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Research Article

A STUDY ON THE EFFECT OF DIFFERENT POLYMERS AND FORMULATION VARIABLES ON ENCAPSULATION EFFICIENCY, MORPHOLOGY AND RELEASE CHARACTERISTICS OF ACECLOFENAC LOADED CALCIUM ALGINATE MICROSPHERES PREPARED BY IONOTROPIC GELATION TECHNIQUE

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ABSTRACT

Aceclofenac loaded alginate microspheres were prepared by the Ionotropic gelation technique for controlling the drug release by using various combinations of blend (Carbopol & HPMC) with Sodium Alginate as anion, Ca^{+2} and Chitosan as cations. The Drug Entrapment Efficiency was found to be ≥86.16%. The effect of Sodium Alginate, Carbopol, HPMC, Chitosan concentration and curing time was evaluated with respect to Entrapment Efficiency, Particle size, Surface Characteristics and In-vitro release behaviors. I.R and D.S.C studies confirmed the absence of drug-polymer interaction. SEM Studies revealed that the drug was molecularly dispersed in the Alginate Microspheres showing rough surface. The mean particle size and Entrapment Efficiency were found to be varied by changing various formulation parameters. The swelling ability of microspheres in pH (5.8 & 6.8) has been found to dependent on polyelectrolyte complexation of microspheres and pH of the media. The In-vitro release profile could be altered significantly by changing various formulation parameters to give a controlled release of drug from the microspheres. Calcium alginate microspheres released ≥ 95% of the drug with 2.5hrs, where as Carbopol blended, HPMC blended released ≥ 95% drug within 5hrs. Similarly Chitosan coated Alginate & Alginate coated Chitosan-Alginate microspheres controlled the drug release and released ≥ 95% drug within 6.5hrs. Drug release from all microspheres changed from case-II (or) anomalous transport mechanism to non-fickian transport as the alginate was replaced in matrix with other polymers (or) coated with Chitosan. It is concluded that Aceclofenac release could be prolonged using binary mixtures (or) coating alginate microspheres with Chitosan.

Keywords: Aceclofenac, Microspheres (Microparticles), Alginate, Carbopol, HPMC, Chitosan.

INTRODUCTION

In the present study a non-steroidal anti-inflammatory drug Aceclofenac has been chosen as model drug [1]. Aceclofenac, (2-[2-[2-(2, 6 – dichlorophenyl) aminophenyl] acetyl] oxyacetic acid) a non-steroidal anti-inflammatory drug [NSAID] has been indicated for various conditions like post-traumatic pain, rheumatoid arthritis, ankylosing spondylitis. The molecule is practically insoluble in water, but almost totally absorbed from gastrointestinal tract, its biological half-life is 4hrs and administered twice daily with single dose of 100mg. To overcome the side effects associated with conventional administration of NSAIDS and increase the patient compliance, controlled release dosage forms have been formulated in the form of Single Unit and Multiunit dosage forms. Compared to Single Unit dosage forms, Multi unit drug delivery system avoid the variations in gastric emptying and different transit rates through the gastrointestinal tract[2], release drugs in a more predictable manner[3], and spread over a large area preventing exposure of the absorbing site to high drug concentration on chronic dosing[4]. Several synthetic polymers have been used to formulate multiunit dosage forms. Recently, much research efforts have been concentrated to develop drug-loaded microspheres using sodium alginate, a natural polymer obtained from marine brown algae, because of simple, mild and eco-friendly preparative conditions.

Materials & Methods

Aceclofenac was received as a gift sample from Rantus Pharma Pvt Ltd., Hyderabad. HPMC (E50LV) was procured from Aurobindo Pharmaceuticals Ltd., Hyderabad. Chitosan Powder was procured from Central Institute of Fisheries, Cochin, and Kerala. All other chemicals and solvents were of analytical grade satisfying pharmacopoeial specifications.

Formulation of Microspheres [5 -19]

Various microspheres were prepared by Ionotropic gelation technique using the formulations as shown in table - 1. In 10ml of aqueous solutions of Sodium Alginate (2% w/v) required amount of Aceclofenac was dispersed uniformly and homogenized for 15min. The dispersion was sonicated for 30min to remove any air bubbles that may have been formed during stirring process. Bubble free dispersion was dropped through a 16 bore glass syringe in a gently agitated calcium chloride solution (2%w/v). After incubating for predetermined time (6Hr & 24 Hr) the gelled microspheres were separated by filtration, washed with 3 × 100ml distilled water, air dried overnight and finally dried at 50°C for 6hrs. Similarly microspheres containing Aceclofenac prepared by employing Sodium Alginate in combination with different concentrations of Carbopol and HPMC incubated for predetermined times were prepared, washed with 3 × 100ml distilled water, air dried overnight and finally dried at 50°C for 6hrs (Formulation code F_1 to H_6). Later Chitosan coated Alginate microspheres were prepared by making solutions of 0.5%w/v and 1%w/v of Chitosan add to distilled water containing 0.5%w/v acetic acid adjusted to pH 5.2 – 5.4 with 0.1N NaOH. The solution was stirred for 1hr. Later this solution was filtered through a muslin cloth to remove impurities. A 2%w/v solution of $CaCl_2$ was added to Chitosan solution. An Alginate/drug solution was added to this solution to form the microspheres. These microspheres were incubated for at different curing times. Later they were decanted, washed with 3 × 100ml distilled water, air dried overnight and finally dried at 50°C for 6hrs (Formulation code E_1, E_2, E_5 & E_6) respectively. The above method, described above to prepare the Aceclofenac loaded Chitosan Coated Alginate Microspheres with 0.5% & 1% w/v of Chitosan was repeated and microspheres so obtained were immediately put in to Sodium Alginate (2% w/v) solution for 15min and then transferred into calcium chloride solution (2%w/v) and cured for 6hr and 24hr. Later they were decanted, washed with 3 × 100ml distilled water, air dried overnight

and finally dried at 50°C for 6hrs (Formulation code E_3, E_4, E_7 & E_8) respectively.

