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Formulation and evaluation of aceclofenac floating microspheres

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ABSTRACT

The floating drug delivery systems (FDDS) have been developed to obtain prolonged and uniform release of drug in the stomach for the development of once-daily formulation. The objective of the present study was to develop once-daily sustained release FDDS of aceclofenac. The study involves preparation and evaluation of floating microspheres with model drug aceclofenac and polymer ethyl cellulose for prolongation of gastric residence time by solvent evaporation method. The microspheres remained buoyant in acidic medium containing surfactant for 8 hours *in vitro*. It was found that at higher polymer concentration, the mean particle size increased and the drug release rate decreased. The shape and surface morphology of microspheres characterized by optical microscopy and scanning electron micrographs, indicated that the microspheres were perfect spheres with porous surface aiding/showing good floating characteristic. The *in vitro* drug release study carried out in pH 1.2 (0.1N HCl) for 8 hours showed slow release in buoyant condition and in small intestine medium - pH 6.8 buffer in a controlled release manner upto 20 hours. *In vitro* drug release studies showed that the drug release was faster in intestinal pH than as compared to gastric pH and drug kinetic analysis suggests that the drug release is a diffusion controlled release from floating microspheres. The *in vivo* anti-inflammatory activity of optimized aceclofenac floating dosage form showed retarded release in comparison to marketed formulation.

KEYWORDS

Floating drug delivery system;
Sustained release;
Aceclofenac;
Ethyl cellulose;
Solvent evaporation method;
Anti-inflammatory activity.

INTRODUCTION

Gastric retention systems are systems, which increase the gastric retention time of the drugs having site-specific absorption in the gastrointestinal tract. Floating drug delivery systems have a bulk density lower than gastric fluids and therefore remain floating in the stomach without affecting the gastric emptying rate for a prolonged period. The drug is released at a desired rate from the floating system and after the complete release, the residual system is expelled from the stomach. The controlled release of the drugs from these systems at preferred absorption sites optimizes delivery of drugs, improves its bioavailability, maximizing its therapeutic benefit, reduces drug waste and reduces side effects by permitting a large portion of the drug to be absorbed before passing through the lower GI tract[1].

Rheumatoid arthritis (RA) is considered a chronic

inflammatory autoimmune disorder that causes the immune system to attack the joints, low-grade fever, weight loss, fatigue, and joint deformities[2]. RA affects extra-articular tissues throughout the body including the skin, blood vessels, heart, lungs, and muscles.It affects between the ages of 40 and 60 years. NSAIDs like asprin, indomethacin, corticosteroids, methotrexate, enbrel (FDA approved drug) are used for the treatment of RA.

Aceclofenac[3] is the most widely used a potent anti-inflammatory agent in comparison to other NSAIDs in the treatment of RA. The oral bioavailability of aceclo fenac is 65%, plasma half life is 2-4 hr. Its pka value is 4.5. It has been determined that aceclofenac is typically absorbed from the stomach and proximal small intestine i.e., it has a narrow absorption window. Sustained release dosage forms are designed to release drugs over an extended period of time and usually through out the gastrointestinal tract. This may result in sub therapeutic blood levels of the drugs, quick termination of the drug action and consequently, ineffective treatment of the patient's condition.

In the present investigation, aceclofenac was selected as a model drug for the design of gastro retentive floating drug delivery systems. The aim of this work is to investigate the possibility of obtaining controlled, prolonged, relatively constant and effective plasma levels of aceclofenac as floating microspheres formulation using ethyl cellulose as carrier. Ethyl cellulose[4] is a chemically modified natural polymer, which has been used in the controlled delivery of drugs.

EXPERIMENTAL

Aceclofenac was gifted by M/s. IPCA laboratories, Mumbai. Ethyl cellulose was obtained from S.D Fine Chem. Ltd., Mumbai, and all other chemicals used were of analytical grade.

Floating microspheres of ethyl cellulose (46cps) [$C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$] loaded with aceclo fenac[(2-[(2, 6-Dichlorophenylamino) phenyl acetoxy acetic acid] were prepared in varying proportion of drug to polymer ratio, keeping solvent ratio constant by solvent evaporation method[5]. The prepared floating microspheres were evaluated for their physico-chemical characteristics like fourier transform infrared spectroscopy, drug loading, entrapment efficiency, particle size analysis, surface morphology, buoyancy test, *in vitro* drug release studies. Kinetic analysis and *In vivo* studies were done for the optimized formula.

