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Emulsion solvent diffusion evaporation technique: Formulation Design Optimization and investigation of Aceclofenac Loaded Ethyl Cellulose Microspheres

Full Length Original Research Paper

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Abstract:

From the past few decades, scientists consider research interest in the area of prepare of drug loaded ethyl cellulose (EC) 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 design optimization and investigation of ACF loaded EC microspheres by 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 characterized by scanning electron microscopy (SEM), percent yield, micrometrics properties, fourier transformer infra red spectroscopy (FTIR), percent entrapment efficiency and percent drug release characteristics. 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 differential scanning calorimetry (DSC) study showed no interaction between drug and polymer; no degradation during microspheres preparation and stable at storage conditions. All microsphere formulations showed various drug releases kinetic but F2 formulation followed first order drug release kinetics and 94.68 ± 0.54 % drug release for prong period of time. From the study, it was investigated that free flowing and spherical microspheres of ACF could be prepared successfully by solvent diffusion evaporation technique with high entrapment efficiency and prolong release profile characteristics.

Keywords: Aceclofenac, Ethyl Cellulose, Differential scanning calorimetry.

Introduction

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 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 [4]. The merits of sustain 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 formulation design optimization and investigation of ACF loaded EC microspheres by emulsion solvent diffusion evaporation technique. 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]. In the same way, several microspheres formulations were prepared by varying the parameters mention in table 1.

Characterization of ACF loaded EC microspheres formulations:

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 [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.

Micromeritics papameters study:

Bulk density and Tapped density:

The sample poured in 10 ml of graduated cylinder, tapped mechanically 50 times and then noted down tapped volume. The experiment was repeated three times for reproducibility of results [13, 14, 15].

Bulk density (BD) = Mass / Bulk volume (1)

Tap density (TD) = Mass / Tapped volume (2)

Carr's index (CI)

Carr's index or Compressibility index (CI) value of microspheres was calculated according to the following equation.

Percent Carr's Index = [(TD – BD) / TD] x 100 (3)

Hausner's ratio (HR):

Hausner's ratio of different microspheres formulations were calculated using following formula when compared the tapped density to bulk density.

Hausner's ratio (= (TD / BD) (4)

Flow property:

For study of flow behavior, weight amount of powder samples to be analyzed poured through the funnel ensure 2.5 cm height of its tip until formed a conical pile on the flat surface of graph and observe the height and radius of pile of base then calculate the tangent of the angle of repose by using following formula-

$$\theta = \tan^{-1}(h / r)$$

Where, θ = Angle of repose, r = Radius of the base of the pile, h = Height of pile

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.

Differential Scanning Calorimetry (DSC) study:

The thermal behavior of ACF, physical mixture of drug-polymer and drug-loaded microspheres were investigated employing differential scanning calorimeter (DSC-60 Instruments, Shimadzu Corporation, Japan). The samples (5mg) were accurately weighed, sealed hermetically into aluminum pans and heating run for each sample kept from 50˚C- 300˚C at a heating rate of 10˚C per min, using in atmosphere of air as blanket gas.

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 [16].

Kinetics and mechanism of drug release study:

The *in-vitro* drug release data were analyzed to understand the drug release mechanism employing various mathematical models such as zero-order kinetics, first-order kinetics, Hixson Crowell's Model and Higuchi model [17, 18, 19, 20].

$A_t = K_0 t$ (1)

Where, K_0 - Release rate constant of Zero order, A_t - Amount of drug release at time (t).

$\ln(A_0-A_t) = \ln A_0-K_1 t$ (2)

Where, K_1- Release rate constant First order, A_0 - Initial amount of drug release, A_t - Amount of drug release at time (t),

$W_o^{1/3} - W_t^{1/3} = K_c\, t$ (3)

Where, K_c - Release rate constant of Hixson Crowell's cube root, W_o - Initial weight ,W_t - Weight remaining at time (t),

$A_t = K_H.\sqrt{t}$ (4)

Where, A_t - Amount of drug release at time (t), K_H - Release rate constant of Higuchi, Square root of time (SQRT) (√t)

$A_t / A_\infty = K_p\, t^n$ (5)

Where, A_t/A_∞ - Fraction of drug released at time (t), A_t and A_∞ - Amount of drug released at time (t) and time (∞) respectively, K_p - Korsmeyer-Peppas power law constant comprising the structural and geometric characteristics of the microspheres, n - Diffusion exponent .

The following graph were plotted for Zero-order kinetic model- $\%A_t$ vs t, First-order kinetic model- log% unreleased vs t, Hixson Crowell's cube root model- ($W_o^{1/3} - W_t^{1/3}$) vs t, Higuchi model- $\%A_t$) vs √t, and Korsmeyer-peppas model- Log percent drug release vs Log t.