Particle Size Analysis [20]

Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Microparticle sizes of each formulations were calculated under 45 x magnifications.

Morphology of Microspheres

The shape and surface morphology of the microspheres were investigated using JOEL, JSM-6360, Scanning Electron Microscope at 15Kv. Prior to examination, samples were mounted onto stubs by using double sided adhesive tape and vacuum coated with gold film using sputter coater (Edwards-150, UK) to render them electrically conductive. The samples include drug loaded Alginate microspheres, Carbopol blended Alginate microspheres, HPMC blended alginate microspheres and Chitosan coated Alginate microspheres before release study. These above mentioned microspheres were not subjected to Scanning Electron Microscope studies after release because they converted to gel type of matrix when dissolution was over.

Swelling Ratio Studies

Swelling ratio of different dried microspheres were determined gravimetrically in slightly agitated phosphate buffer solution of pH (5.8 & 6.8).The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on digital balance (Shimadzu AX-200 corporation, Japan). Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{(W_t - W_0)}{(W_0)} \times 100$$

Where W_0 & W_t are initial weight and Final weight of microspheres respectively.

Determination Of Drug Loading Efficiency

Ten milligrams of drug loaded microspheres from each batch were dissolved in 100ml of Phosphate Buffer solution of pH 7.4 by shaking on a mechanical shaker for 24hrs. The solution was filtered through Whatmann filter paper. An aliquot following suitable dilution was assayed spectrophotometrically (UV-1700 Schimadzu Corporation, Japan) for Aceclofenac at 274nm. Drug loading efficiency was determined by using the following relationship

$$\text{Drug Loading Efficiency:} \quad \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Infrared Spectroscopy

The drug-polymer interactions were studied by infrared spectroscopy. The I.R Spectra were recorded between 500 to 4000 cm^{-1} for Aceclofenac, blank Alginate Microspheres, and Drug loaded Alginate Microspheres, Carbopol blended Alginate Microspheres, HPMC blended Alginate Microspheres and Chitosan coated Alginate Microspheres with KBr Pellets using Fourier Transform infrared (FTIR) spectrophotometer (Shimadzu – 8400, Japan).

Differential Scanning Calorimetry

DSC thermograms were performed by using an automatic thermal analyzer system (NETZSCH, DSC 200 PC). The DSC studies on the samples were performed by heating samples at a heating rate of 10°C/min over a temperature range of 50°C – 200°C in a closed aluminum pans.

Statistical Analysis

Each formulation was prepared in triplicate, and each analysis was triplicated. Effect of formulation variables on DLE and release parameter ($t_{50\%}$) were tested for significance by using analysis of variance (ANOVA: single factor) with the aid of Microsoft1 Excel 2002. Difference was considered significant when $p<0.05$.

In-vitro Release Study

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5°C, 75 rpm) using the USP type – II rotating Paddle method in Phosphate Buffer media (pH 5.8, 900ml). A quantity of accurately weighed microspheres equivalent to 100mg Aceclofenac each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 274nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed phosphate buffer maintaining sink conditions throughout the experiment.

RESULTS AND DISCUSSION

Particle Size Analysis [20]

Table - 1 shows particle size of various Microspheres. Microparticles size tends to increase with increase in the initial drug loading. The increase in the size with increase in the drug loading may be attributed to the presence of insoluble drug in the matrix (Formulations F_1 - F_3, C_1 - C_4, H_1 - H_4). However, decrease in microparticles size with curing time may be attributed to the progressive gelation of alginate with time (Formulations F_4 – F_6, C_5 - C_8, H_5 – H_8).

The increase in the sizes of Cb-ALG, HPMC-ALG with increase in the concentration of Carbopol and HPMC (15 – 50%) may be attributed to the increase in the concentration of non-gelling Carbopol & HPMC in the matrix.

The size of Cs-ALG microparticles coated with 0.5% chitosan (Cs) formulation (E_1) were less, compared to those microparticles coated with 1% Chitosan formulation (E_2) for 6hr. Similarly Cs-ALG microparticles coated with 0.5% Chitosan (formulation E_5) for 24hrs were greater than those coated with 1% Chitosan (formulation E_6) for 24hrs. The increase in the size with increase in the concentration of external chitosan solution may be attributed to thicker coat induced by high viscous solution. The decrease in the particle size with formulation E_1 when compared with E_5 and formulation E_2 with E_6 may be due to increase in the curing time (6hr – 24hr). This may be attributed due to the higher gelation of alginate in Cb-ALG and HPMC-ALG Microparticles. The same effect, i.e increase of chitosan (Cs) concentration in coating solution is responsible for increase in size and increase in curing time is responsible for decreasing size were also replicated in alginate coated chitosan-alginate microparticles(ALG-Cs-ALG) formulations (E_3,E_4,E_7,E_8). The presence of outermost coat of ALG shows increase in the size. It may be due to the presence of a layer of one molecule thickness.

Surface Characterization

Fig - 1 shows the surface morphology of drug loaded alginate (ALG), Carbopol-blended alginate (Cb-ALG), HPMC-blended (HPMC-ALG) & chitosan coated alginate (Cs-ALG). Surface of the alginate microparticles appears to be spherical & rough. Similarly the surface of the carbopol blended alginate microparticles (Cb-ALG), HPMC blended alginate microparticles (HPMC-ALG) & Chitosan coated alginate microparticles appears to be rough having few depression compared to drug loaded alginate microparticles (ALG). The Surface morphology of Alginate coated Chitosan Alginate microspheres were not shown in the Fig-1 due to brittle nature of alginate layer formed on the surface of ALG-Cs-ALG microparticles.

