# Optimization & Comparative Study of Micro-particulate System of Flurbiprofen Prepared by Different Methods

**Prashant Patil [1*], Santosh Singh[2], Girish kashid[3]**

## Abstract

Comparative study of ionic gelation & spray dry methods for preparation of microspheres to deliver drugs has many other advantages over one another, like control-release of the drug, increase bioavailability and target delivery of the drug to the required site. In this research work shows the use of encapsulating sodium alginate, sodium carboxy methyl cellulose in biodegradable microsphere delivery system, to be delivered orally via a capsule, to gives desired therapeutic action. Microsphere formulations have merits 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. Flurbiprofen is a NSAIDs which is used widely in different intestinal diseases ulcerative colitis, Crohn's disease, carcinomas and infections. Flurbiprofen shows maximum absorption in the lower gastrointestinal tract regions, and shows half-life 4 hrs, it shows low bioavailability orally. The microsphere formulations were evaluated for its production yields, actual drug content, encapsulation efficiency, % Swelling Index release study is done by in vitro release analysis, mucoadhesive strength determination in vitro & in vivo methods.

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## Introduction

The idea of mucoadhesive system came from the need to localize drug at a certain site in the body, often as the extent of drug absorption is limited due to the residence time of drug at the absorption site. In oral drug delivery, drug absorption is limited due to the gastrointestinal transit time of the dosage form. To illustrate suppose if a drug dosage form is to deliver a drug in a sustained manner for treating some chronic disease then it is required that the dosage form should remain at the site of absorption which is mainly upper part of the intestine, for a prolonged period of time but this is limited due to the gastrointestinal transit of the dosage form, so mucoadhesive dosage forms are formulated with the purpose of binding with the mucus layer of the GIT and thus increasing the residence time of the drug and also providing the intimate contact between a dosage form and the absorbing tissue and hence enhancing the absorption of the drug1-3.

## Materials and Methods

Flurbiprofen gift sample available from Teva Pharma (Pvt.) Ltd., while Sodium alginate & Sodium carboxy methyl cellulose (Colorcon Ltd., UK). 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 and encapsulation efficiency.

## Microsphere Preparation method

Ionic gelation4-8:
1) Sodium alginate was added to mucoadhesive polymer & dissolved in purified
water forms homogenous polymer solution.

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2) Drug add to polymer alginate mixture stirred to form clear solution resulted solution

was then added drop wise into 5% calcium chloride solution by syringe.

3) Added droplets were retained in calcium chloride solution for 25mins to complete

reaction produce spherical & rigid microspheres.

4) Product wash with water & dried 450c for 12 h. Formulation composition given in Table.4

### Factorial Batches

A 32 factorial design was implanted for optimization of oral controlled release tablet tablets. According to the model it contains two independent variables at three levels +1,0 and -1 (Table.2). According to the model total nine formulations possible. The composition of different formulations is shown in (Table.4). The different independent variables include: amount of HPMC K4 M (X1) & amount of sodium alginate (X2), Where HPMC K4 M (X1) & sodium alginate act as a and controlled release polymers. The different dependent responses include: % drug release at 8 hour(Y1), Time taken to release 50% drug, T50% (Y2 ), Time taken to release 90% drug, (Y3 ).

### Combination Batches for microspheres

#### Table1: Factorial Design for Preparation of Batches

| Batch Code | Variable levels in Coded form | |
|---|---|---|
| | X1 | X2 |
| 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

#### Table2: Factors and their corresponding levels for the construction of 32 factorial design

| Variable levels | Low (-1) | Medium (0) | High (+1) |
|---|---|---|---|
| X1= Concentration of HPMC K4 M (mg) | 60 | 80 | 100 |
| X2= Concentration of sodium alginate (mg) | 60 | 80 | 100 |

### Factorial formulations

#### Table3: Combination batches by using HPMC K4M & sodium alginate in various concentrations according to 32 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  K15M | 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 |
| Total | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 |

## Spray dray technique9-13

Mucoadhesive microspheres were prepared by spray drying technique. An aqueous solution containing different combinations/ratios of the polymers (Table 5) were prepared by dissolving sodium alginate and carboxy methylcellulose in distilled deionized water. The drug (1 g), previously dissolved in 100 ml of absolute methanol, was added to the polymer solution and sonicated using Ultra sonicator (1204 AU-Vibracell, USA) to obtain a homogeneous mixture. A crosslinking agent, glutaraldehyde (0 – 0.30 ml), was added to the homogenized solution and The resultant solution was spray dried using LU-222 ADVANCED lab spray drier (Labultima, India) for preparing microspheres through the nozzle of a spray-dryer (JISL, LSD- 48 mini spray dryer, India) at input temperature of 115 -117 °C, output temperature of 80 – 85 °C at 2 % feed rate and vacuum pressure of 35 psi (2.4 kg/cm2). The resulting microspheres were collected from the spray dryer and kept in a desiccator containing silica gel pending further tests.

## Factorial formulations

**Table4: Combination batches by using Sodium alginate & CMC in various concentrations according to 32 factorial design.**

| CONTENT (Wt in mg.) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| Flurbiprofen : Sodium alginate : Sodium CMC | 1:2:0 | 1:2:1 | 1:2:2 | 1:2:0 | 1:2:1 | 1:2:2 | 1:2:0 | 1:2:1 | 1:2:2 |
| Cross linking agent (%) | 00 | 00 | 00 | 20 | 20 | 20 | 30 | 30 | 30 |

## Factorial Batches10-13

A 32 factorial design was implanted for optimization of oral controlled release tablet tablets. According to the model it contains two independent variables at three levels +1,0 and -1. According to the model total nine formulations possible. The composition of different formulations is shown in (Table.5). The different independent variables include: drug: polymer ratio (X1) &percentage of Cross linking agent (X2), Where carboxyl methyl cellulose & sodium alginate act as an controlled release polymers. The different dependent responses include: % drug release at 8 hour (Y1), Time taken to release 50% drug, T50% (Y2), Time taken to release 90% drug, (Y3 ).

## Combination Batches for microspheres

**Table5: Factorial Design for Preparation of Batches**

| Batch Code | Variable levels in Coded form | |
|---|---|---|
| | X1 | X2 |
| 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 |

X1 : drug: polymer ratio X2 : Concentration of Cross linking agent

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## Evaluations of Microspheres

### Yields of production[14-18]

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.

