

FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF FLURBIPROFEN

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Summary

The objective of the study was to develop mucoadhesive microspheres of Flurbiprofen and to carry out its evaluation. Mucoadhesive microspheres containing Flurbiprofen were prepared by w/o solvent evaporation method using mucoadhesive polymers like Sodium carboxy methyl cellulose (Sod. CMC), Hydroxy propyl methyl cellulose (HPMC), Carbopol 934. The resulting microspheres were in the form of free flowing powder consisting of spherical particles with a rough surface. The particle size was in the range of 29.20 to 38.45 μ m. The percentage entrapment efficiency was in the range of 60.75 to 79.72% and was relatively high in case of HPMC microspheres. The microspheres exhibited good mucoadhesive property in the In-vitro wash off test for mucoadhesion. Flurbiprofen release from these microspheres was slow and extended over longer period of time and depended on the type of polymer used. Drug release followed first order kinetics. In-vivo studies conducted on male Albino rats indicated that the microspheres not only decreased the inflammation to larger magnitude, but also sustained this magnitude for longer period of time as compared to marketed tablets of Flurbiprofen.

Key words: Flurbiprofen, Mucoadhesive, Microspheres, Controlled release

Introduction

Flurbiprofen is a non-steroidal anti-inflammatory drug which is widely used for the treatment of signs and symptoms of Rheumatoid arthritis and Osteo arthritis. It has a short half life ie. 3 to 5.7 hours. Its short half life makes it necessary to administer the drug frequently (3 to 4 times daily) in order to maintain therapeutic concentration, but by slowing down the release of the drug from the dosage form it would be possible to achieve once daily dosage regimen. (20) Thus the development of controlled release dosage form would clearly be advantageous for such drugs. The designing of Mucoadhesive Drug Delivery System for such drugs helps to overcome this problem since the Gastric residence of the drug can be prolonged. The slow release of the drug could also decrease the occurrence of gastro-intestinal side effects which are very common in case of NSAIDs. (2, 3, 8, 11)

Nonsteriodal anti-inflammatory drugs (NSAIDs) are amongst the most commonly prescribed medications in the world. Almost all the NSAIDs available in the market have severe side effects. As awareness of the GI side effects associated with NSAIDs increases, safety becomes a primary requisite in treatment. A trend in NSAID development has been to improve therapeutic efficacy and reduce the severity of GI (Gastric Intestinal) side effects through altering dosage forms by modifying release of the formulations to optimize drug delivery. (10) One such approach is using polymeric microspheres as carriers of drugs. Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000 μm they are made up of polymeric, waxy or other protective materials. Developing microspheres of Flurbiprofen will provide constant and prolonged therapeutic effect, which will reduce the dosing frequency and thereby improve the patient compliance. Apart from oral administration they could also be injected in to the body due to the spherical shape and smaller size. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects. thus the aim of the research is to formulate mucoadhesive microspheres of Flurbiprofen and carry out its evaluation. (4, 6, 23)

Materials and methods

Materials

The drug Flurbiprofen was gift sample from FDC, Mumbai. Polymer HPMC and sodium CMC (high viscosity grade), Carbopol 934 and Liquid Paraffin was purchased from Central Drug House, New Delhi. Ethanol was purchased from Qualigens fine Chemicals, Mumbai.

Methods

Preparation of Mucoadhesive Microspheres

The Microspheres were prepared by w/o emulsification solvent evaporation method. For this drug (Flurbiprofen) was dissolved in minimum amount of ethanol, and then it was added to 2% aqueous polymer solution. A mechanical stirrer was used for rapid mixing of drug solution into aqueous polymer solution for 3 minutes.

The drug and polymer solution was added drop wise to liquid paraffin containing 0.5% Span 80 as emulsifying agent and rapid stirring at 500rpm was done. The constant stirring was carried out using magnetic stirrer. The beaker and its contents were heated at 70 °C for 4.5 hours until aqueous phase was completely removed by evaporation. Then liquid paraffin was decanted and Microspheres were collected, washed three times with n-Hexane filtered and dried in oven at 50 °C for 2 hours. (12, 13, 15, 17) The various Mucoadhesive Microspheres formulations used in this study are listed in (table 1)

