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Full Length Original Research Paper

ACF loaded ethyl Cellulose Microspheres: Formulation Designing, Characterization and *In-Vivo* anti-Inflammatory and Analgesic activities in Albino Wistar Rats

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Abstract:
From the past few decades, scientist interest focused in the area of improvement of therapy and development of microspheres by oil-in-water (o/w) emulsion solvent diffusion evaporation technique. Aceclofenac (ACF) is an analgesic and anti-inflammatory and diarrhoea, dyspepsia, abdominal pain, nausea, indigestion, pancreatitis, constipation the most common side effects. So the aim of the present research work was to formulation designing, characterization and *in-vivo* anti-inflammatory and analgesic activity in rats. ACF loaded EC microspheres were developed by oil-in-water (o/w) emulsion solvent diffusion evaporation technique with different ratio of drug and ethyl cellulose as a polymer in order to achieve high entrapment efficiency and prolonged release characteristics. The prepared microspheres were subjected for characterization by scanning electron microscopy (SEM), percent yield, Fourier transformer infra red spectroscopy (FTIR), X-ray diffraction (XRD), percent entrapment efficiency and percent drug release. The size of microspheres formulations (F1 to F6) were in range of 10±2.1 to 51±2.7 µm, percent yield 75.32±2.21 to 95.43± 1.13%, percent drug entrapment efficiency 55.87±2.03 to 87.53±2.12% and percent drug release 58.36 ± 0.32 to 94.68 ± 0.54 % up to 12 hrs. IR and XRD studies showed no interaction between drug and polymer; no degradation during microspheres preparation and stable at storage conditions. Then compare *in-vivo* activity of optimized microspheres formulations to standard drug in 120-200g of Albino wistar rats of either sex. The results of present study reflect that successfully prepared free flowing ACF loaded EC microspheres by o/w emulsion solvent diffusion evaporation technique and significantly reduction in an inflammation observed when it compared to standard ACF and also showed significant analgesic activity in rats.

Keywords: Analgesic and anti-inflammatory. Aceclofenac, Ethyl Cellulose.

Introduction

Now a day the main goal of any drug therapy to gain a steady-state plasma drug concentration or tissue concentration, nontoxic and therapeutically effective for prolong time period. Many demerits of conventional drug therapy are overcome by modified release drug delivery systems such as controlled release drug delivery system, site specific release drug delivery system, sustained release drug delivery system and delayed release drug delivery system [1]. The merits of sustained release drug delivery therapy like easily administered, enhanced the bioavailability, reduced the side effects, minimized the drug toxicity, increased patient compliance, and enhanced reliability of drug therapy [2].

Aceclofenac (ACF), chemically phenyl acetic acid derivative, effective anti-inflammatory and analgesic drug used in treatment of pain, fever and inflammation in rheumatoid arthritis, ankylosing spondylitis and osteoarthritis [3]. It's half life 3-4 h and prescribes multiple dosing (100 mg twice daily). After oral administration effectively and rapidly absorbed and diarrhea, dyspepsia, abdominal pain, nausea, indigestion, pancreatitis, and constipation are the most common side effects of ACF therapy [4, 5].

One of the novel techniques, microencapsulation used for retarding the drug release from dosage forms and reduced the adverse effects, increased the patient compliance. In this technique, aqueous insoluble core (drugs) coated with an

aqueous insoluble coat (polymer) by emulsion solvent diffusion evaporation technique for sustain release drug delivery system [6].

EC being insoluble in water extensively used for preparation of microencapsule serves as good candidate for water insoluble drug to achieve sustained release drug delivery systems. The study was previously performed using different solvents like dichloromethane, ethyl acetate and chloroform, employed in preparation of microcapsules of diclofenac sodium as a core material to coat with aqueous insoluble EC as a coat material to investigate the effects of solvent on drug release because such solvent enhance the both permeability and drug release profile from microcapsules [7, 8, 9].

Therefore, the objective of the present research work to formulation designing, characterization and *in-vivo* anti-inflammatory and analgesic activity of ACF loaded ethyl cellulose microspheres in albino wistar rats. So we can achieve sustained release drug profile by release rate retarding polymer and reduce the frequency of dose administration result in improve the patient compliance.

MATERIAL AND METHOD

Aceclofenac was obtained as a gift sample from Emcure Pharmaceuticals (Pune, India). Ethyl cellulose and Poly vinyl alcohol of A.R. grade were used as purchased from CDH, Mumbai. All other reagents and solvents employed were of analytical grade.

Method of preparation of ACF loaded EC microsphere:

Emulsion solvent diffusion-evaporation technique was employed to prepare ACF loaded EC microsphere. EC (250mg) and drug (250mg) were dissolved in dichloromethane (10 ml, DCM) as an internal phase. The polymeric solution of drug was then added slowly drop wise manner under stirring in to previous prepared a solution of polyvinyl alcohol (100 ml, 0.5%w/v PVA) in water as an external phase (table 1, Fig 1). The both phase initially forms a milky white emulsion and the resultant mixture was stirred constantly with a propeller type agitator up to 3 hours until complete volatile organic solvent DCM evaporated. The emulsion breaks down to formed tiny microspheres and allowed for settle down. The resulting microspheres were collected after filtration, rinsing thrice with excess of water and then dried overnight at room temperature [10].

