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Effects of formulation and process variables on Aceclofenac Loaded Ethyl Cellulose Microspheres

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Abstract:
Now a day an attempt had been made for treatment of drug effectively employed if drug intercalating in microsphere as a sustain release drug delivery systems by micro-technology. Aceclofenac, 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 and diarrhoea, dyspepsia, abdominal pain, nausea, indigestion, pancreatitis, constipation the most common side effects. So the aim of the present research work was to develop ethyl cellulose microspheres of aceclofenac by oil-in-water (o/w) emulsion solvent diffusion evaporation technique and investigated the effect of internal phase volume (IPV), poly vinyl alcohol (PVA) concentration and external phase volume (EPV) formulation variables and revolution per minute (RPM) process variables on percent yield, size, percent entrapment efficiency and percent *in vitro* drug release profile of aceclofenac microsphere formulations. The size of microspheres formulations were obtained in range of 5±1.3 to 51±2.7 µm, percent yield 75.32±2.21 to 97.87±1.43% and percent drug entrapment efficiency 55.87±2.03 to 89.53±0.93%. Microspheres also investigated for *in vitro* drug release profile and observed t_{50} and t_{70} value in the range of 2.5-10 hrs and 4-12 hrs respectively. Finally concluded, that process and formulation variables play a significant role in particle size and ultimately affect *in vitro* drug release study.

Keywords: Microtechnology, Ethyl Cellulose, Aceclofenac.

Introduction

Worldwide utilized Aceclofenac, 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 [1]. It's half life 3-4 h and prescribes multiple dosing (100 mg twice daily). After oral administration effectively and rapidly absorbed and diarrhoea, dyspepsia, abdominal pain, nausea, indigestion, pancreatitis, and constipation are the most common side effects of ACF therapy [2, 3].

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 of period. Many drawback 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 [4]. The merits of controlled 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 [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 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 was to investigate the effects of formulation and process variables on ACF loaded EC microspheres. So we can achieve sustain release drug profile by release rate retarding polymer for per oral route of administration.

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 (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 [10-11]. In the same way, several microspheres formulations were prepared by varying the formulation and process parameters mention in table 1.

Characterization of ACF loaded EC microspheres formulation

Percent Yield:

The percentage yield of different microsphere formulations was 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 upto 100 ml using pH 7.4 PBS in glass stopper conical flask. The resulting mixture was sonicated, 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 [12] .

Entrapment Efficiency (%) = (A_d/T_d) x 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 [13].

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) spectral 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.

***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 [14].

Result and discussion

Effect of concentration of EC polymer:

The various aceclofenac loaded EC microspheres formulations F1 to F6 were prepared by emulsion solvent evaporation diffusion technique, fig. 1, table 1. In which EC employed as a coat and ACF as a core material used in anti-inflammatory and analgesic activity. First of all for preliminary screening, six ACF loaded EC microspheres formulations i.e. 1:0.5, 1:1.0, 1:1.5, 1:2.0, 1:2.5 and 1:3.0 were developed then subjected for particle size, drug entrapment efficiency and *in vitro* drug release study. 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

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concentration of coat increased the percentage yield increased as well as further increased in coat concentration, decreased in percentage yield. From the SEM analysis size of microspheres formulations F1 to F6 were found in the range of 10±2.1 to 51±2.7 µm and highest percent entrapment efficiency found to be 89.53±0.93% of F2 with core to ratio 1:1.0 with particle size 10±2.1 µm (fig 2), result shown in table 2. So from optimize F2 formulation were observed free flowing and spherical shape microspheres and selected for further preliminary investigation of effect IPV, EPV, PVA concentration and rpm.