Drug Loading Efficiency (DLE)

Drug loading efficiency of microparticles is shown in table - 2. DLE of various formulations was varied depending on the formulation factors such as curing time and initial drug loading. DLE of ALG microparticles (F_1 – F_6) varied from 86.16 ± 1.39 to 94.43 ± 1.87%. Similarly the DLE of Carbopol-blended, HPMC-blended, Chitosan

coated alginate (Cs-ALG) and alginate coated chitosan-alginate microparticles (ALG-Cs-ALG) were found to be ≥ 89.14 ± 2.24%, 89.81± 0.37%, 91.07 ± 1.88% & 91.14 ± 1.55%. The DLE of microparticles prepared by curing 6hr was more compared to those prepared at curing time of 24hrs. Decrease in DLE with increase in curing time may be attributed to higher contact time in calcium chloride solution. Similar decrease in drug loading efficiency (DLE) with increase in curing time was reported by Sankalia etal[7], Halder etal[8]. The DLE of microparticles did not vary ($p>0.05$) with increase in the initial drug loading. But it was found that the DLE of alginate coated chitosan-alginate (ALG-Cs-ALG) microparticles decreased to little extent (≈2%) due to the addition of a small layer of alginate over the chitosan-alginate microparticles. Similarly the DLE of (ALG-Cs-ALG) was not influenced by the increase in the curing time (6hr – 24hr) as the surface of the bead was tightly coated by the chitosan layer.

FTIR

The I.R spectra's of Aceclofenac (A), Alginate microparticles (B), drug loaded alginate microparticles (ALG) (C), Carbopol (Cb) (C), Carbopol-Alginate microparticles(Cb-ALG) (D), HPMC (C), HPMC-Alginate microparticles(HPMC-ALG) (D) Chitosan(Cs) (D), chitosan-coated alginate microparticles (Cs-ALG) (D) were shown in Figs - 13,14,15 & 16 respectively.

I.R spectrum of Aceclofenac shows prominent peaks of secondary amine at 3316.3cm^{-1}, ester stretching at 1770.5cm^{-1}, Carboxylic vibration at 1717.4cm^{-1} and presence of two C-Cl stretching at 749.2cm^{-1} and 662.5cm^{-1}. Comparison of I.R spectrum of drug loaded alginate microparticles (ALG) shows the presence of all the peaks of drug with a little shifting of secondary amine peaks to 3318.3cm^{-1}. It indicates that drug and excipient (polymer) interaction was not seen in the formulation. Similarly other polymers also indicate that the drug was stable in Carbopol blended, HPMC blended and Chitosan coated alginate microparticles.

In- Vitro Drug Release Study

Figs – 2 & 3 shows the dissolution release profiles of alginate microparticles (ALG). The dissolution of drug decreased with increase in the initial drug loading from 10 -50% (Table - 2). $T_{50\%}$ and $T_{80\%}$ values also increased with increase in the initial drug loading. The decrease in the dissolution with increase in the initial drug loading may be attributed to high concentration of insoluble drug in the matrix. As the curing time was increased from 6hr to 24hr, there was a significant ($p<0.05$) increase in the $T_{50\%}$ and $T_{80\%}$ values relative to the results of formulations with different initial drug loading of microparticles cured for 6hr. Increase in the $T_{50\%}$ and $T_{80\%}$ values with increase in the curing time may be due to the penetration of calcium ions to the interior of the microparticles resulting in increased cross linking[7]. Similar results, that decrease in release with increased cross-linking time were reported by Sankalia et al[7]; Halder et al[8]. Similar release profiles of Carbopol-blended (Cb-ALG), HPMC-blended (HPMC-ALG) alginate microparticles in Phosphate buffer pH 5.8 are shown in Figs – 4, 5, 6 & 7. Comparison of $T_{50\%}$ and $T_{80\%}$ values (Table-2) of F_2 formulation with $C_1 – C_6$ & $H_1 – H_6$ formulations indicate that blending of carbopol and HPMC controlled the drug release. Furthermore the release of drug was controlled as the concentration of Carbopol and HPMC was increased in microparticles. The decrease in release of drug from carbopol-blended and HPMC-blended alginate microparticles may be due to the presence of relatively non-ionizing species of Carbopol and HPMC as supported by lower swelling (in pH 5.8 & pH 6.8) of Carbopol-blended and HPMC-blended alginate microparticles (Figs – 10 & 11). Comparison of $T_{50\%}$ and $T_{80\%}$ values of microparticles cured for 6 hr ($C_1 – C_4$) & ($H_1 – H_4$) with those cured for 24hrs ($C_5 – C_8$) & ($H_5 – H_8$) indicate that the release was further retarded due to the increased cross-linking of alginate microparticles. But in case of drug release study of chitosan coated alginate (Cs-ALG) and Alginate coated chitosan alginate microparticles (ALG-Cs-ALG) Thu et al[21] reported that positively charged amino groups of chitosan form membranes through ionic interaction with carboxylic residues of alginate and addition of polycationic polymers to the gelation medium results in reduced microcapsule swelling[22,23] and permeability[24]. In the present study, ALG microparticles were dropped in calcium chloride solution containing at different concentrations of chitosan (0.5 and 1%) and allowed the interaction for two different time intervals (6hr and 24hr). The release profiles of chitosan-alginate (Cs-ALG) and alginate-chitosan-alginate microparticles (ALG-Cs-ALG) are shown in Figs – 8 & 9. Coating of alginate microparticles (F2) prolonged the release to 6hrs depending on the concentration of chitosan in the coating solution (0.5% and 1%). Compared to chitosan-alginate (Cs-ALG) microparticles cross-linked with 0.5% Chitosan solution for 6 hr (E_1), the Cs-ALG microparticles cross-linked with 1% Chitosan solution for 6 hr (E_5) prolonged the release to 5.5hr. Treating the ALG microparticles with chitosan solution (0.5% and 1%) for 24hrs (E5-E6) also prolonged the release to 6 hrs significantly ($p<0.001$). Three types of ionic interactions that contribute to the three dimensional cross-linked networks of chitosan/alginate microspheres i.e., the interaction between opposite charges of the biopolymers, the junction formed by the Ca^{+2} and guluronic and mannuronic acid units and inter chain hydrogen bonds[24,25] are responsible for the stability of microspheres, and hence control the release of drug. This was further conformed by slower swelling of Cs-ALG microparticles. (Fig - 12). However, faster release of drug from ALG microparticles was due to the low stability of the chelating junction in a phosphate buffer above pH 5.0 Dainity et al[26]. With an intention to prolong the release, E1, E2 formulations were further coated with alginate solution (0.2%) for 30 min. The release of drug from alginate-Chitosan-alginate microparticles (E3-E4) with varying concentration of Chitosan (0.5% & 1%) with varying curing times (6hrs & 24hrs) was found to be further prolong the release up to 6 hrs. The additional coating of alginate around Cs-ALG microparticles is believed to be responsible for the prolonged drug release. Following the explanation of Thu et al[21] regarding the formation of ALG-PLL-ALG microparticles and Setty et al[5] regarding the formation of ALG-PEI-ALG microparticles, the un-reacted protonated amine groups of chitosan present at the surface of Cs-ALG microparticles reacted with sodium alginate to form additional membrane. Formation of such a membrane might have increased the thickness of the barrier membrane, plugged the surface pores of Cs-alginate microparticles and led to the decrease in swelling ratios of ALG-Cs-ALG microparticles compared with that of Cs-ALG microparticles (figure - 12). Consequently, the drug release from ALG-Cs-ALG microparticles was considerably prolonged.