Characterization of ACF loaded EC microspheres formulation

Percent Yield:

The percentage yields of different microsphere formulations were determined gravimetrically on the basis of polymer and drug recovery.

% Yield= [Weight of microspheres / Total weight of drug and polymer] x100

Percent Incorporation efficiency:

The drug content in various microsphere formulations were estimated by extracting ACF in 7.4 pH phosphate buffer solution (PBS) after dissolving the microspheres (100mg) in 25 mL ethanol and adjusted the volume up to 100 ml using pH 7.4 PBS in glass stopper conical flask. The resulting mixture was sonicated and agitated on a mechanical shaker for one day, filtered through whatman filter (0.45µm), and then measured the absorbance using a UV/VIS double beam spectrophotometer (Shimadzu UV-1700, Japan) after suitable dilution at 274nm and calculate percent entrapment efficiency (%EE) by using following formula and each determination was made in triplicate [11].

Entrapment Efficiency (%) = $(A_d/T_d) \times 100$

Where, A_d theoretical drug content, A_d actual drug content

Particle size analysis and Scanning Electron Microscopy (SEM) study:

The particle size of microspheres were determined using Scalar-USB Digital scale ver. 1.1 E-Photomicroscope, attached with canon camera (Japan) system based on mean diameter and then calculated size distribution[12].

The surface morphology and shape of microspheres were analyzed by a Scanning Electron Microscopy (SEM, Hitachi Model S-3000H, CECRI, Karaikudi, Tamilnadu, India). During the SEM examination, a drop of microspheres dispersion to be examined was mounted over a SEM stub and dried in desicator. Microspheres were coated with very thin coat of gold employing a vaccum evaporator to make electrically conductive. Then the size of the microspheres was recorded under SEM at a magnification ranging from 500X to 3000X and operated at an accelerating voltage of 20 kV.

Fourier Transformer Infrared (FTIR) spectroscopy study:

Infrared (I.R.) spectrum of drug, physical mixture of drug-polymer and ACF loaded microsphere gives information about the group present in that particular compound. Before I.R. spectra studies, aceclofenac, physical mixture of drug-polymer and ACF loaded microsphere were dried in vaccum for 12 hours. Potassium bromide (KBr) 200mg in 3mg test sample was used to prepared discs, scan under the range 4000 – 400 wave number (cm^{-1}) and % Transmittance employing Perkin Elmer (USA). The above experiments were performed in triplicate manner to confirm the results.

X-Ray Diffraction study

To investigate the effect of microsphere process on crystallinity of the drug carried out X-ray powder diffraction. The XRD patterns of aceclofenac crystals and drug-loaded microspheres were recorded. Before scanning, microspheres were triturated and convert in to fine powder. Powder XRD patterns were determined by using X-ray diffraction (XRD), Philips Analytical X-RD (Model: PW 3710, Holland) using Ni-filtered, CuKa radiation, a voltage of 40 Kv voltages, and a current of 30 mA at room temperature. The samples were loaded on to the diffraction and scanned over range of 2θ values form 10° to 80° at a scan rate of 0.05° /0.4 sec.

***In vitro* Drug Release Profile:**

The *in vitro* dissolution studies were carried out in phosphate buffer solution (PBS), 900 mL of pH 7.4, maintained at 37±0.5°C temperature thermostatic controlled water bath, 100 rpm by employing basket-type dissolution apparatus (United States Pharmacopeia XXIV) of eight station (Electro-lab, Mumbai, India). Microspheres weighed contain 200 mg of ACF were used as test sample. Withdrawn the sample solution (5ml) at predetermined time intervals over a period of 12 hours, filtered through a 0.45 mm membrane filter, diluted suitably, and assessed for drug release at 274nm for ACF by using a UV spectrophotometer (Shimadzu UV-1700, Japan). After each withdraw, immediately supplemented an equal amount of fresh PBS. Each determination was performed thrice and the percent cumulative drug release plotted as the percent drug release in dissolution media Vs time [13].

***In Vivo* Pharmacology study:**

Experimental animals:

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The *in vivo* Anti-inflammatory and analgesic activities was performed by using Carrageenan induced rat paw edema and Eddy hot plate models respectively [14]. The protocol of the present work was approved by Institutional Animal Ethical Committee, Institute of Pharmaceutical Research, GLA University, Mathura, Utter Pradesh, India (Ref. No. GLAUIPR/IAEC/002/CPCSEA). The animals were grouped and housed in standard poly acrylic cages (38x23x10 cm) with not more than four animals per cage and maintained standard laboratory conditions at room temperature (27±2°C; relative humidity 44-56%) with natural dark and light cycle (12 hr light-dark cycle). The rats were given a standard laboratory diet (Golden Feeds, India) and *ad libitum* for one week before and during the experiments. The animals were acclimatized one week before start the activity in laboratory.

