

FABRICATION AND CHARACTERIZATION OF ALGINO-CARBOPOL MICROPARTICULATE SYSTEM OF ACECLOFENAC FOR ORAL SUSTAINED DRUG DELIVERY

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ABSTRACT

Micro-particulate drug delivery of aceclofenac was prepared by gelation technique using a blend of sodium alginate and carbopol 934 as release retardant. All the formulations were investigated for various evaluation parameters like particle size, flow behavior, % swelling, surface morphology study and in vitro drug release etc. All the formulation showed good flow behavior as compared to the pure drug. SEM study revealed that the spheres were almost spherical in shape with rough outer surface. Ex-vivo mucoadhesion study depicts that when the polymer concentration was increased the mucoadhesion property was also increased. FTIR study showed that there was no interaction between drug and polymers. In-vitro drug release study showed that by increasing the polymer concentration the drug release of all the formulation were gradually decreased and the optimized formulation (F7) was able to sustain the drug release for 12 hours. The drug release mechanism showed that all the formulations followed anomalous non-Fickian diffusion mechanism. So, it was concluded that aceclofenac loaded alginocarbopol microspheres can be prepared by gelation technique and used for sustaining the drug release for prolonged period of time.

Keywords: Alginocarbopol microspheres, aceclofenac, flow behavior, ex-vivo mucoadhesion, sustained release.

INTRODUCTION

Aceclofenac is a phenyl acetic acid derivative [2-(2',6'-dichlorophenyl) amino] phenylacetoxycetic acid], and is a novel NSAID indicated for the symptomatic treatment of pain and inflammation¹. It has higher anti-inflammatory action than conventional NSAIDs and works by blocking the action of cyclo-oxygenase that is produced by prostaglandins. Aceclofenac has short biological half-life (about 4 h) and is associated with gastrointestinal disturbances as well as has high frequency of administration that makes it an ideal candidate for sustained release. But in case of sustained release dosage form a major drawback frequently encountered is the inability to increase residence time of the dosage form in the gastrointestinal tract (GIT). The gastric residence of a dosage form is typically short, under fasting condition which is not more than an hour and it is also common for dosage forms to transit rapidly through the small intestine for not more than 3h. Thus rapid gastrointestinal (GI) transit phenomenon may consequently lead to reduction in the extent of absorption of various drugs. As a quite lot number of drugs are absorbed exclusively from small intestine or in a confined segment of intestine, it is therefore advantageous to develop mucoadhesive dosage forms, which can remain in intestinal region for a longer duration of time so as to extend the residence time. Several approaches have been adopted in this direction and one of them is to use oral mucoadhesive drug delivery system^{2,3}. Different polymers have been investigated for mucoadhesion like polyacrylic acid and derivatives⁴, Hydroxypropyl methyl cellulose, various grades of Carbopol as well as natural polymers like sodium alginate, chitosan, starch⁵. Carbopol 934 which is a synthetic high

molecular weight cross-linked polymer of acrylic acid has been investigated extensively by the pharmaceutical researchers as a mucoadhesive polymer because of its high viscosity at low concentration and low toxicity^{6,7}.

So, the objective of the present study was to develop mucoadhesive microsphere of aceclofenac and evaluate the effect of polymer concentration on drug release kinetics. It was also investigated whether these mucoadhesive microspheres were able to restrict the drug release in stomach to prevent the different side effects or not. The gelation technique was selected to prepare microspheres due to its simplicity, low cost and its high entrapment rates achieved with poorly water-soluble drugs.

MATERIALS AND METHODS

Materials

Aceclofenac (AC) was a gift sample from Nicholas Primal India Ltd, India. Sodium alginate (viscosity \approx 3500 cps) was a gift from Signet Chemical Co, India. Carbopol 934 was a gift sample from Noveon, USA. Calcium chloride was purchased from Loba Chem, Pvt. Ltd., India. All other chemicals used were of analytical reagent grade.

Method

Preparation of Na.-alginate microspheres

Sodium alginate microspheres were prepared by ionic cross-linking process (Table I). Sodium alginate was dissolved in distilled water to obtain different concentrations. Then aceclofenac was added to the sodium alginate solution and mixed (Ultratarrax, Jahnke and Kunkel, Germany) for 2 min at 8000 rpm. This



dispersion was added in drop wise to a 5% calcium chloride solution using a 24 G needle with continuous stirring at 200 rpm. The stirring was continued for 30 minutes for complete reaction. After 30 minutes microspheres were collected, washed with distilled water and dried overnight at room temperature.