According to pharmacopial specification buffer solution such as hydrochloric acid (pH 1.2), phosphate buffer (pH 6.8) were prepared and used for experiments[6].

Preformulation study

Preformulation testing is an investigation of physical and chemical properties of a drug and polymer. FTIR spectra of pure drug, polymer, physical mixture of both and optimized formulation of microspheres were obtained in KBr pellets at moderate scanning speed between 450-4500 cm^{-1} in Perkin-Elmer FTIR spectroscopy.

Preparation of floating microspheres (FM)

Three formulations were formulated by taking drug: polymer ratio 1:1, 1:2 and 1:3. The microspheres were prepared by solvent evaporation method and were named as ECL-1,2,3. Ethyl cellulose was dissolved in 15 ml of acetone by using a magnetic stirrer and the powdered aceclofenac was dispersed in the polymer solution. Mixture of 150 ml of liquid paraffin and 1% of span 80 was preheated to 50^0C and at 1000 rpm (Remi motors) stirring condition. The resulting dispersion was then added drop wise with constant stirring for 1h until acetone evaporated completely. The formed micro spheres were collected by filtration under vacuum. The filtered microspheres were washed 4-5 times with copious amount of n-hexane to remove traces of liquid paraffin completely and kept for drying at room temperature for 24h.

For optimization of the formulation various drug: polymer ratio, Span-80 ratio, rotational speed were attempted. Formulae for different batches of floating microsphere are given in the TABLE 1.

Characterisation of microspheres

1. Determination of practical yield

Microspheres were dried at room temperature and weighed. The yield was calculated using the formula:

Practical yield=Amount of microspheres obtained (g)/Theoretical amount (g)×100

2. Determination of drug loading in microspheres[7]

Actual amount of aceclofenac present in different sized microspheres were determined by following method. Accurately weighed amount of ECL floating microspheres (100mg) was dissolved in 100 ml of methanol and assayed spectrometrically at 275 nm us-

TABLE 1: Formulae for different batches of FM

S. no.	Ingredients	ECL-1 (1:1)	ECL-2 (1:2)	ECL-3 (1:3)
1	Aceclofenac(mg)	200	200	200
2	Ethyl cellulose (mg)	200	400	600
3	Light liquid paraffin (ml)	150	150	150
4	Acetone (ml)	15	15	15
5	n-Hexane (ml)	30	30	30
6	Span 80 (ml)	1.0	2.0	2.0

ing UV spstrophotometer.

% Drug loading = Assay value of aceclofenac present/ microspheres weight of microspheres taken for assay × 100

% Entrapment efficiency = Percentage of drug loaded in the microspheres/ Percentage of drug added for loading ×100

3. Size and shape of microspheres

Determination of mean particle size of prepared microspheres was carried out by optical microscopy (Olympus NWF 10x). Required quantity of dried micro spheres was suspended in glycerin and the average particle size of 100 microspheres was determined in each batch and the mean particle size was calculated.

The external morphology studies of microparcticles were examined using scanning electron microscope (SEM Jeol JSM-6400, JAPAN) operating at 15kv. The samples were mounted on a metal stub with double adhesive tape and coated with platinum under vacuum for surface morphology analysis.

4. *In vitro* evaluation of floating ability[7]

In vitro floating study was carried out using simulated gastric fluid 0.1 N HCL in a USP XXIV dissolution apparatus containing 1% Tween 80 as a dispersing medium .100 mg microspheres were suspended in 900 ml of medium at 37±0.5^{0}C. (Paddle, 100 rpm, 12 h in the agitated medium). The floating and settled portions of microspheres were recovered separately. The microspheres were dried and weighed.

%Buoyancy of microspheres= Weight of floating microspheres / Initial weight of microspheres ×100

6. *In vitro* release studies[7,8]

The *in vitro* drug release studies were conducted in 0.1N HCl for 8 hours and in pH 6.8 buffer for 12 hours using USP XXIII, type-II dissolution apparatus under sink conditions. Accurately weighed samples of the microspheres were added in 900ml dissolution medium kept at 37±0.5^{0}C under stirring condition. At preset time intervals aliquots were withdrawn and samples were analyzed spectrophotometrically at 275 nm. The amount of drug present in the filtrate was determined from the calibration curve and cumulative percentage of drug release was calculated.