Effect of PVA concentration and EPV:

The external phase contained poly vinyl alcohol (PVA) act as a stabilizer to stabile the suspension and maintained the microsphere in suspended form. So add aqueous solution of PVA (2 %w/v) as a stabilizer in disperse medium where collection of microspheres was difficult because of higher viscosity of dispersion medium. The stabilizer play significant role during recovery of particles [15]. So select the PVA for addition in different amount (0.1 to 1%w/v) during microsphere formulation. F2 provided size of microsphere 10±2.1µm in aqueous solution of 0.5% w/v PVA but when increased the concentration of PVA (1.0%w/v), increased the size of microspheres (15 ±0.52) due to increase in viscosity of medium and result shown in table 3, fig. 3. Moreover, the volume of EPV also affected size of microspheres. In F2 formulation observed the size of microspheres 10±2.1µm employed 100 ml, 0.5 % PVA solution. When increased the volume of external phase 200 ml, decrease in size of microspheres (5±1.3 µm) was observed or vice versa. *In vitro* drug release also affected by the particle size therefore enhancement in dissolution due to size of microspheres result shown in table 4, fig. 4.

Effect of IPV:

The particle size and its surface morphology were significantly affected by the ratio of internal phase to dispersion medium. When ratio of internal phase decreased, microspheres were rapidly formed with rough surface and larger size 13 ±0.29 µm, as well as increased the ratio of internal phase, obtained microspheres of smaller size 9 ±0.34. The reason behind that higher volume of internal phase responsible for collision between the particles fused and formed large size of microspheres, result shown in table 5 and fig. 5.

Effect of revolution per minute (RPM):

It has been observed that drug release rate depend up on size of microspheres and particle size affected by RPM. So 400, 600 and 800 rpm were used for development of microsphere. F2 formulation provides 10±2.1 µm size of microspheres when rotate at 600 rpm. The size of microspheres was decreased when increase the rpm and ultimately enhance of drug release profile, result shown in table 6 and fig. 6.

Fourier Transformer Infra Red (FTIR) study:

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. 7. 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, result shown in fig. 7.

***In vitro* drug release profile:**

The *in vitro* drug release profile performed in phosphate buffer solution (pH 7.4., PBS) 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 (table 2). All microspheres formulations showed T_{50} with 2.5-10.0 hrs and T_{70} up to 4-12 hrs, reason behind that concentration of polymer increased, decreased in percent drug release due to increase in size particle and decrease in surface area. It reveals that polymer concentration prominent factor responsible for the release of drug. The effect of formulation and process variables on drug release profile showed in fig. 3-6, 8.