Preparation of Sodium alginate microspheres with carbopol

Carbopol and Sodium alginate solutions of different concentrations (Table I) were separately prepared by dissolving both the polymer in water under gentle agitation. Then carbopol solution was added into Sodium alginate solution and mixed at 4000 rpm for uniform mixing. Then, aceclofenac was added to the polymer solution and mixed with an Ultraturrax for 2 min at 8000 rpm. The remaining procedure was as same as in Sodium alginate microspheres.

Evaluation of Microspheres

Production yield (% w/w)

The percentage yield of each batch was calculated on weight basis with respect to the weight of starting material. All experiments were carried out in triplicate.

Particle size analysis

The particle sizes of the prepared microspheres were determined by using optical microscopy⁸ fitted with an ocular micrometer. The ocular micrometer was calibrated with a stage micrometer. The mean diameter reported was obtained from a total of 100 microspheres. All experiments were carried out in triplicate.

Entrapment efficiency

The entrapment efficiency of the prepared formulations was determined by the method of extraction of the drug present in the microsphere. The dried microspheres (100 mg) were taken and extracted in 100 mL of phosphate buffer (pH 6.8) for 4 hours. Then the dispersion of microspheres was sonicated for 30 min (Imeco Sonifier, Imeco Ultrasonics, India) and the solution was filtered through a 0.45 µm filter. Finally, the polymeric debris was washed twice with fresh solvent (phosphate buffer) to extract any adhering drug. The drug content of filtrate and washings was determined spectrophotometrically at 273 nm (UV-2450, Shimadzu, Japan). Each determination was made in triplicate.

Micromeritic properties of microspheres

Flow Properties

The flow properties of microspheres were studied by determining various parameters like the angle of repose, Carr's index⁹, bulk density and tapped density. The angle of repose was determined by the fixed-base cone method. Bulk and tapped densities¹⁰ were determined using digital bulk density apparatus (Electrolab, India). Each experiment was conducted in triplicate.

Swelling Study

A weighed amount of microsphere was placed in 100 ml of phosphate buffer (pH 6.8) and allowed to swell. At each time intervals, the swollen microspheres were removed from the media and weighed. Fluid sorption was calculated from the difference between the initial weight of the microspheres and the weight at the time of determination.

Ex-vivo mucoadhesion study

The mucoadhesive property of the microspheres was evaluated by using phosphate buffer, pH 6.8. The freshly excised pieces of intestinal mucosa (2x3 cm) from goat were mounted onto glass slides with cyanoacrylate glue. About 50 nos. of microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the slides with suitable support were hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid at 37°C contained in a one liter vessel. At different time intervals up to 6 h the machine was stopped and the number of microspheres still adhering to the tissue was counted and % mucoadhesion was calculated¹¹.

In vitro drug release study

In-vitro release profile of aceclofenac from the microspheres was examined in phosphate buffer (pH 6.8) using USP (XXI) six stage dissolution rate test apparatus I (Thermolab®, Mumbai, India). Microspheres equivalent to 100 mg of drug was suspended in dissolution medium at 50 rpm and 37 ± 0.5°C. An aliquot of 5 ml was withdrawn periodically at intervals of one hour and same volume of fresh medium was replaced. The samples were filtered through Whatman filter paper and analyzed spectrophotometrically at 273 nm for amount of drug released^{12,13}.

Analysis of release profiles

The rate and mechanism of release of both drugs from the prepared matrix tablets were analyzed by fitting the dissolution data into the zero-order equation:

$$Q = k_0 t$$

where Q is the amount of drug released at time t and k_0 is the release rate constant, first order equation:

$$\ln(100 - Q) = \ln 100 - k_1 t$$

where k_1 is the release rate constant and Higuchi's equation:

$$Q = k_2 t^{1/2}$$

where k_2 is the diffusion rate constant.

