levels +1,0 and -1 (Table.1). According to the model total nine formulations possible. The composition of different formulations is shown in (Table.3). The different independent variables include: amount of HPMC K4 M (X_1) & amount of sodium alginate (X_2), Where HPMC K4 M (X_1) & sodium alginate act as a and controlled release polymers. The different dependent responses include: % drug release at 8 hour(Y_1), Time taken to release 50% drug, $T_{50\%}$ (Y_2), Time taken to release 90% drug, (Y_3) [6-11].

Combination Batches for microspheres: -

Table.1 Factorial Design for Preparation of Batches

Batch Code	Variable levels in Coded form	
	X_1	X_2
F1	+1	+1
F2	+1	0
F3	+1	-1
F4	0	+1
F5	0	0
F6	0	-1
F7	-1	+1
F8	-1	0
F9	-1	-1

Translation of coded levels in actual units:

Table.2 Factors and their corresponding levels for the construction of 3^2 factorial design

Variable levels	Low (-1)	Medium (0)	High (+1)
X_1= Concentration of HPMC K4 M (mg)	60	80	100
X_2= Concentration of sodium alginate (mg)	60	80	100

Factorial formulations

Table.3 Combination batches by using HPMC K4M & sodium alginate in various concentrations according to 3^2 factorial design.

Batch code/ Content (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Flurbiprofen	100	100	100	100	100	100	100	100	100
HPMC K4M	100	100	100	80	80	80	60	60	60
sodium alginate	60	80	100	60	80	100	60	80	100
Microcrystalline cellulose	80	60	40	60	40	20	40	20	00

***In vitro* release study**

In vitro drug release was studied by dissolution method using dissolution apparatus I (basket). The dissolution was performed in 900 mL (v) phosphate buffer pH 6.8. The temperature was maintained at 37 ± 0.5°C and the speed of basket was kept at 100 rpm during dissolution study. Microspheres filled in capsule and placed in dissolution medium. At appropriate time intervals, 5 mL of the solution was withdrawn, filtered, and the absorbance of samples was measured on UV spectrophotometer (Jasco V-630,Japan) at 247 nm, while an equal volume of fresh dissolution medium was added into the apparatus. Dissolution tests were performed in triplicate. The % drug release was calculated by PCP disso software and reported in results (table.4) [12-13].

Yields of production:

The yields of production microspheres of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation

of microspheres and percent production yields were calculated as per the formula mentioned below and results are reported in results(table.4) [14-16].

Production yield = Practical mass(microspheres) X 100……..1
Theoretical mass(polymer+drug**)**

Actual drug content and encapsulation efficiency:

Wherein the calcium chloride solution in which the microspheres were prepared was estimated for its drug content through UV spectroscopy by taking its absorbance at 247nm and the amount of unloaded drug was estimated, then determined amount of drug was deducted from the total quantity of drug added initially to obtain the amount of drug which is encapsulated. Encapsulation efficiency was determined by direct method wherein the microspheres were immersed in the water for 24 hours with constant shaking which would result in the extraction of drug from the microspheres in water, which is then quantitatively estimated trough UV spectroscopy by taking its absorbance at 247nm and the value thus obtained is used to determine the encapsulation efficiency of the microspheres using the formula mentioned below and encapsulation efficiency values were reported in results(table.4) [17-19].

Percent encapsulation efficiency = Actual drug content(mg) X 100……..2
Total mass of microspheres

Morphology of microspheres:

The shape and size of microspheres of the optimized batches was determined through optical microscope and through SEM (cameca, france model-SV30). Results are reported results(table.6) [20-23].

***In vitro* mucoadhesion strength determination of microparticles:**

A freshly excised sheep's stomach was used. Prior to the study, the mucus surface of the tissue was rinsed with normal saline. The tissue was pinned unto a polyethene support inclined at an angle of 60^{o}. A beaker was placed directly under the base of the polyethene to collect the micropaticles as they got detached from the tissue. A 100 mg quantity of the microparticles formulated with various ratios of the polymers was placed on the trough of the mucus surface of the tissue and allowed to hydrate for 15 min for microparticle–mucin interaction to take place. A 100 ml volume of SGF was allowed to flow over the tissue at the rate of 40 drops/min. The weight of the microparticle detached (washed out) calculated as a percentage of the original weight was used as a measure of mucoadhesion. And results are reported (table.5) [24-25].