In order to define a model, the *in-vitro* drug dissolution data was evaluated by Korsmeyer-peppas mathematical equation represents a best fit for the formulation. The correlation coefficient (R^2) was calculated by least square linear regression method of the above plots and also determine release rate constant of various kinetic models and diffusion exponent [21, 22, 23].

Determination of similarity and difference factor study:

A model independent approach based on determination of difference factor (f_1) and similarity factor (f_2) were evaluated for compare the dissolution profiles. The *in-vitro* drug release data of ACF loaded EC microspheres formulations were compared with marketed formulation (MF) Aceclofenac- Zerodol CR (IPCA) using a statistical tool to investigate the difference factor (f_1) and the similarity factor (f_2) by the following equation-

$f_1 = \{(\sum_{=1}^{n} \mid R_t - T_t \mid) (\sum_{=1}^{n} R_t)\} \times 100$ (6)

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Where, n – No. of samples; R_t and T_t - Drug release data of reference and test sample at the same time point (t) respectively.

The difference factor (f_1) investigates the percent difference between drug release profiles of curves of test and reference samples at the same time and is a measurement of the relative error. If the (f_1) factor between drug release profiles of curves is zero, indicates the identical *in-vitro* dissolution profile.

The similarity factor (f_2) is measurement of the similarity in the percent *in-vitro* dissolution between the test and reference sample profiles by analyzing the average sum of squares. It calculated by using the following formula:

$f_2 = 50 \times \text{Log} \{(1+ (1/n) \sum_{=1}^{n} (R_t - T_t)^2)^{-0.5} \times 100\}$ (7)

The f_2 value (50 to 100 ranges) ensures similarity of the *in-vitro* dissolution profile of test and reference samples [24, 25, 26, 27].

Stability Studies

To find the stable product stability studies were performed under storage conditions. 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 percent drug release profiles 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, percent drug release and physical appearance [28, 29, 30].

Result and discussion

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 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 3.

All microspheres formulations subjected for study of various micromeritics paramaters result shown in table 3. The bulk density, tapped density of all microspheres formulations F1 to F6 were found to be 0.549±0.03 to 0.617±0.02 and 0.631±0.02 to 0.722±0.05 g/cm^3 respectively but F2 showed 0.549±0.03 and 0.631±0.02 g/cm^3 respectively. For study of flow property determined the angle of repose, Hausner's ratio and Carr's index. All F1 to F6 microspheres formulation showed angle of repose 21.31±0.36^0 to 34.54±0.32^0, Carr's index 11.818±0.26 to 15.116±0.11% and Hausner's ratio 1.134±0.005 to 1.178±0.012 respectively but F2 microspheres formulation indicates excellent flow behaviour.

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.

DSC demonstrated a possible interaction between drug and execipient and also provided information on the physical properties of sample and its crystalline or amorphous nature. DSC thermograms showed characteristic sharp endothermic peak of pure ACF at 155.84˚C, which corresponded to its melting point (M.P. 149-156˚C). Thermograms of physical mixture of drug-polymer showed M.P. at 152.96˚C and drug loaded microspheres showed peak at 149.44˚C due to uniform dispersion of drug in microsphere and higher amount of EC. The melting endotherms indicate no considerable change in melting point of drug loaded EC microspheres as compared to drug and indicate no interaction between drug and polymer, result shown in fig. 4. So F2 formulation considered as an ideal formulation, subjected for *in vitro* and stability studies.

The in vitro drug release profile of drug loaded EC microsphere formulations studied in PBS (pH 7.4.) and simultaneously investigated MF for dissolution study. So that compare the *in vitro* dissolution profile of drug loaded EC microspheres formulation to MF and determine the similarity between the formulations. It was observed that all microspheres formulation (F1 to F6) showed drug release 58.36 ± 0.32 to 94.68 ± 0.54% (table 3) but F2 formulation indicated highest drug release 94.68 ± 0.54% up to 12 hrs (fig. 5) as well as concentration of polymer increased, decreased in percent drug release. It reveals that polymer concentration prominent factor that responsible for the drug release profile. MF showed the *in vitro* drug release 92.87± 0.67% up to 12 hrs (table 4, fig. 6) near to similar F2 optimized formulation.