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

### Actual drug content and encapsulation efficiency[14-18]

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.

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

### Morphology of microspheres[14-18]

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.

### Swelling studies[11-14]

The swelling ability of the uncoated microspheres in physiological media was determined by immersing an accurately weighed amount (500 mg) of microspheres in a little excess of 100 ml of phosphate buffer (pH 6.8) and kept for 24 h. equation was to compute the degree of swelling.

$S_{sw}$ = (Ws - Wo/Wo) x 100 ...................... 3

where $S_{sw}$ = percent swelling of microspheres,
Wo = initial weight of microspheres, Ws = weight of microsphere after swelling.

### In vitro release study[19-24]

In vitro drug release was studied by dissolution method using dissolution apparatus I (basket). The dissolution was performed in 900 mL (v) acidic buffer pH 1.2. 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 224 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.

### Study of release mechanism by Curve fitting

Release data were fitted to various mathematical models for describing the release mechanism from buccal tablets; Korsmeyer–Peppas (Eq. (4)), zero-order (Eq. (5)) and Higuchi release models (Eq. (6)). And reported in results.

$M_t/M_\infty$ = $kKP_t^n$ ................................................... 4

Where, $M_t/M_\infty$ = fraction of drug released at time 't';

$kKP$ = release rate constant;
        n = release exponent.

$M_t = M_0 + k_0 t$ ..................................................... 5

Where, $M_t$ = Amount of drug released at time 't';

$M_0$ = concentration of drug in the solution at t=0;

$k_0$ = zero-order release constant.

$M_t$ = $kH$ $t^{1/2}$ ............................................................ 6

Where, $M_t$ = Amount of drug release at time

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'√t';

kH = Higuchi release constant.

All curve fitting, simulation and plotting was carried out by using disso software (PCP V3). The mechanism of the drug release is discussed in results.

## In vitro mucoadhesion strength determination of microparticles[25-29]

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 60o. 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.

## In vivo studies[30-34]

1. Weight count method

In this technique 5 groups of four number of Albino rats fasted overnight & 100mg of microspheres suspension administer to these rats through needle, then these rats sacrifies at an interval of 0, 4, 8, 12 h respectively. Then after dissection their stomach region isolate & cut open longitudinally to note the weight of microspheres adhering to the stomach & intestine region, which give their adhesive strength using the formula given below.

$$\% \text{ adhesive strength} = \frac{N_o - N_s}{N_s} \times 100 \quad \text{.........7}$$

Were, No = Weight of microspheres hydrated with little amount of water

Ns = Weight of microspheres detaching from mucosal surface.

And results are reported.

## Factorial batches dissolution studies for ionic gelation technique

| | | Formulations | | | | |
|---|---|---|---|---|---|---|
| | | 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)

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| | | 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 |

**Table6: Data of release study flurbiprofen from factorial batches**

## Discussion

In vitro dissolution study of the microspheres indicates thatFormulation 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.

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## 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.
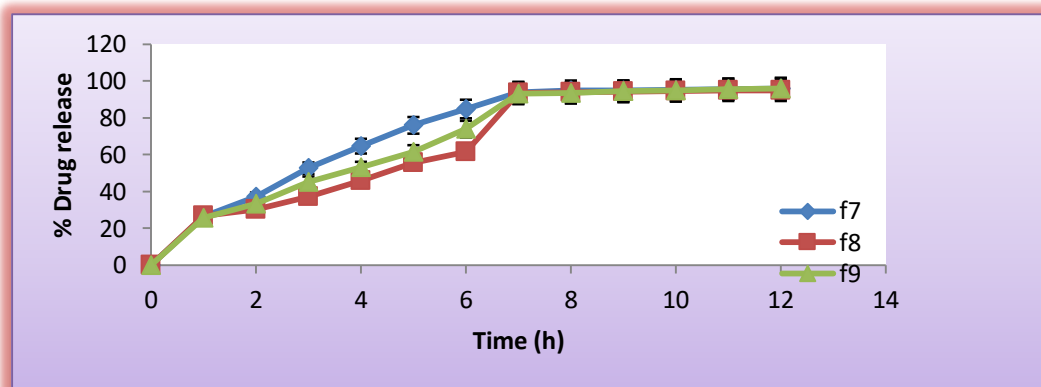
A.



B.

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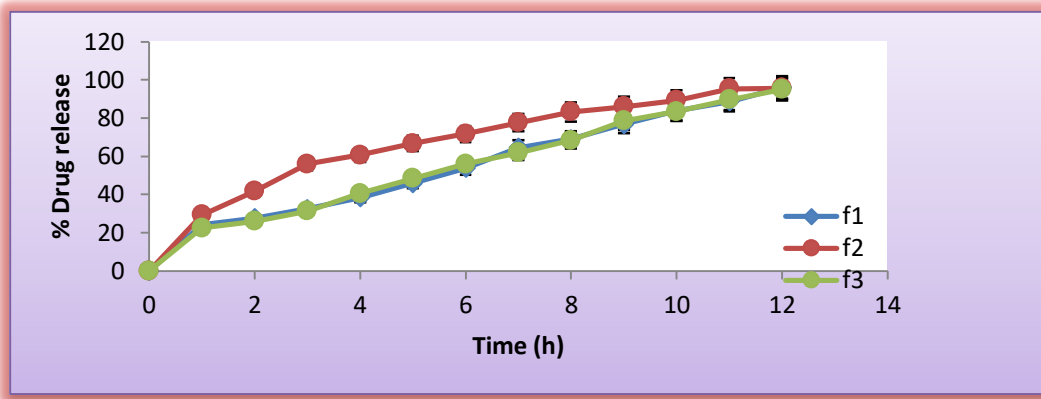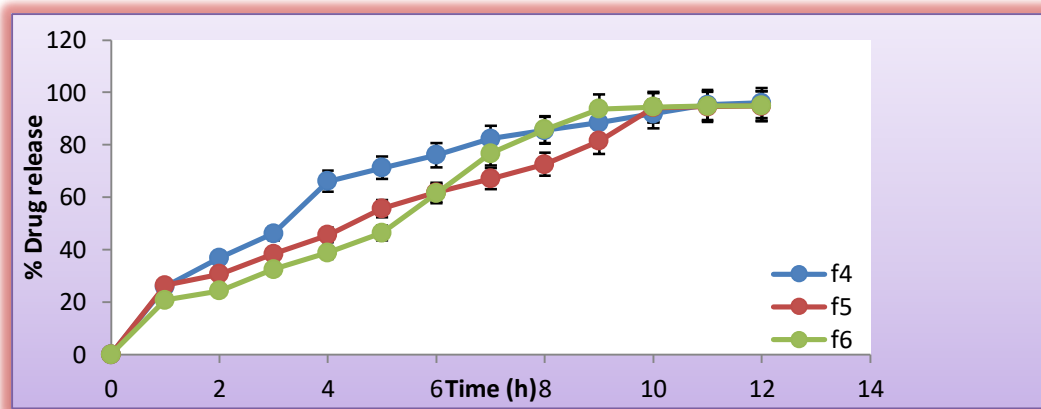


C.