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

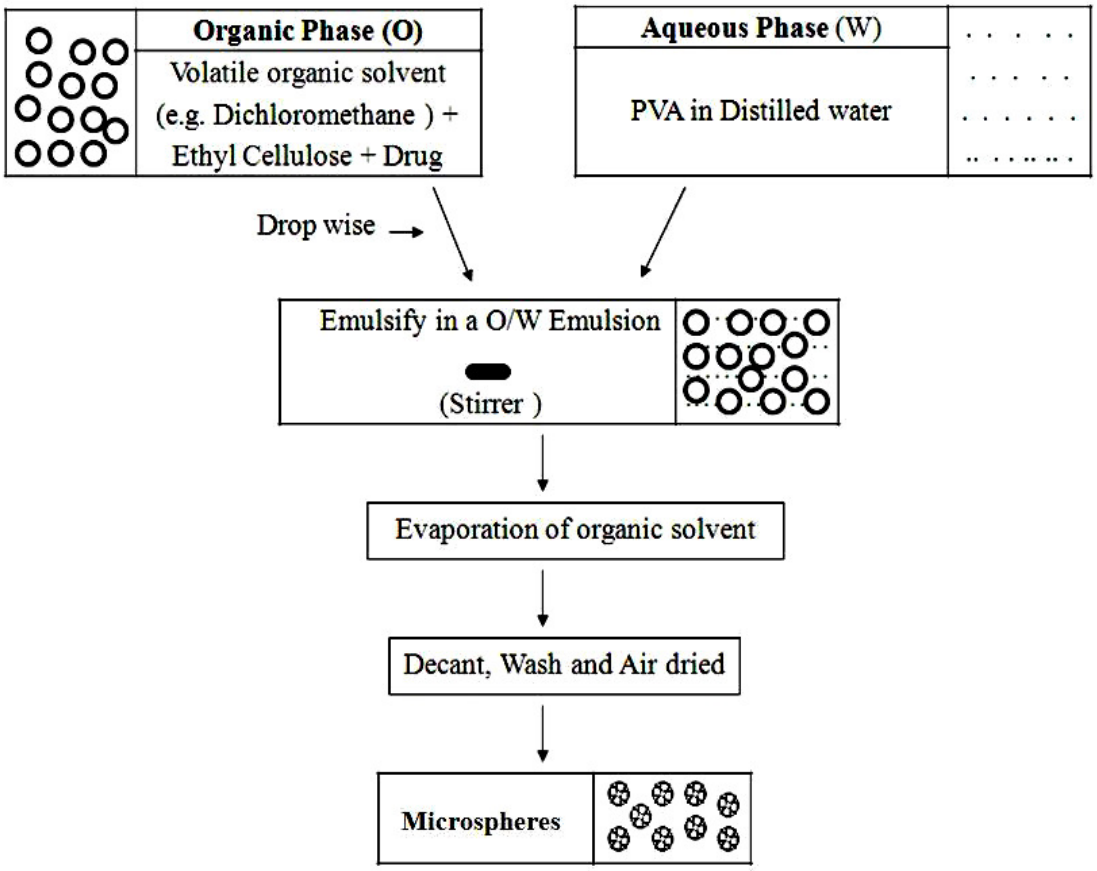

Figure 1: Schematic diagram of Oil-in-Water (o/w) emulsion solvent evaporation diffusion method for preparation of microspheres.

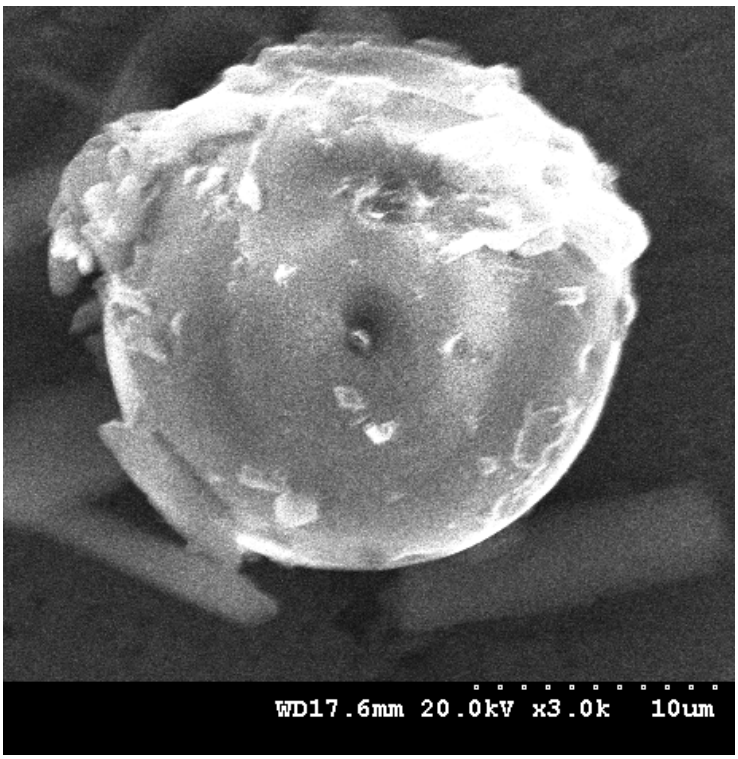

Figure 2: Scanning electron micrograph of ACF loaded EC microsphere.

