ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACEUTICS

FORMULATION AND CHARACTERIZATION OF ACECLOFENAC LOADED BOVINE SERUM ALBUMIN MICROSPHERES

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ABSTRACT

Controlled drug delivery systems designed to deliver drug, at predetermined rates for predefined periods of time, have been used to overcome the shortcoming of conventional drug formulations. Among the microparticulate system, microspheres have a special importance to target drugs and provide controlled release.

Aceclofenac is a potent non-steroidal anti-inflammatory agent, and is widely used in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Aceclofenac is administered in dose of 100mg 2-3 times daily, which leads to fluctuation in the drug blood level and dose related adverse effects. Multiple dosing also often results in poor compliance and inefficient therapy. Formulations of Aceclofenac microsphere prepared by suspension cross-linking method using albumin as polymer to prevent the gastric irritation and to achieve oral sustained / controlled release of the drug.

Preformulation studies revealed that the drug Aceclofenac and the polymer albumin were satisfactorily compatible without any change in the chemical nature of the drug. In present study two formulations were formulated by using bovine serum albumin. The formulations were subjected to various evaluation parameters like % practical yield, actual drug content, entrapment efficiencies, particle size distribution and *in-vitro* release studies. The results of all parameters are tabulated and depicted graphically in the results and discussion section.

The advantages of such controlled release formulations containing non-steroidal anti-inflammatory drugs (NSAIDs) over the conventional dosage forms have been reported. Such formulations minimize the gastric irritant side effects of the conventional NSAID preparations.

KEY WORDS

Aceclofenac, Bovine serum albumin, Microspheres

INTRODUCTION

Controlled and Targeted drug delivery systems are the most successful pharmaceutical and clinical products which involves application of physical and polymer chemistry to dosage form design to produce a well characterized and reproducible drug delivery profile. The main objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug.

Microspheres are polymeric particles ranging in size from 1 - 1000μm, in which the drug is dispersed in the matrix. The mechanism of drug release is either dissolution or diffusion. The release profile from microspheres depend s on the nature of the polymer used in the preparation as well as on the nature of the drug.

Aceclofenac is a Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are a group of heterogeneous compounds of unrelated organic acids that have analgesic, anti-inflammatory and anti-pyretic properties. These are cyclo-oxygenase enzyme inhibitors, which results in direct inhibition of the biosynthesis of prostaglandins and thromboxanes from arachidonic acid.

MATERIALS AND METHODS

Instruments Used U.V. Visible Spectrophotometer, FTIR Spectrometer, Dissolution Test Apparatus USP XXII, Afcoset Electronic Balance, Distillation Unit, Scanning Electron Microscope, JSM-T330A, Magnetic Stirrer, Oven, Humidity control oven, Microscope, Centrifuge.

Reagents and chemicals:

Analytically pure Aceclofenac has been obtained as a gift sample from Lupin Pharma pvt Ltd., Bovine Serum Albumin (BSA) has been obtained from Nice Chemicals

Chemicals like Di-sodium hydrogen phosphate, Potassium dihydrogen phosphate, Acetone, Methanol, Ethanol, Hydrochloric acid, Glutaraldehyde, Liquid paraffin, Petroleum ether L.R. or analytical grade.

METHODS:

Experimental Methods:

Preformulation Studies:

Preformulation study is one of the important prerequisite in development of any drug delivery system. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form.

Hence, preformulation studies on the obtained sample of drug for identification including colour tests, solubility analysis, melting point determination and compatibility studies were performed.

Identification:

The obtained sample was examined by infrared absorption spectral analysis and compared the spectrum with the reference standard I.R. spectrum of Aceclofenac. (Spectra 1)

Solubility Analysis:

Preformulation solubility analysis was done, which include the selection of suitable solvent, to dissolve the respective drug as well as various excipients used for the fabrication of microspheres.

Melting Point Determination:

Melting point determination of the obtained sample was done as it is a good first indication of purity of the sample. The presence of relatively small amount of impurity

can be detected by a lowering as well as widening in the melting point range.

Compatibility Studies:

Compatibility of the drug (Aceclofenac) with excipient bovine serum albumin which was used to produce microspheres was established by infrared absorption spectral analysis. I.R. spectral analysis of pure Aceclofenac, pure bovine serum albumin and combination of Aceclofenac and bovine serum albumin was carried out and observation was made whether changes in chemical constitution of drug after combining it with the excipient occurred.

Standard Plot for Aceclofenac:

i) Acid Buffer (pH 1.2):

Accurately weighed 100 mg of Aceclofenac was dissolved in 10 ml of methanol in a 100 ml of volumetric flask and make up the volume with pH 1.2 buffer solutions. 10 ml of this solution was taken in a 100 ml of volumetric flask and make up the volume with pH 1.2 buffer solutions to get working stock solution having concentration 100 μg/ml.

From this stock solution aliquots 1ml, 2ml, 3ml, 4ml and 5ml were pipetted out into a series of 50ml volumetric flasks and make up to mark with pH 1.2 buffer solution in order to get a concentration within the Beer's range from 2-14 μg/ml.