**Figure1: Dissolution profile of A. F1-F3 B. F4-F6 C. F7-F9 formulations for factorial batches**

### Release mechanism

In order to understand the complex mechanism of drug release from mucoadhesive microspheres, the in vitro flurbiprofen release data fitted to korsmeyer-peppas release model. For non fickian release, the values of n falls between 0.5 to 1; while in case of fickian diffusion, n=0.5; For zero order release (case II transport), n=1; and for super case II transport, n>1(15).The values of n were between 0.5-1 (Table III), indicating that the release of flurbiprofen was found to be non-fickian diffusion. These formulations follows korsmeyer-peppas, zero order, andhiguchi model. The table no. shows the R2 (correlation coefficient) and constants for the korsmeyer-peppas, zero order, andhiguchi models.

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### Table7: Data for study of release mechanism by curve fitting analysis

| Formulation | Korsmeyer-Peppas | | Zero order | | Higuchi | |
|---|---|---|---|---|---|---|
| | *Kkp (h-n) | *R2 | *K0 (h1) | *R2 | *KH (h1/2) | *R2 |
| F1 | 19.2353 ± 1.065 | 0.9717 ±0.001 | 8.5290 ± 0.335 | 0.9729 ±0.002 | 20.5343 ±0.687 | 0.9734 ±0.002 |
| F2 | 30.9127 ± 2.600 | 0.9958 ±0.003 | 9.7815 ±0.319 | 0.8041 ±0.003 | 27.4527 ±0.784 | 0.9823 ±0.008 |
| F3 | 19.4774 ± 1.104 | 0.9735 ±0.001 | 8.5003 ±0.244 | 0.9697 ±0.006 | 23.6584 ±0.538 | 0.9788 ±0.007 |
| F4 | 27.0174 ± 0.969 | 0.9854 ±0.002 | 10.0069 ±0.172 | 0.8348 ±0.011 | 29.0911 ±0.895 | 0.9864 ±0.001 |
| F5 | 22.5523 ± 2.315 | 0.9824 ±0.010 | 9.1331 ± 0.145 | 0.9467 ±0.010 | 22.4401 ±0.623 | 0.9763 ±0.013 |
| F6 | 16.5141 ± 0.256 | 0.9732 ±0.009 | 9.3929 ± 0.348 | 0.9664 ±0.008 | 20.2178 ±0.879 | 0.9725 ±0.014 |
| F7 | 28.3458 ± 0.775 | 0.9734 ±0.002 | 10.5075 ±0.162 | 0.7907 ±0.002 | 18.3309 ±0.997 | 0.9467 ±0.011 |
| F8 | 22.2536 ±3.363 | 0.9628 ±0.001 | 9.8672 ± 0.210 | 0.9152 ±0.001 | 18.8960 ±0.994 | 0.9489 ±0.009 |
| F9 | 24.2871 ± 0.969 | 0.9825 ±0.002 | 10.1515 ±0.172 | 0.8842 ±0.002 | 19.0023 ±0.978 | 0.9633 ±0.006 |

*Represents mean ± S.D. (n = 3)

## Factorial batches dissolution studies spray dry

### Table8: Data of release study flurbiprofen from factorial batches

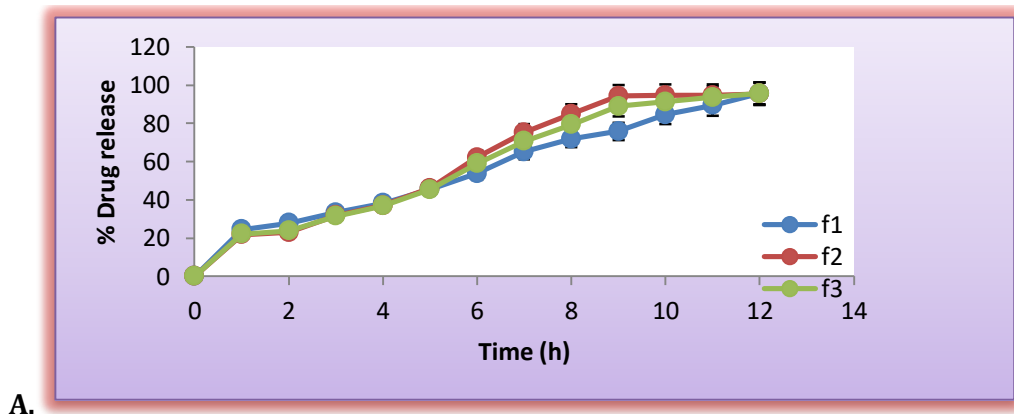| | | Formulations | | | | |
|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 |
| * Percent drug release | 1 | 24.753 ±0.21 | 26.674 ±0.21 | 29.548 ±1.45 | 21.81±0.39 | 27.813 ±0.54 |
| | 2 | 31.346 ±0.20 | 33.218 ±0.35 | 41.863 ±1.54 | 24.91±0.34 | 30.546 ±0.34 |
| | 3 | 39.293 ±0.28 | 46.423 ±0.28 | 57.134 ±0.46 | 32.74±0.33 | 39.293 ±0.33 |
| | 4 | 46.876 ±0.12 | 54.834 ±0.18 | 61.909 ±0.20 | 39.40±0.17 | 45.886 ±0.17 |
| | 5 | 55.592 ±0.26 | 61.853 ±0.19 | 66.800 ±0.38 | 45.81±0.45 | 56.492 ±0.45 |
| | 6 | 62.555 ±0.65 | 75.354 ±0.55 | 71.621 ±0.54 | 61.51±0.31 | 63.955 ±0.79 |
| | 7 | 93.121 ±0.29 | 83.11 ±0.54 | 78.383 ±1.05 | 77.21±1.20 | 67.765 ±1.49 |
| | 8 | 93.726 ±1.07 | 93.982 ±1.28 | 83.467 ±0.89 | 85.72±0.32 | 72.633 ±1.02 |
| | 9 | 94.035 ±0.67 | 94.184 ±1.40 | 95.255 ±0.44 | 93.82±0.29 | 82.102 ±0.99 |
| | 10 | 94.545 ±0.66 | 94.742 ±1.23 | 95.310 ±0.32 | 94.20±1.08 | 94.401 ±0.42 |
| | 11 | 94.931 ±1.17 | 94.949 ±0.74 | 95.422 ±0.40 | 94.72±1.21 | 94.719 ±0.18 |
| | 12 | 94.960 ±0.43 | 95.558 ±0.55 | 95.556 ±1.64 | 94.78±0.82 | 94.849 ±0.14 |
| Production yield (%) | | 31.55 | 35.25 | 44.25 | 33.74 | 42.98 |
| Encapsulation efficiency (%) | | 60.24 | 71.44 | 80.11 | 72.14 | 79.55 |
| Swelling index (%) | | 204±8 | 212±6 | 260±5 | 158±4 | 169±4 |

