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### Original Article

# Preparation of Sustained Release Microspheres of Aceclofenac: Characterization & *in-vivo* studies

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**Keywords:** Aceclofenac, Carbopol, Sustained Release, Mucoadhesive, Dissolution.

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**ABSTRACT: Objective:** To explore the sustained drug release model of Aceclofenac from microspheres to establish the result of hydrogel forming mucoadhesive polymers on drug release.

**Methods:** Microspheres were produced by Emulsion solvent evaporation method using Carboxy Methyl cellulose (CMC) and Carbopol 934P (CP) as hydrogel forming polymers.

**Results and Discussion:** Mucoadhesive sustained release microspheres of Aceclofenac were prepared using carboxymethyl cellulose sodium and carbomer 934P hydrogel forming polymers. Carboxymethyl cellulose sodium and Carbomer 934P were used in 1/3 and 1/6w/w proportion in the preparation. Scanning electron microscopy revealed the uneven outer surface of the microspheres. *In vitro* dissolution test was carried out in simulated gastric fluid pH 1.2 and simulated intestinal fluid pH 6.8. Release of Aceclofenac from microspheres was found sustained, dependent on the erosion of crosslinking of carbopol 934P. *In vitro* mucoadhesive study was done on fresh stomach mucosa of chicken and chicken egg shell membrane. *In vivo* gastroenteric conveyance of microspheres was done in Male Sprague-Dawley rats.

**Conclusion:** *In vitro* drug release is sustained for the period of 14 hrs. *In vitro* Mucoadhesion evaluation can be done either on chicken stomach mucous membrane or chicken eggshell membrane.

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## INTRODUCTION

The superiority of the stream mucoadhesive polymers are polyacrylic acid derivatives, cellulose derivatives, chitosan and gums such as guar, xanthan, pectin [1]. The procedure of mucoadhesion cover wetting and swelling of the polymer in contact with the tissue, interpenetration between the polymer and the mucin chains and construction of weak chemical bonds [2,3]. The presence of noble amount of hydrogen-bonding analytical groups, high polymer molecular weight and high polymer bond flexibility enhance the mucoadhesive properties of the polymer [4]. Hydrogels are indissoluble polymer mesh that can expand in water and physiologic fluids. The cross-links present in the chemical structure prevent their dissolution and maintained their physical integrity [5]. Hydrogels have been extensively used as excipients in the preparation of dosage forms for peroral dispensation [6,7].

Aceclofenac is highly lipotropic biopharmaceutical classification system (BCS) class II drug, extensively used non-steroidal anti-inflammatory drug in the management of arthritis. As it is under the class II of the BCS, polymers can be used to prolong its tenor of operation in body, thereby increasing the residence time of drug in the gastroenteric tract. This will reduce the frequency of administration there by lower adverse effects; therefore, a better composed patient compliance is expected, for that a sustained release formulation of the drug is demand.

It causes annoyance in the gastroenteric mucous membrane and has fast dissolution at enteric fluid pH 7.2 [8, 9,13]. In the instant investigation, an attempt has been made to provide mucoadhesive sustained release microspheres of Aceclofenac with confederacy of Semi-synthetic and synthetic hydrogel forming polymers; carboxymethyl cellulose sodium and carbomer 934P.

The sustained drug release model of Aceclofenac from microspheres was to be investigated and attempt will be to establish the result of hydrogel forming mucoadhesive polymers on drug release.

## MATERIALS AND METHODS

Aceclofenac (ACF) and Carbopol 934P (CP) were purchased from Alfa Aesar, GmbH & Co KG, Germany. Carboxy Methyl cellulose sodium (CMC); Molecular Weight 90,000 was purchased from BE, New jersey, USA. Dibasic sodium phosphate and monobasic sodium phosphate were purchased from Sigma-Aldrich. The liquids used in the study were of HPLC grade. All the chemicals were used as received.