Estimation of Flurbiprofen

UV (Ultra Violet) Spectral Analysis was carried out for the identification of Flurbiprofen. For this 100mg of drug was dissolved in 100mg of PBS (Phosphate Buffer Saline) pH 7.4. Suitable dilutions were made. Different concentrations of Flurbiprofen in the range 1-10 µg/ml were scanned for λ_{max} between 200-400 nm using Double Beam Spectrophotometer (Shimadzu UV-1700 series). The λ_{max} was found to be 247 nm. The scan is shown in (fig. no.1)

Production Yield

The percentage of production yield was calculated from the weight of dried Microspheres (W1) and the sum of initial dry weight of starting materials (W2) as the following equation:

$$\% \text{ Production Yield} = \frac{W1}{W2} \times 100$$

The production yield of various formulations are listed in (table. 2)

Entrapment efficiency

Microspheres (20 mg) were crushed in a glass mortar and pestle and the powdered microspheres were dispersed in 10 ml of Phosphate buffer (pH 7.4). After 24 hours, the dispersion was vortexed repeatedly to break up the microspheres completely and cause them to discharge their contents. It was then filtered and the filtrate was analysed for the drug content spectrophotometrically after suitable dilutions using a digital UV- spectrophotometer (Shimadzu). The determinations were done in triplicate. The Drug Entrapment Efficiency (DEE) was determined as:

$$DEE = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

The entrapment efficiency of formulations F1 to F6 are listed in (table no 3).

Particle size analysis

The particle size of the microspheres was determined by using an Optical microscope (Magnus MLX-DX, Olympus). Approximately 200-300 microspheres were counted for particle size using a calibrated optical microscope. Listed in (table no 3).

Morphology

The morphology of microspheres was examined by Scanning Electron Microscopy. The inner and outer surface was observed using Scanning Electron Microscope (LEO-430, UK). (8, 9, 21) Shown in (fig.2)

In-vitro drug release

In-vitro drug release in 0.1 N HCl pH 1.2

The USP Paddle Type method (XXIII) was adopted in this study. The release medium consisted of 900 ml of 0.1 N HCl (pH 1.2), without pepsin, maintained at $37\pm0.5^{\circ}\text{C}$. A quantity of microspheres equivalent to 50mg Flurbiprofen from each batch was tied to the paddle and agitated at 50 rpm. At predetermined time intervals, 5 ml portions of the release medium were withdrawn, filtered through Wattman filter paper, appropriately diluted and analysed for drug content spectrophotometrically at 247 nm. The volume of the release medium was kept constant by replacing it with 5 ml of 0.1 N HCl after each withdrawal. The release studies were performed in triplicate. (16, 18)

In-vitro drug release in PBS pH 7.4

Flurbiprofen release in PBS pH 7.4 was carried out using same procedure only dissolution medium was replaced with PBS pH 7.4.

In-vitro wash off test for mucoadhesion

The Mucoadhesive property of Microspheres was evaluated by in-vitro wash off test for mucoadhesion. Pieces of intestinal mucosa ($3\text{cm}\times2\text{cm}$) were mounted onto glass slides using cyanoacrylate glue. Some Microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of USP Disintegration Apparatus. By operating the Disintegration Test machine, the tissue specimen was given a regular up and down movement in 0.1 N HCl/ PBS pH 7.4 at 37°C taken in a 1 litre vessel of the machine. At the end of every hour the tissue specimen was viewed to find out whether the microspheres were still adhering to the tissue specimen or not and the time of adhesion of every formulation was noted down.

In-vivo studies

Carrageenan induced Paw Edema Method

Carrageenan induced paw edema method was used to study the In-vivo performance of the prepared drug delivery system, and the study was approved by Institutional Animal Ethical Committee. Anti-inflammatory activity was determined by measuring change in the volume of inflamed paw, produced by carrageenan (0.1ml of 1% w/v) using Plethysmometer.