RESULTS

Drug release study

Factorial batches dissolution studies for ionic gelation technique

Table.4 Data of release study flurbiprofen from factorial batches

		Formulations				
	Time (Hr.)	F1	F2	F3	F4	F5
*** Percent drug release**	1	24.119 ±0.83	29.556 ±1.62	24.212 ±1.06	20.81±0.39	26.833 ±0.39
	2	27.921 ±0.52	41.863 ±1.52	28.033 ±0.41	23.91±0.34	30.446 ±0.34
	3	32.521 ±1.37	56.034 ±0.46	33.022 ±0.25	32.54±0.33	38.293 ±0.33
	4	38.257 ±0.41	60.909 ±0.20	38.419 ±0.17	38.40±0.17	45.876 ±0.17
	5	45.655 ±0.65	66.800 ±0.38	46.391 ±0.24	46.81±0.45	55.492 ±0.45
	6	53.740 ±0.79	71.621 ±0.54	53.962 ±0.92	60.51±0.31	61.955 ±0.79
	7	64.740 ±1.49	77.383 ±1.05	64.778 ±1.03	76.21±1.20	67.165 ±1.49
	8	68.594 ±1.02	83.267 ±0.89	68.706 ±0.35	85.82±0.32	72.633 ±1.02
	9	76.941 ±0.99	85.950 ±1.27	77.017 ±1.06	**93.72±0.29**	81.002 ±0.99
	10	83.795 ±0.41	89.145 ±0.45	83.732 ±0.39	94.30±1.08	94.202 ±0.41
	11	88.924 ±0.18	**95.115 ±0.40**	**95.005 ±0.76**	94.82±1.21	94.619 ±0.18
	12	**96.006 ±0.35**	95.845 ±1.64	95.073 ±1.02	94.88±0.92	94.849 ±0.54
Production yield (%)		84.01	82.80	78.40	82.60	81.00
Actual drug content(%)		77.22	76.19	79.89	80.14	79.63
Encapsulation efficiency (%)		76.89	77.49	78.13	82.10	77.93

*Represents mean ± S.D. (n = 3)

		Formulations			
	Time (Hr.)	F6	F7	F8	F9
***Percent drug release**	1	25.93±0.34	26.833 ±0.56	26.833 ±0.22	25.574 ±0.11
	2	37.00±0.31	37.013 ±0.80	30.446 ±0.23	33.208 ±0.38
	3	46.71±0.34	52.213 ±1.04	38.293 ±0.29	45.423 ±0.28
	4	66.36±0.42	66.403 ±0.29	45.876 ±0.12	53.834 ±0.18
	5	71.18±0.08	76.012 ±0.23	55.492 ±0.22	61.993 ±0.19
	6	76.95±0.54	85.607 ±0.17	61.955 ±0.56	74.354 ±0.50
	7	82.83±0.31	**94.410 ±0.90**	**93.107 ±0.29**	82.11 ±0.54
	8	85.51±1.64	93.989 ±0.57	93.626 ±1.07	**93.775 ±1.29**
	9	88.71±0.59	94.498 ±0.62	94.135 ±0.67	94.284 ±1.40
	10	91.86±0.87	95.006 ±0.64	94.645 ±0.63	94.812 ±1.23
	11	**95.39±0.59**	95.423 ±0.61	94.951 ±1.17	95.349 ±0.74
	12	95.90±0.89	95.838 ±0.54	94.959 ±0.41	95.858 ±0.45
Production yield (%)		79.41	80.71	82.70	81.72
Actual drug content(%)		77.44	81.22	78.51	81.59
Encapsulation efficiency (%)		78.86	82.25	77.29	79.13

DISCUSSION

In vitro dissolution study of the microspheres indicates that Formulation f1 is combination of 100:100 HPMC K4M and sodium alginate shows 100% release upto 12 h. f2 is combination of 100:80 HPMC K4M and sodium alginate shows 100% release upto 11 h. f3 is combination of 100:60 HPMC K4M and sodium alginate shows 100% release upto 11 h.

Formulation f4 is combination of 80:100 HPMC K4M and sodium alginate shows 100% release upto 11 h f5 is combination of 80:80 HPMC K4M and sodium alginate shows 100% release upto 10 h f6 is combination of 80:60 HPMC K4M and sodium alginate shows 100% release upto 9 h.

