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Formulation and evaluation of mucoadhesive microspheres of macromolecular polymers using flurbiprofen as a model drug

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ABSTRACT

Among modified-release oral dosage form increasing interest has currently turned to systems designed to achieve prolonged retention at the site of drug delivery. Amongst them, mucoadhesive microspheres offer better retention and controlled release. To overcome inherent drawbacks associated with conventional dosage forms of Flurbiprofen, an attempt was made to develop an alternative drug delivery system in the form of mucoadhesive microspheres. The objective of the present study was to formulate and evaluate mucoadhesive microspheres of Flurbiprofen. In the present study, 6 formulations (F1, F2, F3, F4, F5, F6) with variable concentrations of polymers (Sodium CMC, Carbopol & HPMC) were formulated and evaluated for physico-chemical, preformulation and formulation parameters, in vitro release studies and results obtained in in vitro release studies were plotted in different models of data treatment. Compatibility studies by FTIR proved that there was no interaction between Flurbiprofen and the polymers used. The mean particle size of microspheres of each batch ranged between 289.43 to 387.75 µm which ensured good handling characteristics of all batches. The percentage drug entrapment efficiency of all formulations was found to be between 86.94% and 92.06%. The percentage drug loading of all the formulations were found to be between 19.56% and 21.35%. All the six batches were subjected to in vitro release studies with 0.1 N HCl (pH 1.2) and phosphate buffer pH 7.4. All the formulations had shown adequate release of the drug, however, the optimum release was observed with formulation F_1.

Key words: Flurbiprofen, Mucoadhesive microspheres, Sodium CMC, Carbopol, HPMC, Controlled release.

INTRODUCTION

Controlled drug delivery systems have acquired a centre stage in the area of pharmaceutical R & D sector [1]. Such systems offer temporal &/or spatial control over the release of drug and grant a new lease of life to a drug molecule in terms of controlled drug delivery systems for obvious advantages of oral route of drug administration. Most of the oral dosage forms possess several physiological limitations such as variable gastrointestinal transit, because of variable gastric emptying leading to non-uniform absorption profiles, incomplete drug release and shorter residence time of the dosage form in the stomach [2]. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body promptly and then maintain the desired drug concentration in the body over an entire period of treatment. This is possible through administration of conventional dosage form in a particular dose and particular frequency to provide a prompt release of drug. Therefore to achieve and maintain the concentration within the therapeutically effective range needs repeated administration in a day. This results in a significant fluctuation in a plasma drug level, leads to several undesirable toxic effects, and poor patient compliance [3]. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems. The success of these microspheres is limited due to the short residence time at the site of absorption. It would therefore advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres [4] [5].

Flurbiprofen [1,1’-biphenyl]-4-acetic acid, 2-fluoro-alpha-methyl-, is an important analgesic and non-steroidal anti-inflammatory drug (NSAID) also with anti-pyretic properties whose mechanism of action is inhibition of prostaglandin synthesis. It is used in the therapy of rheumatoid disorders. Flurbiprofen is rapidly eliminated from the blood and its plasma elimination half-life is 3-6 hours. In order to maintain therapeutic plasma levels the drug must be administered approximately 150-200mg daily by oral in divided doses [6]**.**

To overcome inherent drawbacks associated with conventional dosage forms of Flurbiprofen, an attempt is being made to develop an alternative drug delivery system in the form of mucoadhesive microspheres.

MATERIALS AND METHODS

2.1 Materials
Flurbiprofen was obtained as gift sample from Micro Labs Bangalore and Carbopol 934, HPMC, Sodium CMC were of pharmaceutical grade.

2.2. Preparation of Mucoadhesive microspheres: Emulsification-solvent evaporation [7] [8]
For the present study, mucoadhesive polymers Carbopol 934, HPMC and Sodium CMC were used in different ratios with the active ingredient for the preparation of mucoadhesive microspheres. These polymers were employed for the fact that they possess good biocompatibility, non-irritant and non-toxic.