Male Albino rats (Wistar strain) selected for the study were weighed and marks were made on the right hind paw just behind the tibia-tarsal junction on each animal. Thus every time the paw was dipped in the plethysmograph (mercury displacement method) up to the fixed mark to

ensure constant paw volume. The rats were divided into 3 groups including one controlled group with each group comprising of 3 animals. The paw volume was noted at 0, 1, 2, 4, 6, 8, 12, 24 hr. First group (Standard) was administered marketed tablet of Flurbiprofen orally according to the dose of 3.8 mg/kg. The second group (Test group) was administered formulation i.e. microspheres of flurbiprofen while the controlled group animals were given saline (0.9% NaCl) containing no drug. After 30 minutes of oral administration of formulation and plain Flurbiprofen, 0.1 ml of 1% w/v carrageenan (in 0.9% normal saline) was injected in the sub planter region of the right hind paw of rats. The initial reading just after injection and subsequent paw volume was measured up to 24 hrs. The % inhibition of edema induced by carrageenan was calculated for each group using the following equation:

$$\% \text{Inhibition of Edema} = \frac{V_{\text{control}} - V_{\text{treated}}}{V_{\text{control}}} \times 100$$

Where, V_{control} = mean edema volume of rats in controlled group and V_{treated} = edema volume of each rat in test group. (16)

Results and discussion

The mucoadhesive microspheres consisting of mucoadhesive polymer could be prepared by w/o solvent evaporation method. The microspheres were found to be discrete, spherical, free flowing. The microspheres were uniform in size, with size range of 29.20-38.45 μm .

Morphology

The morphology of the Microspheres was examined by scanning electron microscopy. The SEM (Scanning Electron Microscope) photographs indicated that the microspheres were spherical with a rough surface and completely covered with the coat polymer. The loading of the drug did not cause any significant change in morphology. Shown in (fig no 2).

Production yield

The percentage of production yield of the prepared mucoadhesive microspheres of Flurbiprofen was in the range of 66.0 – 78.0 % (table no.2). Being highest for formulation F2 (HPMC + Drug) and lowest for formulation F3 (Carbopol + drug). Listed in (table no 2).

Entrapment efficiency

The percentage entrapment efficiency was in the range of 60.75-79.72 % being highest for F2 (drug + HPMC) and lowest for F5 (drug + Carbopol). Depending on the type of polymer used. Listed in (table no 3).

In-vitro drug release

Drug release from the microspheres was studied in phosphate buffer (pH 7.4) and in 0.1 N HCl (pH 1.2). Drug release from the microspheres was slow and dependent on the type of polymer

used. Sodium CMC microspheres gave relatively fast release as compared to others. The order of microspheres showing increasing release rate was F6(Drug+HPMC+Carbopol) < F4(Drug +sod. CMC+HPMC) < F2 (Drug+HPMC) < F5 (Drug+Sod. CMC+Carbopol) < F3 (Drug+Carbopol) < F1 (Drug+Sod CMC). It was also observed that the drug release was faster in PBS than in 0.1N HCl which was perhaps due to the greater solubility of the drug in the former. The graph of cumulative % release of various formulations in 0.1 N HCL pH 1.2 and in PBS pH 7.4 are shown in (fig. 3 and fig. 4) respectively.

To find out the kinetics and mechanism of drug released from all the formulations of Flurbiprofen Mucoadhesive Microspheres, the data were treated according to zero order, first order, Higuchi square root law and Korsmeyer's equation pattern. It indicated that, the correlation coefficient value of all the formulations showed that the formulations did not follow zero order release pattern. When the data were plotted according to the first order equation, the formulations show a fair linearity, with correlation coefficient values between 0.976 and 0.995. (5, 14)

In-vitro wash off test for mucoadhesion

Microspheres consisting of mucoadhesive polymers exhibited good mucoadhesive properties in the In vitro Wash off Test for mucoadhesion. The wash off was slow in case of microspheres containing mucoadhesive polymers. Formulations containing Carbopol showed better mucoadhesive properties than formulations containing HPMC or Sodium CMC which exhibit more or less equivalent mucoadhesiveness. Listed (table. 4)

The wash off was faster at intestinal pH than at gastric pH. The rapid wash off observed at intestinal pH was due to ionization of carboxyl and other functional groups in the polymers at this pH which increases their solubility and also reduces adhesive strength. (5, 7, 19)

In-vivo studies

The in-vivo performance of selected formulation was carried out in carrageenan-induced rat paw edema model. Carrageenan elicits a time dependent increase in paw depth, which consists of a significant increase after 1hr and a maximum inflammation occurring at 4hr post injection. The formulation not only decreased the inflammation to larger magnitude, but also sustained this magnitude. In the formulation maximum inhibition was observed at 8th hour with higher value (83.33%), up to 12hr inhibition was maintained above 65%, and even after 24 hr, 16.66% inhibition was observed.