**Method of preparation:** Microspheres were produced by Emulsion solvent evaporation method and described as follows [10, 11]. 3g of Carboxy Methyl cellulose (CMC) and Carbopol 934P (CP) in different (1part of CMC/3 parts of CP) ratio 1/3 and 1/6w/w were dissolved in 20 ml of ethanol containing 1g of ACF and then poured into 150ml of paraffin containing 1% span 80. The mixture was mechanically stirred at room temperature (2000rpm) to form a w/o emulsion. After 45 minutes the solution was heated at 70°C under continuous agitation (600rpm) to evaporate acetone. Then the solution was gradually cooled at 20°C and then decanted off. The removal of residual oil was performed by washing the microspheres with petroleum ether for 3 times. The microspheres were dried under vacuum at room temperature. The percentage yield was determined. Placebo microspheres were prepared by the same method under similar conditions using CMC-CP 1/6w/w ratio.

**Drug loading determination:** Drug content of the microspheres was determined by HPLC. Mobile phase was prepared by mixing 0.01M dibasic sodium phosphate in acetonitrile and 0.01M monobasic sodium phosphate in water in the ratio of 1:1. Weigh accurately the microspheres containing ACF equivalent to 100mg and transfer to a 100 ml volumetric flask. Dissolved and diluted to 100ml with mobile phase.

Pipette 10ml of this solution into a 100ml volumetric flask, diluted with mobile phase to volume and mix. Standard solution of USP ACF RS 0.1mg/ml concentration in mobile phase was used. The UV detector with 254nm detector, sample volume 20µml was used. Mobile phase flow rate 1ml/min was maintained [12].

$$\% \text{Drug loading efficiency} = \frac{\text{weight of the drug in microcapsules}}{\text{Weight of the drug added}} \times 100$$

**Stability of Aceclofenac:** The stability of ACF microcapsules during the preparation was investigated. The ACF-CMC-CP (1/3) (1/6) formulation was used in the stability study. Certain amount of microcapsules were powdered, 0.5 g of it was taken and dissolve in 50ml of ethanol. The solution was maintained at 70°C for 120 minutes to remove the ethanol. The dried sample was dissolve in mobile phase and filtered with a membrane filter (pore size 0.45µm) and drug contents were determined by HPLC (Agilent Technologies)

**Particle size, morphology and flow properties:** The particle size of the microspheres was determined by using optical microscopy. The surface and inner part of the microspheres were observed respectively by scanning electron microscopy on S3400N SEM from Hitachi. Flow properties of the prepared microspheres were evaluated by determination of angle of repose and hausner's ratio.

**Differential Scanning Calorimetry:** Thermal properties of the Microspheres were investigated using the differential scanning calorimetry (Modulated DSC VI.1A). 10mg of the sample was analyzed in an aluminium pan at a scanning rate of 10°C/min between 0°C and 300°C under the inert environment of nitrogen. The thermograms of ACF, ACF microspheres and placebo microspheres were obtained.

**In-vitro Drug Release:** The invitro release of ACF from microspheres was measured using Erweka Basket type dissolution test apparatus. ACF microspheres containing equivalent to 50 mg of drug were filled in the hard gelatin capsules were used in the study. Dissolution media used was simulated gastric fluid USP pH 1.2(SGF) and simulated intestinal fluid USP pH 6.8(SIF). The volume of the dissolution media was 900ml and maintained at 37°C±0.5 at a rotation speed of 50 rpm. An aliquot of 10ml of the solution was withdrawn at predetermined time intervals diluted to 50ml with respective dissolution media, filtered through 0.45µm and replaced by 10 ml of fresh dissolution media immediately. The samples were assayed via UV spectrophotometry (Thermo fisher scientific model Evolution 60S) at 320 nm. The content of ACF was calculated taking 193 as the specific absorbance at 320 nm [13].

The specific absorbance ( $A_{1cm}^{1\%}$ ) of ACF solution (10mg/ml) at 320nm was reported 193. Mathematically the absorbance of 0.0133mg/ml solution of ACF ( $A_m$ ) was calculated as follows:

$$\left( \frac{10}{193} = \frac{0.0133}{A_m} \right); A_m = 0.256$$

$$\% \text{ ACF Dissolved} = A_t / A_m \times 100$$

The data obtained were analyzed by Hixson-Crowell, Higuchi and Korsmeyer-Peppas drug release models. The equations were as follows

$$\text{Hixson-Crowell: } \sqrt[3]{Q_0} - \sqrt[3]{Q_t} = k_{HC} t$$

$$\text{Higuchi: } Q_t = k_H t^{1/2}$$

$$\text{Korsmeyer-Peppas: } \frac{Q_t}{Q_0} = K_m t^n$$

Where  $Q_0$  initial amount of drug ( $t=0$ ) and  $Q_t$  is the drug dissolved in time  $t$ .  $k_{HC}$ ,  $k_H$  and  $K_m$  are the release constants. Release exponent 'n' indicates the mechanism of release, calculated from slope.