Drug release data was further analyzed by the Peppas equation^{14, 15}:



$$\frac{M_t}{M_\infty} = kt^n$$

where n is the release exponent indicative of the mechanism of release, M_t/M_∞ is the fractional release of the drug, t is the release time, k is the kinetic constant.

Morphological Examination

The texture of surface and shape of the microspheres were investigated by using Scanning electron microscopy (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan). The sample (optimized formulation F7) was spread on stub and coated for 120 s with a layer of gold using a sputter coater. Afterwards, the stub containing the sample was placed in the scanning electron microscope chamber. The scanning electron photomicrograph image was taken at the acceleration voltage of 20 kV, chamber pressure of 0.6 mm Hg.

Drug Polymer Interaction (FTIR) Study

FTIR spectra of pure drug, pure polymers and formulations containing both drug and polymers were performed to study the drug polymer interaction. FTIR study was performed by using Fourier transformed

infrared spectrophotometer (Prestige-21, Shimadzu, Japan).

Statistical analysis

In vitro drug release data was subjected to one way analysis of variance (one-way ANOVA). Statistical analysis of the data was performed using the software PRISM (Graph pad, San Diego, CA, USA).

RESULTS AND DISCUSSION

All the microspheres were prepared by ionic gelation process. Calcium chloride was selected as cross linking agent to prepare the microspheres. This method was selected to formulate the microspheres as because it is quick, easy and cost effective. The composition of all prepared formulations is depicted in Table-1.

Production yield

The yield of all the formulations was within the range of 90.7 ± 3.01 to 98.8 ± 2.55 . The low percentage yield in some formulation (F1 and F2) may be due to microspheres lost during the washing process and recovering process. The values of production yield are depicted in Table-1.

Table 1: Composition of prepared microspheres

Formulation Code	Polymer level (% w/w)	Drug level (% w/w)	Cross- linking agent/level (%w/w)	Yield (%)
F1	Sodium alginate, 1%	5	CaCl ₂ /5%	90.7 ± 3.01
F2	Sodium alginate, 2%	5	CaCl ₂ /5%	92.5 ± 2.17
F3	Sodium alginate, 3%	5	CaCl ₂ /5%	98.8 ± 2.55
F4	Sodium alginate, 4%	5	CaCl ₂ /5%	96.3 ± 3.18
F5	Sodium alginate, 1% + Carbopol, 1%	5	CaCl ₂ /5%	98.4 ± 2.98
F6	Sodium alginate, 1% + Carbopol, 2%	5	CaCl ₂ /5%	97.7 ± 3.11
F7	Sodium alginate, 1% + Carbopol, 3%	5	CaCl ₂ /5%	95.6 ± 4.03
F8	Sodium alginate, 1% + Carbopol, 4%	5	CaCl ₂ /5%	96.7 ± 2.16

Mean ± SD, n=3

Table 2. Micromeritic study of prepared microsphere formulations

Formulation	Bulk density (g mL ⁻¹)	Tapped density (g mL ⁻¹)	Angle of repose (°)	Carr's index
PD	0.61 ± 0.12	0.98 ± 0.13	40.2 ± 1.1	37.8 ± 1.3
F1	0.45 ± 0.08	0.56 ± 0.05	19.5 ± 1.2	19.6 ± 1.4
F2	0.53 ± 0.18	0.65 ± 0.13	18.2 ± 1.1	18.5 ± 1.3
F3	0.55 ± 0.03	0.64 ± 0.11	18.7 ± 2.1	14.1 ± 2.0
F4	0.56 ± 0.11	0.65 ± 0.07	17.6 ± 2.3	13.8 ± 1.1
F5	0.65 ± 0.06	0.74 ± 0.07	16.1 ± 1.4	12.2 ± 1.0
F6	0.46 ± 0.07	0.52 ± 0.08	15.0 ± 1.3	11.5 ± 1.3
F7	0.49 ± 0.10	0.54 ± 0.05	14.2 ± 2.0	9.26 ± 1.2
F8	0.51 ± 0.09	0.56 ± 0.07	12.4 ± 1.7	8.92 ± 0.8

Mean ± SD, n=3, PD = Pure Drug.