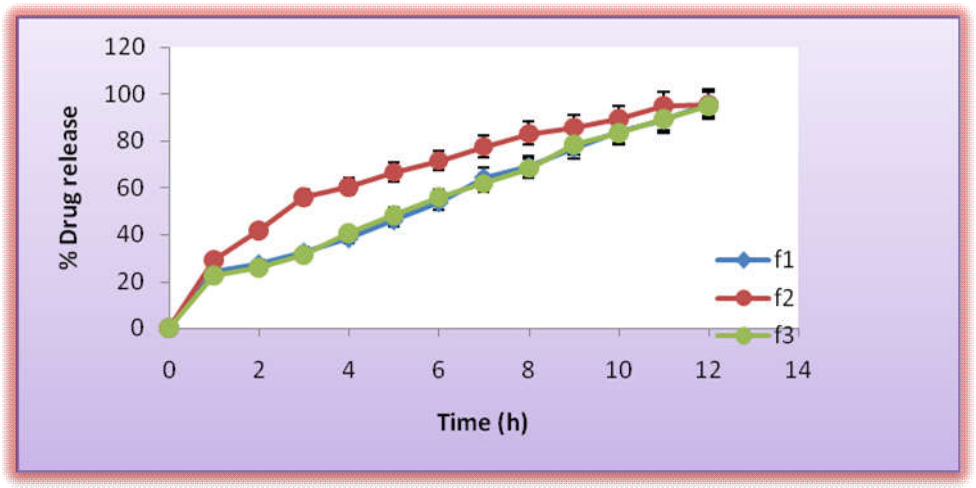
Formulation f7 is combination of 60:100 HPMC K4M and sodium alginate shows 100% release upto 7h f8 is combination of 60:80 HPMC K4M and sodium alginate shows 100% release upto 7h f9 is combination of 60:60 HPMC K4M and sodium alginate shows 100% release upto 7h. From above discussion it was clear that the as we increases the concentration of polymer HPMC K4M release of drug was retarded.

From above discussion formulation f1 was the optimized formulation.

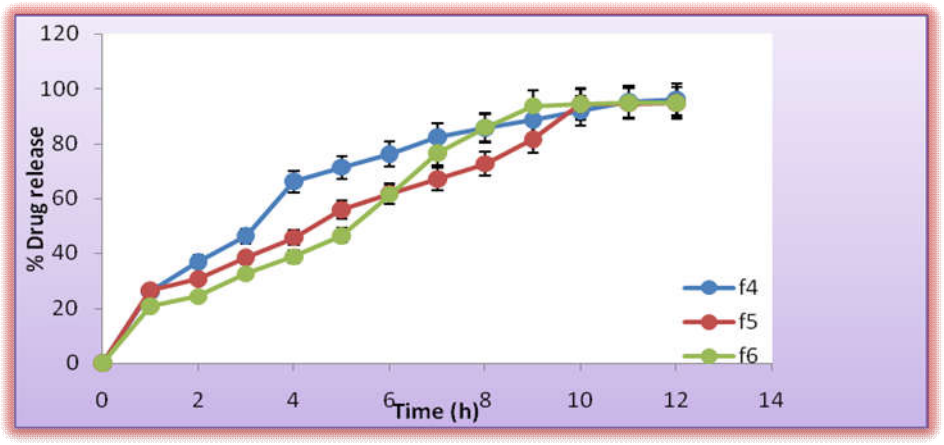
Yield of production, Actual drug content and encapsulation efficiency

The production yields of microspheres prepared through the ionic gelation technique is found in the range of 78-84%. Actual drug content and encapsulation efficiency or drug entrapment efficiency of the microspheres prepared by ionic gelation technique was found to be 75-85%. It is not up to 100% because in ionic gelation technique microspheres prepared in external aqueous solution of calcium chloride and since drug is water soluble, most of the drug gets diffused in this aqueous solution.