7. *In vivo* evaluation[8]

The optimized formulation of microspheres was subjected for *in vivo* studies. Carrageenan induced paw edema method was used to assess the anti-inflammatory activity.

Wistar albino rats of either sex (180-200gms) were taken for experiment. The studies were approved by the CPCSEA and local ethics committee. Animals were divided into two groups of five rats each. One group as standard i.e., carrageenan induced and treated with pure aceclofenac (Dose equivalent to 2mg/ kg body wt. of rat) and another group was carrageenan induced and treated with optimized formulation (ECL-3) (micro sphere quantity equivalent to 2mg/kg body wt. of rat).

8. Kinetic analysis of dissolution data[9]

The release profile of aceclofenac for the optimized formulation was fitted to different kinetic models such as zero order, first order, Higuchi and Peppas for Kinetic analysis.

RESULTS AND DISCUSION

Characterization of floating microspheres

Three batches of microsphere formulations of different ratios of aceclofenac and ethyl cellulose (ECL-1, ECL-2 and ECL-3) were prepared and effect of process variables were observed (TABLE 3) parameters like practical yield, percentage of drug loading, percentage of entrapment efficiency of microspheres were evaluated and are tabulated. (TABLE 4)

The percentage of drug loading of microspheres ranges from 26.7-35.5%. It decreased as the polymer concentration increases. Similarly, the entrapment efficiency decreased as the polymer concentration increases.

Mean particle size

Particle size was determined by optical microscopy. The results indicate that the mean particle size increased with increase in polymer concentration. These results showed that the viscosity of polymer is an important

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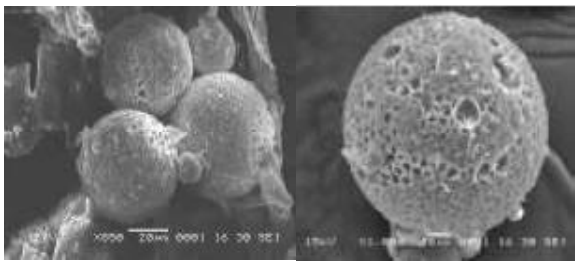

Figure 1: SEM of ECL-2

factor, which determines the particle size of micro spheres. As the viscosity increases particle size also increases. The average particle sizes for all batches of microspheres were given in TABLE 5.

Scanning electron microscopy (SEM)

The floating microspheres prepared by solvent evaporation method showed good sphericity, with porous surface and the particles are distributed uniformly without any lumps. The characteristic porous surface indicated the floating behavior of ethyl cellulose microspheres. The scanning electron micrographs are given in figure 1.

In vitro evaluation of floating ability

Floating ability or % of buoyancy for all the three formulations was determined TABLE 6. The formulation ECL-2 showed 87.6% buoyancy in comparison to ECL-1 (79.9%) and ECL-3 (73.5%). The floating ability of ECL-2 was found to be better incomparison to ECL-1 and ECL-3, which could be due to the porous nature of ethyl cellulose as evident by SEM photographs.

In vitro release of ECL floating microspheres

From the *in vitro* results it was observed till 8th hour in 0.1N HCl medium the drug release was only 10% and the restricted drug release could be due to the floating behavior and ethyl cellulose coating. The formulations ECL-1, ECL-2 and ECL-3 batches released 85.6%, 90.40% and 79.66% respectively at the end of 12th hour in pH 6.8 buffer medium. In stomach medium the microspheres showed good floating property and slow release in comparison to intestinal medium. In all the batches the drug release rate was decreased with increase in polymer concentration and results are graphically represented in figures 2 and 3.

In vivo animal studies

The optimized formulation ECL-2 was taken for *in vivo* study. Carrageenan induced paw edema method was used to study anti-inflammatory activity. Standard aceclofenac produced 54% inhibition of inflammation in comparison to ECL-2 formulation (82% inhibition of inflammation) TABLE 7. The animal study confirmed

TABLE 2: Process variables involved in optimization of FM

S.no	Process parameters	Variables involved
1	Drug: Polymer ratio	1:1, 1:2, 1:3
2	Span-80	0.5%, 1%, 2%
3	Stirring speed during solvent evaporation process	800,1000 and 1200 (rpm)

TABLE 3: Influence of process variables on morphology and size range of the microspheres