*Represents mean ± S.D. (n = 3)

| | | Formulations | | | |
|---|---|---|---|---|---|
| | | F6 | F7 | F8 | F9 |
| *Percent drug release | 1 | 25.33±0.31 | 26.003 ±0.14 | 24.512 ±1.16 | 24.619 ±0.45 |
| | 2 | 36.00±0.32 | 37.253 ±0.80 | 29.013 ±0.41 | 27.721 ±0.52 |
| | 3 | 46.81±0.34 | 53.213 ±1.04 | 33.332 ±0.25 | 32.561 ±1.37 |
| | 4 | 67.36±0.42 | 65.403 ±0.29 | 38.429 ±0.17 | 38.557 ±0.41 |
| | 5 | 71.18±0.08 | 76.212 ±0.23 | 47.391 ±0.24 | 46.655 ±0.65 |
| | 6 | 76.85±0.51 | 84.624 ±0.17 | 53.882 ±0.92 | 53.780 ±0.79 |
| | 7 | 81.83±0.31 | 94.110 ±0.77 | 65.778 ±1.23 | 65.721 ±1.49 |
| | 8 | 85.11±1.64 | 94.252 ±0.27 | 69.706 ±0.35 | 69.594 ±1.02 |
| | 9 | 86.71±0.59 | 94.457 ±0.62 | 77.517 ±1.06 | 76.741 ±0.99 |
| | 10 | 95.59±0.59 | 95.106 ±0.44 | 83.532 ±0.39 | 83.195 ±0.41 |
| | 11 | 95.66±0.54 | 95.553 ±0.61 | 95.273 ±1.87 | 88.544 ±0.24 |
| | 12 | 95.80±0.19 | 95.778 ±0.44 | 95.410 ±1.51 | 96.146 ±0.45 |
| Production yield (%) | | 51.65 | 39.54 | 55.64 | 62.95 |
| Encapsulation efficiency (%) | | 88.12 | 73.25 | 80.54 | 92.57 |
| Swelling index (%) | | 175±6 | 118±5 | 130±4 | 148±4 |

## Discussion

In vitro dissolution study of the microspheres indicates thatFormulation f1 is combination of 1:2:0 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 0.0 % shows 100% release upto 7.0 h. f2 is combination of 1:2:1 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 0.0 % shows 100% release upto 8.0 h. f3 is combination of 1:2:2 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 0.0 % shows 100% release upto 9.0 h.

Formulation f4 is combination of 1:2:0 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 20.0 % shows 100% release upto 9.0 h f5 is combination of 1:2:1 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 20.0 % shows 100% release upto 10 h f6 is combination of 1:2:2 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 20.0 % shows 100% release upto 10 h.

Formulation f7 is combination of 1:2:0 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 30.0 % shows 100% release upto 7h f8 is combination of 1:2:1 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 30.0 % shows 100% release upto 11h f9 is combination of 1:2:2 Flurbiprofen : Sodium alginate : Sodium CMC & cross linking agent 30.0 % shows 100% release upto 12h. From above discussion it was clear that the as we increases the concentration of polymer & cross linking agent release of drug was retarded.

From above discussion formulation f9 was the optimized formulations.
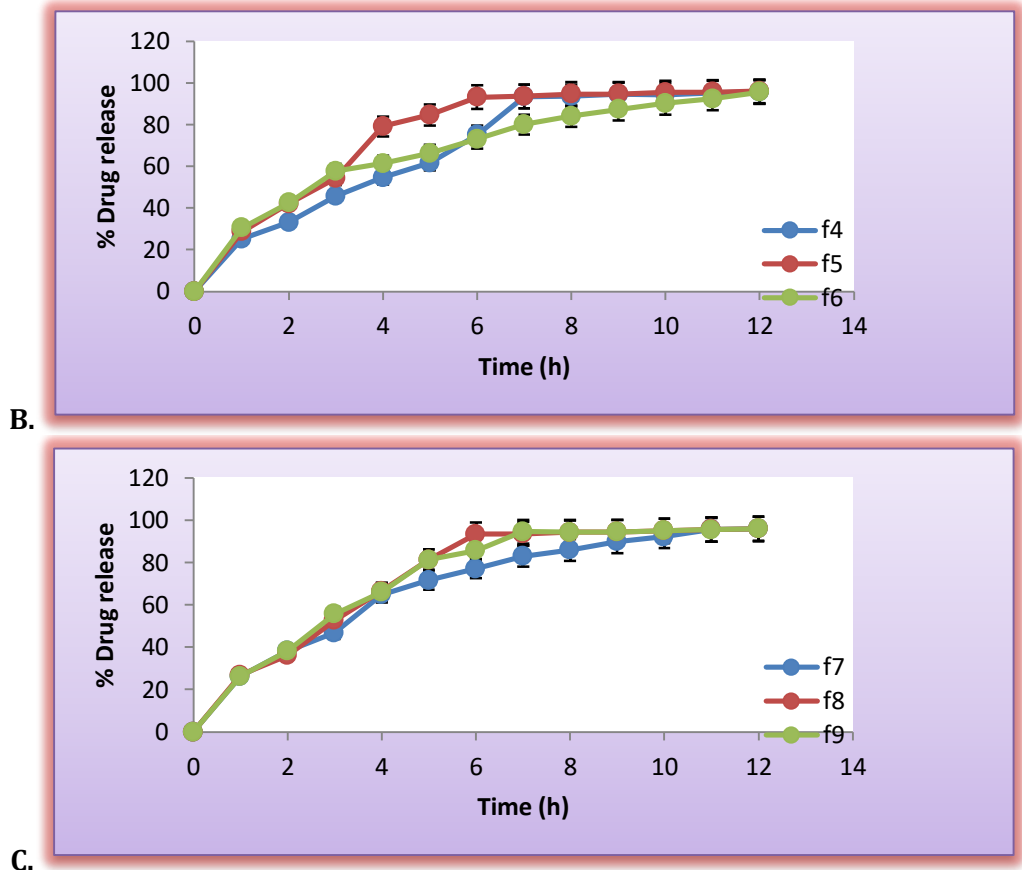
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A.

