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Preparation and In-vitro evaluation of mucoadhesive microspheres of Aceclofenac.

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Abstract

Aceclofenac (ACE) is NSAIDs of a phenyl acetic acid class. It is indicated in arthritis and osteoarthritis, rheumatoid arthritis, ankylosing spondylitis. It has short elimination half life of 4 hours and many side effects. The objective of the study is to design, characterize and evaluate mucoadhesive microspheres of ACE employing carbopol (CP) as mucoadhesive polymer and ethylcellulose (EC) as matrix polymer. Mucoadhesive microspheres of ACE were prepared by an emulsification/evaporation method. The prepared microspheres were free flowing and spherical in shape and characterized for drug loading, *in-vitro* wash-off test, infrared spectroscopy (IR), differential scanning colorimetry (DSC) and scanning electron microscopy (SEM). The *in-vitro* release studies were performed using pH 6.8 phosphate buffer. The drug loaded microspheres in a ratio of 1:1.5 showed 62% of drug entrapment, percentage mucoadhesion after 1 hour was 78% and 88% release in 12 h. The infrared spectra and DSC showed stable character of aceclofenac in the drug loaded microspheres and revealed the absence of drug-polymer interactions. SEM studies showed that the microspheres are spherical and porous in nature. The in vitro release profiles from microspheres of different polymer-drug ratios followed Korsmeyer Peppas model.

Keywords: Aceclofenac, mucoadhesive, microspheres, Carbopol, emulsification/ evaporation method

Introduction

Controlled release multiple unit dosage forms have advantages over single unit ones as they can spread out in a more uniform manner over a large surface area in the gastrointestinal tract (Lee et al., 2000). This can reduce a local irritation of the gastrointestinal tract by some drugs and can provide a large area for drug absorption. Microsphers form an important part of novel drug delivery system which can precisely control release rates and target drugs to a specific body sites. Mucoadhesion is a topic of interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with underlying absorption surface to improve and enhance the bioavailability of drugs.

Aceclofenac (ACE), phenyl acetic acid derivative 2-[(2,6-Dichlorophenyl)amino] phenyl acetoxy acetic acid, is a novel NSAID indicated in the symptomatic treatment of pain and inflammation with a reduced side effect profile especially regarding gastrointestinal complications (Parfitt, 1999; Brogden et al., 1996). Recommended dose is 200 mg daily in

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divided doses. The successful treatment of arthritis depends on the maintenance of effective drug concentration in the body for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow release rate over an extended period of time and achieve this objective. The mean plasma elimination half life of aceclofenac is 4 hours (Maryaele et al., 1996). To reduce the dosing frequency and adverse effects during prolong treatment it is needed to formulate in long acting dosage form. Different workers have attempted to prepare sustained release oral formulations of aceclofenac like sustained release tablet and microparticulate system (Mutalik et al., 2007; Dashora et al., 2006). Preparation of mucoadhesive microspheres would be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes (Patel et al, 2004). This can be achieved by coupling mucoadhesion characteristic to microspheres and developing mucoadhesive microsphere.

Ethylcellulose (EC), non-biodegradable and biocompatible polymer, is an extensively studied encapsulating material for the controlled release of pharmaceuticals(Ramarao et al.,2005). Carbopol 934 (CP) selected as polymer in the production of mucoadhesive microspheres due to its mucoadhesive properties (Harikarnapakdee et al., 2006). The purpose of present work was to design, characterize and evaluate mucoadhesive microspheres of ACE employing carbopol as mucoadhesive polymer.

Materials and Methods

Materials

Aceclofenac (ACE) was obtained as a gift sample from Comed Chemicals Limited (Vadodara, India). Ethyl cellulose (EC) and Carbopol 934 (CP) were purchased from Central Drug House, India. All other reagents and solvent used were of analytical grade.