Drug release follows fickian diffusion in case  $n < 0.45$ ; a non-fickian transport in case  $0.43 < n < 0.85$ . In case 'n' is more than 0.85 indicates released by erosion [14].

**In-vitro Mucoadhesive study:** The Mucoadhesive properties of microspheres were evaluated using isolated chicken stomach (Broilers; *Gallus domesticus*) and egg shell membrane. The chicken GIT was procured from the local chicken culling facility; was preserved in normal saline and used in the study within 3 hr. The stomach mucosa was rinsed with normal saline. 100 particles of ACF microspheres with different CMC and CP ratio (1/3, 1/6) were scattered uniformly on the surface of the stomach mucosa maintained at 93% relative humidity. After 30 minutes, the tissue was taken out and fixed on a plate at an angle of 45°. The mucosa was rinsed with simulated gastric fluid pH 1.2, without enzymes for 5 minutes [15].

Simultaneously eggshell membranes were employed as a substitute model for invitro mucoadhesion evaluation. Fresh chicken eggs were used to obtain eggshell membranes. Similar procedure was carried out as mice mucosa to measure the invitro mucoadhesion of the microspheres [16]. The microspheres remaining at the surface of stomach mucosa and on the eggshell membrane were counted and percentage adhering was calculated using following equation.

$$\text{Adhering \%} = \frac{100 - \sum_{t=0} N_t}{100} \times 100$$

$N_t$  – number of unbounded microspheres at time 't'

**In-vivo Gastrointestinal Transit of Microspheres:** The *in-vivo* experimental investigation was carried out in the pharmacology test facility Lic no: 1606/PO/a/12/CPCSEA, of our organization under the supervision of Institutional Ethical Committee for the safe use of animals established under CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines.

Male Sprague-Dawley rats weighing 250-300g were fasted for 24h before the experiments. 100 microspheres were orally administered to each animal using the polyethylene tube with 0.4ml of purified water. At specific time intervals in 1, 3 and 7h, the rats were sacrificed with ether. Stomach and small intestine was removed. The small intestine was further cut into three segments and opened longitudinally. The microspheres in the stomach and the intestine were counted [17-19].

## RESULTS AND DISCUSSION

The drug loading efficiency of microspheres was 60.35% for 1/3 w/w ratio and 62.36% for 1/6 w/w ratio respectively (n=3). Content stability of ACF microspheres is shown in figure 1. The ethanol in the sample was removed by evaporation and dry solid was maintained at 70°C for 120 minutes, almost 100% of the drug remained in the preparations of ACF microspheres.

Surface morphology of the microsphere formulation was presented in figure 2. Size of ACF microspheres was relatively closely diversified, with 86% of capsules being 70-110 µm in diameter, and a mean particle size of 80 µm microspheres are spherical with rough surface. Crystals of ACF was seen as deposits on the surface; these crystals remained insoluble in SGF and were visible in the dissolution media during the conduct of drug release experiments.

The angle of repose and hausner's ratio was found 32° (n=3) and 0.98(n=3) respectively for 1/3 Msp. For 1/6 Msp the angle of repose was 34° and hausner's ratio was 105 respectively; indicated that the flow characteristics of the microspheres was found poor and was independent of the concentration and type of polymer used in the investigation.