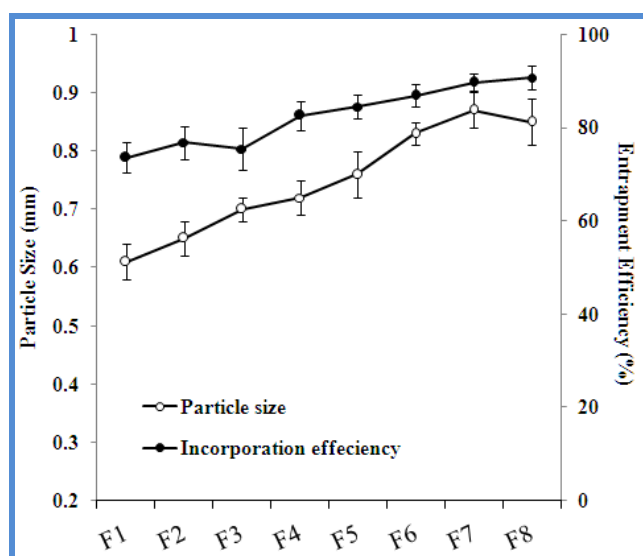
Table 3: Kinetics of drug release from aceclofenac microspheres

Formulation	Drug release kinetics Coefficient of determination (R ²)				Release exponent (n)
	Zero Order (R ²)	First Order (R ²)	Higuchi (R ²)	Korsmeyer-Peppas (R ²)	
F1	0.993	0.932	0.979	0.999	0.843
F2	0.990	0.956	0.983	0.996	0.821
F3	0.987	0.977	0.978	0.993	0.744
F4	0.983	0.962	0.973	0.995	0.762
F5	0.989	0.958	0.984	0.992	0.675
F6	0.992	0.978	0.991	0.996	0.762
F7	0.994	0.975	0.989	0.997	0.611
F8	0.991	0.958	0.979	0.994	0.572

Particle size and entrapment efficiency

The effects of polymer concentration on the particle size and entrapment efficiency of the prepared microspheres are shown in Figure-1. It was observed that the size of the prepared microspheres were in the size range of $0.63 \pm 0.03 \mu\text{m}$ to $0.87 \pm 0.04 \mu\text{m}$. The entrapment efficiency was found within the range of $73.6 \pm 3.21\%$ to $90.7 \pm 2.48\%$.

Figure 1: Effect of Polymer Concentration on Particle size and Entrapment Efficiency



It was found that when the polymer concentration was increases, particle size as well as encapsulation efficiency increased. It may be due to higher concentration of the polymer producing much larger particles. Higher concentration of the polymer increases the viscosity of the medium as well as greater availability of calcium binding sites in the polymeric chains. As a result degree of cross-linking was increases¹⁶ and larger droplets were formed which entrapping greater amount of drug.

Micromeritic properties of microspheres

Bulk density and Tap density

Interparticulate interaction is one of the most important parameter that affects the bulk and flow characteristics of powder¹⁷. The studies of flow characteristics suggested

that an improvement was found in values of bulk density, tap density as described in Table-2 on developing microsphere formulation of aceclofenac, and indicated that the microspheres had good pack ability and enhanced flow ability.

Angle of repose, Carr's index

Angle of repose and Carr's index of pure drug and prepared formulations are summarized in Table-2. Angle of repose and Carr's index of pure drug was found $41.5 \pm 2.4^\circ$ and 37.8 ± 1.3 respectively which indicated poor flow property of the pure drug. The angle of repose and Carr's index of all the formulations (F1 to F8) were found from 13.9 ± 2.1 to 20.3 ± 2.7 and 8.92 ± 0.8 to 19.6 ± 1.4 respectively. So it is concluded that by formulating microsphere formulation of the drug, the flow property of the poorly flowing drug can be improved.

Swelling Study

Figure-2 shows the percentage swelling of different microsphere formulations at different time intervals. The results revealed that all the formulations swelled rapidly when immersed in phosphate buffer (pH 6.8). The adhesive and cohesive properties of mucoadhesive polymers are generally affected by their swelling behaviour which has been reported¹⁸. It is also expected that mucoadhesive polymers take up solvent from the rudimentary mucosal surface by capillary effect and swell up¹⁹. The percent swelling of alginate microsphere (Figure-3a) (F1 to F4) was found within the range of $86.9 \pm 4.71\%$ to $117.8 \pm 3.79\%$ whereas in case of alginate-chitosan microsphere it was found from $108.7 \pm 3.56\%$ to $153.7 \pm 4.66\%$ (Figure-3b). It was found that by increasing the polymer concentration, swelling of all the formulation was also increasing. This could be due to high ionization of carbopol at pH 6.8, which is capable of absorbing a high amount of solvent²⁰.