Accurately weighted amount of the polymers Carbopol, HPMC and Sodium CMC as shown in Table-1 were dissolved in 50ml of acetone to form a homogenous polymers solution. Flurbiprofen was then dispersed in it and mixed thoroughly. This organic phase containing drug was slowly poured at 150°C into liquid paraffin (50 ml) containing 1% (w/w) of Span-80 with stirring at 1000 rpm to form a uniform emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone evaporated and smooth-walled, rigid and discrete microspheres were formed. The microspheres were collected by decantation and the product was washed with petroleum ether or n- hexane and stored in desiccators over fused calcium chloride.

Table 1: Formulation design of Mucoadhesive microspheres

Sl. No	Ingredients	Formulation code					
		F_1	F_2	F_3	F_4	F_5	F_6
1	Drug (mg)	200	200	200	200	200	200
2	Sodium CMC (mg)	800	-	-	400	400	-
3	HPMC (mg)	-	800	-	400	-	400
4	Carbopol 934 (mg)	-	-	800	-	400	400
5	Liquid Paraffin (ml)	50	50	50	50	50	50
6	Span 80 (ml)	1	1	1	1	1	1
7	Acetone (ml)	50	50	50	50	50	50

2.3 Evaluation of prepared Mucoadhesive microspheres
2.3.1 Particle size [9]
Determination of average particle size of Mucoadhesive microspheres loaded with flurbiprofen was carried out by using optical microscopy. A minute quantity of microspheres was spread on a clean glass slide and average size of 300 microspheres was determined in each batch.

2.3.2 Percentage yield [10] [11] [12]
The measured weight was divided by total amount of all non-volatile components which were used for the preparation of microsphere. Percentage yield can be calculated using the formula

% yield = Total weight of excipient and drug / Actual weight of product x 100

2.3.3 Encapsulation Efficiency and Drug Loading [13] [14]
To determine the amount of drug encapsulated in Mucoadhesive microspheres, a weighed amount (50 mg) of microspheres was suspended into 50 ml of ethanol and sonicated for 15 min in order to extract the entrapped drug completely. The solution was filtered and 1 ml of this solution was withdrawn and diluted to 50 ml with pH 7.4 phosphate buffer solution. This solution was assayed for drug content by UV spectrophotometer at 247 nm. Calculating this concentration with the dilution factor we get the percentage drug content.

a. Encapsulation efficiency was calculated as [15]

EE (%) = Actual Drug Content / Theoretical Drug Content X 100

b. Drug loading was calculated as [16]

DL (%) = Actual Drug Content / Weight of Powdered Microspheres X 100

2.3.4 Degree of Swelling [17]
The swell ability of Mucoadhesive microspheres in physiological media was determined by swelling them in the PBS pH 7.4. Accurately weighed 100 mg of microspheres were immersed in little excess of PBS pH 7.4 for 24 hrs and washed.

The degree of swelling was calculated using following formula:

$$\alpha = (Ws - Wo) / Wo$$

α is the degree of swelling; Wo is the weight of microspheres before swelling; Ws is the weight of microspheres after swelling.

2.3.5 *In vitro* Mucoadhesion Studies [18] [19] [20]
A small portion of the sheep intestinal mucosa was mounted on a glass slide and accurately weighed microspheres were sprinkled on the mucosa. This glass slide was kept in desiccator for 15 min to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle of 45º. Phosphate buffer solution pH 7.4, previously warmed to 37 ± 5 ºC was circulated all over the microspheres and membrane at the rate of 1 ml/min. Washings were collected at different time intervals and microspheres were collected by centrifugation followed by drying at 50 ºC. The weight of washed out microspheres was determined and percentage mucoadhesion was calculated by following formula:

$$\% \text{ Mucoadhesion} = (Wa - W_l) \times 100 / Wa$$

Where, Wa = weight of microspheres applied; W_l = weight of microspheres leached out.

2.3.6 Scanning Electron Microscopy [21]
Dry microspheres are kept in a brass stub coated with gold in an ion sputter. Then picture of microspheres were taken by random scanning of the stub. The SEM analysis of the mucoadhesive microspheres was carried out by using JEOL–6360A analytical scanning electron microscope.