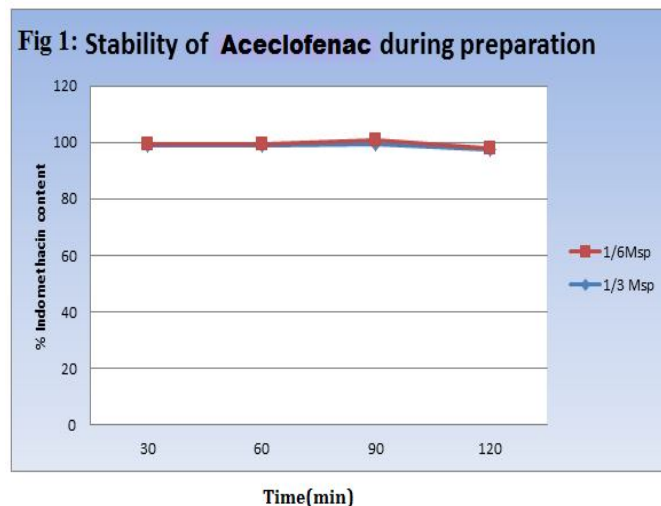


Fig. 1: Stability of Aceclofenac during preparation

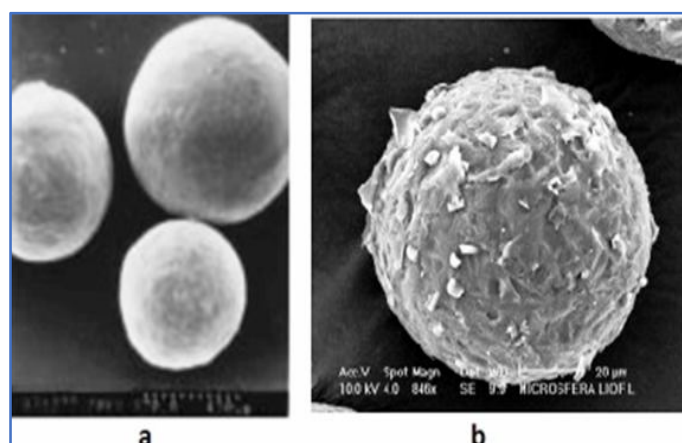


Fig. 2: Optical and Scanning electron micrograph of Aceclofenac microspheres. (a) under optical microscope (100x); (b) surface of the microsphere under SEM (200x)

DSC analysis was done to examine the thermal behavior of microsphere formulation Figure 3. A sharp endothermic peak corresponding to the melting of crystalline ACF was seen at 158.93°C. A broad low intensity peak with glass transition temperature 140.56°C was observed in case of 1/3 and at 145.3°C for 1/6 microspheres.

There was no peak detected in placebo microspheres. This revealed that ACF was in amorphous state in the polymer matrix and is uniformly dispersed between the macromolecular chains of the polymer within microspheres.

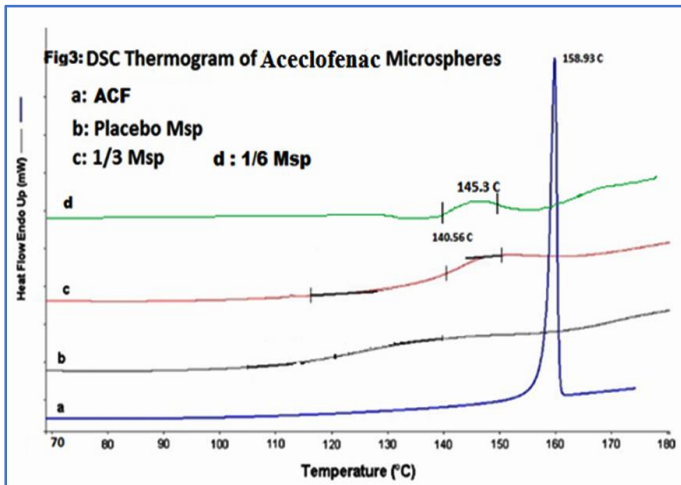


Fig. 3: DSC thermogram of Aceclofenac microspheres

The *in-vitro* release rate of ACF from mucoadhesive microspheres in SGF pH 1.2 and SIF pH 6.8 dissolution media was determined and compared with the marketed ACF capsule preparation, is shown in figure 4. The dissolution testing was performed first in SGF pH 1.2 from 0-3 hours, succeed by SIF pH 6.8 from 3 to 14 hours. More than 80% of Aceclofenac was dissolved from the marketed capsules within an hour in SIF pH 6.8 while less than 10% was dissolved in SGF pH 1.2 in the first 3 hours of the testing from 0-3 hours. ACF release from the microspheres was almost similar with the marketed formulation of around 10% in SGF pH 1.2 dissolution media.