In order to study the mechanism of ACF release from the ACF loaded EC microspheres, the *in-vitro* drug release data of various drug to polymer ratio for EC microspheres were analyzed by using various mathematical model to describe drug release, i.e. zero order, first order, Higuchi model, Hixson Crowell's cube root model and Korsmeyer-peppas model. The correlation coefficients (R^2) of all release kinetic models were determined, results shown in table 5, fig. 7-11. From table 5, in first order model, the R^2 of various F1, F2 and F3 microsphere formulations were obtained 0.983, 0.992 and 0.971 respectively and in Higuchi model obtained 0.961, 0.975 and 0.966 of F4, F5 and F6 respectively. The various microsphere formulations containing different drug to polymer ratio were obtained the highest correlation coefficient in first order model than Higuchi order followed by zero order. The microsphere formulation F2 found highest correlation coefficient (R^2=0.992) of first order release plot. It confirmed that rate of drug release depend upon amount of drug present in microspheres. The diffusion exponent (n) value from Peppas model was found 0.759-0.868 range for various drug to polymer ratio (1:0.5 to 1:3) indicating that all microsphere formulations follow non Fickian (Anomalous transport) diffusion controlled release. Amongst the all microsphere formulations, the highest correlation coefficient containing formulation gives idea about model best fitted to the release data. The *in vitro* drug release profile of MF as a reference standard and F2 optimized microsphere formulation as a test sample was compared, result shown in Fig. 6. The similarity factor (f_2) was determined by the equation 6 between MF and F2 optimized microsphere formulation as reported earlier. It was observed that optimized microspheres F2 formulation have similarity factor more than 50 and confirmed the similarity of dissolution profiles with that of MF. If F2 optimized microsphere formulation as a reference sample to compare with other drug loaded EC microsphere formulations as test samples individually, the similarity factor were obtained between 30.83 to 54.70 and difference factor 13.57 to 42.11 respectively.

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, percent drug release profiles and physical appearance of drug loaded EC microspheres formulations, result shown in table 6.

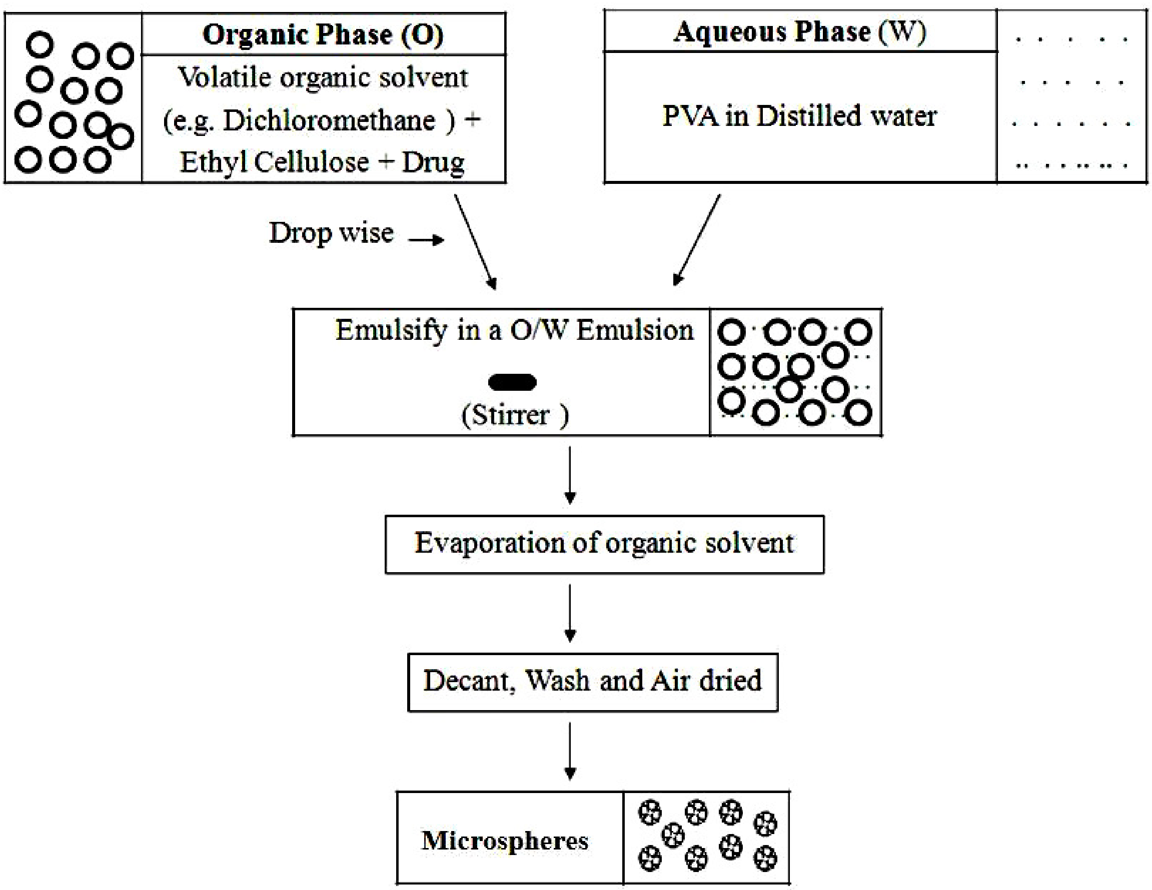

Figure 1: Schematic diagram of Oil-in-Water (o/w) emulsion solvent evaporation diffusion method for preparation of microspheres.