Preparation of mucoadhesive microspheres

The ACE mucoadhesive microspheres were prepared by an emulsification/ evaporation method (Liu et al., 2005). EC was dissolved in 10 ml of acetone; ACE was added and dissolved in polymer solution. Then, 50 mg of CP powder was added and stirred under magnetic stirring for 2 hour. Then the suspension was slowly dispersed in 100 ml light liquid paraffin containing 3 % Span 80 at a stirring rate of 600 rpm using mechanical stirrer (Lab stirrer, Remi motors, India). After 60 min of emulsification, acetone was completely evaporated. The hardened microspheres were collected by filtration and washed with three portions of petroleum ether and air dried at room temperature for 12 hours. Batches were prepared in triplicate to obtain reproducible results. The composition of various formulations is shown in Table 1.

Table 1. Composition of mucoadhesive microspheres formulations

Batch	Drug(g)	EC(g)	CP(g)	Acetone (ml)	Span-80 (%)	Liquid paraffin light (ml)	n-Hexane (ml)
B1	0.500	0.250	0.050	10	3.0	100	10
B2	0.500	0.500	0.050	10	3.0	100	10
B3	0.500	0.750	0.050	10	3.0	100	10
B4	0.500	1.000	0.050	10	3.0	100	10
B5	0.500	1.250	0.050	10	3.0	100	10

Encapsulation efficiency

To determine encapsulation efficiency, 100 mg of accurately weighed drug loaded mucoadhesive microspheres were added to 100 ml of methanol. The resulting mixture was kept shaking on a mechanical shaker for 24 h. Then, after the solution was filtered and 1 ml of this solution was appropriately diluted with methanol and analyzed with spectrophotometrically at 275 nm using a Shimazdu UV-1700 (UV/VIS double beam spectrophotometer, Kyoto, Japan). The drug encapsulation efficiency was calculated using the following formula: (Practical drug content/Theoretical Drug content) × 100.

Particle Size

The particle size of microsphere was determined by using optical microscopy method (Dandagi et al., 2007). Approximately 100 microspheres were counted for particle size using a calibrated optical microscope fitted with an ocular micrometer and stage micrometer (ALMICRO, Ambala, India). The average particle size was determined by using Edmondson's equation $D_{Mean}=\Sigma nd/\Sigma n$, where n=number of microspheres observed and d=mean size range.

Swelling index

Swelling index was determined by measuring the extent of swelling of microspheres in phosphate buffer pH 6.8 (Dandagi et al., 2007). To ensure complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in phosphate buffer pH 6.8 for 12 h. The excess surface adhered liquid drops were removed by blotting and swollen microspheres were weighed by using microbalance. The hydrogel microspheres then dried in oven at 60°C for 5 h until there was no change in dried mass of sample. The swelling index of microspheres were calculated by using the formula Swelling index= (mass of swollen microspheres- mass of dry microspheres/ mass of dried microspheres) ×100.

In-Vitro wash off test

The mucoadhesive properties of the microspheres were evaluated by in vitro adhesion testing method known as the wash-off method (Chowdary et al., 2003). Freshly excised pieces of intestinal mucosa (2×2 cm) from rat were mounted onto glass slide (3×1 inch) using thread. Approximately 50 Microspheres were spread onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the Phosphate buffer pH 6.8. At the end of 30 minutes, 1 hour, and at hourly intervals up to 8 hours, the number of microspheres still adhering onto the tissue was counted. Adhesion number for mucoadhesive microspheres is determined as the ratio of the number of particles attached to the surface to the total number of applied particles, expressed as a percentage.

Scanning electron microscope (SEM)

A scanning electron microscope (ESEM TMP with EDAX, Philips, Holland) was used to characterize the surface topography of the microscope. The microspheres were placed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum. The surface was scanned and photographs were taken at 30kV accelerating voltage for the drug loaded microspheres.

Fourier transform infrared spectroscopy (FTIR)

The spectra were recorded for pure drug, drug loaded microspheres and blank microspheres using FTIR (Perkin-Elmer, Spectrum GX, USA). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400 – 4000 cm^{-1} and the resolution was 2 cm^{-1}.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) scans of aceclofenac, blank microspheres and drug loaded microspheres were performed using DSC-PYRIS-1(Perkin-Elmer, USA). The analysis was performed with a heating range of 50- 300°C and a rate of 10 °C min^{-1}.