KINETICS AND MECHANISM OF DRUG RELEASE

In general, the release date from swellable systems can be analyzed according to the following power law expression (Korsmeyer 1983)[28].

$$\frac{M_t}{M_\infty} = kt^n \quad \text{----- (1)}$$

Where M_t/M_∞ is the fraction of drug released at time, t, 'k' is the proportional constant which accounts for the structural and geometrical properties of the matrix, and 'n' is the diffusional exponent indicative of the mechanism of drug release. The exponent, n, depends on the polymer swelling characteristics and the relaxation rate at the swelling front[5]. The values of release parameters, n and k are inversely related. A higher value of k may suggest burst drug release from the matrix. According to the criteria for release kinetics from swellable systems, a value of release exponent $n=0.45$, $0.45<n>0.89$ and $0.89<n>1.0$ indicates fickian (case-I) diffusion, non-fickian (anomalous) diffusion and zero order (case-II) transport, respectively[29]. The initial dissolution profiles (≤ 60%) of the formulations were fitted into equation (1). Using least square procedure the values of 'n' and 'k' for all the systems were calculated and the results along with the values of correlation coefficients (r^2) are presented in table - 2. The 'n' values for ALG microparticles (F1-F6) were between 0.8-1.2. This indicates that the drug release from ALG microparticles followed case-II transport mechanism due to the rapid swelling and erosion of the microparticles. The drug release data of carbopol blended alginate microparticles (Cb-ALG) (C1-C8) also fitted well in the power law of expression and the values of 'n' were between 0.6-0.9 indicating that drug release followed the anomalous transport (or) non-fickian kinetics. The presence of non-ionizing carbopol in Cb-ALG might

have controlled erosion. Similarly, the calculated values of 'n' for HPMC blended alginate microparticles (HPMC-ALG) were found to be between 0.6-0.8 indicating anomalous transport due to the presence of HPMC in the matrix which controlled the erosion of microparticles. In case of chitosan formulation (E1-E8) the calculated values of 'n' were between 0.6-0.7 indicating that the swelling was much controlled and the drug release followed the anomalous transport (or) non-fickian kinetics. The drug release studies were conducted in pH 5.8 and the calculated values 'n', k and r^2 are presented in the table - 2. In case of ALG microparticles the calculated 'n' values were around 1.0 indicating that the release followed case-II transport due to the rapid swelling and erosion of the microparticles. The calculated 'n' values for carbopol blended

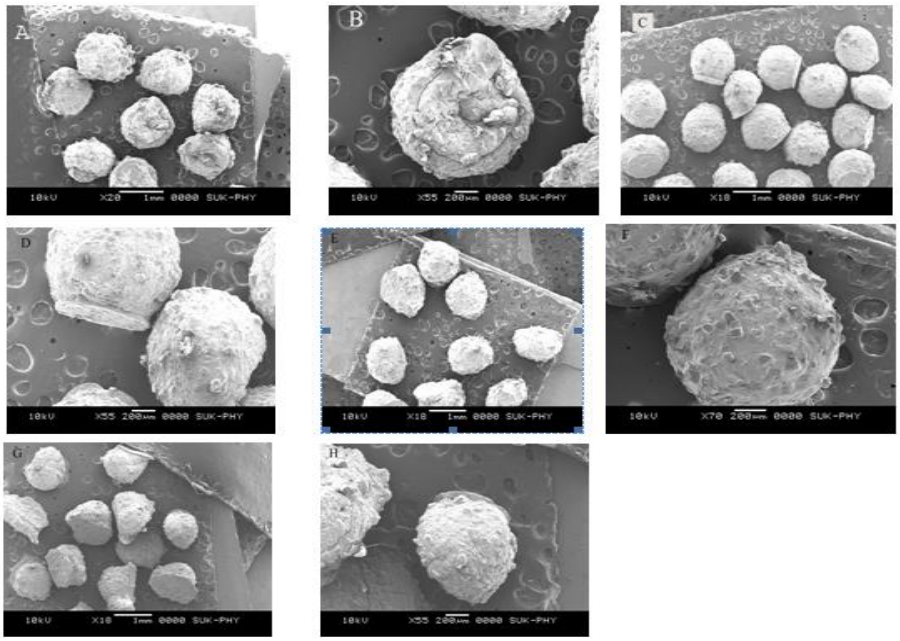

Figure 1: Scanning Electron Micrographs of (A) Drug loaded alginate microparticles (ALG); (B) Drug loaded alginate micro particles at 55x; (C) carbopol-alginate micro particles; (D) carbopol-alginate micro particles at 55x.Micrographs of (E) chitosan coated alginate micro particles (Cs-ALG); (F) Alginate coated chitosan alginate microparticles at 70x; (G) HPMC-alginate micro particles; (H) HPMC-alginate micro particles at 55x.

