

FORMULATION AND EVALUATION OF ALBUMIN MICROSPHERES CONTAINING ACECLOFENAC

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ABSTRACT

Microencapsulation is one of the novel methods for retarding drug release from dosage forms and minimizing the adverse effects thereby increasing the patient compliance. Microspheres form homogeneous, monolithic particles which improve the treatment by providing localization of the drug at the site of action and by prolonging the drug release. The objective of the present study was to formulate sustained release microspheres of aceclofenac using egg albumin as release retarding agent. The results of FTIR spectral and DSC studies showed that there was no significant interaction between the drug and polymer. The maximum yield of the microspheres was found to be 96.99% and the encapsulation efficiency was found to be 65.2%. The prepared albumin microspheres released the drug completely within 10 hours at lower drug to polymer ratio. At ratio of more than 1:2, the drug release was sustained over a period of 12 hours. The microspheres showed similar release profile as compared to the marketed sample. The microspheres were discrete, spherical and uniform in shape. The particle size of the microspheres was found to be 99.6 μm . The drug does not cause any toxic symptoms up to the dose of 300mg/kg. The optimized formulation showed good analgesic and anti inflammatory activity when compared to the standard drug. The prepared microspheres showed minor changes in particle size only under long term stability study with no appreciable change in drug content proving good stability of the product conducted both in accelerated and long term stability studies. The present study signifies the utility of microspheres in retarding the drug release. This may in turn reduces the frequency of dosing, there by improving the patient compliance.

Keywords: Microspheres, Aceclofenac, Albumin, Microencapsulation.

INTRODUCTION

Aceclofenac is a nonsteroidal anti-inflammatory drug, which is a phenyl acetic acid derivative, having potent analgesic anti-inflammatory property that is mainly used in osteoarthritis and ankylosing spondylitis¹. Aceclofenac is rapidly and efficiently absorbed after oral administration but has short half life of 3-4 hours and requires multiple dosing for maintaining therapeutic effect throughout the day². The goal of any drug therapy is to achieve the desired drug concentration in blood which is therapeutically effective over a extended period of time. This can be achieved by proper design of sustained release dosage regimen^{3,4}. Microencapsulation is one of the novel methods for retarding drug release from dosage forms and minimizing the adverse effects thereby increasing the patient compliance. Heat denaturation is one such microencapsulation method that can be used to coat a drug with a polymer for sustaining the drug release⁵. Microspheres are homogeneous, monolithic particles which improve the treatment by providing localization of the drug at the site of action and by prolonging the drug release⁶. The objective of the present study was to formulate sustained release microspheres of aceclofenac using egg albumin as release retarding agent.

MATERIALS AND METHODS

Aceclofenac drug was obtained as gift sample from Meyer Health care ltd, Bangalore, India. Egg Albumin and Sodium carboxy methyl cellulose was purchased from BDH Chemicals, Mumbai, India. Chloroform, Methanol and

Dichloromethane were purchased from Merck Ltd. All other chemical used were of AR grade.

Preparation of Microspheres:

Albumin microspheres were prepared by heat denaturation method. A solution of albumin (1 g in 25ml) was prepared and the drug (1g) was added to the albumin solution. The contents were slowly added to a beaker containing 100 ml of preheated (60°C) liquid paraffin containing Tween 80 as emulsifying agent and stirred for 1h. The temperature was reduced to 40°C for hardening process and was maintained for 25min. The resulting microspheres were stabilized using glutaraldehyde solution (25%v/v) for a period of 15min. The microspheres were collected by decantation and washed with n-hexane and dried at room temperature. The microsphere formulations were carried out in different ratio as per the Table1.

Table 1: Composition of formulations of Aceclofenac albumin microspheres

Formulation Code	Drug-Polymer Ratio	
	Aceclofenac	Egg Albumin
AAF1	1	1
AAF2	1	1.5
AAF3	1	2
AAF4	1	2.5
AAF5	1	3
AAF6	1	4
AAF7	1	5
AAF8	1	6



Evaluation of Microspheres:

Drug Polymer Interaction Studies: Drug-polymer interactions were studied by FT-IR spectroscopy. The spectra were recorded for Aceclofenac, egg albumin and physical mixture of Aceclofenac: albumin (1:1). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of $5.2 \tau \text{ cm}^{-2}$ for 3 minutes. The scanning range was $400\text{--}4000 \text{ cm}^{-1}$ and the resolution was 4 cm^{-1} .