Table 2: Effect of concentration of EC polymer on various parameters of ACF loaded EC microspheres.

Formulation	Drug : Polymer	Percent yield#	Entrapment Efficiency (%)#	Mean Particle Size (µm) #	T_{50} (hrs)	T_{70} (hrs)
F1	1:0.5	80.37±1.37	73.12±1.33	22±1.7	3.5	8
F2	1:1.0	95.43±1.13	89.53±0.93	10±2.1	3	5
F3	1:1.5	89.56±2.16	78.47±1.57	27±1.3	4	8
F4	1:2.0	85.92±1.19	71.35±0.98	34±4.2	5	12
F5	1:2.5	75.32±2.21	67.69±1.13	42±3.4	8	-
F6	1:3.0	78.09±1.10	55.87±2.03	51±2.7	10	-

#N=3±S.D., T_{50} and T_{70}- Time at which 50 and 70 percent amount of drug release respectively.

Table 3: Effect of PVA concentration (%w/v) on percent yield, particle size, percentage entrapment efficiency, T_{50} and T_{70}.

Formulation	PVA (%w/v)	Yield (%)*	Particle Size (µm)*	Entrapment Efficiency (%)*	T_{50} (hrs)	T_{70} (hrs)
F2	0.5	95.43±1.13	10±2.1	89.53±0.93	3	5
F7	0.1	96.67±1.25	11 ±0.97	85.49±1.77	3	4.5
F8	1.0	97.87±1.43	15 ±0.52	86.03±1.47	4	7

*N=3 ± S.D.

Table 4: Effect of EPV on percent yield, particle size, percentage entrapment efficiency, T_{50} and T_{70}.

Formulation	EPV (mL)	Yield (%)ª	Particle Size (µm)ª	Entrapment Efficiency (%)ª	T_{50} (hrs)	T_{70} (hrs)
F9	50	91.82±2.21	14±1.5	85.67±2.05	4	6
F2	100	95.43±1.13	10±2.1	89.53±0.93	3	5
F10	200	88.31±1.27	5±1.3	79.33±1.35	2.5	4

ªN=3 ± S.D.

Table 5: Effect of IPV on percent yield, particle size, percentage entrapment efficiency, T_{50} and T_{70}.

Formulation	IPV (mL)	Yield (%)[b]	Particle Size (µm)[b]	Entrapment Efficiency (%)[b]	T_{50} (hrs)	T_{70} (hrs)
F2	10	95.43±1.13	10±2.1	89.53±0.93	3	5
F11	5	97.82±1.20	13 ±0.29	85.44±1.14	3	7
F12	20	87.64±2.54	9 ±0.34	78.87±2.71	3.5	6

[b]N=3±S.D.

Table 6: Effect of rotation per minutes (rpm) on percent yield, particle size, percentage entrapment efficiency, T_{50} and T_{70}.

Formulation	RPM	Yield (%)[c]	Particle Size (µm)[c]	Entrapment Efficiency (%)[c]	T_{50} (hrs)	T_{70} (hrs)
F2	600	95.43±1.13	10±2.1	89.53±0.93	3	5
F13	400	96.72±1.19	16 ±0.77	80.21±1.25	4.5	6.5
F14	800	94.53±1.34	6 ±0.34	84.11±1.11	3.5	5.5