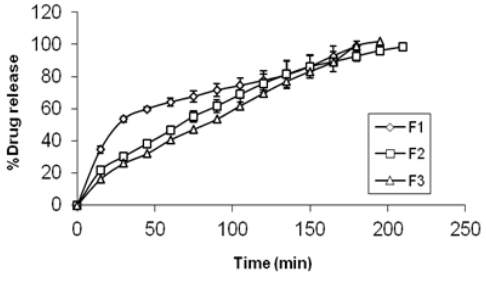

Figure 2: Effect of drug loading on the Release Profiles of from Aceclofenac from Alginate Micro particles (ALG) (Curing time 6hr) in Phosphate Buffer pH 5.8.

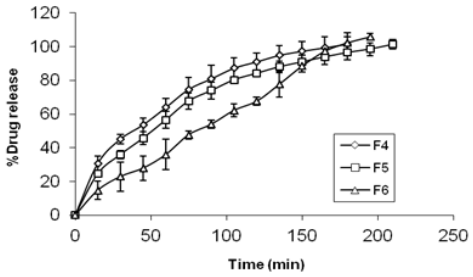

Figure 3: Effect of drug loading on the Release Profiles of Aceclofenac from Alginate Micro particles (ALG) (curing time 24hr) Phosphate Buffer pH 5.8.

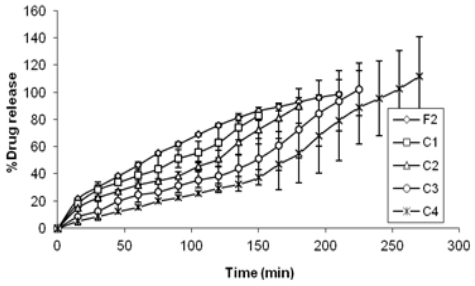

Figure 4: Effect of Carbopol Concentration on the Release Profiles Of Cb-ALG Microparticles (curing time 6hr) in Phosphate Buffer pH 5.8

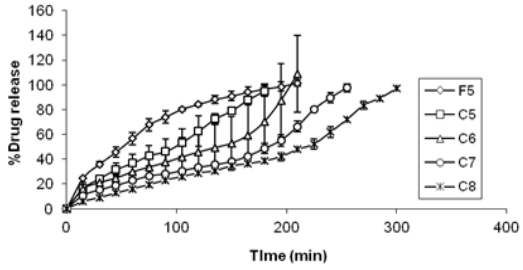

Figure 5: Effect of Carbopol Concentration on the Release Profiles of Cb-ALG Micro particles (curing time 24hr) in Phosphate Buffer pH 5.8.

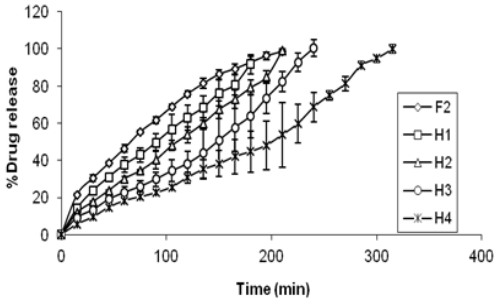

Figure 6: Effect of HPMC Concentration on the Release Profiles of HPMC-ALG Microparticles (curing time 6hr) in Phosphate Buffer pH 5.8.

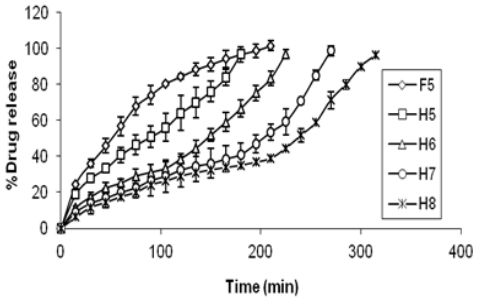

Figure7: Effect of HPMC Concentration on the Release Profiles of HPMC-ALG micro particles (curing time 24hr) in Phosphate Buffer pH 5.8.

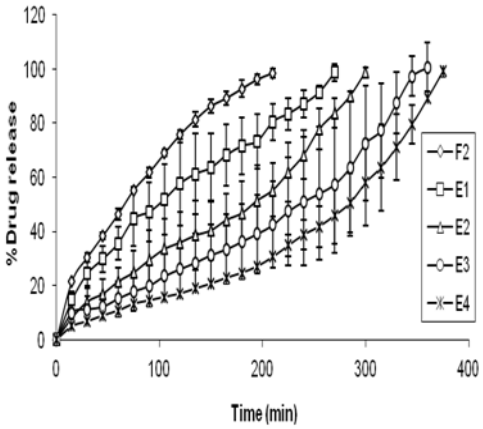

Figure 8: Effect of Chitosan Concentration on the Release Profiles of Cs-ALG Microparticles (curing time 6hr) in Phosphate Buffer

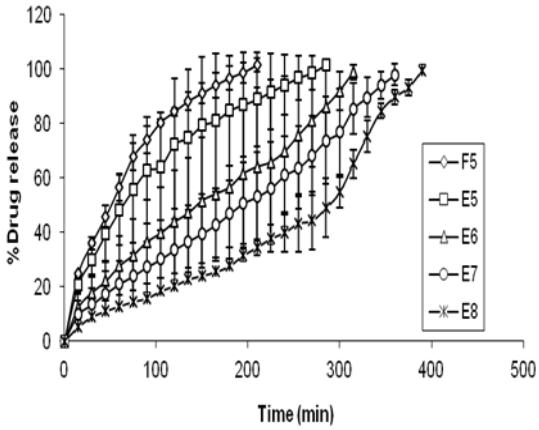

Figure9: Effect of Chitosan Concentration on the Release Profiles of Cs-ALG Micro particles (curing time 24hr) in Phosphate Buffer

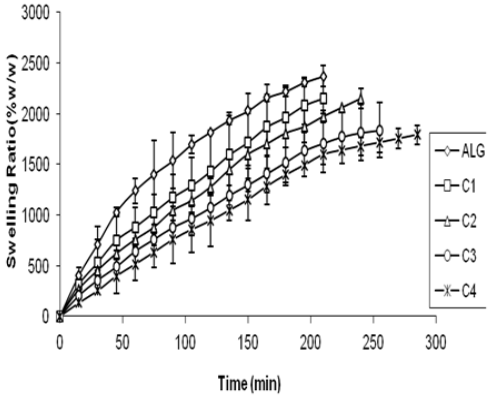

Figure10: Swelling ratio-time profiles of Drug Loaded Alginate Micro particles (ALG);Carbopol-Alginate Micro particles (Cb-ALG).