2.3.7 *In vitro* dissolution study [22]
Mucoadhesive microspheres equivalent to 100 mg of Flurbiprofen was loaded into the basket of the dissolution apparatus. Dissolution study carried out for 12 hrs in two different media of 0.1 N HCl pH 1.2 and pH 7.4 phosphate buffers. 1 ml of the sample was withdrawn from the dissolution media at suitable time intervals and diluted to 10 ml using pH 7.4 phosphate buffer and 0.1 N HCl of pH 1.2 (separately) and the same amount was replaced with fresh buffer. The absorbance was measured at 247 nm by using Shimadzu 1700 UV spectrophotometer, against a blank solution.

2.3.8 Stability study [23] [24]
From the six batches of Mucoadhesive microspheres, formulation F_1, F_2 and F_3 were tested for stability studies. All the formulations were divided into 3 sample sets and stored at 4 ± 1°C; 25± 2°C and 60 ± 5% RH; 37± 2°C and 65 ± 5% RH. After 30 days, the drug release of selected formulations was determined by the method discussed previously in *in vitro* drug release.

RESULTS AND DISCUSSION

In the current research, gastroretentive drug delivery system containing mucoadhesive microspheres of Flurbiprofen were developed and evaluated.

3.1 FTIR Studies
The FTIR studies revealed no chemical interaction between the drug molecule and polymers.

3.2 Particle size
With increase in polymer concentration, the mean particle size of the microspheres significantly increased and range was between 289.43 to 387.75µm. (Table 2)

Table 2: Particle size analysisFlurbiprofen Microspheres

Formulation	Particle Size (μm)
F_1	387.75
F_2	320.45
F_3	346.00
F_4	340.08
F_5	310.54
F_6	289.43

3.3 Percentage Yield
Percentage yield of the formulations were carried out and was found to be within the range between 85.12 to 91.44 % (Table 3).

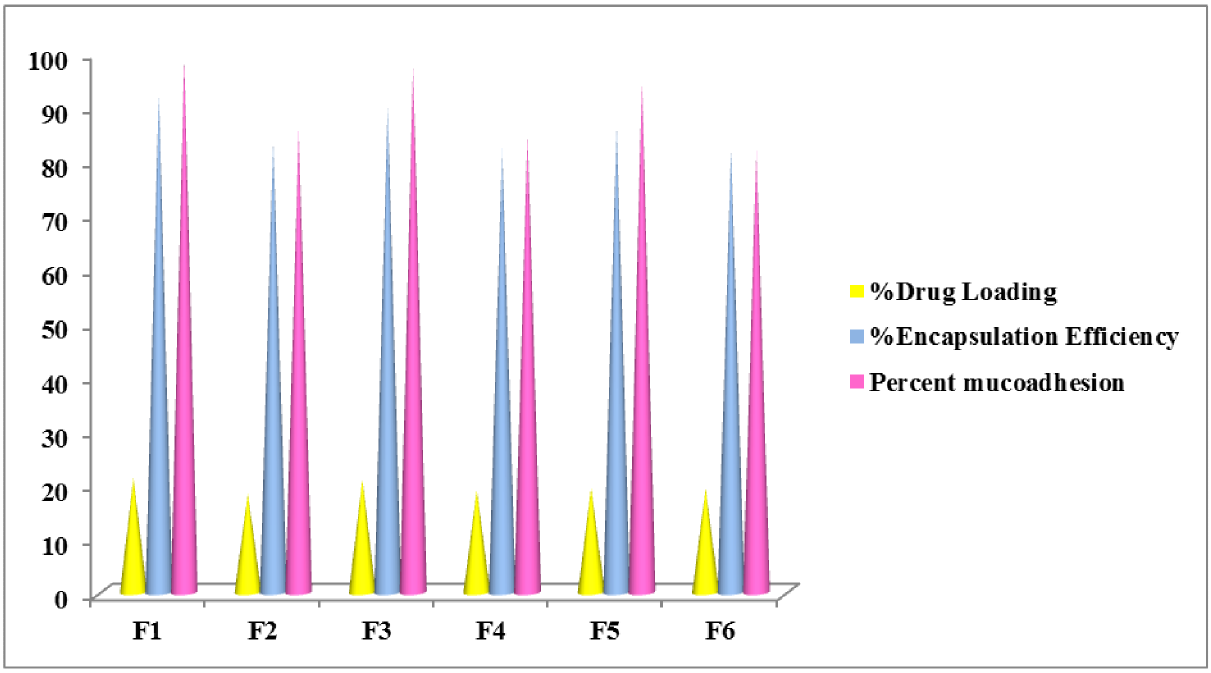
3.4 Percentage Encapsulation Efficiency & Percentage Drug Loading
Percent Encapsulation Efficiency and Percent Drug Loading of the formulations were found to be within the range between 81.66 to 91.86% and 18.21 to 21.28%. (Fig 3)