In Vivo Anti-inflammatory study:

The *in vivo* Anti-inflammatory and analgesic activities were performed by employing Carrageenan induced rat paw edema models. The animals were randomly selected divided into six groups each containing six Albino rats Wistar strain of either sex of 6–8 weeks of age, each weighing 120-200g fasted over night and marked just beyond the tibiotarsal junction on the left hind paw so that every time the paw was dipped in the fluid column up to fixed mark to ensure constant paw volume. The experiment was performed at constant temperature [15] care was taken that the animals have minimal stress. [16, 17,18].

Group I serve as control received 0.9% normal saline in 3% Tween 80, group II was administered pure ACF (10mg/k body weight p.o.) as a standard [19]. Group III, IV and V serve as treated group and administered optimized microsphere formulations (F1, F2, F3 p.o.) after disperse into 0.9% normal saline containing 3% Tween 80 respectively. After 30 minutes, carrageenan 0.1ml of 1%w/v in distilled water injected in the sub-plantar region of the left paw to both control and treated groups. The volumes (ml) of paw edema in each animal of both control and treated groups were measured using plethysmometer (PM 70, Rolex, India) at different intervals of 0.5, 1, 2, 4, 6, 8, 10 and 12 hrs after carrageenan administration. The percent inhibition produced by microspheres formulations treated groups were calculated against the respective control group as follows [10].

% Edema Inhibition = $[1-(V_t/V_c)] \times 100$

Where:

V_t - Difference in mean paw volume of treated group (ml)

V_c - Difference in mean paw volume of control group (ml)

In Vivo Analgesic activity study:

The *in-vivo* Analgesic activity was carried out by Eddy's hot plate method. The animals were randomly selected and divided into six groups each containing six Albino rats wistar strain of either sex of 6–8 weeks of age, each weighing 120-200g fasted over night and marked. Group I serve as control, administered 0.9% normal saline containing 3% Tween 80 and Group II serve as standard received standard ACF (10mg/kg body weight p.o). Group III, IV and V serve as treated group, the optimized microsphere formulations (F1, F2, F3) were administered p.o after disperse into 0.9% normal saline containing 3% Tween 80 respectively. All animals individually of each group were placed on the hot plate maintained at 55±0.5°C, one hour after their respective treatments. The latency(s) period or reaction time was noted at the different time intervals of

0.5,1,2,4,6,8,10 and 12 hrs as the time at which animals reacted to the pain stimulus either by jump response or paw licking, whichever appeared first. 15 seconds cut off period for the reaction was maintained. Rat with baseline latencies higher than 10 second was eliminated from the study [11, 20]. The percent analgesic activity was calculated as follow.

% Analgesic activity = $[1-(L_t/ L_c)] \times 100$

Where:

T_t - Mean latency(s) period or reaction time of treated group (sec.)

T_c - Mean latency(s) period or reaction time of control group (sec.)

Statistical analysis

The results of pharmacological studies were expressed as Mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's Test. The result were considered statistically significant when P value less than 0.05 ($P<0.05$) vs control.

Stability Studies

As per ICH guidelines, optimized drug loaded microspheres formulation subjected to stability studies and stability protocol was designed to find the effect of percent RH (relative humidity) and temperature. Optimized drug loaded microspheres formulations in hermetically sealed tubes were exposed at 5±2°C, 25±2°C/60±5% RH and 40±2°C/75±5% RH to check the effects of temperature and RH on percent entrapment efficiency and physical appearance for a period of six months at 2 months interval. At the end of prescribed time period, the microspheres evaluated for determination of percent encapsulation efficiency and physical appearance [21, 22, 23].

Result and discussion

The various aceclofenac loaded EC microspheres formulations F1 to F6 were prepared by emulsion solvent evaporation diffusion technique. In which EC employed as a polymer and ACF as a core material used in therapy of anti inflammatory and analgesic activity.

The percent yield of all microspheres formulations F1 to F6 was found to be 75.32±2.21 to 95.43±1.13%. Out of six formulations, F2 formulation showed highest yield (95.43±1.13%). The reason behind that concentration of coat increased the percentage yield increased as well as further increased in coat concentration, decreased in percentage yield. In the similar way, highest percent entrapment efficiency of F2 microspheres formulation was found to be 89.53±0.93%, result shown in table 2.

From the SEM investigation (fig 2) free flowing and spherical shape microspheres were found and indicate 10±2.1µm particles size. The particle size of various microspheres formulations were depicted in table 2.

FTIR analysis study was used for interaction between the drug and polymer. I.R. spectra of pure ACF, physical mixture of drug-polymer and ACF loaded EC microspheres shown in Fig. 3. I.R. spectra of pure ACF showed the prominent characteristic peaks at 3331 nm indicating the NH– stretching, two peaks at 3070 nm and 3026 nm indicating aromatic –CH stretching, peak at 2821 nm indicating aliphatic –CH stretching, peak at 1770 nm indicating –C=O stretching of –COO, peak at 1717 nm indicating –C=O stretching of –

COOH, peak at 1589 nm indicating –C=N stretching, two peaks at 1481nm, 1454 nm indicating aromatic –C=C stretching and another two peaks at 750 nm, 717 nm indicating C-Cl stretching. I.R. spectra of drug loaded microspheres showed the prominent characteristic peaks of pure aceclofenac that confirms the presence of drug in microsphere without any interaction with polymer.