However, in case of oral administration of marketed tablet of flurbiprofen (standard), maximum inhibition was displayed at 2hr with magnitude of 80% and just after 4hrs it scored below 20 % (fig. 5). The possible reason could be the drug concentration in the blood, which was maintained for longer duration in case of mucoadhesive microspheres of Flurbiprofen in comparison to marketed Flurbiprofen. Thus the anti-inflammatory activity of the formulation was maintained for longer period of time due to slow release of the drug from the mucoadhesive polymers. (16, 22)

Table1: Formulation design

Ingredients	F1	F2	F3	F4	F5	F6
Drug	200mg	200mg	200mg	200mg	200mg	200mg
Sod.CMC	800mg	-	-	400mg	400mg	-
HPMC	-	800mg	-	400mg	-	400mg
Carbopol	-	-	800mg	-	400mg	400mg

F=Formulation

Table2: Production yield

Formulation code	W1 (mg)	W2 (mg)	% Production yield
F1	762	1000	76.2 (± 1.50)
F2	780	1000	78.0 (± 0.96)
F3	660	1000	66.0 (± 1.23)
F4	748	1000	74.8 (± 0.87)
F5	721	1000	72.1 (± 1.43)
F6	730	1000	73.0 (± 1.05)

W1=weight of dried microspheres, W2=weight of starting material. Results have been expressed as Mean \pm S.D. where n=3

Table 3: Showing the percentage entrapment efficiency and particle size of various formulations of mucoadhesive microspheres

Formulation code	% Entrapment efficiency	Particle size(μm)
F1	67.7	38.45 (± 3.33)
F2	79.72	29.20 (± 5.67)
F3	72.77	36.54 (± 2.84)
F4	65.8	30.62 (± 4.35)
F5	60.75	34.67 (± 5.22)
F6	74.35	31.66 (± 5.21)

Results have been expressed as Mean \pm S.D. where n=3

Table 4: Comparative Results of in-vitro wash off test to assess mucoadhesiveness of Microspheres in 0.1N HCl and in PBS pH 7.4

Formulation Code	Adhesion Time (hours) In 0.1N HCl pH 1.2	Adhesion Time (hours) in PBS pH 7.4
F1	7.5	6
F2	7	5.5
F3	8.5	7
F4	7	6.5
F5	8	6.5
F6	7.5	7

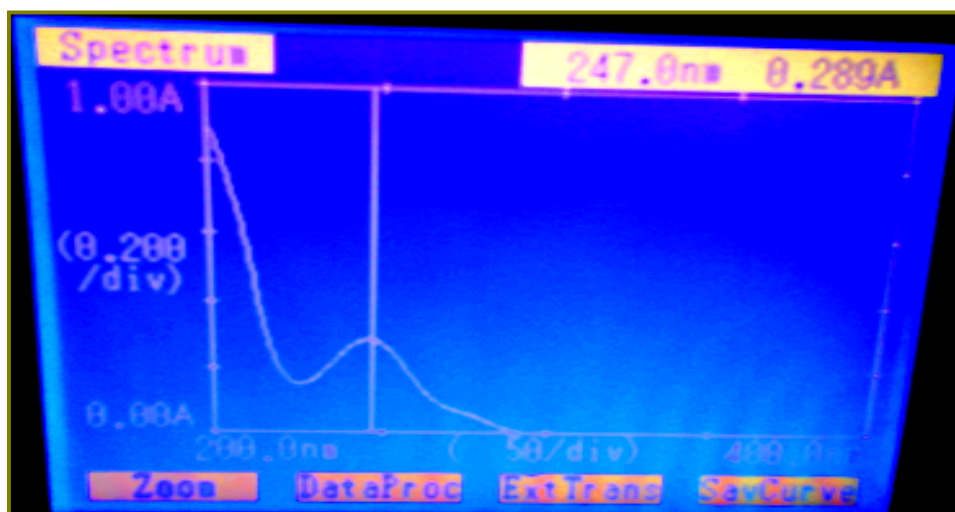
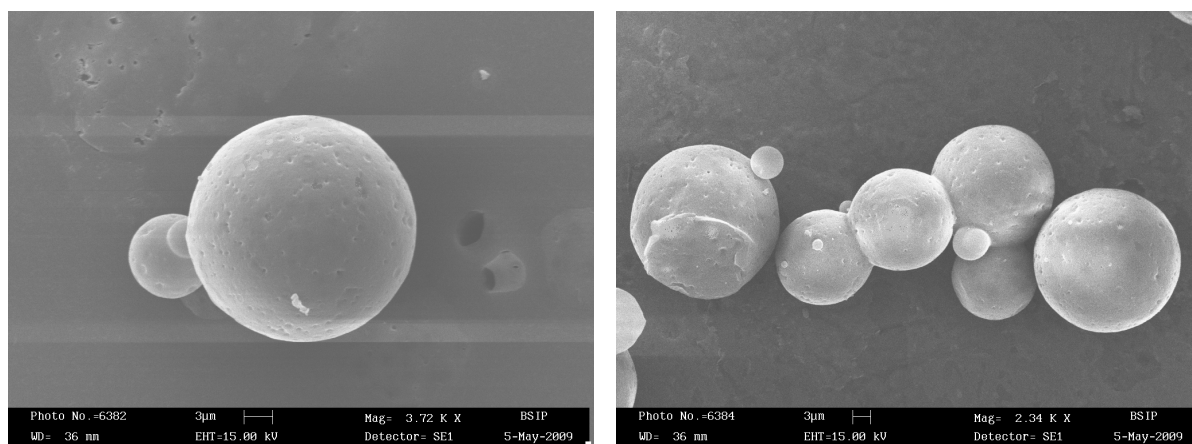