3.5 Degree of Swelling & percent mucoadhesion
Degree of swelling and percentage mucoadhesion of the formulations were carried out and were found to be within the range between 1.03 to 1.63 and 81.6 to 98.5% (Table 3 & Fig 3).

Table 3: percentage yield, percent encapsulation, percent drug loading of microspheres, degree of swelling, percent mucoadhesion

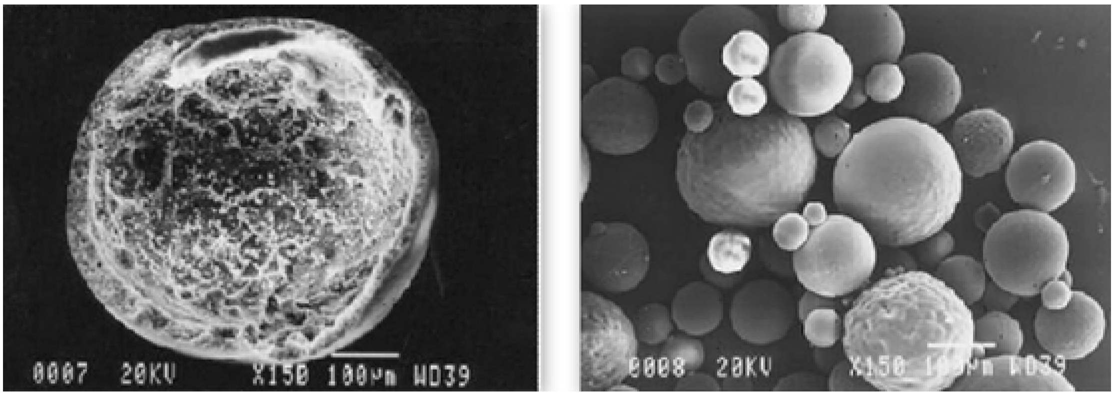
Formulation	Percentage yield	%Drug Loading	%Encapsulation Efficiency	Degree of swelling	Percent mucoadhesion
F_1	86.22	21.28	91.86	1.63	98.5
F_2	91.44	18.21	83.35	1.16	85.3
F_3	86.46	20.79	90.06	1.61	97.2
F_4	87.42	18.76	82.11	1.10	84.7
F_5	87.70	19.22	86.26	1.57	94.1
F_6	85.12	19.16	81.66	1.03	81.6

Fig 3: Percent drug loading, Percent encapsulation & Percent mucoadhesion



3.6 Scanning Electron Microscopy
Scanning electron microscopy confirms the outer surface of F_2 formulation was smooth and dense, while the internal surface was porous. The shell of microspheres also showed some porous structure it may be caused by evaporation of solvent entrapped within the shell of microspheres after forming smooth and dense layer (Fig 4).