After 7<sup>th</sup> hour in SIF pH 6.8; 64.3% & 78.5% of ACF<sup>th</sup> released was observed in microspheres 1/6 and 1/3 respectively. Sustained release of ACF form microspheres was observed in SIF with more than 90% ACF released from both 1/3 and 1/6 CMC-CP microspheres from 5 to 14 hours of the dissolution test. This is because both CMC and CP form gel in the aqueous media with carbopol can swell up to 1000 times its original volume above pH 4 to 6 [20,21].

In Figure 4; the correlation coefficients ( $R^2$ ) obtained from different release models of ACF microspheres with distinct CMC/CP ratio of 1/3 and 1/6 at SIF pH 6.8 was presented. The value of  $R^2$  found in stroll of 0.90 to 0.99, indicates a closely perfect linear correlation observed in the dissolution profile of Aceclofenac from 1/3 and 1/6 types of microspheres. The drug release is due to both dissolution and diffusion. The Korsmeyer-Peppas model 'n' value was found above 0.89 indicated the erosion of polymeric chain dominates the drug release.

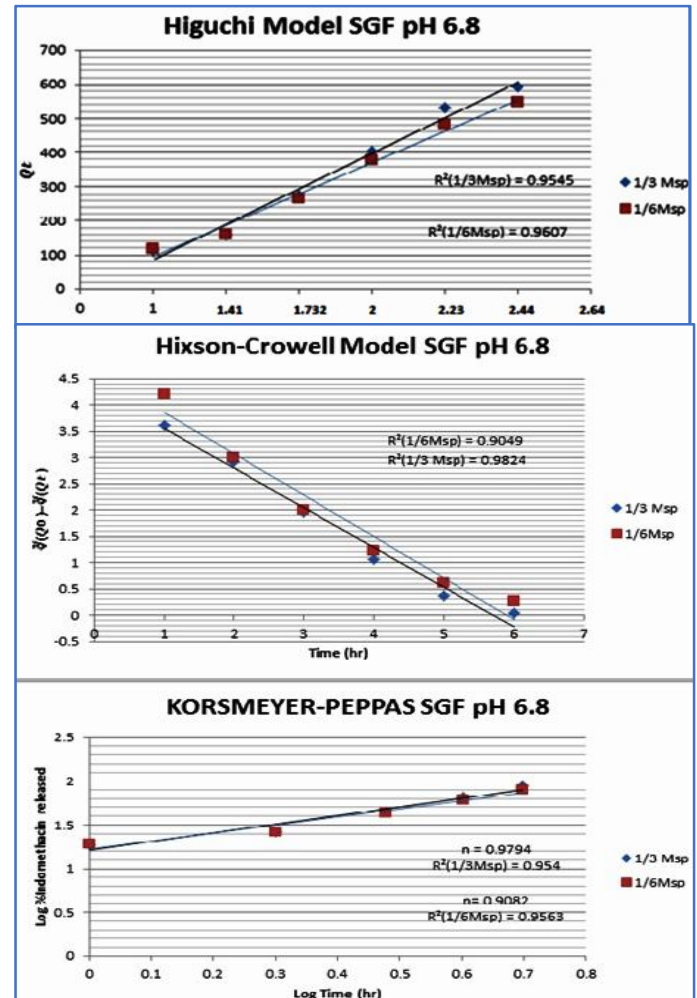
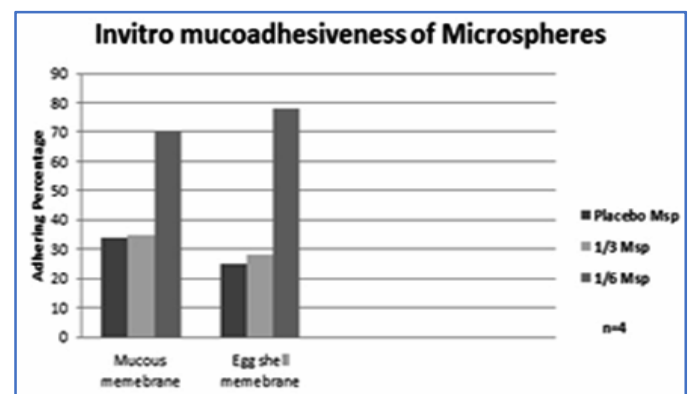
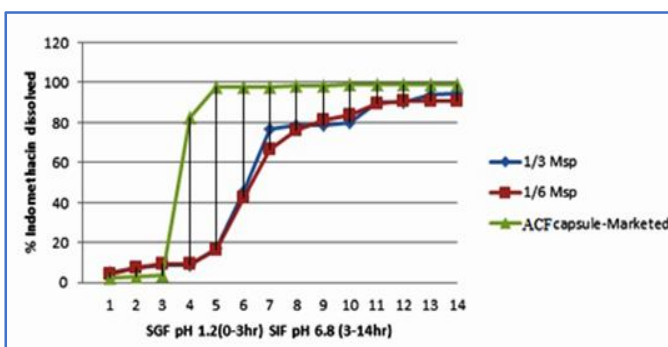
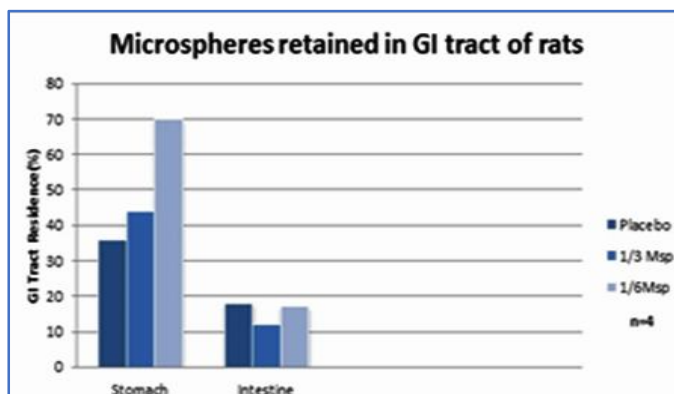