B.



C.

**Figure2: Dissolution profile of A. F1-F3 B. F4-F6 C. F7-F9 formulations for factorial batches**

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### Yield of production, Actual drug content and encapsulation efficiency

The production yields of microspheres prepared through the spray dry technique is found in the range of 30-62%. Actual drug content and encapsulation efficiency or drug entrapment efficiency of the microspheres prepared by spray dry technique was found to be 60-92%.

### In vitro mucoadhesive strength determination

**Table9: 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 |
| F9 (Spray dry) | 44 | 41 | 35 | 32 | 76.00 |

## Ex vivo mucoadhesive strength determination

**Table10:  In vivo data for mucoadhesive strength determination**

| Sr.no | Weight (mg) of microspheres remaining on the rat stomach | | | | % mucoadhesive strength |
|---|---|---|---|---|---|
| | Time (h) | | | | |
| Optimized | 0 | 4 | 8 | 12 | |
| Ionic gelation | 98.30 | 81.31 | 72.31 | 67.44 | 79.84 |
| Spray dry | 98.12 | 80.34 | 74.21 | 64.32 | 78.24 |

From both in vitro and in vivo mucoadhesive strength determination tests it was cleared that in ionic gelation & Spray dry technique optimized formulations shows 79.84% & 78.245 mucoadhesive strength respectively. Ionic gelation formulation comparising of 100:60 of HPMC K4M:sodium alginate & Spray dry formulation comparising of 1:2:2 ratio of flurbiprofen: Sodium alginate: Sodium CMC 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.3 and size shown in table no.9
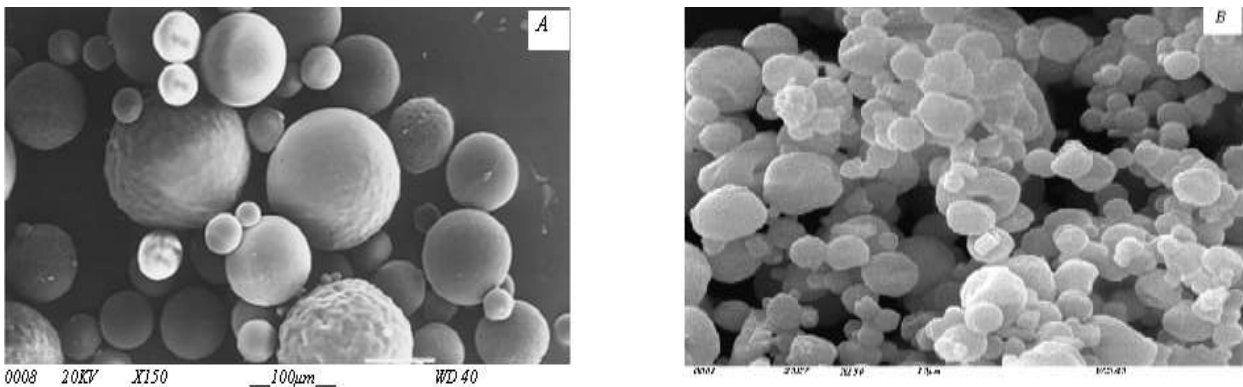


**Fig3: Morphology of microspheres A. Ionotropic gelation  B. Spray dry**

**Table11: Size of optimized microspheres**

| FORMULATIONS | SIZE in μm | SHAPE |
|---|---|---|
| SIZE in μm(Gelation) | 55.32-67.12 | Almost spherical |
| SIZE in μm(Spray Dry) | 11.32-12.50 | Almost spherical |

## Optimization of mucoadhesive microspheres formulations(Ionic Gelation Method)

### Effect of formulation variables.

### Effect of formulation variables on T50%

The model terms for response Y1 (T50%) 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 T50% can be written as;

 $Y1 = 2.96 + 0.53X1 - 0.29 X2 + 0.27 X1 X2 + 0.46 X12 + 1.10 X22$

 As the amount of X1 and X2 increases the corresponding T50% (time required to release 50% of the drug) also increases The Fig 10 shows the response surface plot. It indicates at all the high levels of X1 and X2 the T50% 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 T50% value increases, while HPMC K4M forms pores in the formed matrix and will increases the drug release thus decreases the T50% value. The Fig 11 shows the graph of predicted verses actual data.
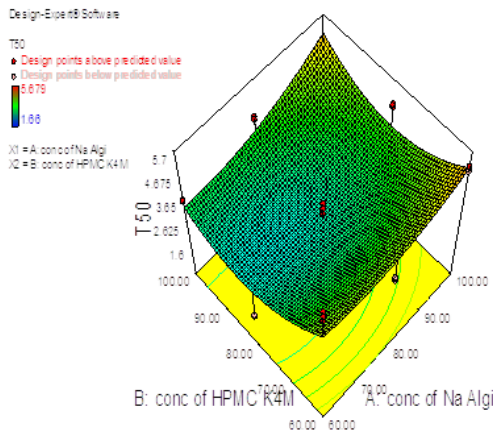
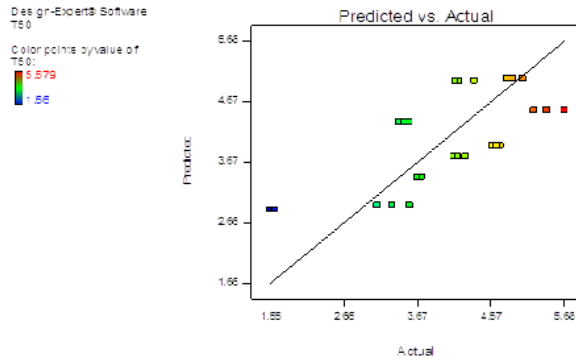Figure 10 Response surface plot showing effect of formulation variables on $T_{50\%}$