Fig 4: SEM Photograph of Mucoadhesive microspheres (F_1)



3.7 In vitro release studies

The *In vitro* release studies of Mucoadhesive microspheres were carried out in pH 1.2 and pH 7.4 buffers as a dissolution medium for a period of 12 & 8 hrs respectively. The release showed a biphasic release with an initial burst effect. At the end of first 30 min drug release was 21.6%, 15.48%, 17.55%, 15.12%, 16.74 and 13.59% for F_1 to F_6 respectively in pH 1.2 buffer. The cumulative % release for F_1, F_2, F_3, F_4, F_5 and F_6 were found to be 95.5%, 84.0%, 90.08%, 82.27%, 85.04% and 79.35% in 1.2 pH buffer at the end of 12^{th} hrs. The cumulative % release for F_1, F_2, F_3,F_4, F_5 and F_6 in phosphate buffer pH 7.4 were found to be 97.31%, 86.26%, 93.56%, 84.42%, 83.49% and 89.0% at the end of 8^{th} hrs. (Table 4 & Fig 5, 6)

Table 4: *In vitro* release of Mucoadhesive microspheres in different media

Formulation	%CDR in pH 1.2 buffer at 12^{th} hour	%CDR in pH 7.4 buffer at 8^{th} hour
F_1	95.51	97.31
F_2	84.00	86.26
F_3	90.08	93.56
F_4	82.27	84.42
F_5	85.04	83.49
F_6	79.35	89.00

Fig 5: In vitro dissolution profile of Mucoadhesive microspheres in pH 1.2 buffer

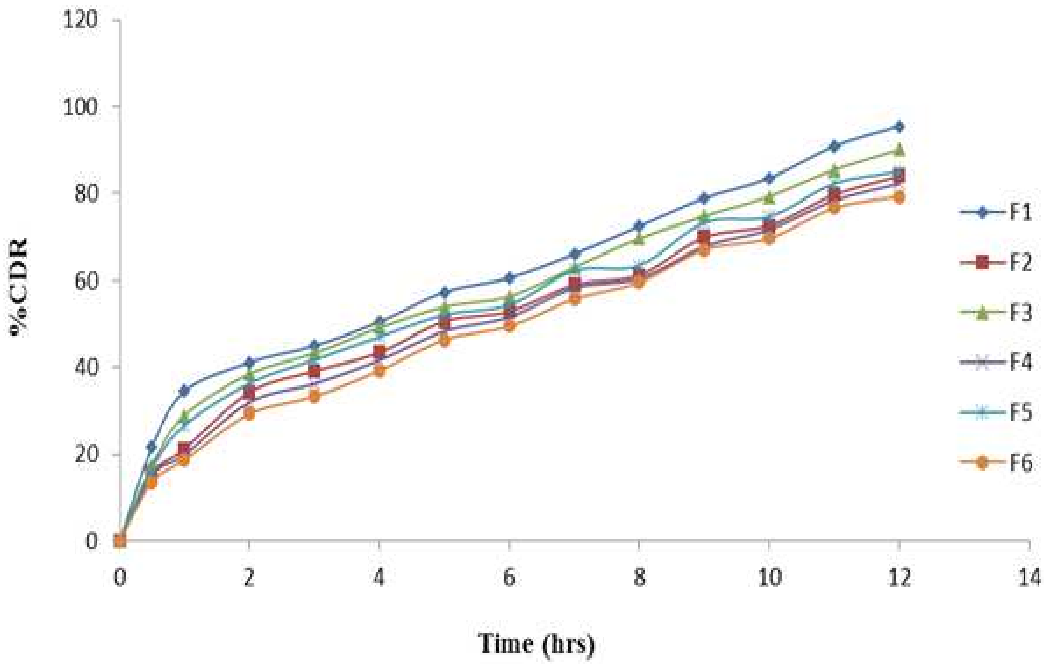
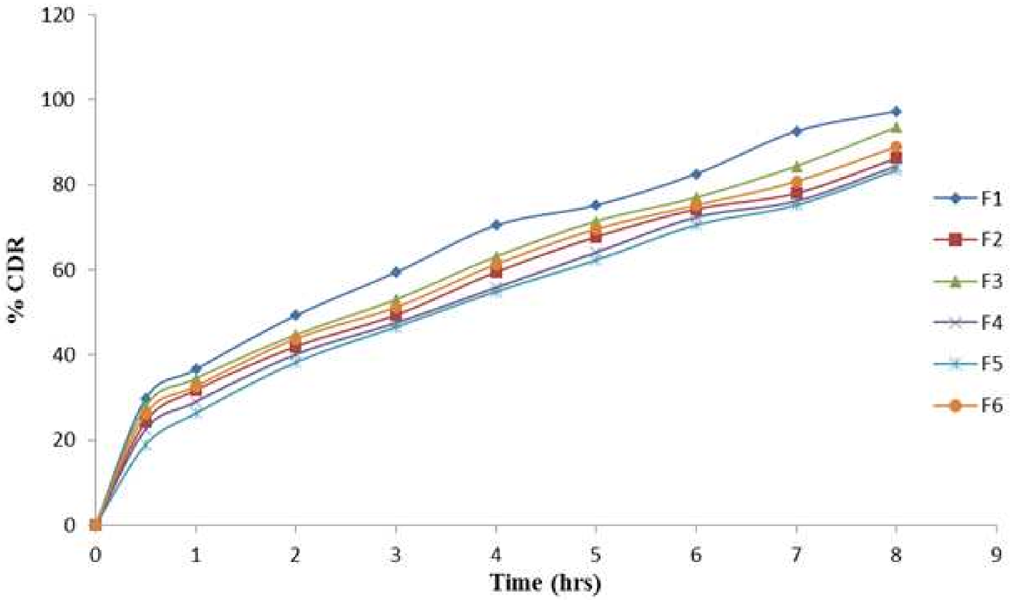