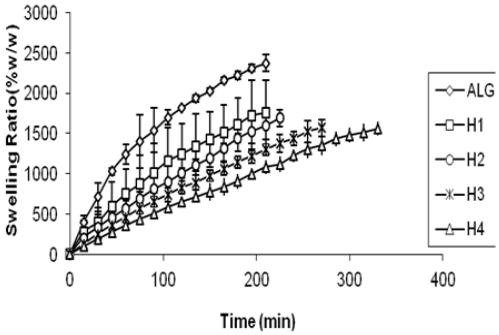

Figure 11: Swelling ratio-time profiles of drug loaded Alginate Micro particles (ALG) HPMC-Alginate Micro particles (HPMC-ALG).

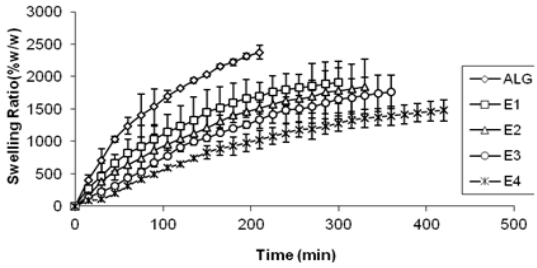

Figure12: Swelling ratio-time profiles of drug loaded alginate microparticles (ALG); chitosan-alginate microparticles (Cs- ALG); Alginate coated chitosan-alginate microparticles (ALG-Cs-ALG).

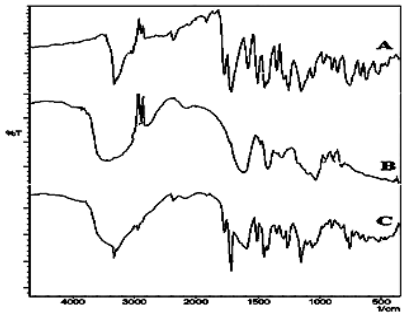

Figure13: FTIR spectra of (A) drug; (B) alginate micro particles; (C) drug loaded alginate micro particles (ALG).

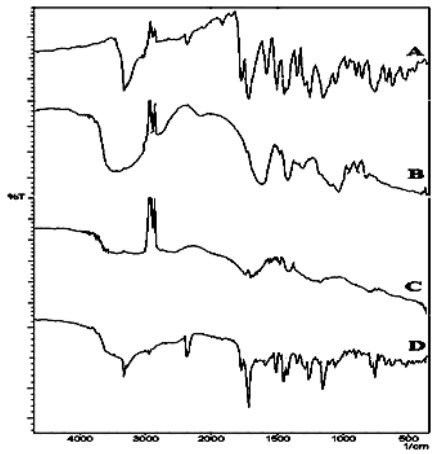

Figure14: FTIR Spectra of (A) Drug; (B) Alginate Micro particles; (C) Carbopol (Cb) (D) Carbopol-Alginate Micro particles (Cb-ALG).

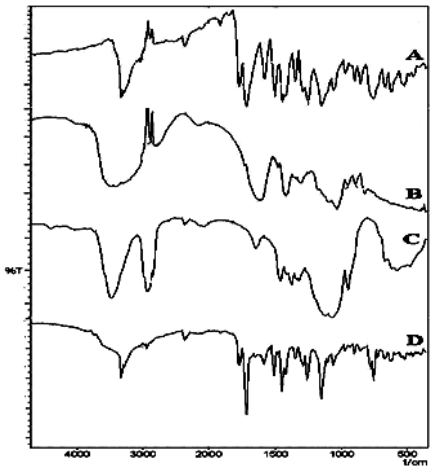

Figure 15: FTIR Spectra of (A) Drug; (B) Alginate Micro particles; (C) HPMC (D) HPMC-Alginate Micro particles (HPMC-ALG).

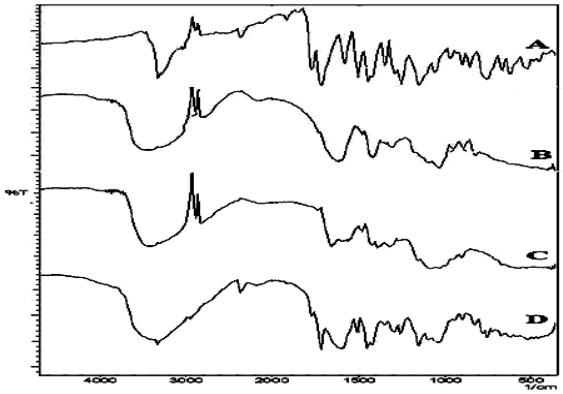

Figure16: FTIR Spectra of (A) Drug; (B) Alginate Micro particles; (C) Chitosan (Cs) (D) Chitosan-Coated Alginate Micro particles (Cs-ALG).

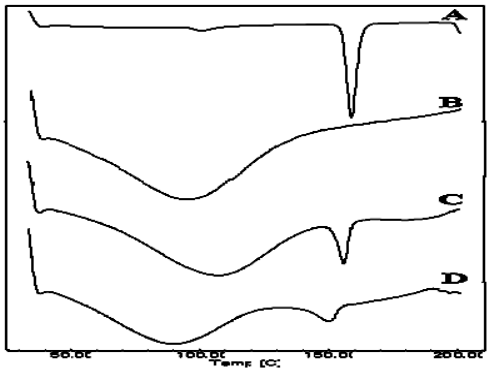

Figure17: DSC Thermogram of (A) Drug; (B) Alginate Micro particles; (C) Drug Loaded Alginate Micro particles (ALG); (D) Chitosan-Coated Alginate Micro particles (CS-ALG).