Figure 2a: Swelling Study of alginate microsphere

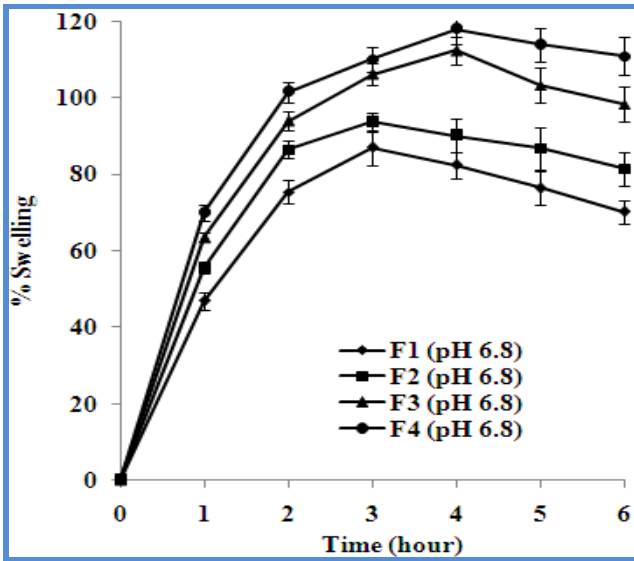


Figure 3b: Mucoadhesion Study of alginato-carbopol microspheres

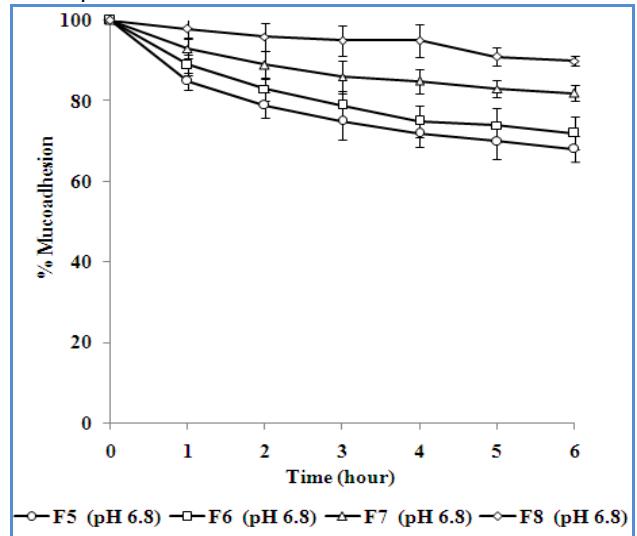


Figure 2b: Swelling Study of alginato-carbopol microsphere

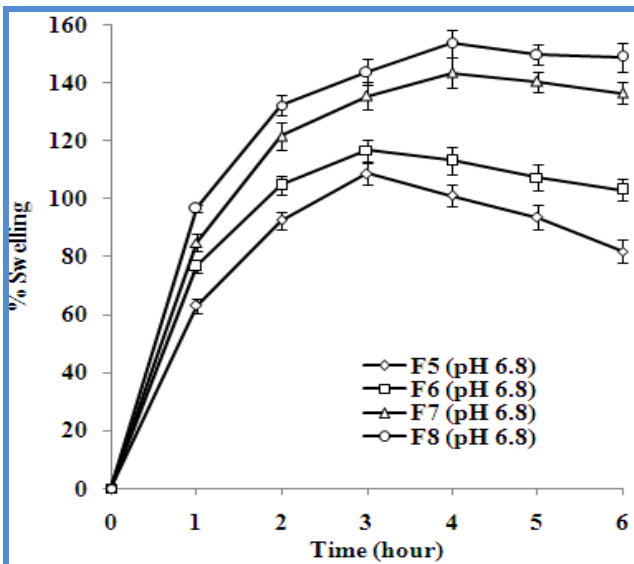


Figure 4a: In-vitro drug release study of alginate microspheres

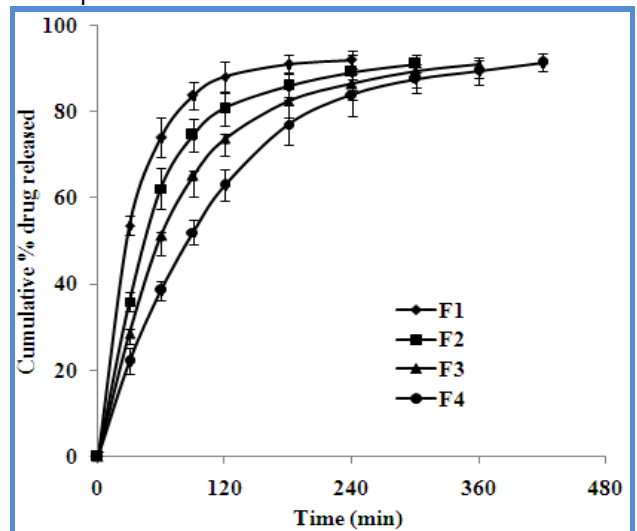


Figure 3a: Mucoadhesion Study of alginate microspheres

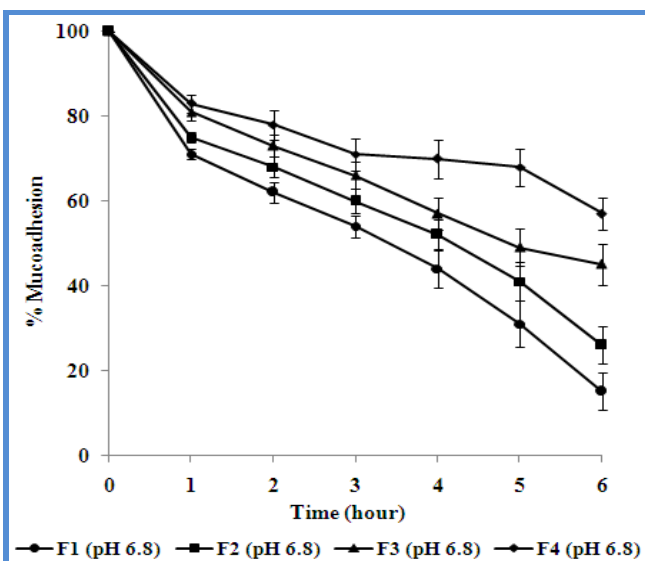
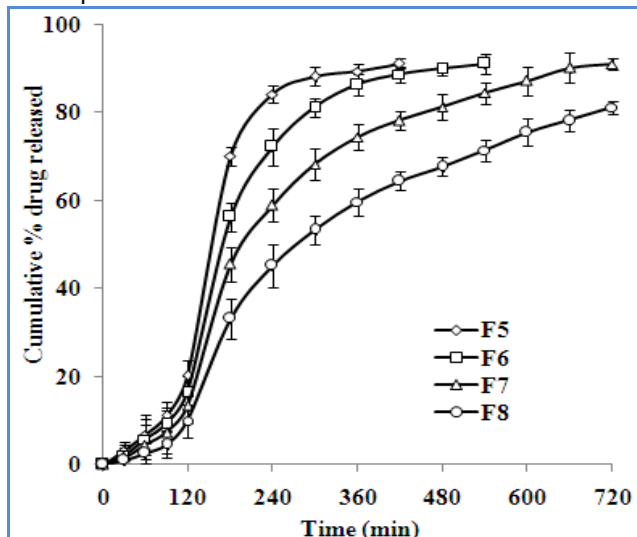


Figure 4b: In-vitro drug release study of alginato-carbopol microspheres



Ex-vivo mucoadhesion study of microspheres

The results of the *ex-vivo* mucoadhesion study indicated that all microspheres had good mucoadhesive properties. The percentage of microspheres attached to the mucosa upto 6 hours has been shown in Figure-3a and 3b. It was found that Sodium alginate microspheres (F1-F4) show less mucoadhesion property (Figure-3a) as compared to algino-carbopol microspheres (Figure-3b). Because in simulated intestinal fluid Sodium alginate solubility, hydration and mucoadhesive property was increased due to ionization of carboxyl acid group present in the polymer. This increases its solubility and shows less mucoadhesion property as compared to algino-carbopol microspheres. Because combination of both the polymers (F5-F8) increases the viscosity, produce more viscous gel which helps to increase adhesion with intestinal mucosa. Therefore, it is suggested that prepared microspheres adhere to the intestinal mucosa for a prolonged period where they release drug in a sustained manner before being eroded off.