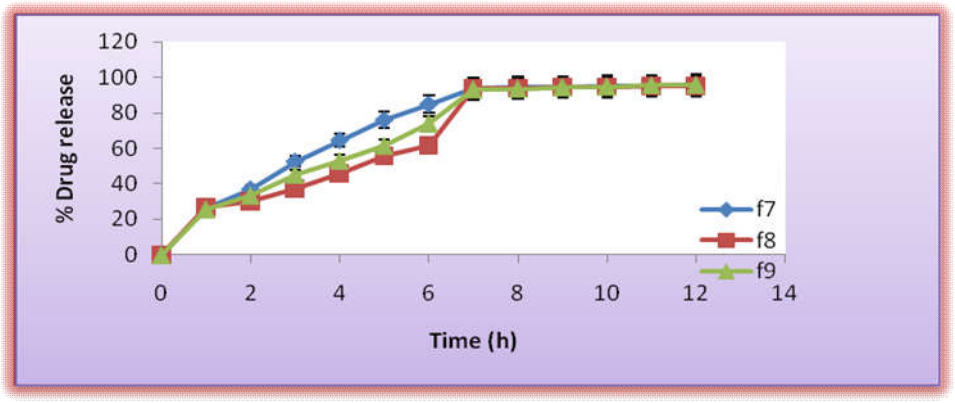
Figure.1 Dissolution profile of A. F1-F3 B. F4-F6 C. F7-F9 formulations for factorial batches



A.



B.



C.

***In vitro* mucoadhesive strength determination**

Table.5 *In vitro* data for mucoadhesive strength determination

SR. NO	WEIGHT (mg) OF MICROSPHERES REMAINING ON GASTRIC MUCOSA				% MUCOADHESIVE STRENGTH
	3h	6h	9h	12h	
F1 (Ionic gelation)	45	41	37	34	78.50

From *in vitro* mucoadhesive strength determination tests it was cleared that in ionic gelation technique optimized formulations shows 79.84% mucoadhesive strength respectively. Ionic gelation formulation comparising of 100:60 of HPMC K4M:sodium alginate it retard the release of drug up to 12 hours due to high mucoadhesive strength

Morphology of microspheres

Morphological study of microspheres done using SEM & microspheres was studied which shows shape of microspheres almost spherical shown in fig no.2 and size shown in table no.6

Figure.2 Morphology of microspheres prepared by Ionotropic gelation (F-1)

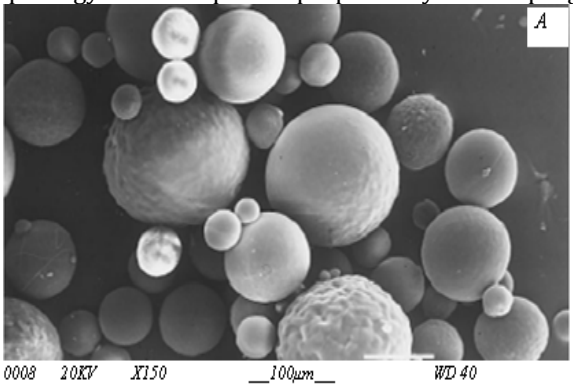


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Table.6 Shape and size of optimized formulations.

FORMULATIONS	SIZE in µm	SHAPE
SIZE in µm(Gelation)	55.32-67.12	Almost spherical

Optimization of mucoadhesive microspheres formulations:

Results of release parameters as $T_{50\%}$, $T_{90\%}$ and flurbiprofen release at 8h for ionic gelation method

Table.7 Results of release parameters

Formulation	$T_{90\%}$ (h)± SD (n=3)	$T_{50\%}$ (h)± SD (n=3)	Flurbiprofen release at 8h (%)± SD (n=3)
F1	5.679± 0.88	5.251 ± 1.05	69.211 ±0.115
F2	3.487 ± 1.45	4.583 ± 0.71	83.267 ±0.264
F3	4.965 ± 0.77	4.94 ± 0.98	68.706 ± 0.115
F4	1.71 ± 1.47	1.66 ± 1.81	85.517 ±0.057
F5	3.329 ± 0.92	4.204 ± 0.54	72.633 ±0.208
F6	4.75 ± 0.88	7.416 ± 1.59	85.825 ±0.264
F7	3.671 ± 1.19	4.926 ± 0.90	94.763 ± 0.115
F8	4.223 ± 0.64	6.09 ±1.35	93.626 ± 0.115
F9	4.236 ± 0.69	6.106 ± 1.41	93.248± 0.1244

Effect of formulation variables.