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 of various formulations of ACF loaded EC microspheres.

Formulation Code	Drug : Polymer	Percent yield#	Entrapment Efficiency (%)#
F1	1:0.5	80.37 ± 1.37	73.12 ± 1.33
F2	1:1.0	95.43 ± 1.13	89.53 ± 0.93
F3	1:1.5	89.56 ± 2.16	78.47 ± 1.57
F4	1:2.0	85.92 ± 1.19	71.35 ± 0.98
F5	1:2.5	75.32 ± 2.21	67.69 ± 1.13
F6	1:3.0	78.09 ± 1.10	55.87 ± 2.03

#N=3±S.D.

Table 3: Micromeritic properties and percent drug release of various drug loaded EC microspheres formulations.

Formulation Code	Bulk Density (g/cm^3)#	Tapped Density (g/cm^3)#	Hausner's Ratio#	Carr's Index (%)#	Angle Repose (^{0})#	Particle Size (μm)#	Cumulative Drug Release (%)#
F1	0.568±0.04	0.658±0.06	1.158±0.003	13.636±0.23	23.15±0.54	22±1.7	82.11 ± 0.56
F2	0.549±0.03	0.631±0.02	1.148±0.007	12.857±0.14	21.31±0.36	10±2.1	94.68 ± 0.54
F3	0.562±0.05	0.649±0.04	1.156±0.003	13.483±0.54	26.23±0.27	27±1.3	77.47 ± 0.21
F4	0.568±0.03	0.644±0.02	1.134±0.005	11.818±0.26	28.11±0.67	34±4.2	69.99 ± 0.15
F5	0.581±0.06	0.685±0.03	1.178±0.012	15.116±0.11	31.49±0.41	42±3.4	67.32 ± 0.23
F6	0.617±0.02	0.722±0.05	1.169±0.006	14.444±0.35	34.54±0.32	51±2.7	58.36 ± 0.32

#N=3± S.D.

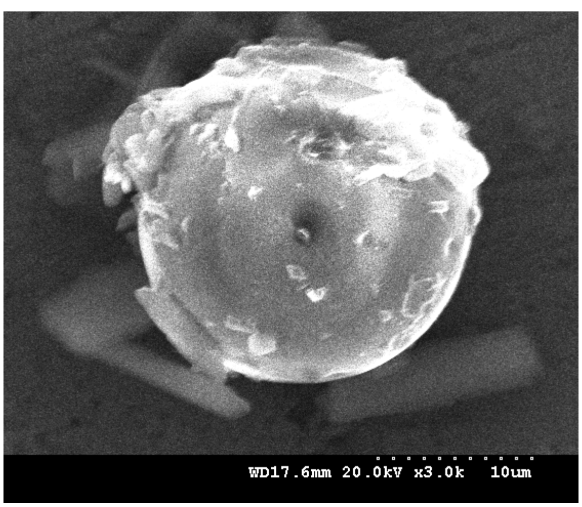

Figure 2: Scanning electron micrograph of ACF loaded EC microsphere.

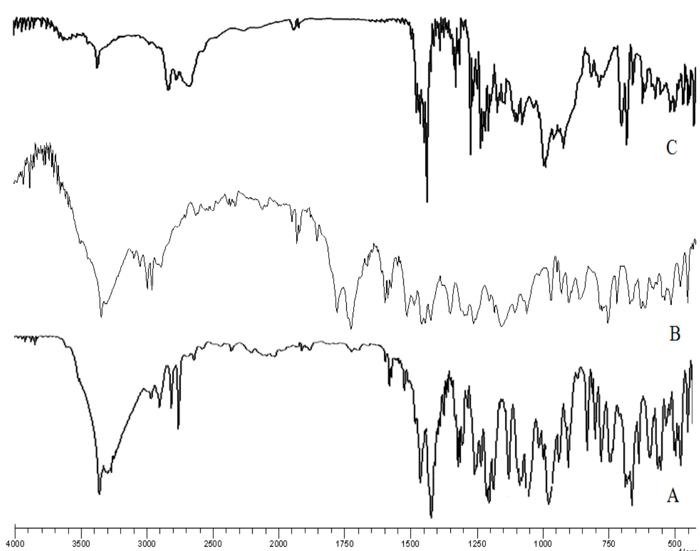

Figure 3: FTIR spectrum of pure aceclofenac (A), Physical mixture of drug-EC polymer (B) and Drug loaded EC microsphere formulation (C)