For study of crystalline change of drug employed XRD analysis. XRD of pure ACF, drug loaded EC microspheres and EC polymer shown in Fig 4. The diffraction spectrum of pure ACF showed that the drug was of crystalline nature as demonstrated by numerous characteristic prominent sharp peaks at 2θ of 17.6489, 18.5845, 19.5423, 22.3030, 24.5837 and 26.0176 etc. XRD patterns of pure drug compared to XRD patterns of drug loaded EC microspheres, found that the observe peaks of drug loaded EC microspheres formulation was similar to peak of pure drug but of low intensity, confirm the presence of drug in polymer matrix either entrapped or dispersed.

The *in vitro* drug release profile performed in PBS pH 7.4 to investigate the sustain release behavior EC containing ACF. It was observed that different optimized microspheres formulations showed drug release 58.36 ± 0.32 to 94.68 ± 0.54% within 12 hrs (Fig. 5, table 2) but F2 formulation showed highest drug release 94.68 ± 0.54% for prolong period of time. So on the basis of above result F2 formulation considered as a best and further subjected for *in-vivo* study.

From *in vivo* carrageenan induce rat paw edema model, percent edema inhibition (PEI) was determined and then compare anti-inflammatory activity of optimized ACF loaded EC microspheres formulations with standard ACF. However optimized ACF loaded EC microspheres formulations F1, F2 and F3 at a dose of 10 mg/kg, p.o. exhibited PEI 68.04%, 87.83% and 59.45±2.3% at 12 hrs respectively but F2 showed significant inhibitory effect on increase in paw volume in comparison to control and standard ACF at similar dose and same time, result shown in table 3. and fig. 6. Similarly in case of analgesic activity F1, F2 and F3 microspheres formulation showed 11.58±0.4, 12.83±0.5 and 10.62±0.3 sec latency(s) period or reaction time at 12 hrs, result shown in table 4 and fig. 7 but F2 indicated highest percent increase latency period or reaction time 12.83±0.5 sec. at 12 hrs and dose of 10 mg/kg than standard ACF (5.41±0.3 sec.) at same time and similar dose. Finally from fig. 6, PEI vs time concluded that highest PEI of F2 was achieved for prolong period of time than the PEI of standard drug. Similarly from fig. 7, latency period vs time, the latency period of F2 was achieved for prolong duration than standard drug. In order to make stable sustained product, tubes were evaluated at the end of prescribed time interval. There was no significant difference observe in their percent entrapment efficiency and physical appearance of drug loaded EC microspheres formulations.