In vitro drug release study

The effect of polymer concentration on the release of aceclofenac from the prepared microspheres was studied (F1-F8). Alginate formulation F1, F2, F3 and F4 were able to sustain the drug release for 4, 5, 6 and 7 hours (Figure-4a), respectively whereas algino-carbopol microspheres (F5 to F7) were able to sustain the drug release for more than 7 to 12 hours (Figure-4b). In case of formulation F8 (carbopol 4 %), the release of the drug was too slow and only 81.2 % of the drug was released after 12 hours. Formulations F1 to F4 (Na.-alginate alone) undergo erosion before complete swelling could take place, resulting in faster release of drug. Algino-carbopol microspheres (F5 to F8) were more efficient in sustaining the drug release as compared with alginate microspheres because carbopol could form rigid coat as compared to Sodium alginate. Among all the formulations F7 showed better dissolution profile (more than 90 % drug was

released in 12 hours), so it was selected as optimized formulation for further study.

The coefficient of determination (R^2) (Table-3) of all the formulations reached higher coefficient of determination ($R^2 = 0.992$ to 0.999) with the Korsmeyer-Peppas model whereas release exponent value (n) ranged from 0.572 to 0.843. So, it can be suggested all the formulation follows anomalous non-Fickian diffusion mechanism. In conclusion a combined release mechanism of drug diffusion and spheres erosion might be appropriate.

Morphological Examination

The morphological evaluation of the optimized microsphere formulation (F7) was done by scanning electron microscopy (Figure-5a, 5b). SEM study revealed that the microspheres were almost spherical in shape with rough outer surface.

Drug Polymer Interaction (FTIR) Study

FTIR spectra are shown in Figure-6. Pure aceclofenac showed different characteristic peaks at 3319.2, 3278.9, 1716.8, 1508.1 & 1280.6 cm^{-1} . Among which some major peaks are 3319.2 for stretching vibration of OH of COOH group, 3278.9 for stretching vibration of NH group, 1716.8 for stretching vibration of C=O attached with methylene group and ether, 1280.6 for C-N stretching vibration of secondary aromatic amine, It was observed that all the major peaks of aceclofenac were intact when it was incorporated in the microspheres formulation and no considerable changes in the IR peaks were observed. So FTIR spectra indicate the stable nature of aceclofenac in the prepared formulations.

Statistical analysis

One way analysis of variance suggested that a significant difference was present among all the formulations F1 to F8 with respect to *In vitro* drug release.

Figure 5: SEM Micrographs of optimized formulation (F7)