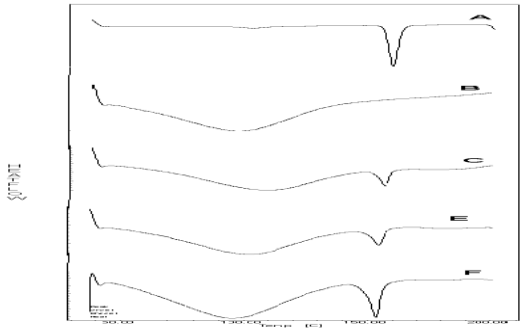

Figure18: DSC thermogram of (A) drug; (B) alginate Micro particles(C) drug loaded alginate micro particles (ALG); (E) HPMC-alginate Micro particles (HPMC-ALG); (F) carbopol-alginate micro particles(Cb-ALG).

TABLE1: FORMULATION AND PARTICLE SIZES OF VARIOUS MICROSPHERES

FORMULAE	DRUG (mg)	SODIUM ALGINATE	CARBOPOL (mg)	HPMC (mg)	CHITOSAN (mg)	GLACIAL ACETIC ACID	CALCIUM CHLORIDE	CURING TIME (hr)	PARTICLE SIZE (±SD)µm
F_1	22.22	2000	---	---	---	---	2	6	803.3±23.0
F_2	85.7	2000	---	---	---	---	2	6	828.5±33.4
F_3	200	2000	---	---	---	---	2	6	888.3±47.6
F_4	22.22	2000	---	---	---	---	2	24	646.0±15.4
F_5	85.7	2000	---	---	---	---	2	24	785.3±6.8
F_6	200	2000	---	---	---	---	2	24	702.2±47.0
C_1	85.7	170	30	---	---	---	2	6	1118.8±28.3
C_2	85.7	160	40	---	---	---	2	6	1129.0±27.5
C_3	85.7	150	50	---	---	---	2	6	1220.5±17.6
C_4	85.7	100	100	---	---	---	2	6	1251.0±30.2
C_5	85.7	170	30	---	---	---	2	24	1054.3±8.5
C_6	85.7	160	40	---	---	---	2	24	1126.0±104
C_7	85.7	150	50	---	---	---	2	24	1176.6±10.0
C_8	85.7	100	100	---	---	---	2	24	1236.3±9.07
H_1	85.7	170	---	30	---	---	2	6	1161.0±18.9
H_2	85.7	160	---	40	---	---	2	6	1149.9±8.7
H_3	85.7	150	---	50	---	---	2	6	1214.4±5.0
H_4	85.7	100	---	100	---	---	2	6	1230.5±33.1
H_5	85.7	170	---	30	---	---	2	24	1110.6±17.4
H_6	85.7	160	---	40	---	---	2	24	1135.3±22.5
H_7	85.7	150	---	50	---	---	2	24	1174.0±13.8
H_8	85.7	100	---	100	---	---	2	24	1214.4±5.1
E_1	85.7	2000	---	---	500	0.5	2	6	1256.4±9.5
E_2	85.7	2000	---	---	1000	1.0	2	6	1358.4±47.9
E_3	85.7	2000	---	---	500	0.5	2	6	1256.2±12.0
E_4	85.7	2000	---	---	1000	1.0	2	6	1278.0±13.0
E_5	85.7	2000	---	---	500	0.5	2	24	1225.3±18.5
E_6	85.7	2000	---	---	1000	1.0	2	24	1344.3±7.5
E_7	85.7	2000	---	---	500	0.5	2	24	1246.0±9.1
E_8	85.7	2000	---	---	1000	1.0	2	24	1253.9±7.9

DSC: The DSC thermograms of drug (A), Alginate microparticles (B), Drug loaded alginate microparticles (ALG) (C), Chitosan-Coated Alginate Microparticles (Cs-ALG) (D), Drug loaded alginate microparticles (ALG) (C), HPMC-alginate microparticles (HPMC-ALG) (E), Carbopol-alginate microparticles (Cb-ALG) (F) were shown in Figs – 17 & 18 respectively. Figs – 17 & 18 shows a sharp endothermic peak at 157.03^{0}c which was slightly decreased to 153.24^{0}c in drug loaded carbopol, HPMC & chitosan coated alginate microparticles. It may be due to the presence of amorphous alginate. Thus it is confirmed that the drug was stable in carbopol-blended (Cb-ALG), HPMC-blended (HPMC-ALG), chitosan coated alginate formulations (Cs-ALG).

Table 2: DLE, Dissolution Parameters ($T_{50\%}$ & $T_{80\%}$) & Kinetic Parameters of Dissolution Data in Phosphate Buffer pH 5.8 described by Korsmeyer-Peppas equation