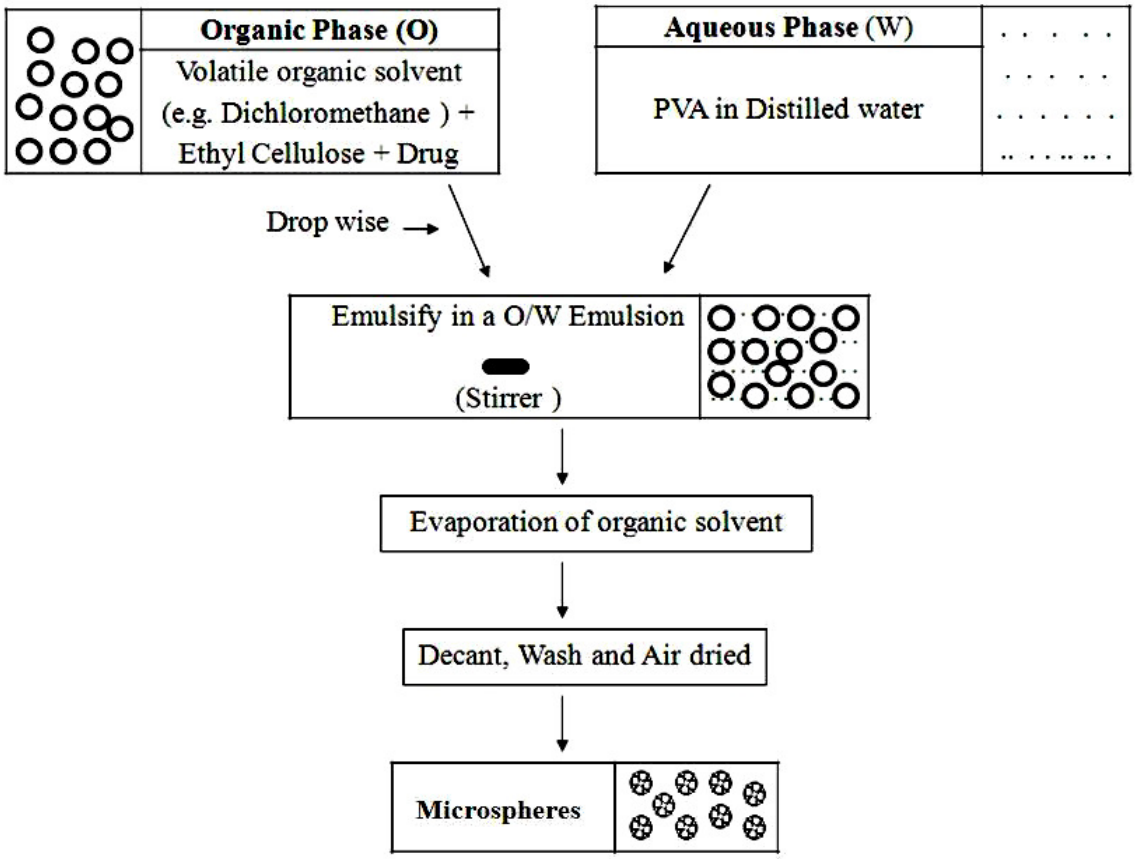

Figure 1: Schematic diagram of Oil-in-Water (o/w) emulsion solvent evaporation diffusion method for preparation of microspheres.

Table 1: Composition of various ACF loaded EC microsphere formulations.

Formulation Code	Drug : Polymer	IPV (ml) (DCM)	PVA (%w/v)	EPV (ml)
F1	1:0.5	10	0.5	100
F2	1:1.0	10	0.5	100
F3	1:1.5	10	0.5	100
F4	1:2.0	10	0.5	100
F5	1:2.5	10	0.5	100
F6	1:3.0	10	0.5	100

IPV- Internal Phase Volume (ml), EPV- External Phase Volume, DCM- Dichloro methane, PVA- Poly vinyl alcohol

Table 2: Percentage yield and percent entrapment efficiency, mean particle size and percent cumulative drug release of various ACF loaded EC microspheres formulations

Formulation	Percent yield[#]	Entrapment Efficiency (%)[#]	Mean Particle Size (µm)[#]	Cumulative Drug Release (%)[#]
F1	80.37±1.37	73.12±1.33	22±1.7	82.11 ± 0.56
F2	95.43±1.13	89.53±0.93	10±2.1	94.68 ± 0.54
F3	89.56±2.16	78.47±1.57	27±1.3	77.47 ± 0.21
F4	85.92±1.19	71.35±0.98	34±4.2	69.99 ± 0.15
F5	75.32±2.21	67.69±1.13	42±3.4	67.32 ± 0.23
F6	78.09±1.10	55.87±2.03	51±2.7	58.36 ± 0.32

[#]N=3±S.D.

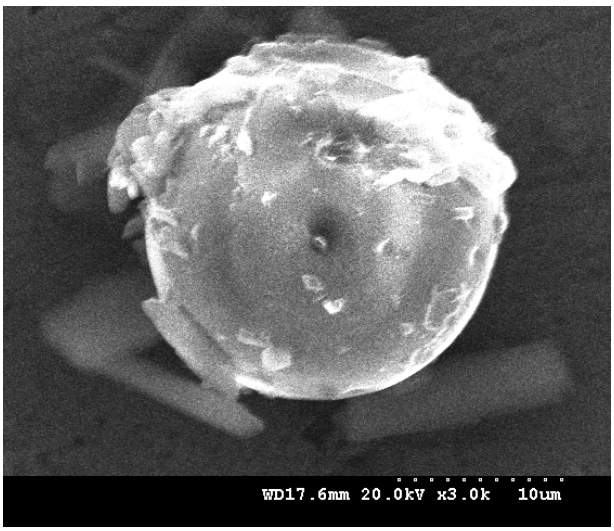

Figure 2: Scanning electron micrograph of ACF loaded EC microsphere.

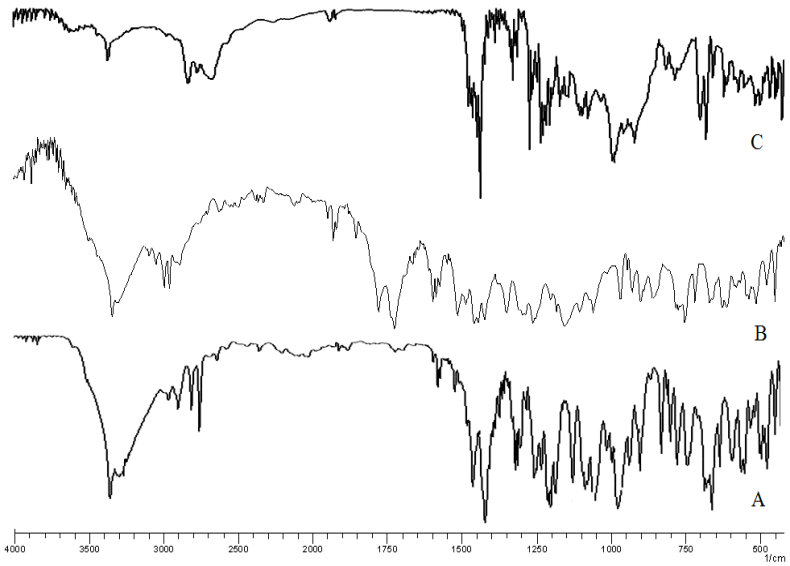

Figure 3: FTIR spectrum of pure aceclofenac (A), Physical mixture of drug-EC polymer (B) and Drug loaded EC microsphere formulation (C)