Differential Scanning Calorimetry: The thermal behavior of the microspheres was investigated using Metler DSC-7 Differential Scanning Calorimeter. Samples of about 5mg were placed in 50 μl perforated aluminium pans and sealed. Heat runs for each sample were set from 5 to 300°C , using nitrogen as blanket gas. The apparatus was Indium-Cyclohexane calibrated.

Surface morphology: The microspheres were coated with gold vacuum at high voltage (800-1500V) using ion coater. Samples were examined with scanning electron microscope.

Micromeritic properties: The average particle size of the microspheres was determined by using optical microscope. The flow properties and packing properties were investigated by measuring the angle of repose, tapped density and bulk density⁷.

Drug entrapment: Accurately weighed microspheres equivalent to 200mg of drug was suspended in 25ml of methanol and sonicated for 3 mins. The solution was then filtered, diluted suitably and analyzed for drug content spectrophotometrically at 275 nm^8 . The percentage drug entrapment was calculated as,

$\% \text{ Drug Entrapment} = \text{Practical drug loading} / \text{Theoretical drug loading} \times 100$

Dissolution studies: Dissolution test was performed in USP XXIII dissolution test apparatus by paddle method. The dissolution media used was 900ml of phosphate buffer pH 7.4 maintained at $37 \pm 0.5^\circ\text{C}$ and rotated at 100 r/min. Aliquots samples were withdrawn at specified time intervals and replaced with same volume of fresh media, filtered and analyzed spectrophotometrically (Shimadzu 1600) at 260.5 nm for cumulative drug release.

Acute Toxicity studies: The animal experiments were carried out with prior permission from the Institutional Animal ethics Committee approval (IAEC NO: MSRCP/P-07/2008). The acute toxicity studies were carried out as per OECD guidelines for testing of Chemicals – No. 423. Female Albino rats of Wistar strain weighing 170-190g were taken for the study. The selected rats were housed in acrylic cages under laboratory conditions. They were fasted overnight but had access to water. The test procedure was started with a dose of 5 mg/kg administered orally and the animals were observed for 7 days. Based on the mortality rate the dose was subsequently increased up to 300mg/kg and the animals were observed for any toxic symptoms. On the 8th day,

the animals were sacrificed, blood samples were withdrawn and analyzed for haematological and biochemical parameters. The tissues were subjected to histopathological study⁹.

Anti inflammatory activity: The study was carried out by Carrageenan induced rat paw edema method. The animals were divided into 3 groups, each group containing 6 animals. Albino rats Wistar strain of either sex weighing 170-190g were taken for the study. To the first group normal saline was injected. To the second group 9mg/kg of standard Aceclofenac was administered orally and to the third group the optimized final microsphere formulation was administered orally. After 30minutes, 0.05ml of 1%w/v carrageenan was injected in the sub plantar region of the left paw to both control and treated groups. The paw volumes of both control and treated groups were measured at the intervals of 15, 30, 60, 120, 180, 240, 300 and 360min after Carrageenan administration. Percentage difference in the paw volumes of each animal of control and treated groups were calculated¹⁰.

Analgesic activity: The study was carried out by Writhing stimulus method. The animals were divided into 3 groups, each group containing 6 animals. Swiss albino mice of either sex were taken for the study. Animals are injected intraperitoneally with 1ml/100g of 0.6% acetic acid solution. Animals that do not exhibit writhing within 30 seconds are discarded from the test. The drug is administered subcutaneously 15 to 20 min prior to administering acetic acid solution. Animals showing no response are analgesic positive. The percentage protection at each dose level is calculated for each group¹¹.

Stability Studies: The stability protocol was designed based on the ICH 'Q1AR2' guidelines. The microspheres formulations chosen were stored at $30 \pm 2^\circ\text{C}$ and $65 \pm 5\% \text{ RH}$ for a period of 12 months and at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\% \text{ RH}$ for a period of 6 months. The stored samples were tested for their drug content and for any physical change. The testing was carried out at 0, 2, 4 & 6 months for accelerated storage condition and at 3-month intervals for a period of 12 months for long-term storage condition¹².