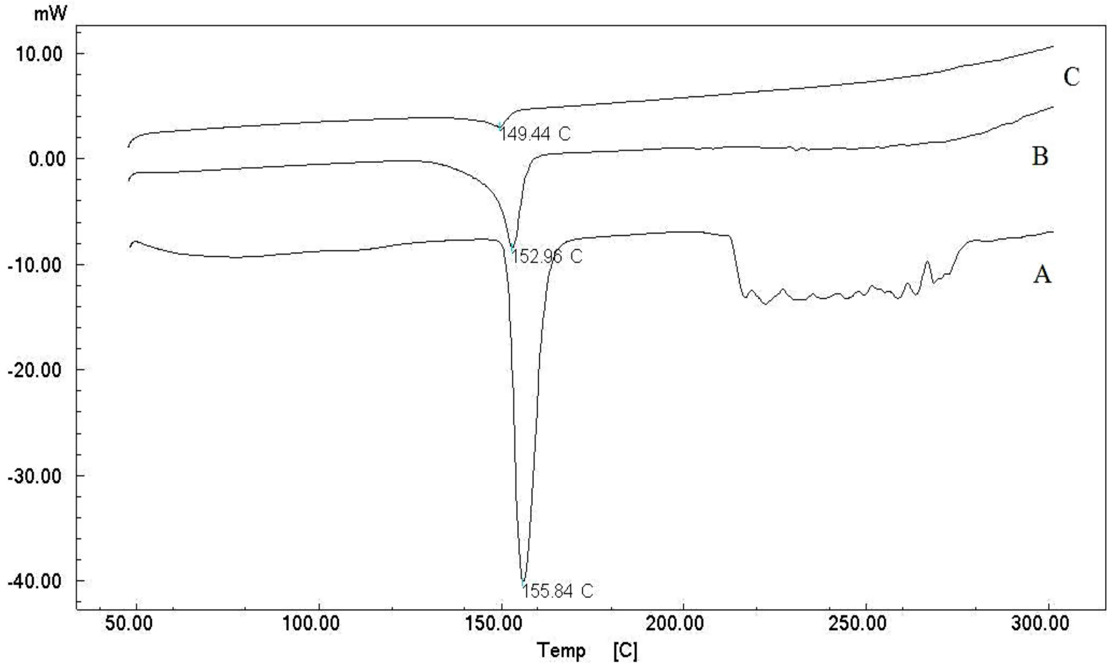

Figure 4: Comparative DSC thermogram of Pure ACF (A), Physical mixture drug-polymer (B), ACF loaded EC microspheres (C).

Table 4: *In-vitro* percent cumulative drug release of F2 microsphere formulation (Test sample) and marketed product (MP as a reference)

Time (h)	Percent drug release of Test sample (T_t)#	Percent drug release of Reference sample (R_t)#
0	0	0
0.50	7.27 ± 0.89	6.72±0.37
1.00	15.08 ± 0.24	14.10±0.54
1.50	24.48 ± 0.11	22.35±0.89
2.00	33.76 ± 0.87	31.93±0.42
2.50	41.04 ± 0.44	39.17±0.27
3.00	50.84 ± 0.78	49.02±0.10
4.00	62.32 ± 0.61	61.11±0.48
6.00	73.08 ± 0.42	72.58±0.87
8.00	82.08 ± 0.54	80.83±0.22
10.00	88.92 ± 0.89	87.69±0.13
12.00	94.68 ± 0.54	92.87±0.67

#N=3± S.D.

Table 5: Drug release kinetic parameters of different ACF loaded EC microspheres formulations.

Formulation Code	Zero order	First order	Higuchi	Hixson Crowell	Korsmeyer–Peppas	
	R^2 Value	R^2 Value	R^2 Value	R^2 Value	R^2 Value	n value
F1	0.89	0.983	0.967	0.75	0.94	0.788
F2	0.893	0.992	0.96	0.757	0.949	0.794
F3	0.884	0.971	0.965	0.751	0.945	0.759
F4	0.875	0.956	0.961	0.731	0.931	0.786
F5	0.913	0.969	0.975	0.754	0.932	0.812
F6	0.891	0.948	0.966	0.712	0.903	0.868