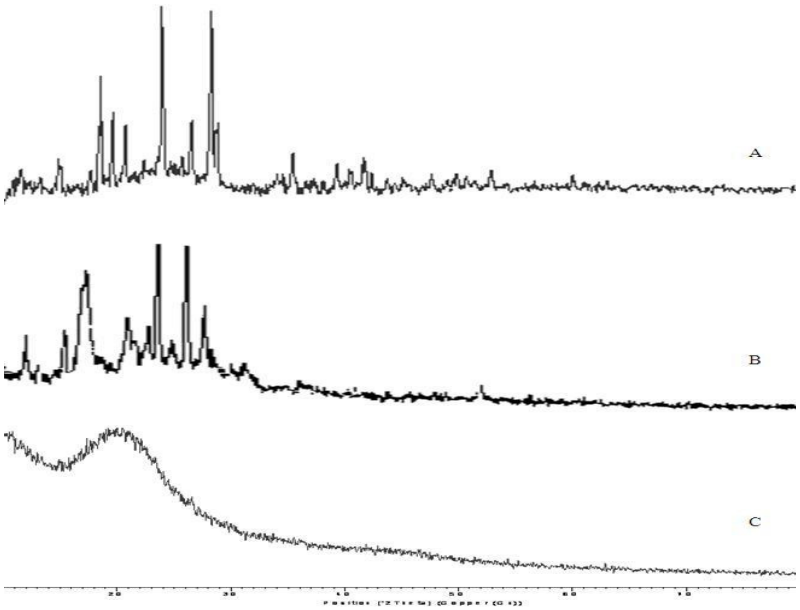

Figure 4: XRD spectra of pure drug (A), drug loaded EC microspheres (B), EC polymer (C)

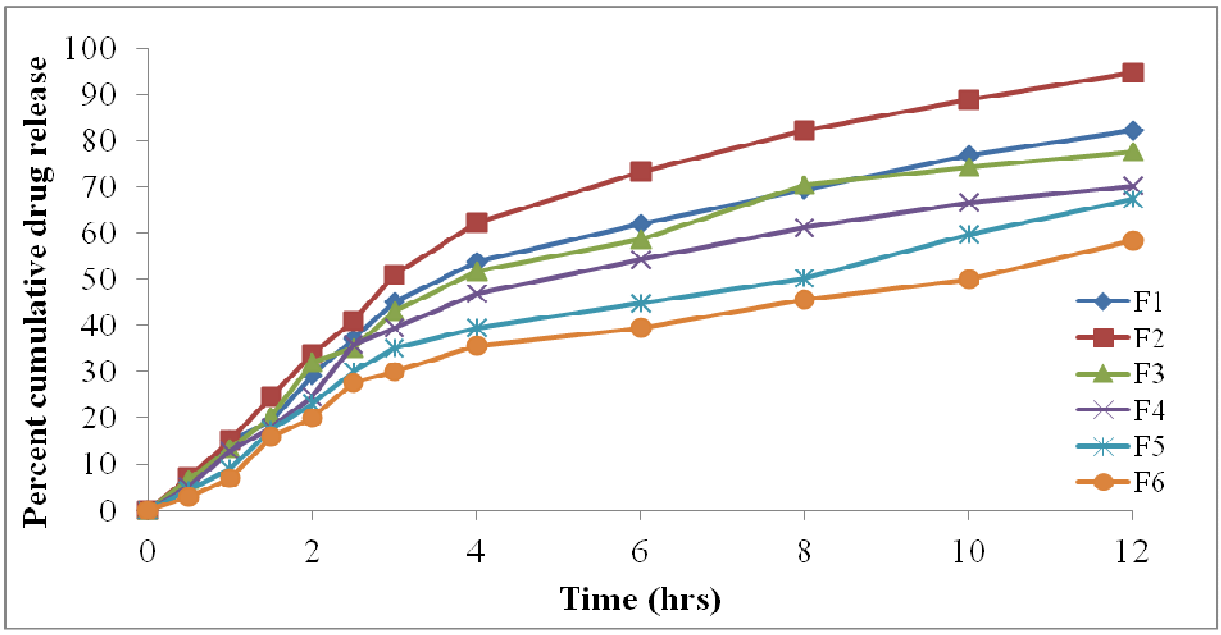

Figure 5: Comparative *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations.

Table 3: Comparative study of effect of different ACF loaded microspheres formulations on edema induced by Carrageenan induced rat paw edema model in rats.