Figure 11 Linear correlation plots between actual and predicted values for $T_{50\%}$ (Y1)

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## Effect of formulation variables on T90%

The model terms for response Y2 (T90%) 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 T50% can be written as;

Y2 =     -5.79 + 0.68X1 - 14.83X2 + 0.99 X1X2 + 15.32 X12 + 16.12  X22

As the amount of X1 and X2 increases the corresponding T90% (time required to release 90% of the drug) also increases The Fig 12 shows the response surface plot. It indicates at all the high levels of X1 and X2 the T50% 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 T50% value increases, while HPMC K4M forms pores in the formed matrix and will increases the drug release thus decreases the T90% value. The Fig 13 shows the graph of predicted verses actual data.
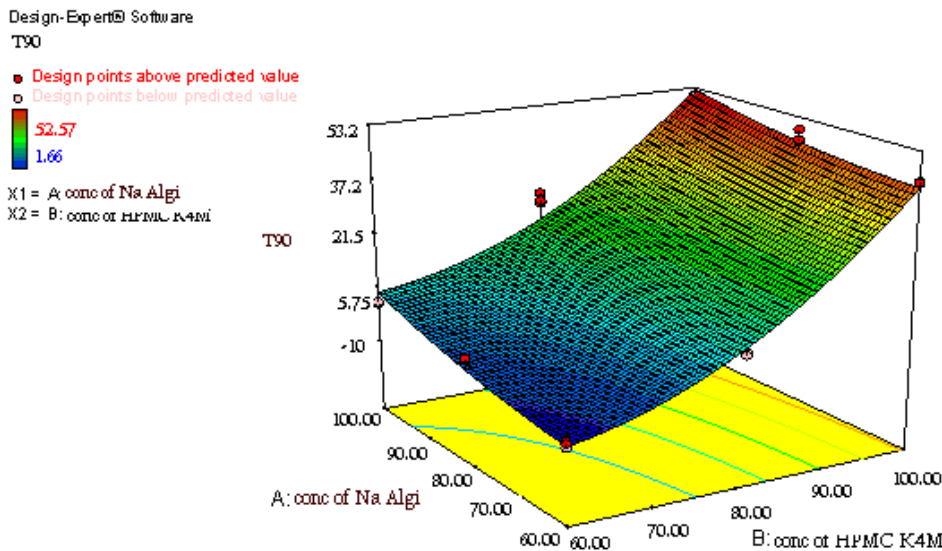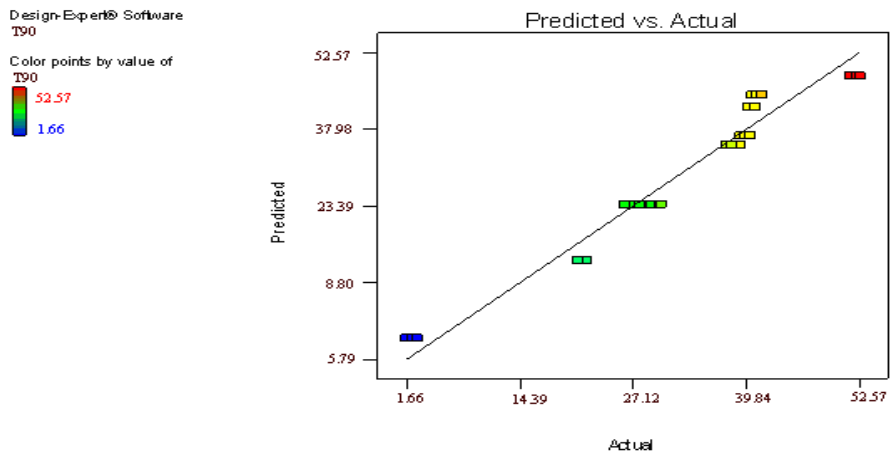


**Figure 12: Response surface plot showing effect of formulation variables on T90%**

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**Figure 13: Linear correlation plots between actual and predicted values for T90% (Y2)**

## Effect of formulation variables on the drug release at 8 hr. (Y3)

The quadratic model was found to be significant with an F value 27.44 (P<0.0001). In this case X1, X2 was found to be significant and the model describes the percent flurbiprofen release at 8h can be written as;

Y3 = 82.91 - 0.30X1 + 10.17 X2

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 X1, X2 shown in response surface plot (Fig 14) 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 X1 and X2 have negative effect on the drug release. The Fig 15 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.
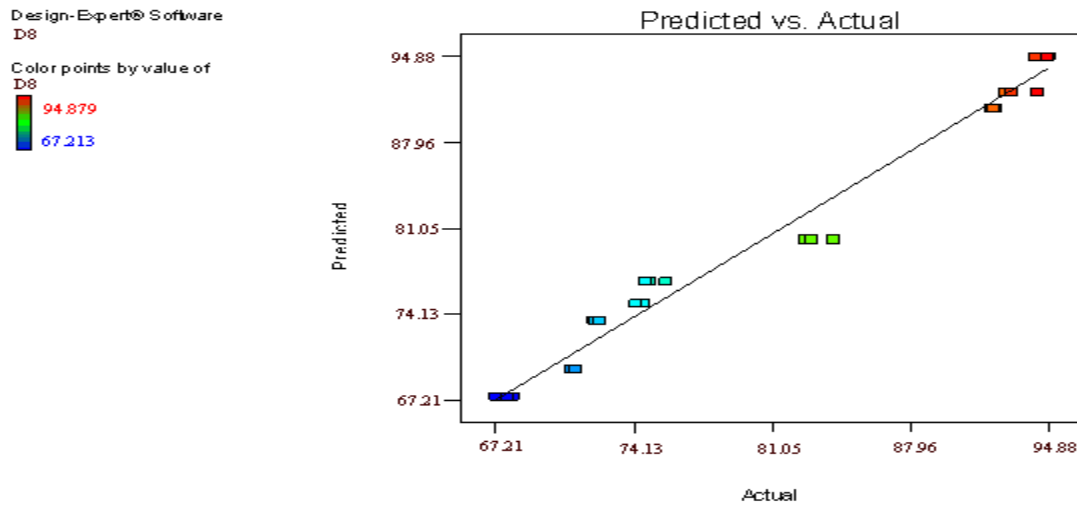
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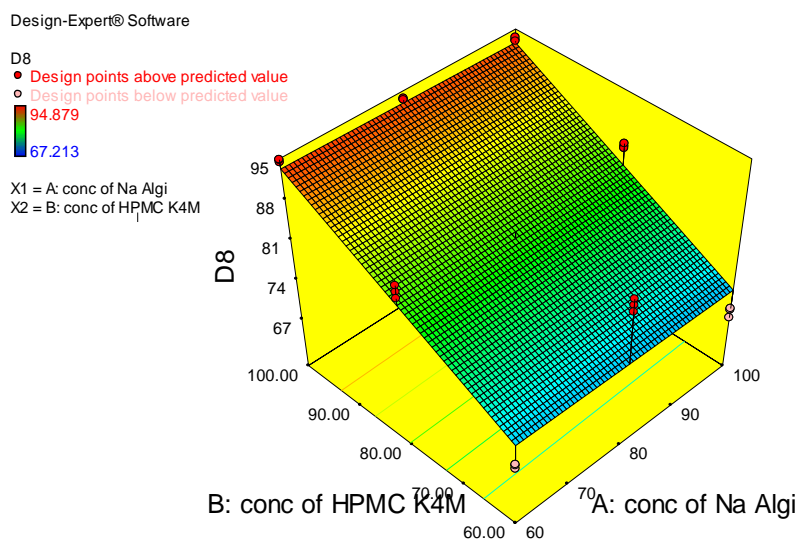