A. Effect of formulation variables on $T_{50\%}$

The model terms for response Y_1 ($T_{50\%}$) were found to be significant with F value of 4.73 ($p<0.0047$). In this case all the factors were found to be significant and the model describing $T_{50\%}$ can be written as;

$$Y_1 = 2.96 + 0.53X_1 - 0.29X_2 + 0.27X_1X_2 + 0.46X_1^2 + 1.10X_2^2$$

As the amount of X_1 and X_2 increases the corresponding $T_{50\%}$ (time required to release 50% of the drug) also increases The **Fig 3** shows the response surface plot. It indicates at all the high levels of X_1 and X_2 the $T_{50\%}$ value is high, As discussed above this behavior is due to increase in amount of sodium alginate and HPMC K4M forms a high viscous gel matrix and thus decreases the drug release and hence $T_{50\%}$ value increases, while HPMC K4M forms pores in the formed matrix and will increases the drug release thus decreases the $T_{50\%}$ value. The **Fig 4** shows the graph of predicted verses actual data.

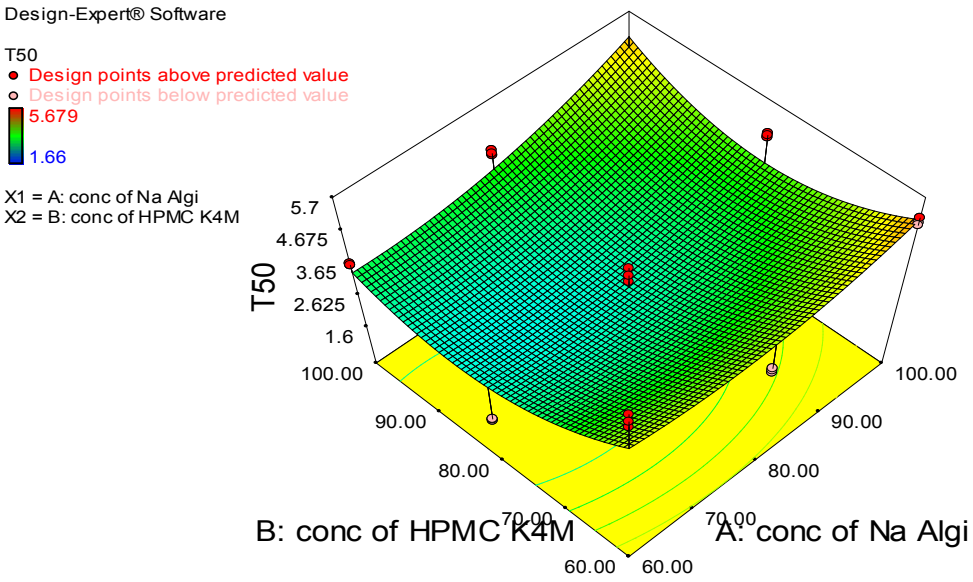

Figure 3 Response surface plot showing effect of formulation variables on $T_{50\%}$

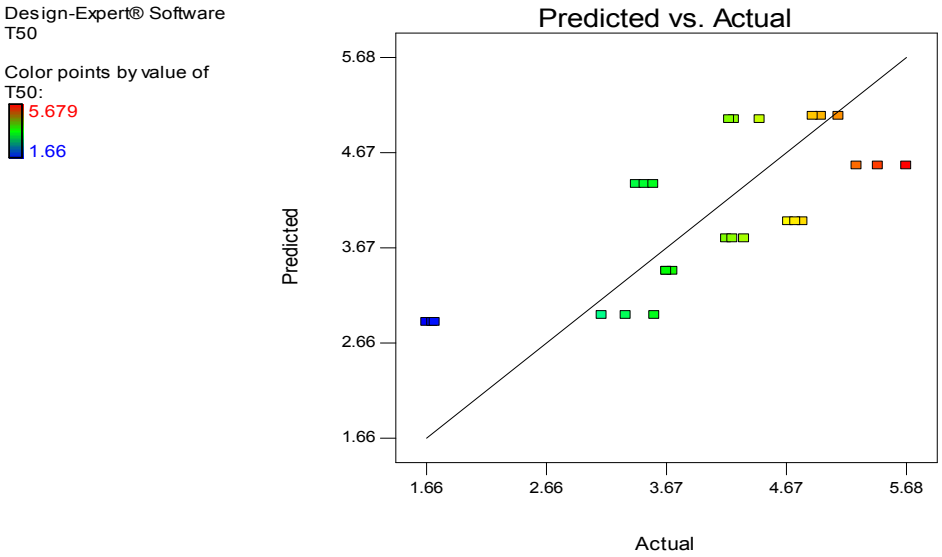

Figure 4 Linear correlation plots between actual and predicted values for $T_{50\%}$ (Y1)