Fig. 4: Dissolution profile of Aceclofenac microspheres

The *in-vitro* mucoadhesive properties of microspheres were evaluated on two distinct membranes; chicken stomach mucosa and chicken egg shell membrane; portrayed in figure 5. The adhering percentage was notably increased with enhance in carbopol incorporated in the microspheres, shown that carbopol has a stout ability to engage with mucus. The correlation coefficient between both methods used in the mucoadhesion studies was also examined. The coefficients were more than 0.96 between the two methods; on stomach mucous membrane or eggshell membrane which shown that egg shell membrane could be a necessity as surrogate of animal mucous membrane to measurement *in-vitro* mucoadhesion.





**Fig. 5:** *In vitro* and *in vivo* evaluation of Aceclofenac microspheres

*In-vivo* mucoadhesive appraisalment of microspheres of different formulation was conducted in rats. The microspheres stay in the gastroenteric tract were studied after being orally dispense to rats. The arrangement of the microspheres in the gastroenteric tract of rats was explore at 2, 4 and 6 hr after oral dispensation as shown in figure 5. The remaining percentages of microspheres (CMC-CP1/6) in the stomach at each time interval were all way higher than that of placebo and CMC-CP1/3. This shown that carbopol incorporated in the microspheres induced forcible adherence to the stomach mucus layer. Most of the microspheres had vanished through the stomach in 2hr after dispensation. However, the small amount of microspheres residual in the stomach, had a slow rate of gastric conveyance which could be due to the small particle sized microspheres being entrap in the stomach.

## CONCLUSION

In the present study, mucoadhesive CMC-CP microspheres of ACF were prepared by emulsion solvent evaporation method. *In vitro* dissolution studies indicated the drug release is sustained for the period of 14 hrs due to the erosion of the polymer chains and is independent of the concentration of the polymer. For *in vitro* mucoadhesion evaluation either chicken stomach mucous membrane or chicken eggshell membrane can be used. Mucoadhesion of the microspheres was dependent on the amount of polymer.

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