**Figure 14: Response surface plot showing effect of formulation variables on percent drug release at 8 h**

**Figure 15: Linear correlation plots between actual and predicted values for percent drug release at 8 h (Y3)**

## Optimization of mucoadhesive microspheres formulations(Spray Dry Method)

### Effect of formulation variables.

### Effect of formulation variables on T50%

The model terms for response Y1 (T50%) were found to be significant with F value of 4.88 (p<0.0048). In this case all the factors were found to be significant and the model describing T50% can be written as;

$Y1 = 2.87 + 0.51X1 - 0.28 X2 + 0.26 X1 X2 + 0.48 X12 + 1.14 X22$

As the amount of X1 and X2 increases the corresponding T50% (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 X1 and X2 the T50% value is high, As discussed above this behavior is due to increase in amount of polymers (sodium alginate and Sodium CMC) & cross linking agent forms a high viscous gel matrix and thus decreases the drug release and hence T50% value increases, while Sodium CMC forms pores in the formed matrix and will increases the drug release thus decreases the T50% value. The Fig 4 shows the graph of predicted verses actual data.
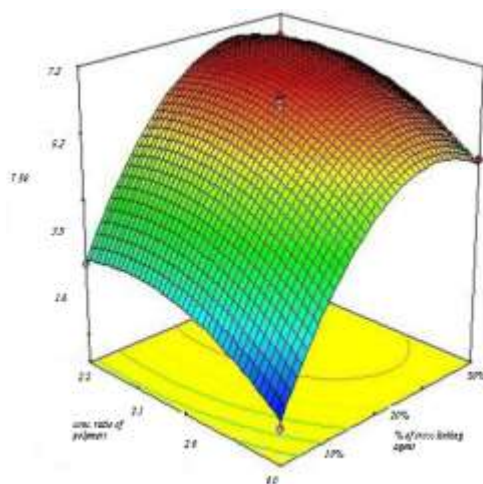


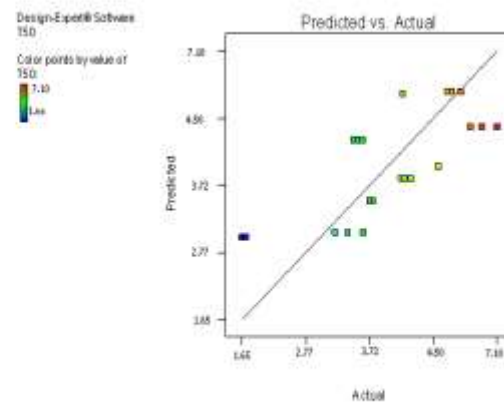Figure 3 Response surface plot showing effect of formulation variables on T₅₀%



Figure 4 Linear correlation plots between actual and predicted values for T₅₀% (Y1)

### Effect of formulation variables on T90%

The model terms for response Y2 (T90%) were found to be significant with F value of 10.11 (p<0.0001). In this case all the factors were found to be significant and the model describing T50% can be written as;

$Y2 = -5.79 + 0.68X1 - 14.83X2 + 0.99 X1X2 + 15.32 X12 + 16.12 X22$

As the amount of X1 and X2 increases the corresponding T90% (time required to release

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90% of the drug) also increases The Fig 5 shows the response surface plot. It indicates at all the high levels of X1 and X2 the T50% value is high, As discussed above this behavior is due to increase in amount of polymers (sodium alginate and Sodium CMC) & cross linking agent forms a high viscous gel matrix and thus decreases the drug release and hence T50% value increases, while Sodium CMC forms pores in the formed matrix and will increases the drug release thus decreases the T90% value. The Fig 6 shows the graph of predicted verses actual data.
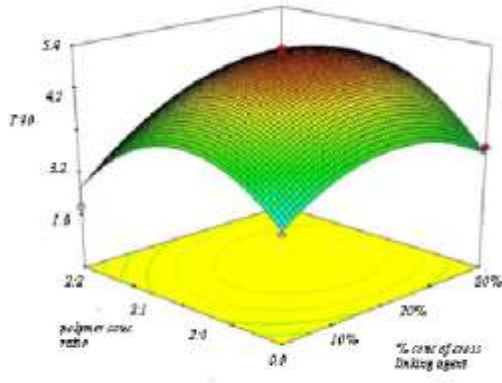


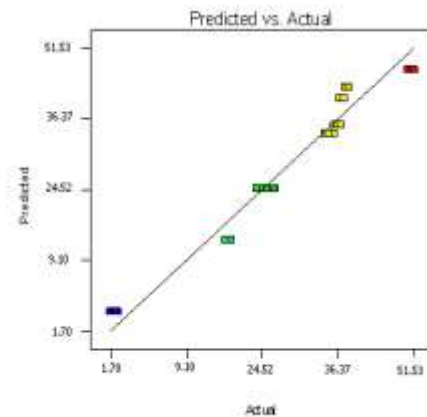Figure 5 Response surface plot showing effect of formulation variables on T₉₀%