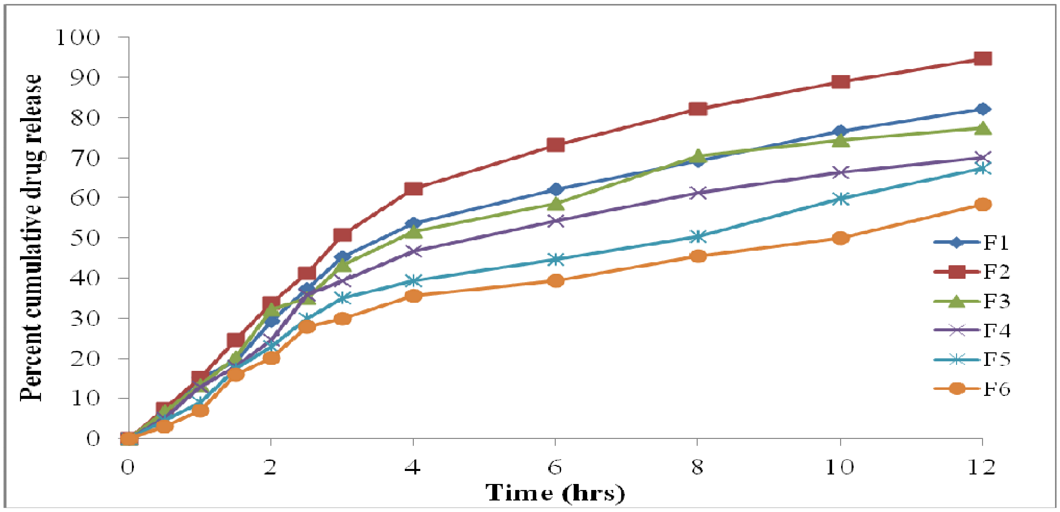

Figure 5: Comparative *in vitro* percent cumulative drug release profile of various ACF loaded EC microspheres formulations

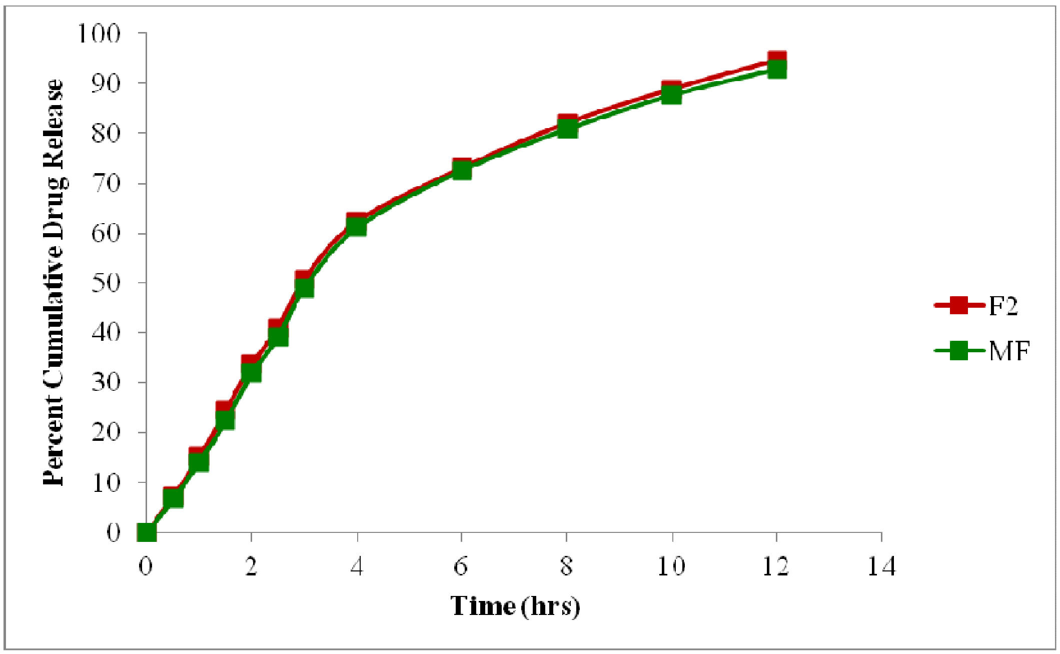

Figure 6: Comparative *in-vitro* dissolution study of F2 optimized microspheres formulation and MF.

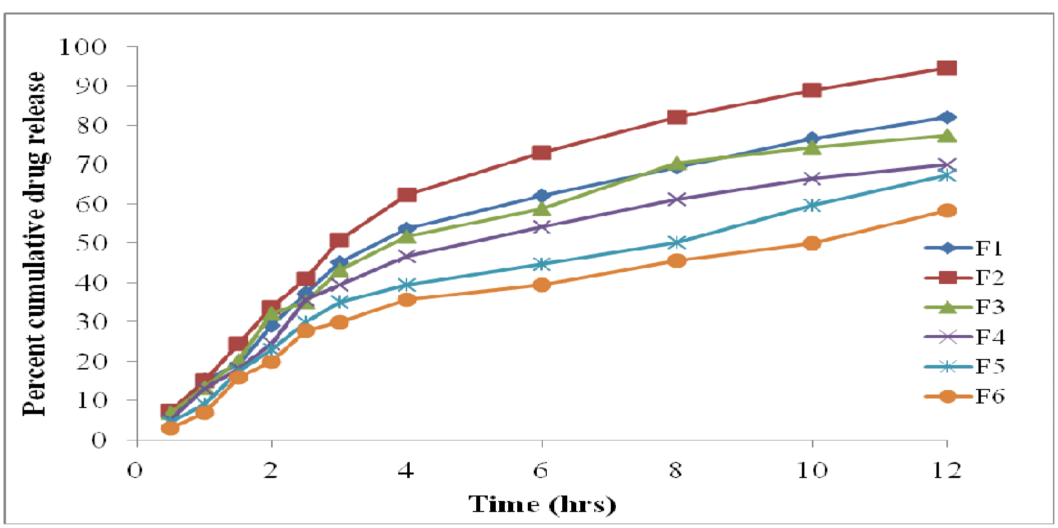

Figure 7: Zero order release model of ACF from ACF loaded EC microspheres.