Formulation	Drug loading Efficiency (DLE) (%w/w)(±SD, n=4)	Phosphate buffer pH 5.8		Phosphate buffer pH 5.8		
		$T_{50\%}$ (min) (± SD) n=4	$T_{80\%}$ (min) (± SD) n=4	n	k	r^2
F1	92.46(0.47)	26.00 (1.63)	81.75 (1.70)	0.8614	0.3826	0.9941
F2	93.72(1.46)	66.00 (1.63)	133.00 (2.58)	0.9003	1.090	0.9963
F3	94.43(1.87)	85.00 (2.58)	146.00 (1.63)	1.1522	0.1471	0.9953
F4	88.89(1.11)	38.00 (1.63)	94.00 (1.63)	0.9253	0.1178	0.9901
F5	87.72(1.44)	52.50 (1.91)	135.00 (1.29)	0.9517	1.030	0.9922
F6	86.16(1.39)	86.00 (1.63)	147.50 (1.29)	1.2973	0.3938	0.9922
C1	93.49(3.48)	96.00 (1.63)	143.50 (1.91)	0.6067	0.8903	0.9903
C2	94.74(2.49)	112.75 (1.50)	153.00 (1.15)	0.6056	0.8706	0.9909
C3	95.50(2.90)	164.25 (1.70)	206.00 (1.63)	0.9096	0.0735	0.9949
C4	96.90(0.99)	188.25 (1.70)	241.50 (1.91)	0.8764	0.0798	0.9915
C5	89.14(2.24)	112.00 (1.63)	149.00 (1.15)	0.6023	0.8867	0.9913
C6	90.74(1.12)	172.25 (1.25)	202.50 (1.29)	0.6352	0.6365	0.9954
C7	91.87(1.71)	183.00 (0.81)	224.25 (3.30)	0.6013	0.7305	0.9903
C8	92.66(1.56)	205.00 (1.00)	263.50 (1.91)	0.7948	0.2524	0.9985
H1	91.62(1.56)	96.25 (3.09)	162.00 (1.63)	0.6470	0.8399	0.9904
H2	92.35(1.27)	112.25 (1.25)	183.50 (1.91)	0.7205	0.6305	0.9943
H3	93.45(1.74)	164.25 (1.70)	206.50 (1.91)	0.8579	0.1951	0.9902
H4	95.05(1.99)	219.25 (2.50)	267.50 (0.57)	0.7453	0.3387	0.9913
H5	89.81(0.37)	101.00 (2.58)	168.00 (2.82)	0.6009	0.9389	0.9929
H6	90.25(0.43)	146.25 (1.70)	207.25 (2.50)	0.6331	0.7437	0.9891
H7	91.75(0.86)	203.25 (2.21)	248.75 (2.50)	0.7200	0.3997	0.9860
H8	92.24(1.54)	236.25 (1.25)	285.00 (1.15)	0.6916	0.4464	0.9907
E1	93.40(1.81)	144.00 (1.63)	236.25 (1.25)	0.6003	0.7357	0.9964
E2	94.41(3.75)	231.50 (1.91)	270.00 (1.63)	0.7672	0.3190	0.9921
E3	91.14(1.55)	284.50 (2.08)	331.00 (1.15)	0.7295	0.2226	0.9952
E4	92.97(1.53)	309.25 (0.95)	348.00 (4.32)	0.7766	0.0919	0.9900
E5	91.77(2.50)	180.50 (1.91)	245.00 (2.58)	0.6029	0.7389	0.9982
E6	91.07(1.88)	246.00 (1.63)	285.50 (1.00)	0.6067	0.6029	0.9952
E7	91.46(2.69)	289.50 (1.91)	333.25 (1.91)	0.6734	0.2895	0.9890
E8	93.47(2.25)	317.75 (1.70)	353.50 (1.91)	0.6904	0.3286	0.9912

DISSCUSION

In the first part of the work, Aceclofenac loaded alginate microparticles (ALG) were prepared by varying the curing time (6hr and 24hr) and drug loading (10-50%). The microparticles were spherical with roughness on the surface as shown by scanning electron microscopy studies. The particle size increased with increase in the initial drug loading and decreased slightly with increase in the curing time. Drug loading efficiency was ≥ 86% and was dependent on the formulation variables. The release of ALG formulation was studied in phosphate buffer pH 5.8. The release decreased with increasing the initial drug loading as well as curing time. The release of the drug was always found to be more in phosphate buffer pH 5.8. FTIR and DSC studies did not show any remarkable changes in the drug properties indicating that the drug was stable. Neither increase in curing time nor initial drug loading prolonged the drug release and drug release completed within 2.5hr. It was thought that rapid ionization of calcium alginate has not been controlled and hence other polymers were blended with alginate. Carbopol-blended alginate microparticles (Cb-ALG) were prepared by replacing a portion of alginate with carbopol. Scanning electron microscopy analysis showed that carbopol-blended alginate (Cb-ALG) microparticles were spherical having smooth surface. The particle size increased with increase in the concentration of carbopol. Drug loading efficiency (DLE) was ≥ 89% and was found to increase with increase in concentration of carbopol. The release of drug from Cb-ALG microparticles decreased with increase in the concentration of carbopol and curing time (6hrs & 24hrs). The release studies indicated that, the drug release was prolonged to (4.5 & 5 hrs).. FTIR and DSC studies showed that aceclofenac was stable in carbopol-blended alginate (Cb-ALG) microparticles.

With an intention to study the effect of HPMC on the drug release, a portion of alginate was replaced by HPMC. Scanning electron microscopy analysis showed that HPMC-blended alginate microparticles (HPMC-ALG) were spherical having rough surface. The particle size increased with increase in the concentration of HPMC in the microparticles. Drug loading efficiency (DLE) of HPMC microparticles was ≥ 89.18% and found to increase with increase in the concentration of HPMC and decreased with increase in the curing time (6hrs & 24hrs). The release studies indicated that, the drug release was prolonged to (4.5 & 5 hrs). FTIR and DSC studies of HPMC-blended alginate microparticles (HPMC- ALG) showed that Aceclofenac was stable in the formulation.

In the last part of the work chitosan coated ALG microparticles (Cs-ALG) and alginate coated Cs-ALG microparticles were prepared. Scanning electron microscopy showed that the microparticles were spherical having rough surface with depressions. The particle size of Cs-ALG microparticles and ALG-Cs-ALG microparticles increased with increase in the coating. The drug loading efficiency (DLE) were ≥ 91% and the DLE of ALG-Cs-ALG decreased to a little extent (≈ 2%) due to the addition of small amount of alginate layer. The release of the drug from Cs-ALG microparticles was 6hrs (in pH 5.8) and that from ALG-Cs-ALG microparticles prolonged to 6.5hrs (in pH 5.8) indicating that the surface pores were plugged and controlled the drug release. FTIR and DSC studies showed that the drug was stable in both Cs-ALG and ALG-Cs-ALG microparticles.

CONCLUSION

In conclusion, the ALG microparticles alone cannot prolong the release from weakly acidic drug aceclofenac. The blending of alginate with relatively non-ionizing polymers or formation of polyelectrolyte complex membrane can prolong the drug release in alkaline phosphate buffers of pH 5.8.

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