[c]N=3±S.D.
RPM- Rotation per minute

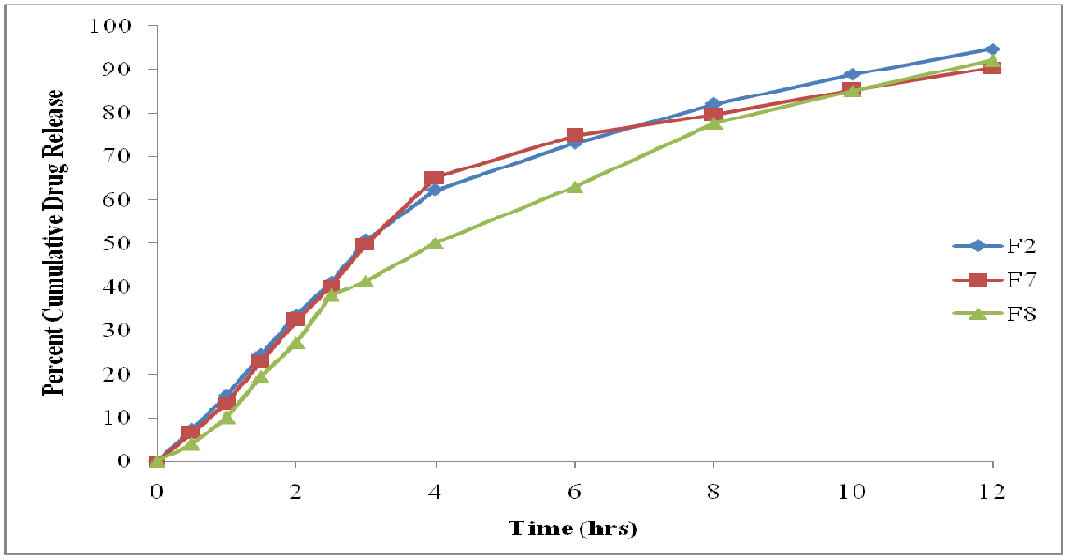

Figure 3: Effect of concentration (%w/v) of PVA on *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations.

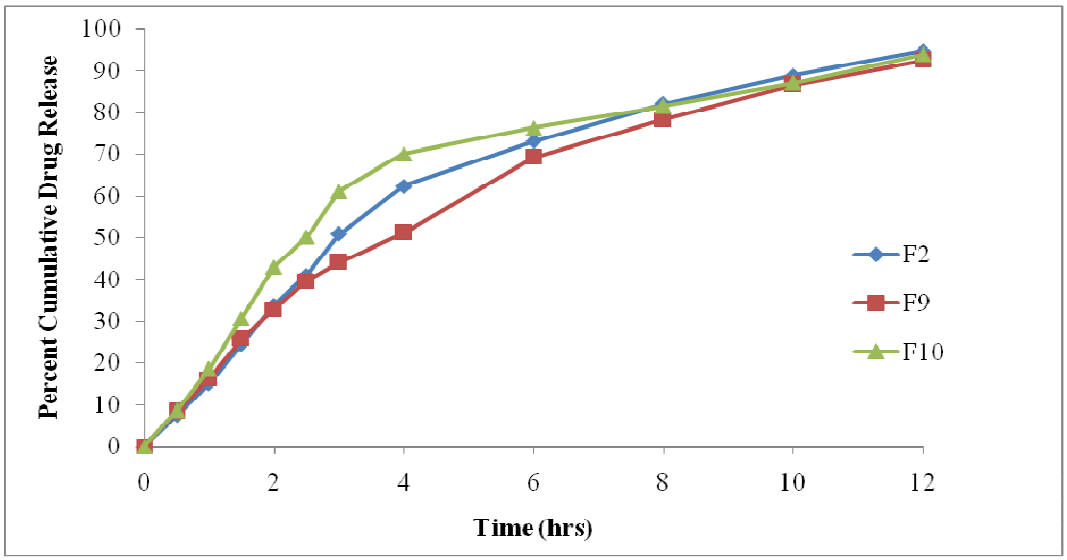

Figure 4: Effect of EPV on *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations

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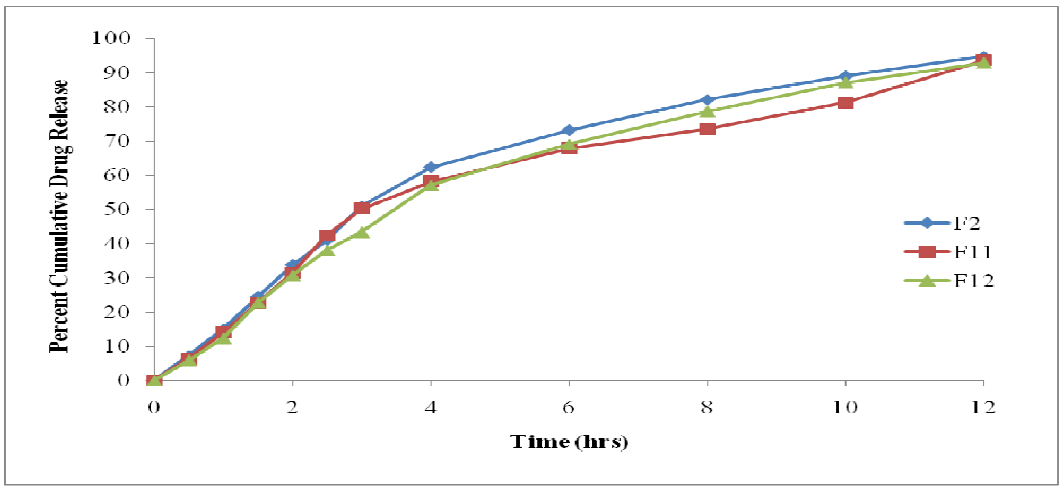

Figure 5: Effect of IPV on *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations.

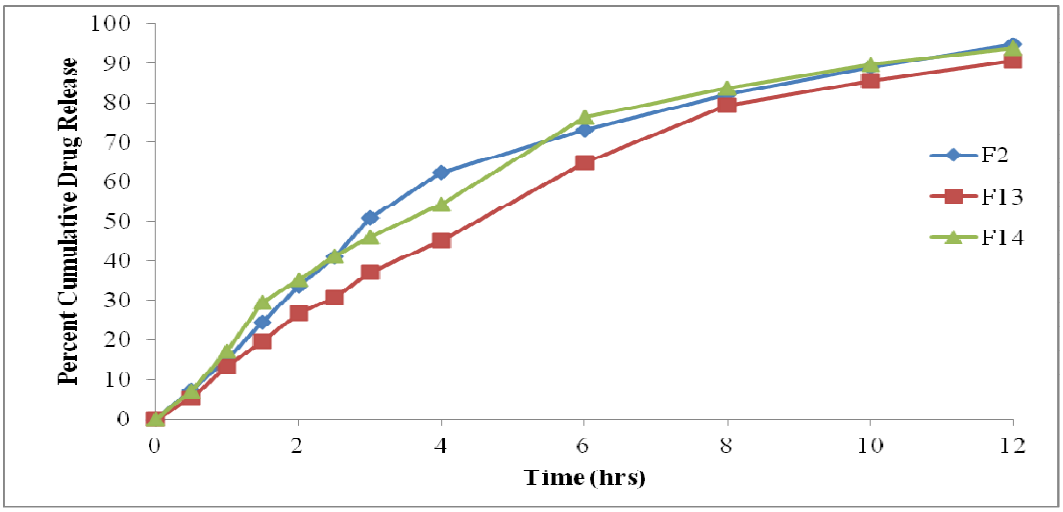

Figure 6: Effect of RPM on *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations.