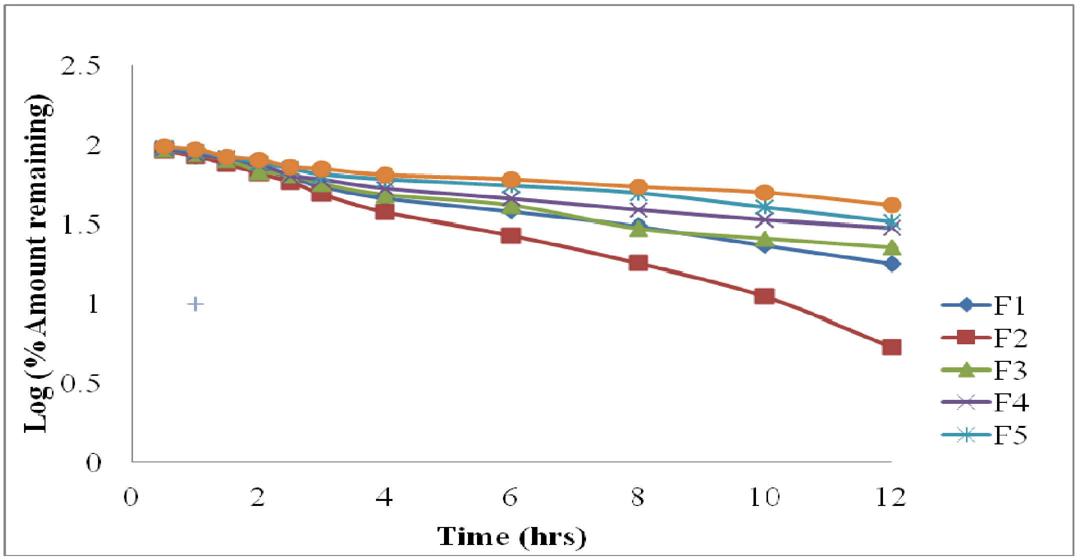

Figure 8: First order release model of ACF from ACF loaded EC microspheres.

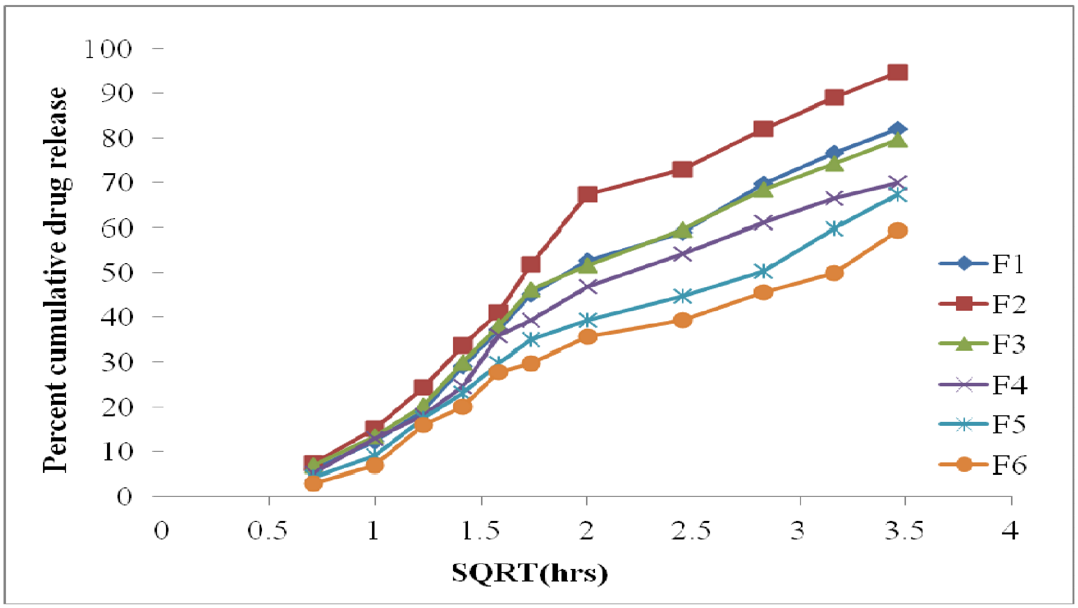

Figure 9: Higuchi release model of ACF from ACF loaded EC microspheres.