Drug release study

The drug release study was performed using USP XXIV basket apparatus (Electrolab, TDT-06T, Mumbai, India) at 37°C and at 50 rpm using 900 mL of phosphate buffer (pH 6.8) as a dissolution medium up to 12 hr (Mutalik et al., 2007). Microspheres equivalent to 100 mg of Aceclofenac were used for the test. Five ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 mm membrane filter, diluted suitably, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation.

Release kinetics

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equations like zero order(% release vs t), first order (log% unrelease vs t) , Higuchi matrix (% release vs square root of time), Hixion Crowell (cube root of % release vs time) (Yadav et al., 2007). In order to define a model which will represent a better fit for the formulation, drug release data further analysed by Peppas equation, $Mt/M\infty=kt^n$, where Mt is the amount of drug released at time t and $M\infty$ is the amount released at time ∞, the $Mt/M\infty$ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. r^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

Results and discussion

Effect of experimental variables on particle size

The processing variables like drug to polymer ratio, stirring speed, stabilizer concentration affect the particle size of microspheres. The drug to polymer ratio appeared to influence on particle size distribution of microspheres (Table 2). When drug to polymer ratio was increased from 1:05 to 1:2 and 1:2.5, the proportion of larger particles formed became higher, because this may be due to increase in viscosity of the solvent with increase in polymer to drug ratio. The mean particle size ranged from 15 to 240 μm. The minimum concentration of span 80 required to form stable emulsion was found to be 3%. Changing the stirring speed during emulsification process seems to influence the mean particle size of the microspheres. When the stirring speed was kept below 600 rpm, the mean particle size of the microspheres was increased and they became large and aggregated.

Table 2. Mean Particle size, Encapsulation efficiency

Batch	Theoritical drug content	Practical drug content	Encapsulation efficiency (%)	Mean Particle size (μm)
B1	62.50	23.72	37.95±0.801	89.20±13.10
B2	47.62	20.43	42.90±1.181	95.55±15.54
B3	38.46	18.83	58.38±0.997	99.05±17.76
B4	32.25	20.77	64.43±0.343	126.00±19.87
B5	27.77	15.53	55.92±0.241	155.57±21.072

* Each observation is the mean (±SD) of three determinations

Encapsulation efficiency

The drug entrapment efficiency within microspheres produced using the solvent evaporatior method is of fundamental importance as failure to achieve acceptable drug loadings may preclude the use of this method for economic reasons (Jones et al., 1995).The entrapmen

efficiency of various formulations was found to be in the range of 37 to 64% as shown in Table 2. The entrapment efficiency less than expected may be due to solubility of the drug in the solvent. Because of its solubility, the drug may be migrated to the processing medium during extraction and evaporation process of acetone.

Swelling index

The most promising approach to achieving gastro retention is that of creating a swelling or expanding system *in situ* (Davis SS, 2005). Figure 1 depicts the swelling index of microspheres. It is evident from the figure that all obtained microspheres rapidly swelled in phosphate buffer pH 6.8. The high swelling property of CP could be attributed to high molecular weight and their ionized ability to uncoil polymer into an extended structure.