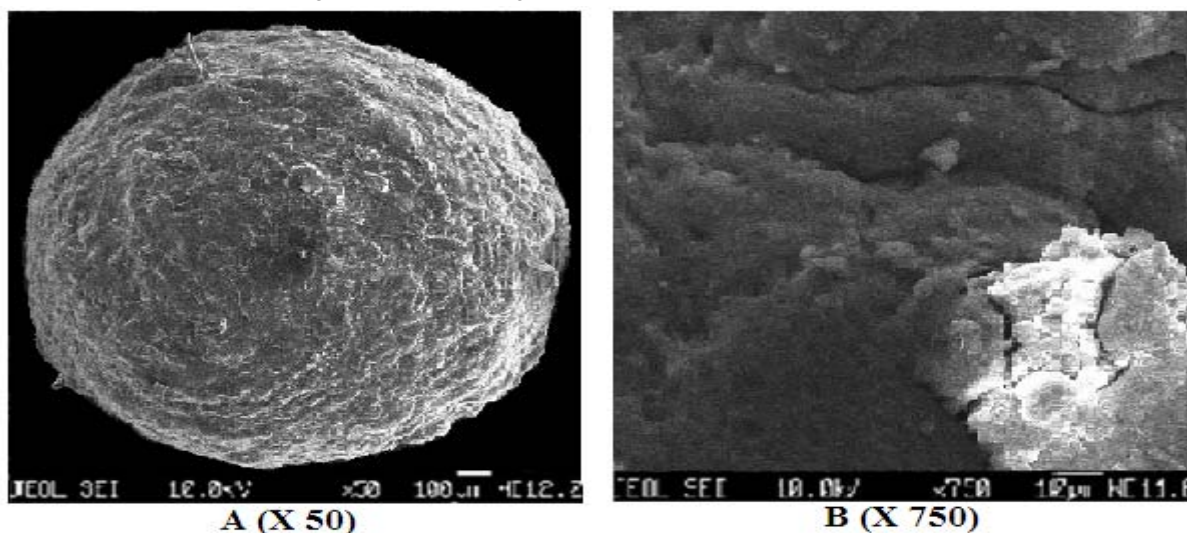
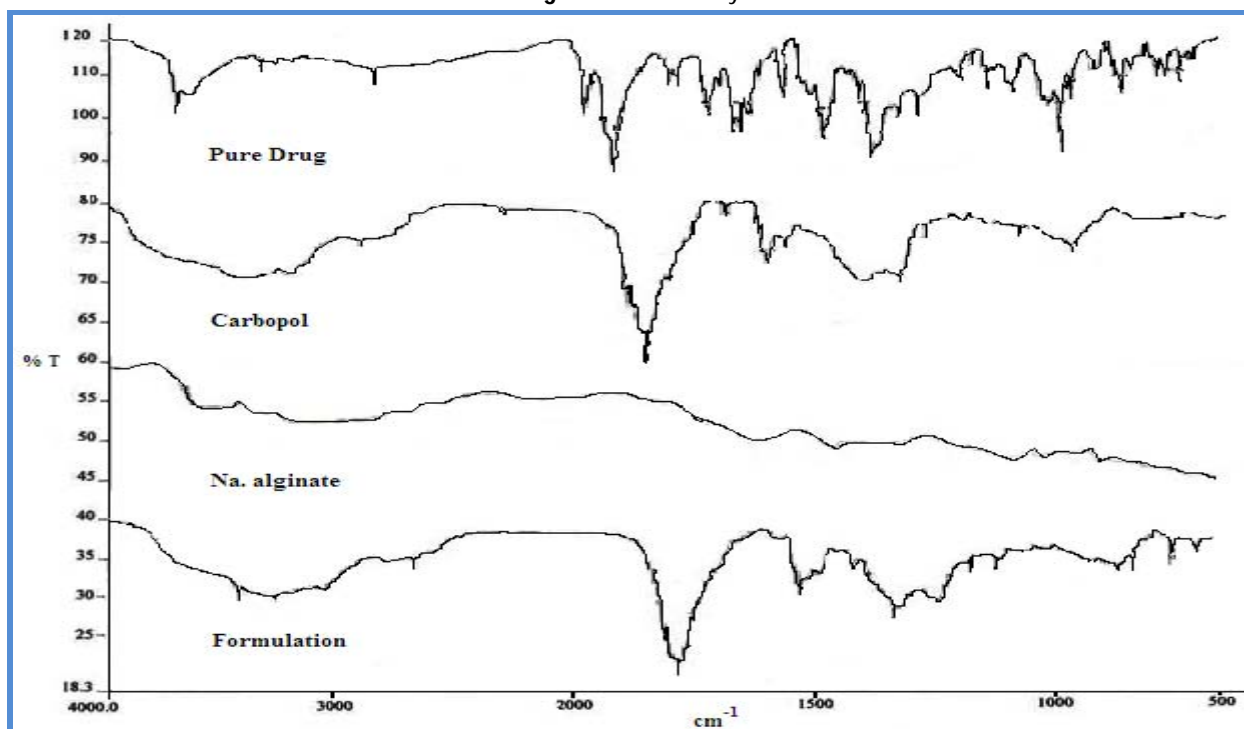


Figure 6: FTIR Study



CONCLUSIONS

In conclusion, the algino-carbopol microspheres can widely use for controlled release for aceclofenac. The mucoadhesive microspheres was also able to restrict the drug release in stomach and adhere themselves in to the intestinal region. The drug release mechanism shows indicated that drug release from the microspheres was triggered by drug diffusion and spheres erosion.

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