RESULTS AND DISCUSSION

The results of FTIR spectral and DSC studies showed that there was no significant interaction between the drug and polymer. This was confirmed by the characteristic peaks of pure drug aceclofenac in FTIR spectra (Fig.1-3). The DSC graph showed a characteristic sharp endothermic peak corresponding to its melting point $150\text{--}158^\circ\text{C}$ for the pure drug sample as well as the physical mixture. The thermograms were shown in figures 4-6. The experiments were carried out and the results of % yield of albumin microspheres were 83.86% to maximum of 96.99%. The maximum yield was obtained with formulation F5. On further analysis of drug encapsulation of albumin



microspheres, the encapsulation efficiency was found to be between 36.3-65.2%. As the polymer concentration increases the drug encapsulation was found to be increasing in albumin microspheres. The results of percentage yield and drug entrapment were shown in table 2.

Table 2: Data for % yield & Drug encapsulation efficiency of Aceclofenac albumin microspheres

Formula Code	Percentage yield (%)	Drug encapsulation efficiency (%)
F1	90.40	36.35
F2	94.86	44.86
F3	86.52	52.60
F4	91.23	62.55
F5	96.99	65.21
F6	84.30	67.50
F7	86.94	69.85
F8	83.86	74.35

So based on this study, the drug release profile of all the formulations were carried out in Phosphate buffer pH 7.4. Parallely a marketed formulation was also subjected for dissolution studies so as to compare the dissolution profile of the prepared microspheres and to confirm the similarity between the products. The dissolution studies revealed that albumin microspheres released the drug completely within 10 hours at lower drug to polymer ratio. At ratio of more than 1:2, the drug release was

sustained over a period of 12 hours. The marketed product showed a cumulative drug release of 95% at 12 hours. The data for In-vitro drug release profile of aceclofenac albumin microspheres is shown in table 3 and the comparative release profile of these formulations was shown in figure 7. Based on the similarity of the dissolution profile of the prepared microspheres with the marketed sample, formulation AAF5 was taken for further studies. The optimized formulation was coded as ACFAL.

The SEM photomicrographs of the optimized formulation ACFAL indicated that the microspheres were discrete, spherical and uniform in shape (Fig.8). The optimized formulation ACFAL was assessed for parameters angle of repose, bulk density, Carr's index and the values are indicated in table 4.

Table 4: Micromeritic properties of ACFAL Microspheres:

Parameters	Observed Values*
Particle size	99.6±0.03 µm
Angle of Repose	17.4±0.03
Bulk Density	0.712± 0.04g/ml
True density	0.843 ±0.05g/ml
Carr's index	12.1±0.02
Hausner's Ratio	1.12±0.01

* - Average of three determinations

Table 3: Data for In-vitro drug release profile of Aceclofenac Albumin microspheres

Time in Hrs	Cumulative % Drug Release*								
	F1	F2	F3	F4	F5	F6	F7	F8	Market sample
0.5	39.4	40.3	48.9	39.9	30.3	33.2	31.6	30.7	26.6
1	51.3	58.1	66.6	50.4	46.4	41.5	37.4	36.1	39.9
2	66.5	69.5	71.2	64.7	53.7	51.3	43.3	52.0	46.4
3	78.5	80.1	78.6	73.3	59.4	55.6	53.1	57.3	52.5
4	90.8	89.6	83.5	77.6	64.5	61.4	59.6	64.7	61.1
6	93.6	94.3	89.6	84	73.6	68.4	66.8	73.6	68.2
8	97.8	98.6	94.4	88.5	82.4	73.3	74.2	82.2	77.3
10	101.5	100.5	98.9	95.3	91.5	80.7	83.7	86.4	84.5
12	---	---	100.8	99.9	96.3	88.8	89.7	92.3	95.1

* - Average of three determinations

Table 5: Anti inflammatory of the optimized aceclofenac formulation (ACFAL)

Treatment groups (n=6)	Dose (mg/kg)	Paw volume in ml after 6 hr (mean±SEM)	% inhibition of paw oedema after 6 hr
Control (Normal saline)	10ml/kg	0.51±0.03	--
Standard Aceclofenac	9.0	0.13±0.03*	65.8
ACFAL	9.0	0.13±0.05*	64.8

* p < 0.01 compared to control

Table 6: Analgesic activity optimized aceclofenac formulation (ACFAL)