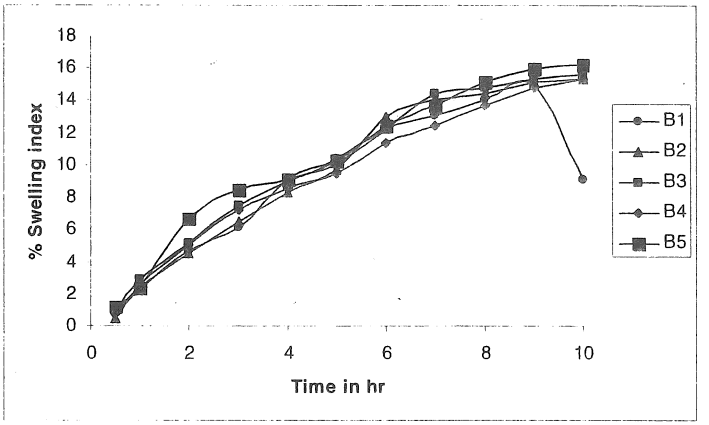


Figure 1. The Profiles of percentage swelling index with time of microspheres

In-vitro wash off test

In the mucoadhesion process, it is necessary for swelling and expansion of the polymer chain since interpenetration and entanglement of the polymers and the mucous networks are considered to be responsible for adhesion (Ponchel et al., 1997). Therefore, bioadhesives should swell and expand rapidly when they come in contact with water. A high percentage of adhesion indicates that microspheres have excellent mucoadhesion to mucosal tissue. Percentages of muoadhesion are given in Table 3. It can be seen that the microspheres had good mucoadhesive properties and could adequately adhere to intestinal mucosa. The results also showed that with increasing polymer to drug ratio, the % mucoadhesion also increases.

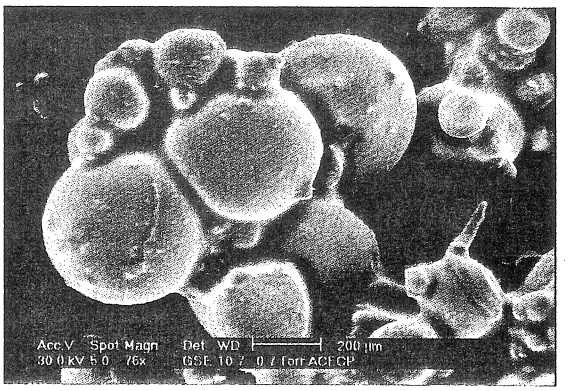
Table 3. In-Vitro Wash off test

Batch	Percentage of mucoadhesive microsphere adhering to tissue after (hr)				
	1	2	4	6	8
B1	78 (1.1)	70 (0.60)	42 (1.54)	26 (2.21)	-
B2	74 (0.83)	62 (1.14)	44 (0.45)	22 (2.1)	-
B3	78 (1.34)	70 (1.30)	56 (0.58)	42 (1.81)	30 (0.79)
B4	76 (1.25)	66 (0.37)	52 (2.15)	38 (1.25)	20 (0.73)
B5	68 (1.65)	56 (1.55)	36 (0.47)	24 (1.55)	12 (2.14)

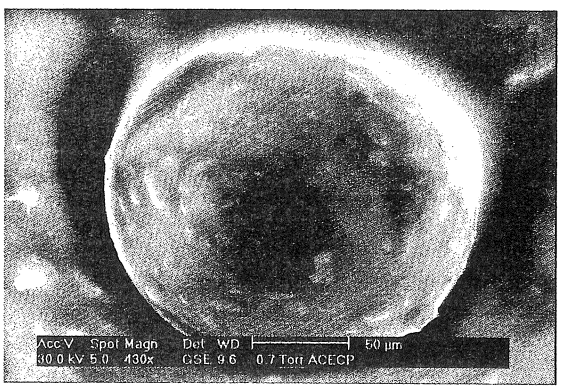
* Figures in parentheses are coefficient of variation values.

Scanning Electron Microscopy

Surface morphology of microspheres and the morphological changes produced through polymer degradation can be investigated and documented using scanning electron microscopy (SEM). The morphological surfaces changes occurring due to the hydrolytic degradation of the polymers From Scanning Electron Microscopy (SEM) study, it was found that microspheres were spherical as shown in Figure 2. The surface of microspheres was rough. The study of drug loaded microspheres shows the presence of drug particles on the surface, which may be responsible for an initial burst release of the drug during dissolution.