S. no	Variables studied	General aspects observed	Analyzed size range (µm)
1.	Stirring speed (opted during solvent evaporation) (rpm)		
	a. 500	a. Large irregular particles.	a. ≥ 500
	b. 1000	b.Discrete homogenous spheres were recovered	b. 100-200
	c. 1500	c. Smaller spheres along with aggregated particles.	c. ≤ 50
2.	Span-80 concentration (%)		
	a. 0.5	a. Irregular aggregated particles	a. -
	b. 1.0	b. Discrete and homogenous spheres	b.100-200
	c. 2.0	c. Small aggregated particles	c. ≤ 50
3.	Drug: Polymer ratio (%)		
	a. 1:1	a.Irregular particles were observed	a. -
	b. 1:2	b.Intact,discrete spheres were obtained	b. 100-200
	c. 1:3	c. Larger particles, oversized.	c. ≥ 500

TABLE 4: Percentage of drug loading and entrapment efficiency

Batches	Drug : polymer	Practical yield (mg)	% Loading	% Entrapment
ECL-1	1:1	138	30.5	70.3
ECL-2	1:2	160	35.5	81.4
ECL-3	1:3	132	26.7	68.3

TABLE 5: Mean particle size of microspheres

S. no	Formulations	Mean particle size(µm)
1	ECL-1	55.5
2	ECL-2	146.6
3	ECL-3	196

TABLE 6: % Buoyancy of ECL microspheres

S.no	Formulations	% of buoyancy
1	ECL-1	79.9
2	ECL-2	87.6
3	ECL-3	73.5

TABLE 7: *In vivo* anti-inflammatory activity of ECL-2 and standard drug

S.no	Treatment	Dose	% of inhibition at time (hours)					
			0	1	3	6	9	12
1	Standard	0.59mg/kg	63	71	75	72	58	54
2	ECL	3.3mg/kg	16	22	56	79	81	82

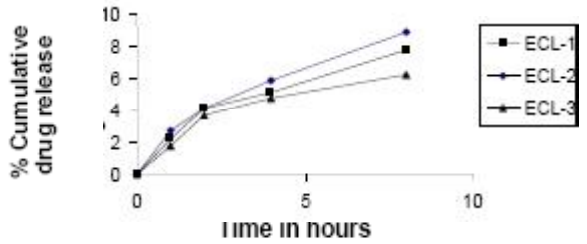

Figure 2: *In vitro* release profiles of ECL microspheres (0.1 N HCl)

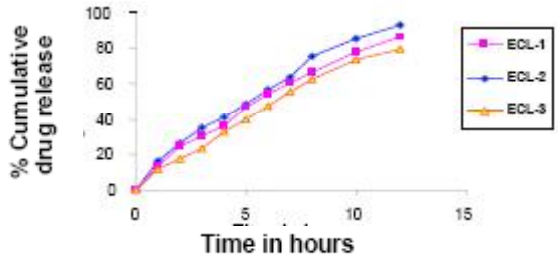

Figure 3: *In vitro* release of ECL microspheres (pH 6.8 buffer)

that ECL-2 produced good anti-inflammatory activity in comparison to standard aceclofenac.

Kinetic analysis of dissolution data

The release profiles of aceclofenac from different batches of ECL floating microspheres were fitted to the Higuchi equation. It follows the first order kinetic and followed the Higuchi model and diffusion drug release.

CONCLUSION

Floating microspheres of aceclofenac were prepared with ethyl cellulose by solvent evaporation technique. The microspheres obtained were porous and buoyant. It could be concluded from the above investigation that the experimental design supported product development and optimization of procedure yielded the desired microspheres. To maintain the desired micellar size range throughout the process, drug: polymer ratio, span-80 ratio, homogenization speed was optimized to produce homogenous spheres of desired range with drug release equivalent to those of the marketed formulation.

In vitro data obtained for the floating microspheres of aceclofenac showed good floatability, buoyancy, and prolonged drug release aiding patient compliance in comparison to the conventional dosage forms. The optimized formulation *in vivo* showed better anti-inflammatory activity in comparison to pure drug aceclofenac.

The short biological half-life of aceclofenac requires prolonged gastric retention of the dosage form extending the time for absorption. Aceclofenac FDDS promises to be a potential approach (once daily) for gastric retention used in the treatment of rheumatoid arthritis.

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