Time (hrs)	Paw volume in mL# (Mean ± S.E.M.)					Percent Edema Inhibition (PEI)			
	Group I	Group II	Group III	Group IV	Group V	Group II	Group III	Group IV	Group V
	Control	Standard	F 1	F 2	F 3	Standard	F 1	F 2	F 3
0.5	0.31±0.013	0.22±0.018*	0.27±0.011*	0.26±0.009*	0.29±0.017*	29.03	12.90	16.12	6.45
1	0.36±0.012	0.2±0.007*	0.26±0.014*	0.24±0.006*	0.28±0.018*	44.44	27.77	33.33	22.22
2	0.44±0.010	0.18±0.012*	0.25±0.018*	0.23±0.010*	0.27±0.014*	59.09	43.18	47.72	38.63
4	0.57±0.008	0.08±0.010*	0.23±0.013**	0.21±0.015**	0.25±0.016*	85.96	59.64	63.15	56.14
6	0.52±0.009	0.18±0.013*	0.19±0.015*	0.13±0.014*	0.22±0.011*	65.38	63.46	74.42	57.69
8	0.43±0.013	0.26±0.014*	0.141±0.017*	0.08±0.012*	0.18±0.008*	39.53	67.20	81.39	58.13
10	0.41±0.018	0.34±0.011*	0.131±0.009*	0.06±0.011*	0.17±0.007*	17.07	68.04	85.36	58.53
12	0.37±0.007	0.35±0.005**	0.115±0.011*	0.04±0.050***	0.15±0.012*	5.41	68.91	87.83	59.45

#Mean ± SEM, N=6,
*$p < 0.05$, **$p < 0.01$, ***$p<0.001$ for comparison of treated groups vs control

Table 4: Comparative study of analgesic effect of different ACF loaded microspheres formulations employing Eddy hot plate model in rats.

Time (hrs)	Latency Period or Reaction Time (Sec)#				
	Group I	Group II	Group III	Group IV	Group V
	Control	Standard	F 1	F 2	F 3
0.5	5.03±0.4	6.16±0.3*	5.57±0.2*	5.98±0.4*	5.73±0.2*
1	5.17±0.3	8.12±0.1*	8.93±0.1*	10.27±0.5*	7.34±0.3*
2	5.14±0.1	11.02±0.2*	9.22±0.3*	11.21±0.3*	8.44±0.2*
4	5.13±0.09	12.54±0.3***	10.35±0.2*	11.86±0.6***	9.02±0.3*
6	5.15±0.2	8.39±0.1*	11.16±0.4*	12.09±0.4*	10.11±0.6*
8	5.16±0.11	7.12±0.3**	11.35±0.3**	12.52±0.5***	10.47±0.4**
10	5.18±0.1	6.36±0.2*	11.43±0.5*	12.69±0.3*	10.53±0.2*
12	5.19±0.3	5.41±0.3***	11.58±0.4**	12.83±0.5***	10.62±0.3**

#Mean ± SEM, N=6,
*$p < 0.05$, **$p < 0.01$, ***$p<0.001$ for comparison of treated groups vs control

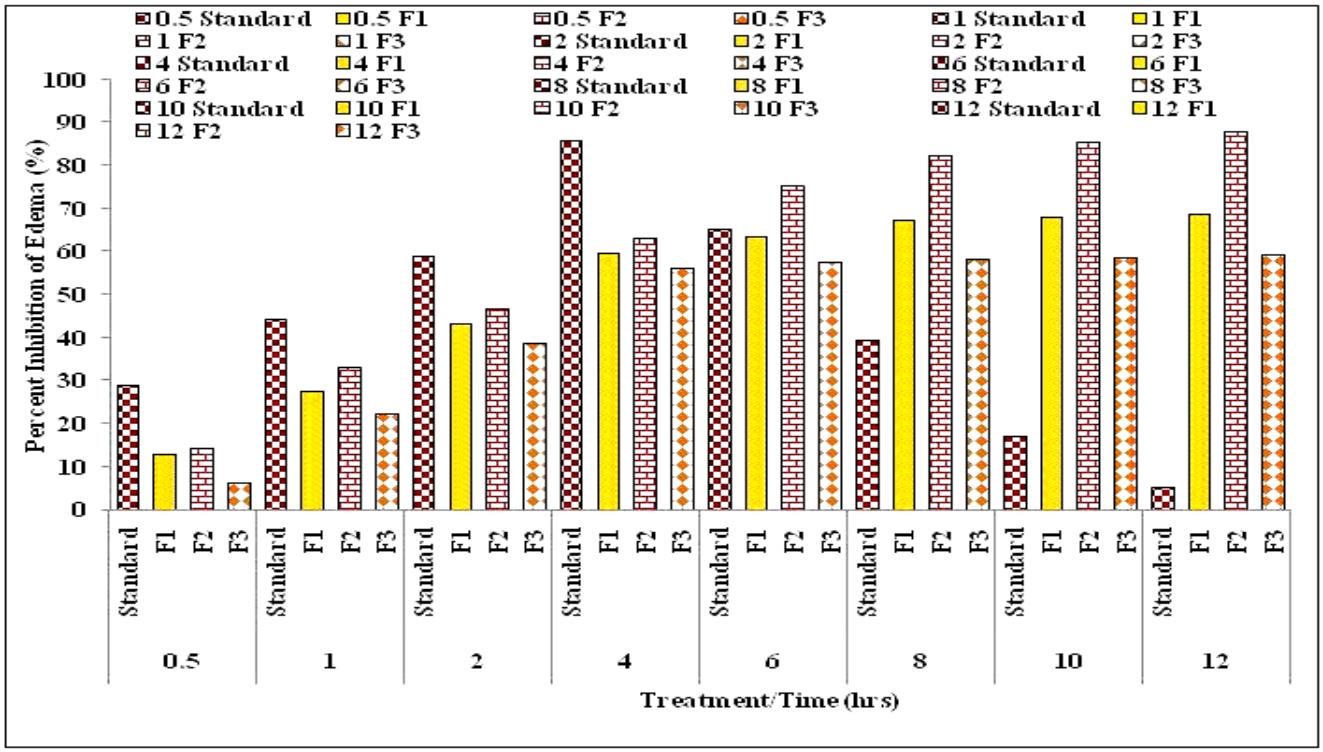

Figure 6. Comparative study of effect of various ACF loaded EC microspheres formulations on edema induced by Carrageenan induced rat paw edema model in rats.