B. Effect of formulation variables on $T_{90\%}$

The model terms for response Y_2 ($T_{90\%}$) were found to be significant with F value of 10.06 ($p<0.0001$). In this case all the factors were found to be significant and the model describing $T_{50\%}$ can be written as;

$$Y_2 = -5.79 + 0.68X_1 - 14.83X_2 + 0.99\,X_1X_2 + 15.32\,X_1^2 + 16.12\,X_2^2$$

As the amount of X_1 and X_2 increases the corresponding $T_{90\%}$ (time required to release 90% of the drug) also increases The **Fig 5** shows the response surface plot. It indicates at all the high levels of X_1 and X_2 the $T_{50\%}$ value is high, As discussed above this behavior is due to increase in amount of sodium alginate and HPMC K4M forms a high viscous gel matrix and thus decreases the drug release and hence $T_{50\%}$ value increases, while HPMC K4M forms pores in the formed matrix and will increases the drug release thus decreases the $T_{90\%}$ value. The **Fig 6** shows the graph of predicted verses actual data.

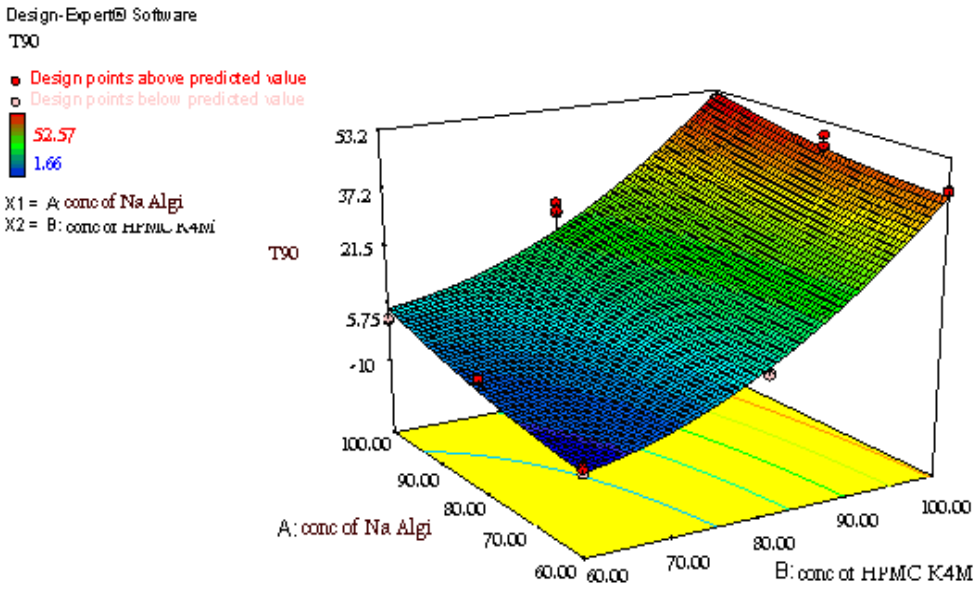

Figure 5 Response surface plot showing effect of formulation variables on $T_{90\%}$

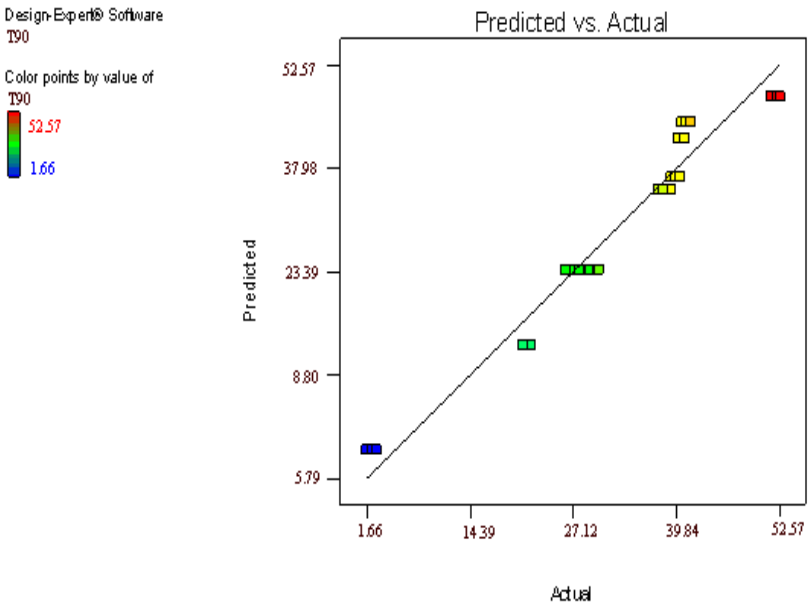

Figure 6 Linear correlation plots between actual and predicted values for $T_{90\%}$ (Y_2)