Treatment groups (n=6)	Dose (mg/kg)	No. of writhings (mean±SEM)	% inhibition of writhing
Control (Normal saline)	10ml/kg	17.1±0.30	--
Standard Aceclofenac	9.0	8.83±0.30	48.3*
ACFAL	9.0	10.16±0.30	40.5 ⁺

* p < 0.001 compared to control + p < 0.05



Table 7: Stability data for the optimized formulation

Microspheres Formulations	Accelerated storage Condition (40 ± 2°C and 75 ± 5% RH)*				Real time storage condition (30 ± 2°C and 60 ± 5% RH)*				
	Drug Content (%)				Drug Content (%)				
	Months				Months				
	0	2	4	6	0	3	6	9	12
ACFAL	99.43	98.89	97.45	96.53	99.34	98.56	97.86	96.92	95.99

* - Average of three determinations

Table 8: Regression co-efficients and rate constants for release of optimized aceclofenac formulation (ACFAL)

Formulation code	Zero order		First order		Higuchi matrix		Peppas koresmeyer		Hixon Crowell	
	r	k1	r	k2	r	k3	r	k4	r	k5
ACFAL	0.7588	3.295	0.7589	3.296	0.8871	5.435	0.9770	12.950	0.7912	3.293

Figure 1: FTIR spectra of Albumin

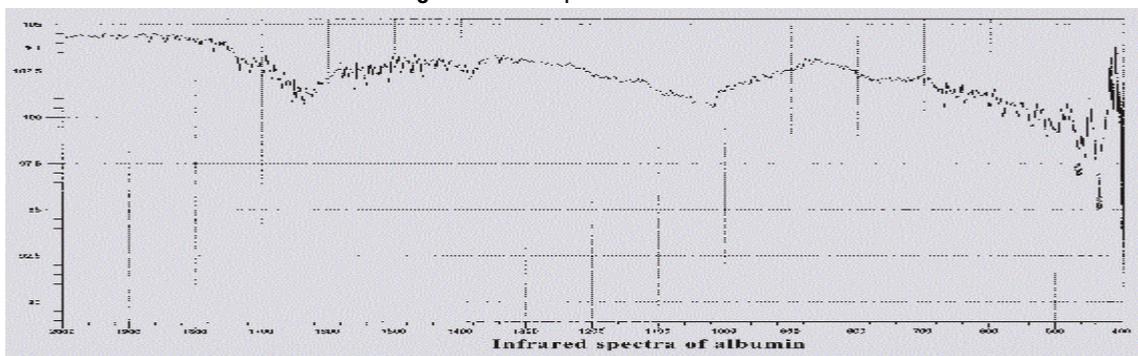


Figure 2: FTIR spectra of drug Aceclofenac

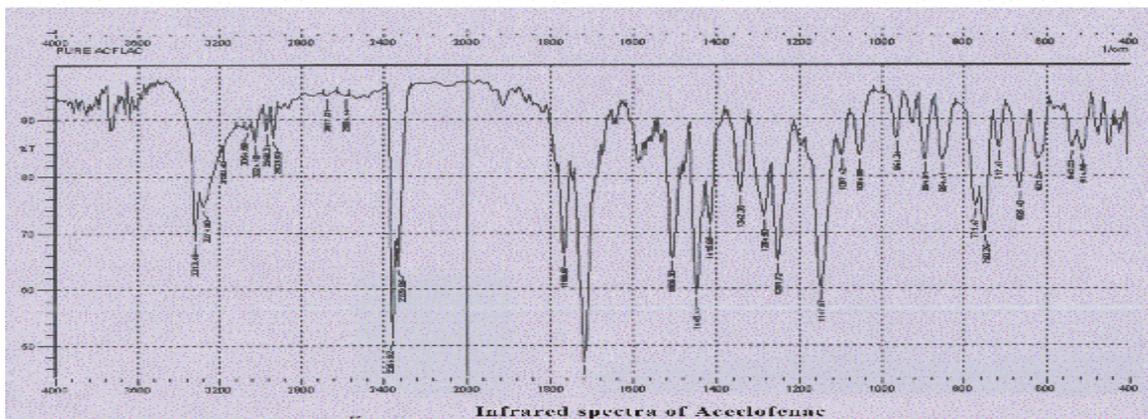
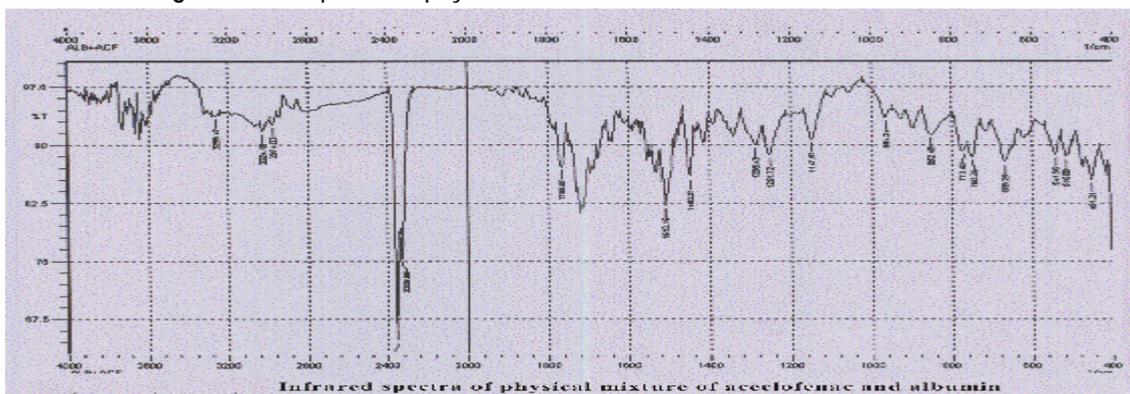
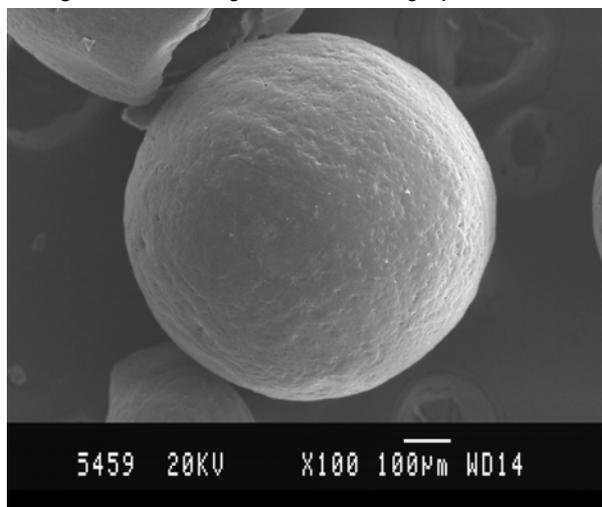


Figure 3: FTIR spectra of physical mixture of Aceclofenac and albumin



The particle size of the microspheres was found to be 99.6 μm and the size of the microspheres was found to increase with increased polymer loads which may be due to increase in viscosity of polymer solutions at higher concentration. In the acute toxicity studies there were no deaths observed during the treatment period up to the dose of 300mg/kg for aceclofenac. Based on this the animals were administered 9mg/kg of aceclofenac and the microsphere formulations containing the said amount of drug. At this dose there was no change in general behavior or other physiological activities of the animals during the study. Haematological and biochemical parameters showed that there were no significant changes in these values which proved that the prepared formulation does not exhibit any toxicity. This confirms that the drug or polymer do not have any interaction in the biological fluid. The optimized formulation showed good analgesic and anti-inflammatory activity when compared to the standard drug. The results of activities studied were indicated in Table 5 & 6. The optimized formulation ACFAL showed minor changes in particle size only under long term stability study with no appreciable change in drug content proving good stability of the product conducted both in accelerated and long term stability studies (Table 7). The drug release kinetics studied for ACFAL using PCP Dissolv software is indicated in Figure.9. Table 8 showed that the R^2 value is 0.9770 that confirms the best fit model was found to be peppas model.

Figure 8: Scanning electron micrograph of ACFAL



CONCLUSION

An emulsion solvent evaporation technique has been successfully employed to produce aceclofenac loaded egg albumin microspheres with maximum drug encapsulation and desirable release profile. The formulation variable drug-polymer ratio exerted a significant influence on the drug encapsulation. The present study signifies the utility of microspheres in retarding the drug release. This may in

turn reduce the frequency of dosing, thereby improving the patient compliance.

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