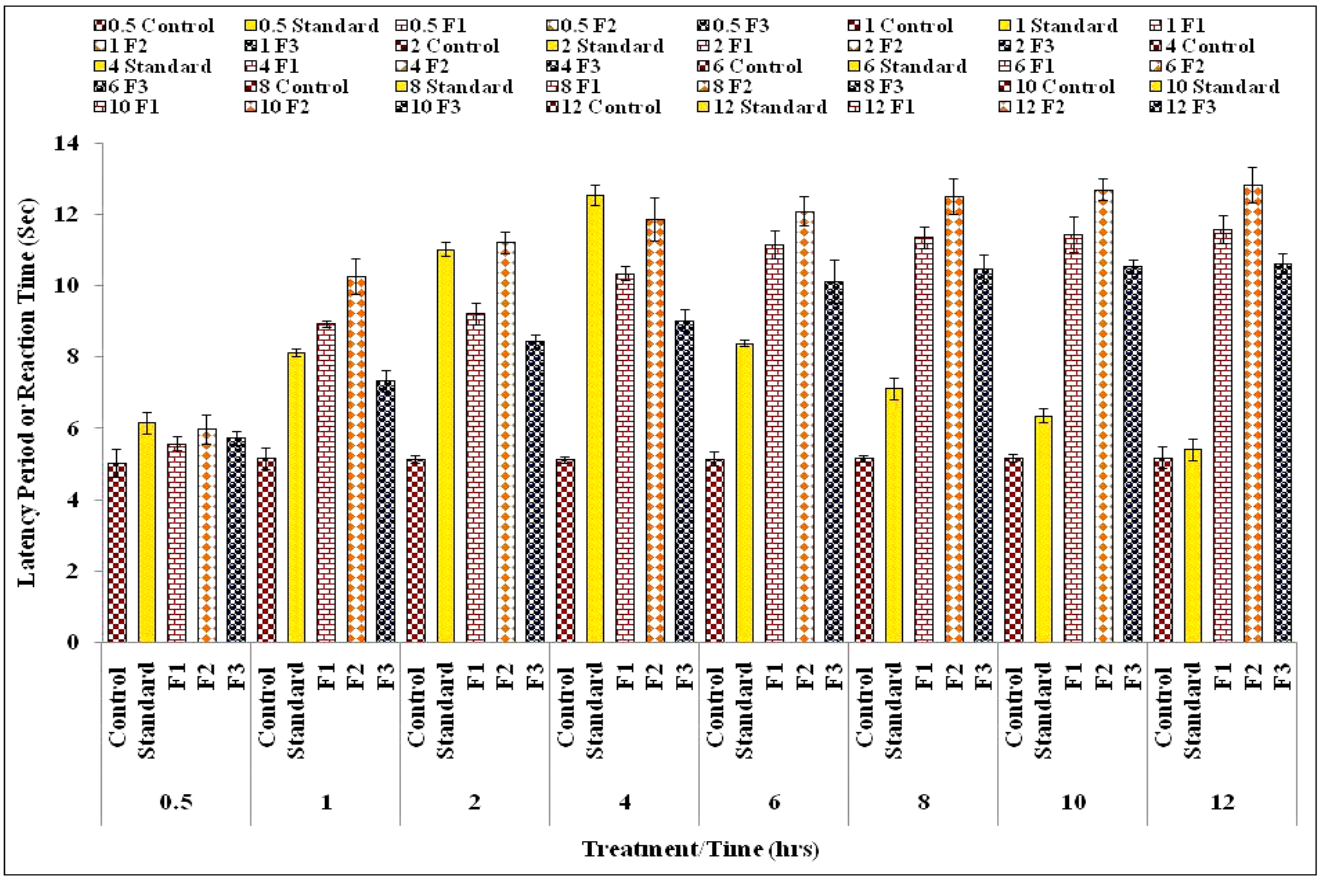

Figure 7. Comparative study of analgesic effect of different ACF loaded microspheres formulations employing Eddy hot plate model in rats.

Conclusion

Among the six formulations, F2 microspheres formulation provided reliable, reproducible results when compare to other microspheres formulations with respect to percent entrapment efficiency, *in-vitro* release profile of drug for prolong period of time, stability study and also assured from output of results of *in vivo* anti-inflammatory and analgesic activity employing EC polymer for preparing ACF microspheres by emulsion solvent diffusion evaporation technique which provides PEI and latency period for prolong period of time. So the present o/w technique significantly employed to retard the *in vitro* drug release this may result in reduce the frequency of dose administration and improve the patient compliance.

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