Fig1: UV Spectrum of Flurbiprofen



(A) Surface morphology of single Microspheres. (B) Microspheres in a cluster

Fig 2: Scanning electron microscopy of Flurbiprofen Microspheres.

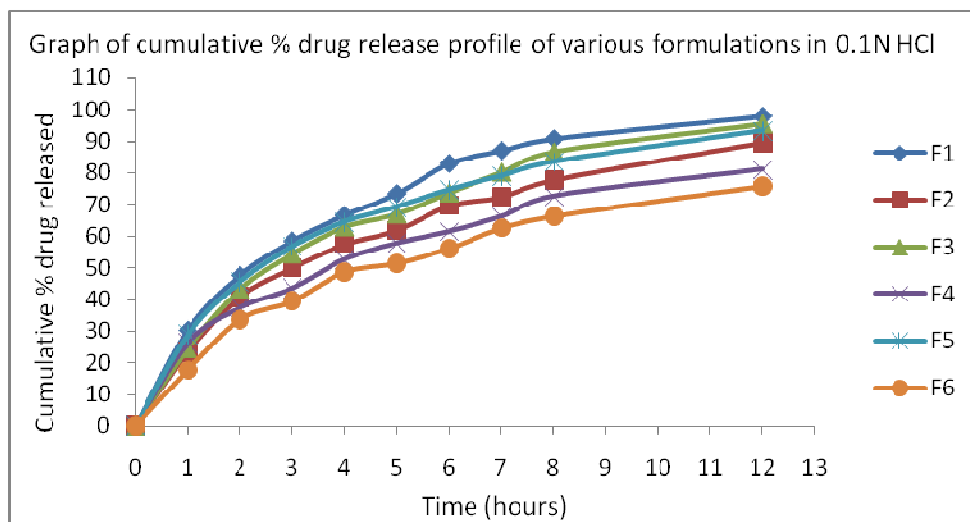


Fig 3 : Graph of cumulative % drug release profile of formulation F1, F2, F3, F4, F5 and F6 in 0.1N HCl pH 1.2