Fig 6: In vitro dissolution profile of Mucoadhesive microspheres in pH 7.4 phosphate buffer



3.8 Stability studies

These studies revealed that, there is a reduction in entrapment efficiency after storage for one month at 4 ± 1°C, 25 ± 2°C & 60 ± 5% RH and 37 ± 2°C & 65 ± 5% RH. It was also revealed that formulations maintained at 4±1°C showed maximum entrapment followed by the storage at 25±2°C; 60±5% RH and 37±2°C; 65±5% RH conditions. Formulations F_1, F_2 and F_3 maintained at 4±1°C showed 91.42%, 87.64% and 89.98% drug release respectively. Formulations maintained at 25±2°C & 60±5% RH showed 93.58%, 89.09% & 91.16%. Formulations stored at 37±2°C 65± 5% RH showed 99.71%, 94.16% & 96.87% drug release after 10 hours for F_1, F_2 & F_3 respectively. These results indicate that the drug release from the formulations maintained at 4±1°C was lowest followed by formulation maintained at 25±2°C; 60±5% RH and 37±2°C; 65±5% RH (Table 5).

On comparing this data with the previous release data of F_1, F_2 & F_3, it was observed that there was no much difference in the drug release of formulation maintained at 4±1°C. There was a slight increase in drug release for formulation maintained at 25±2°C & 60±5% RH and 37±2°C & 65±5% RH. These results may be attributed to erosion of polymer matrix to some extent during storage.

Table 5: stability studies - Percentage entrapment efficiency and *In vitro* release

Formulation code	4°C± 1		25 ± 2°C & 60 ± 5% RH		37 ± 2°C & 65 ± 5% RH	
	%EE	%CDR	%EE	%CDR	%EE	%CDR
F_1	88.16	91.42	87.98	93.58	85.83	99.71
F_2	82.94	87.64	80.62	89.09	79.98	94.16
F_3	87.37	89.98	85.34	91.16	84.51	96.87

CONCLUSION

By studying all the experimental results it was conclusively demonstrated that Mucoahesive microspheres loaded with macromolecular bioadhesive polymers can be successfully formulated by emulsification solvent evaporation method. Formulations employing individual polymers as well as their combinations showed optimum results of which formulation containing sodium CMC showed the best results in the evaluated parameters.

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