C. Effect of formulation variables on the drug release at 8 hr. (Y_3)

The quadratic model was found to be significant with an F value 27.44 (P<0.0001). In this case X_1, X_2 was found to be significant and the model describes the percent flurbiprofen release at 8h can be written as;

$$Y_3 = 82.91 - 0.30X_1 + 10.17\,X_2$$

As the concentration of mucoadhesive polymer (sodium alginate and HPMC K4M) increased it causes an increase in viscosity of swollen gel matrix, which contributes more hindrance for drug diffusion and thus decreases the release rate. The combined effect of X_1, X_2 shown in response surface plot (**Fig 7**) In this plots it was observed that the increasing amount of sodium alginate and HPMC K4M causes the decreases in the drug release, due to formation of high viscous gel matrix. Thus the factors X_1 and X_2 have negative effect on the drug release. The **Fig 8** Shows a graph of observed verses predicted values. The sodium alginate and HPMC K4M have negative effect on drug release, due to increased viscosity and gel strength. The swelling of sodium alginate may be due to uncharged –COOH group which forms hydrogen bonds with imbibing water and also holds water inside the gel matrix. Increasing amount of HPMC K4M which contains –OH groups will may increases the formation of hydrogen bonding and form a gel matrix network with sodium alginate.

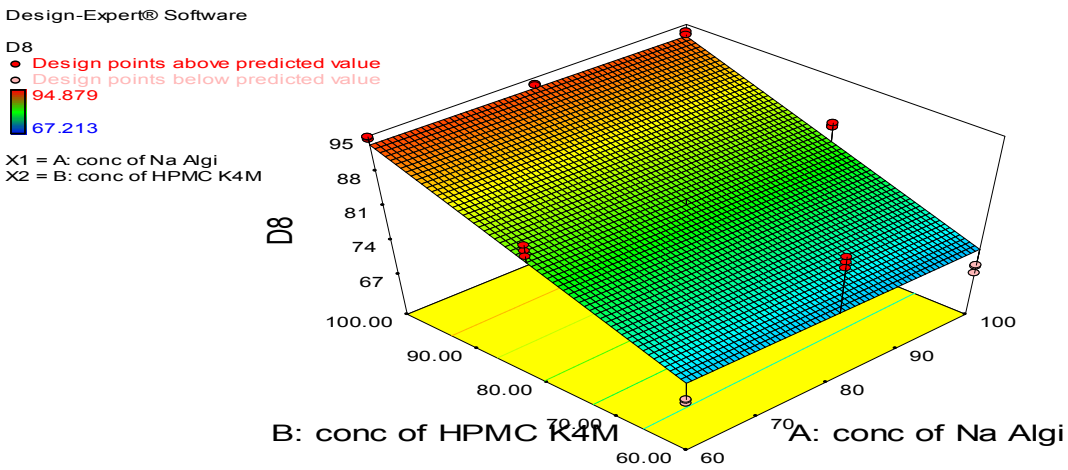

Figure 7 Response surface plot showing effect of formulation variables on percent drug release at 8 h