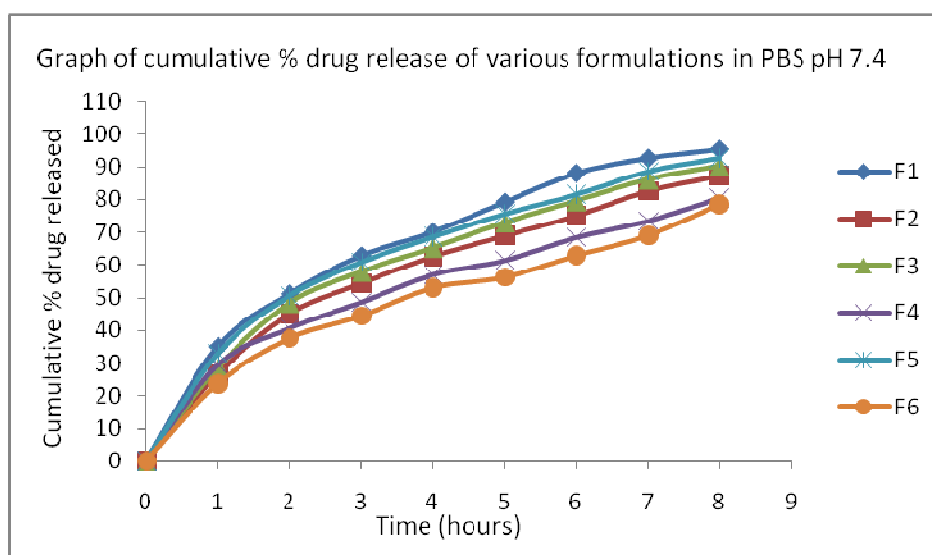


Fig 4 : Graph of cumulative % drug release profile of formulation F1, F2, F3, F4, F5 and F6 in PBS pH 7.4

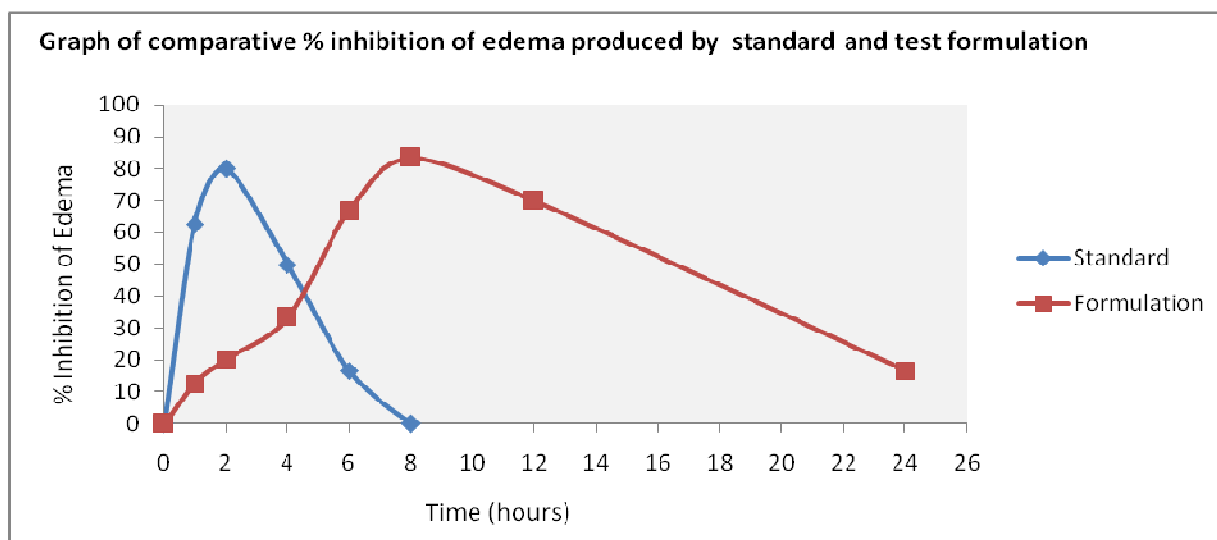


Fig 5 : Showing comparative % inhibition of edema produced by standard (marketed tablet of Flurbiprofen) and prepared formulation of mucoadhesive microspheres of Flurbiprofen.

Conclusions

Thus, spherical microspheres consisting of mucoadhesive polymer (sodium CMC, Carbopol, or HPMC) could be prepared by w/o solvent evaporation method. The microspheres exhibited good mucoadhesive properties in an in vitro test. Flurbiprofen release from these mucoadhesive microspheres was slow and extended over longer periods of time and depended on composition of the coat. Drug release followed first-order kinetics. In the in vivo evaluation indicated that the Flurbiprofen microspheres not only decreased the inflammation to larger magnitude, but also sustained this magnitude for longer period of time as compared to marketed tablets of Flurbiprofen. These mucoadhesive microspheres are, thus, suitable for oral controlled release of Flurbiprofen.

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