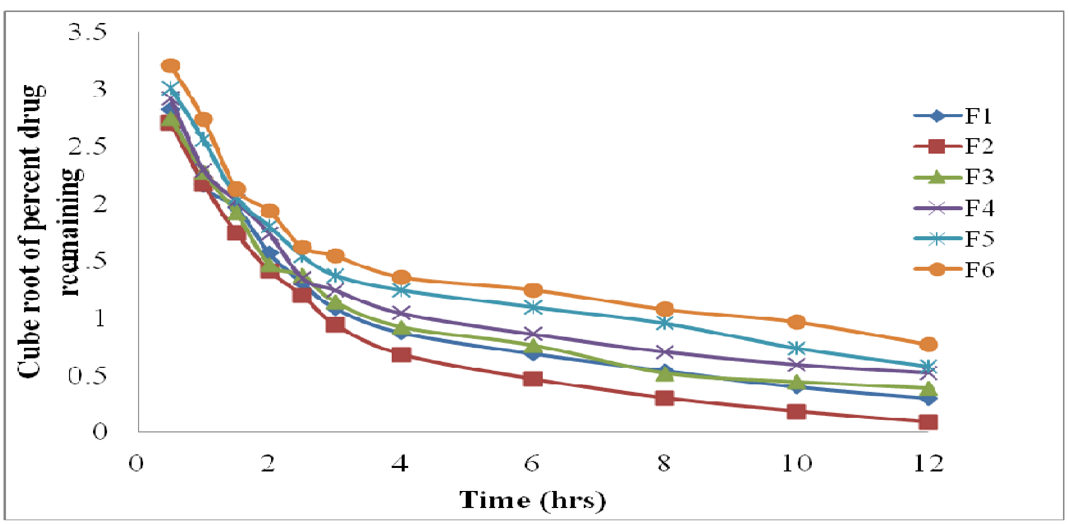

Figure 10: Hixson-Crowell cube root release model of ACF from ACF loaded EC microspheres.

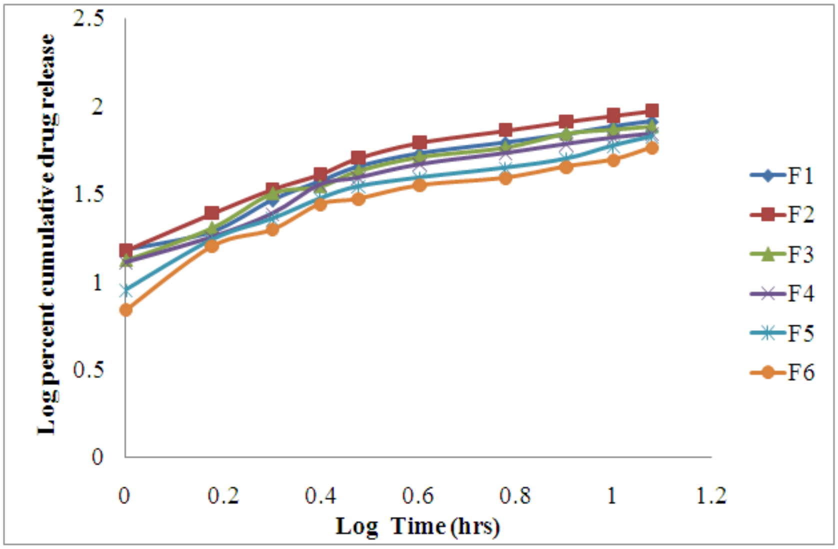

Figure 11: Korsmeyer-Peppas release model of ACF from ACF loaded EC microspheres.

Table 6: Stability data for optimized ACF loaded EC microspheres formulation.

Time Period (Months)	% Entrapment Efficiency#			% Drug Release (at 12 hrs)#			Physical appearance#		
	5± 2°C	25±2°C, 60±5% RH	40±2°C, 75±5% RH	5± 2°C	25±2°C, 60±5% RH	40±2°C, 75±5% RH	5± 2° C	25±2°C, 60±5% RH	40±2°C, 75±5% RH
0	89.53	89.53	89.53	94.68	94.68	94.68	-	-	-
2	89.47	89.42	89.39	94.54	94.50	94.47	-	-	-
3	89.39	89.33	89.30	94.43	94.31	92.92	-	-	-
4	89.27	89.18	89.04	94.37	93.97	92.50	-	-	-
6	89.20	88.97	89.65	94.15	93.73	91.89	-	+	+

N=3, (-) No change, (+) Slight change

Conclusion

Among the six formulations, F2 microspheres formulation provided reliable, reproducible results when compare to other microspheres formulations and MF with respect to percent entrapment efficiency, *in-vitro* release profile of drug for prolong period of time and stability study and also assured from output of results of kinetics of drug release employing EC polymer is suitable for preparing ACF microspheres by emulsion solvent diffusion evaporation technique which provides first order drug release kinetics. 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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