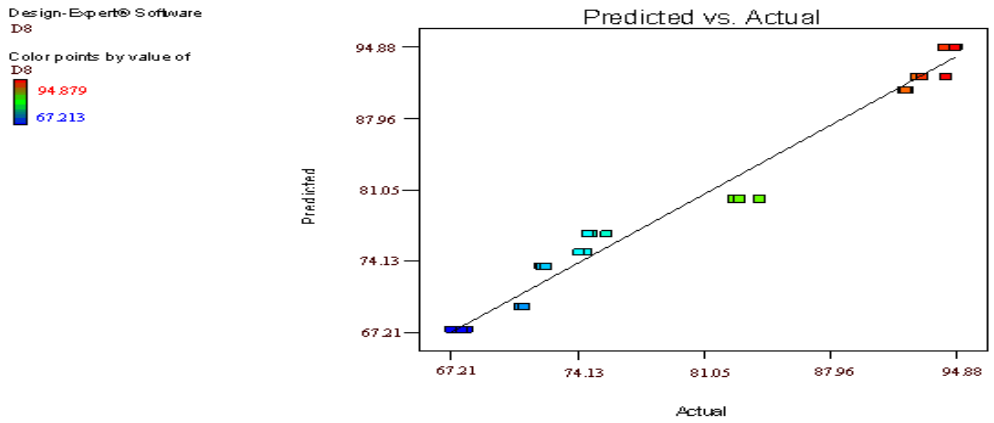

Figure 8 Linear correlation plots between actual and predicted values for percent drug release at 8 h (Y_3).

ANOVA, Pure error, Lack of fit:

The results of ANOVA in **Table 8** for the dependent variables demonstrate that the model was significant for all response variables. Regression analysis was carried out to obtain the regression coefficient (**Table 9**) and effects as follows; all factors found to be significant for response Y_1, similarly only X_1, X_2 and X_1X_2 were found for Y_2, the X_1, X_2 were found significant for Y_3. The above results conveyed us that the amount of sodium alginate, HPMC K4M plays important role in formulation of mucoadhesive microspheres of flurbiprofen. Thus appropriate range of these variables yields an optimized mucoadhesive microspheres with good bioadhesive strength and drug release. The data of pure error and lack of fit are summarized in **Table 8** The residuals are the difference in the observed and predicted value. Since computed F values were respectively less than critical F values, denotes non-significance of lack of fit.

Table.8 Data of ANOVA study for dependent variables from 3^2 factorial design

Source	d.f.	Sum square	Mean square	F value	Probability
$T_{50\%}$ (h)					
X_1	1	5.08	5.08	7.56	0.0120
X_2	1	1.48	1.48	2.20	0.1526
X_1X_2	1	0.87	0.87	1.30	0.2677
$T_{90\%}$ (h)					
X_1	1	8.28	8.28	0.060	<0.0001
X_2	1	3959.58	3959.58	28.66	<0.0001
X_1X_2	1	11.80	11.80	0.085	0.0009
NF release at 8 h (%)	1	1.60	1.60	0.047	0.8298
X_1	1	1862.21	1862.21	54.83	<0.0001
X_2					

Table.9 Data of ANOVA study for results in analyzing lack of fit and pure

Source	d.f.	Sum square	Mean square	F value	Probability
$T_{50\%}$ (h)					
Model	5	15.89	3.18	4.73	0.0047
Residual	21	14.10	0.67	-----	-----
Total	26	30.00	-----	-----	-----
Lack of fit	3	13.82	4.61	295.79	<0.0001
Pure error	18	0.28	0.016	-----	
$T_{90\%}$ (h)					
Model	5	6948.06	1389.61	10.06	<0.0001 *
Residual	21	2901.00	138.14	-----	-----
Total	26	9849.06	-----	-----	-----
Lack of fit	3	2900.00	966.67	17347.34	<0.0001
Pure error	18	1.00	0.056	-----	

NF release at 8 h (%)	2	1863.81	931.91	27.44	<0.0001 *
Model	24	815.18	33.97	-----	-----
Residual	26	2678.99	-----	-----	-----
Total	6	804.28	134.05	221.14	<0.0001
Lack of fit	18	10.90	0.61	-----	
Pure error					

Optimization:

A numerical optimization technique by the desirability approach was used to generate the optimum settings for formulation. The process was optimized for dependent variables Y_1-Y_4. The optimized formula arrived by targeting the Y_1 was targeted at 6 h, , Y_2 was targeted at 10 h,Y_3 was kept at range 70-80% drug release. The optimized results obtained to give 7 results out of that one formula is shown in **Table 10**. The results of optimized formula were compared with the predicted values and it was shown in **Table 11** which showed good relationship between experimented and predicted values, which confirms the practicability and validity of the model.

Table.10 Composition of optimized formulation

Ingredients	Quantities (mg)
Drug	50
Sodium alginate	100
HPMC K4M	60

Table 11:- Comparison between the experimented and predicted values for most probable optimal formulation

Dependent variables	Optimized formulation	
	*Experimented value	Predicted value
Sodium alginate	98.908 ± 2.48	98.225
HPMC K4M	57.23 ± 0.11	57.3833

*Represents mean ± S.D. (n = 3)

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