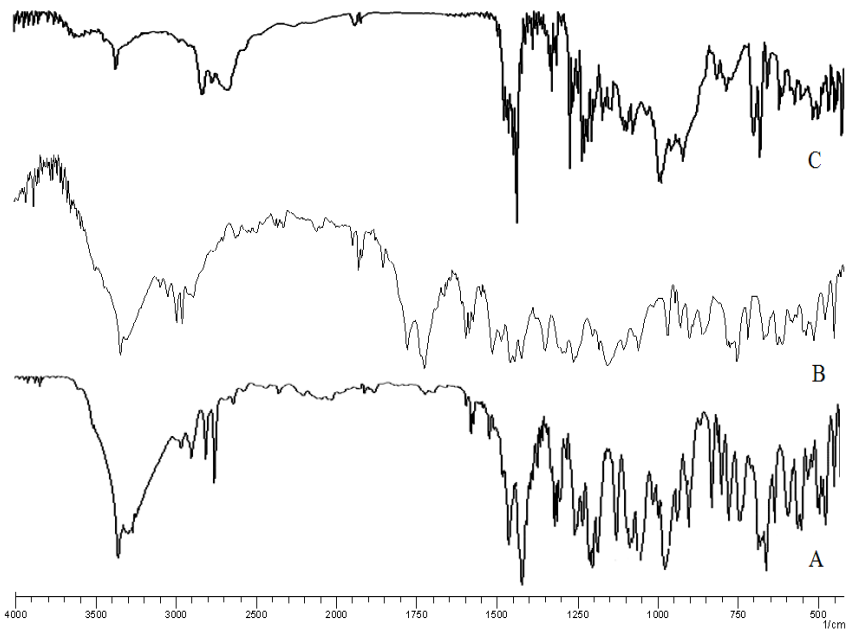

Figure 7: FTIR spectrum of pure aceclofenac (A), Physical mixture of drug-EC polymer (B) and Drug loaded EC microsphere formulation (C)

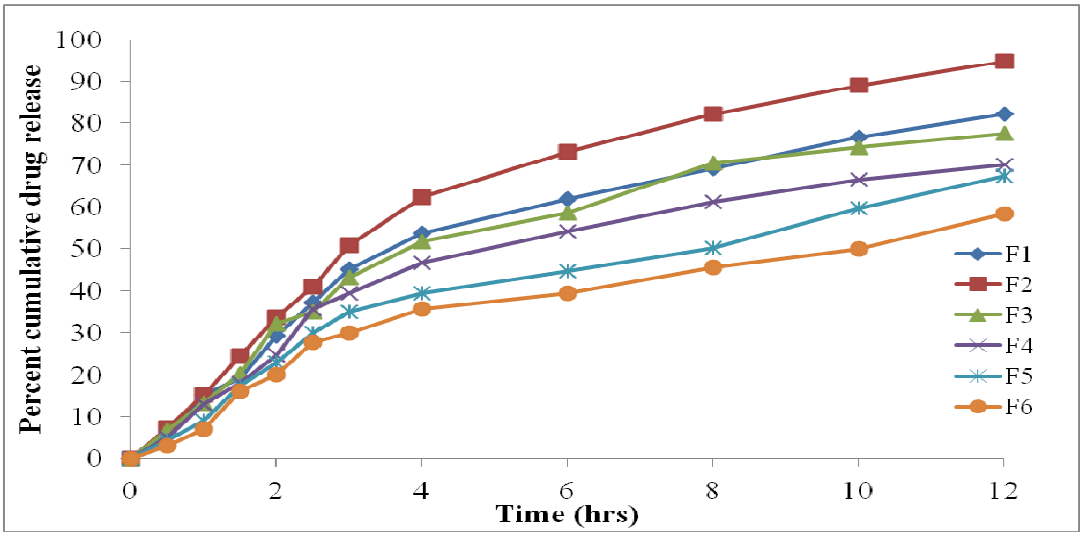

Figure 8: Comparative *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations

Conclusion

Now present day an attempt had been made for treatment of drug effectively employed if drug intercalating in microsphere as a sustain release drug delivery systems by mictrotechnology. The percent yield, particle size, surface morphology, shape, entrapment efficiency were significantly influenced by both formulation and process variables. Finally conclude that internal phase and dispersion medium played significant role in development of microspheres. ACF loaded EC microspheres were obtained spherical free flowing and smooth and showed sustain release of drug for prolong period of time.

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