(a)

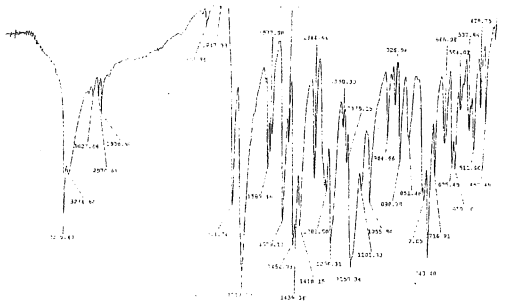


(b)

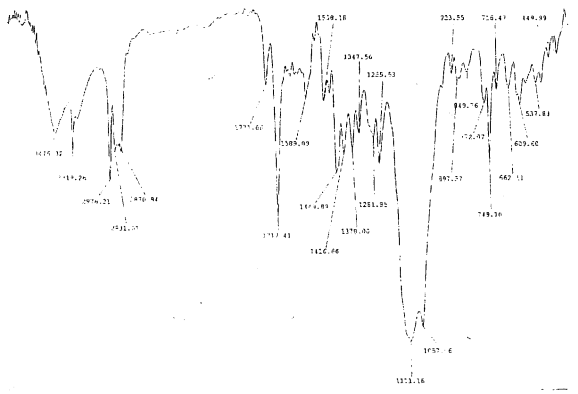
Figure 2. SEM photographs of microspheres. (a) different size of microspheres, (b) single microsphere.

Infrared Spectroscopy

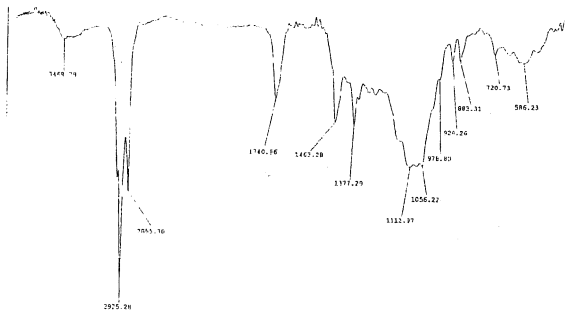
The IR spectra of pure aceclofenac, drug loaded microsphere and blank microsphere are shown in the Figure 3. The peak at 3319 nm indicating the –NH stretching, two peaks at 1771 nm and 1717 nm for the –C=O stretching of –COO and –COOH group respectively. The peaks at 1589 nm, 1281 nm, and 749 nm show as major peaks for drug. All the above are peaks presents in drug loaded microspheres that confirms the presence of drug in the polymer without any interaction.



(A)



(B)

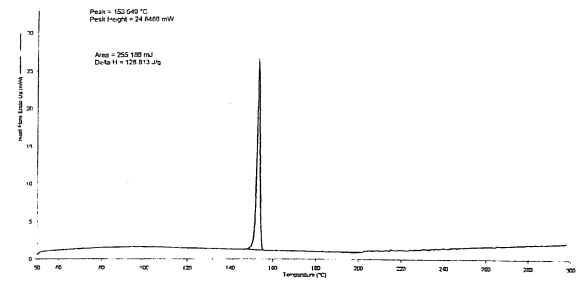


(C)

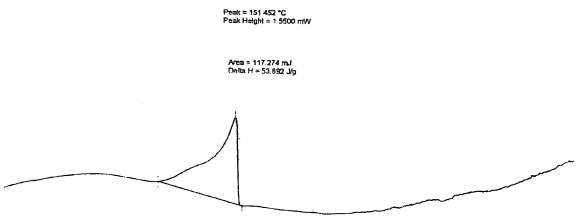
Figure 3. FT-IR spectra of pure Aceclofenac (A), Drug loded microspheres (B), Blank microspheres (C)

Differential Scanning Colorimetry study

The results of DSC study are given in Figure 4. DSC thermograms showed endothermic peak of ACE at 153°C, which corresponded to its melting point. Thermograms of drug loaded microspheres showed peak at 151°C and blank microspheres showed at 140°C. No interaction was observed between drug and polymers.