Figure 6 Linear correlation plots between actual and predicted values for T₉₀% (Y₂)

## Effect of formulation variables on the drug release at 8 hr. (Y3)

The quadratic model was found to be significant with an F value 28.22 (P<0.0001). In this case X1, X2 was found to be significant and the model describes the percent flurbiprofen release at 8h can be written as;

Y3 = 81.76 - 0.29X1 + 11.45 X2

As the concentration of mucoadhesive polymer (sodium alginate and Sodium CMC) 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 X1, X2 shown in response surface plot (Fig 7) In this plots it was observed that the increasing amount of Sodium CMC causes the decreases in the drug release, due to formation of high viscous gel matrix. Thus the factors X1 and X2 have negative effect on the drug release. The Fig 8 Shows a graph of observed verses predicted values. The sodium alginate and Sodium CMC 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 Sodium CMC which form a gel matrix network with sodium alginate.
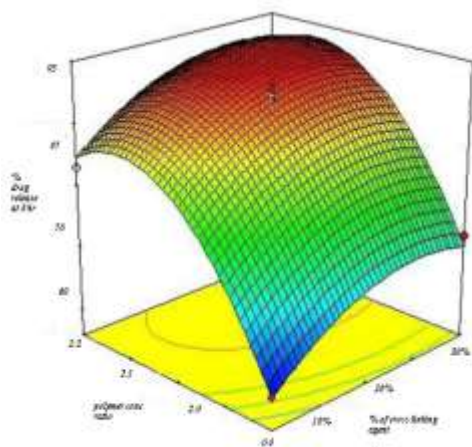
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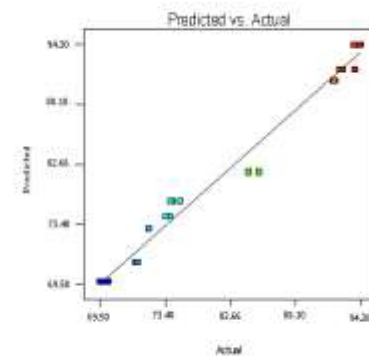Figure 7 Response surface plot showing effect of formulation variables on percent drug release at 8 h



Figure 8 Linear correlation plots between actual and predicted values for percent drug release at 8 h (Y₃)

## ANOVA, Pure error, Lack of fit

The results of ANOVA in Table 14 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 15) and effects as follows; all factors found to be significant for response Y1, similarly only X1, X2 and X1X2 were found for Y2, the X1, X2 were found significant for Y3. 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 optimiz

ed mucoadhesive microspheres with good bioadhesive strength and drug release. The data of pure error and lack of fit are summarized in Table 14 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.

| Source | d.f. | Sum square | Mean square | F value | Probability |
|---|---|---|---|---|---|
| T50% (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 | ----- | |
| T90% (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 (%) | | | | | |
| Model | 2 | 1863.81 | 931.91 | 27.44 | <0.0001 * |
| Residual | 24 | 815.18 | 33.97 | ----- | ----- |
| Total | 26 | 2678.99 | ----- | ----- | ----- |
| Lack of fit | 6 | 804.28 | 134.05 | 221.14 | <0.0001 |
| Pure error | 18 | 10.90 | 0.61 | ----- | |

**Table11: Data of ANOVA study for dependent variables from 32 factorial design**

## Table 12:  Data of ANOVA study for results in analyzing lack of fit and pure

### 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 Y1-Y4. The optimized formula arrived by targeting the Y1 was targeted at 6 h, , Y2 was targeted at 10 h,Y3 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 16. The results of optimized formula were compared with the predicted values and it was shown in Table 17 which showed good relationship between experimented and predicted values, which confirms the practicability and validity of the model.

### Table13: Composition of optimized formulation

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

### Table 14: 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)

| Source | d.f. | Sum square | Mean square | F value | Probability |
|---|---|---|---|---|---|
| T50% (h) | 1 | 5.08 | 5.08 | 7.56 | 0.0120 |
| X1 | 1 | 1.48 | 1.48 | 2.20 | 0.1526 |
| X2 | 1 | 0.87 | 0.87 | 1.30 | 0.2677 |
| X1X2 | | | | | |
| T90% (h) | 1 | 8.28 | 8.28 | 0.060 | <0.0001 |
| X1 | 1 | 3959.58 | 3959.58 | 28.66 | <0.0001 |
| X2 | 1 | 11.80 | 11.80 | 0.085 | 0.0009 |
| X1X2 | | | | | |
| NF release at 8 h (%) | 1 | 1.60 | 1.60 | 0.047 | 0.8298 |
| X1 | 1 | 1862.21 | 1862.21 | 54.83 | <0.0001 |
| X2 | | | | | |

### Summery & Conclusion

The results so far obtained during this investigation encouraged us to derive the following conclusions
The production yield of microspheres prepared by ionic gelation technique was found in the range of 78-84% which is reliable.
The production yield of microspheres prepared by spry drying technique was found in the range of 30-62 % which is reliable

The encapsulation efficiency of microspheres prepared by ionic gelation technique was found in the range of 75-85% it is not 100% because during preparation of microspheres some drug lost in external media.

The encapsulation efficiency of microspheres prepared by spry drying technique was found in the range of 60-92% it is not 100% because during preparation of microspheres some drug lost in external media.

The in vitro release profile of Flurbiprofen from optimized formulations in ionic gelation technique were F1 shows retardation of release up to 12 hours shows good controlled release.

The in vitro release profile of Flurbiprofen from optimized formulations in spray drying technique were F9 shows retardation of release up to 12 hours shows good controlled release.

The in vitro Flurbiprofen release data fitted to korsmeyer-peppas release model also shows zero order and higuchi model.

The in vitro mucoadhesive strength of optimized formulations of ionic gelation technique were for F1 78.50% & F3 76.50% which shows good mucoadhesion.

The ex vivo mucoadhesive strength of optimized formulations of ionic gelation & spray drying technique were for F1 79.84% & F9 78.24% which shows good mucoadhesion.

The size of microspheres prepared by ionic gelation & spray drying technique was found for F1 is 55.32-67.12 μm & for F9 11.32-12.50μm

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