(A)



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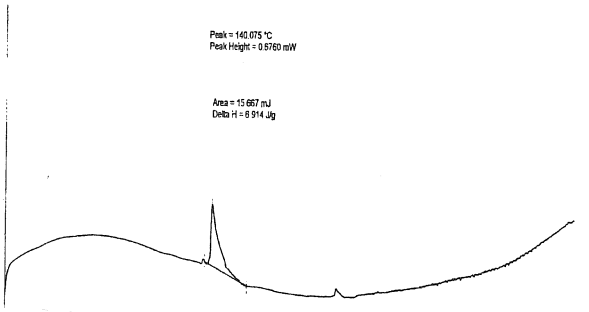

e (C)

Figure 4. DSC thermograms of pure Aceclofenac (A), Drug loaded microspheres (B), Blank microspheres (C)

In-Vitro release study

In vitro release profiles of ACE microspheres are shown in Figure 5. The release profiles of the formulations appear to be slow release with negligible burst effect. The burst effect corresponds to the release of the drug located on or near surface of the microspheres or release of poorly entrapped drug. The rate of release of drug from the mucoadhesive microspheres was slow and found to further decrease with increase in EC ratio. The slow release may be due to the medium being diffused in the polymer matrix and the drug diffusing out of the microspheres.

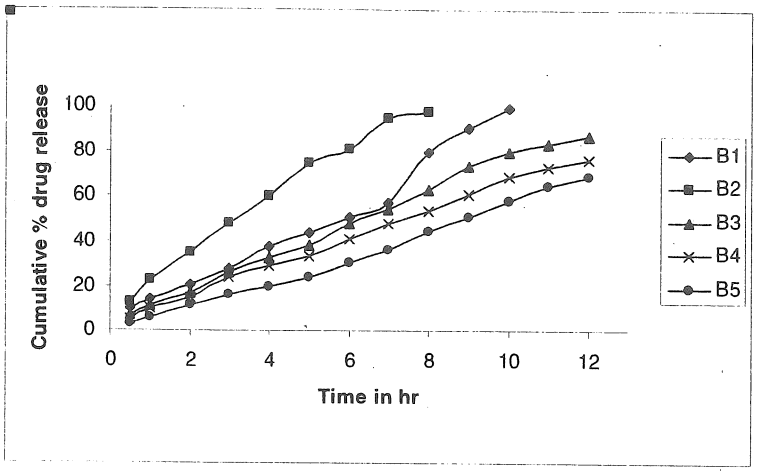

Figure 5. Cumulative percent release of aceclofenac (n=3) from different mucoadhesive microspheres prepared with different drug:polymer ratio.

Release kinetics

The In vitro release profile was analyzed by various kinetic models. The kinetic models used were Higuchi, zero order, first order, Hixion Crowell and Krosmeyer Peppas equations (Table 4). The release constants were calculated from the slope of the respective plots. Higher correlation was observed in the Peppas equation. For planer geometry, the value of n=0.5 indicates a Fickian diffusion mechanism, for 0.5<n<1.0, indicates anomalous (non-fickian) transport, and n=1 implies case II (relaxation controlled) transport. In the present systems, the value for n was found to be in the range of 0.74 to 1.012, indicating that the release mechanisms followed anomalous (non-fickian) transport as well as case II transport. The optimized batch B3 was having n=0.864, indicating that the release mechanism followed is anomalous (non-fickian).

Table 4. Various parameters of the model equations on the in vitro release kinetics

Batch	Higuchi model		Zero order		First order		Hixion Crowell		Krosemeyer peppes model	
	r^2	K_h	r^2	K_0	r^2	K_1	r^2	K_c	r^2	n
B1	0.918	35.91	0.972	9.36	0.972	-9.36	0.825	-0.314	0.968	0.780
B2	0.990	41.88	0.985	11.61	0.985	-9.11	0.965	-0.409	0.995	0.742
B3	0.973	31.21	0.992	07.34	0.992	-7.34	0.976	-0.195	0.996	0.818
B4	0.975	26.97	0.996	06.33	0.994	-6.33	0.987	-0.148	0.993	0.864
B5	0.949	24.44	0.992	05.82	0.992	-5.82	0.974	-0.124	0.992	1.012

In attempt to prepare mucoadhesive microspheres of Aceclofenac using emulsification/evaporation method, the microspheres were at a suitable size and had good mucoadhesive property. The entrapment efficiency was in the range of 37-64%. Aceclofenac release from these mucoadhesive microspheres was slow and extended over longer periods of time and depended on composition of the coat. Drug release was non-fickian. Further, the desired goals can be obtained by systemic evaluation of mucoadhesive microsphers in animals and/or human volunteers.

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