The absorbance of the resulting solution was then measured at 275 nm using UV spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance Vs. concentration in μg/ml.

ii) Phosphate Buffer (pH 7.4):

In this the pH 7.4 phosphate buffer solution was used to preparing different concentration solutions and also as a blank solution.

Preparation of Albumin loaded Aceclofenac Microspheres :

Method: - Suspension cross linking method

Abumin microspheres were prepared by using Suspension cross linking method. The required amount of BSA was dissolved in little quantity of distilled water at room temperature and the drug (100 mg) was added in it.

This dispersion was heated to 60°C for 2 min and then homogenized for 5 min. This dispersion was transferred to 500 ml beaker containing 100ml of light liquid paraffin which contain 1% of Tween 80. After 20 min of stirring, 1ml of glutaraldehyde (25% solution, as cross linking agent) was added and stirring was continued for 3 hours. Microspheres thus formed were separated by filtration, washed with petroleum ether to remove oil, and finally washed with water to remove excess of glutaraldehyde. Microspheres were then air dried at room temperature.

Title	Aceclofenac : BSA
AM – 1	1:1 (100mg : 100mg)
AM – 2	1:2 (100mg : 200mg)

% Practical Yield:

Microspheres were collected and weighed to determine production yield (PY) from the following equation.

$$PY(\%) = \frac{\text{Practical Mass (Microspheres)}}{\text{Theoretical Mass (Polymer + Drug)}} \times 100$$

Particle size analysis:

Determination of average particle size of Aceclofenac microspheres was carried out by optical microscopy, fitted with an ocular micrometer and a stage micrometer. The particle diameter of more than 100 microspheres was measured randomly by optical microscope.

The average particle size was determined by using the Edmondson's equation.

$$D_{mean} = \Sigma nd/ \Sigma n$$

Where n = no. of microspheres observed

D = mean size range

Study of flow properties of microspheres:

Flow properties of microspheres were studied by measuring the angle of repose of the formulation by employing fixed funnel method.

Microspheres were weighted, passed through the funnel, which was kept at a height 'h' from the horizontal surface. The passed microspheres formed a pile of a height 'H' above the horizontal surface and the radius 'r' of the pile was measured and the angle of repose was determined for all the batches by using the formula…

$$\text{Angle of repose } (\theta) = \tan^{-1} (h/r)$$

h = Height of the pile r = Radius of the pile

Study of shape and surface morphology:

The scanning electron microscopy has been used to determine shape, particle size distribution, and surface topography to examine the morphology of fractured or sectioned surface.

SEM studies were carried out by using JEOL JSM T-330 scanning electron microscopy (Japan). Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres were taken by random scanning of the stub.

Drug content and encapsulation efficiency:

A microsphere sample (10 mg) was dissolved in 10 ml of 0.1N HCl (1:1 v/v) mixture with ultrasonication for 4 h at 30°C. The samples were filtered using 0.2 μm membrane filter and absorbances of samples were scanned at 275 nm using UV spectrophotometer.

Actual drug content and encapsulation efficiency were calculated in duplicate for all batches using the equation as follows:

$$\text{Drug content \%} = \frac{M_{act}}{M_{MS}} \times 100$$

$$= \frac{\text{Actual aceclofenac content in weight quantity of microspheres}}{\text{Weighed quantity of sample microsphere}} \times 100$$

$$\text{Encapsulation efficiency} = \frac{M_{act}}{M_{The}} \times 100$$

$$= \frac{\text{Actual aceclofenac content in weight quantity of microspheres}}{\text{Theoretical amount of aceclofenac in microspheres}} \times 100$$

In vitro dissolution study:

In vitro release profile of Aceclofenac microspheres was examined in pH 1.2 buffer from 0 to 2 hr, , and in phosphate buffer of pH 7.4 from 2 to 8 hr using rotating basket method specified in USP XXIII at 100 rpm. Microspheres equivalent to 50 mg of Aceclofenac were taken in the dialysis bags in the basket and rotated at a constant speed of 100 rpm. The medium was maintained at 37°C ± 0.5°C. Aliquots of samples were withdrawn after predetermined periods of time and the same volume of fresh medium was added immediately to the test medium. The withdrawal samples were filtered through a 0.45 μm membrane filter.

The concentration of the drug release at different time intervals was then determined by measuring the absorbance at 275 nm spectrophotometrically using Shimadzu 1201 UV-visible spectrophotometer. Corresponding concentrations in the sample were calculated from standard plot and calculated cumulative percentage of drug release from each formulations.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES:

Identification: The IR spectrum of the obtained sample complied with the reference standard I.R. spectrum of Aceclofenac which indicates that the obtained sample is Aceclofenac. (Spectra 1)

Solubility Analysis: Aceclofenac is found to be practically insoluble in water, freely soluble in acetone and in dimethyl-formamide, soluble in alcohol and methanol.

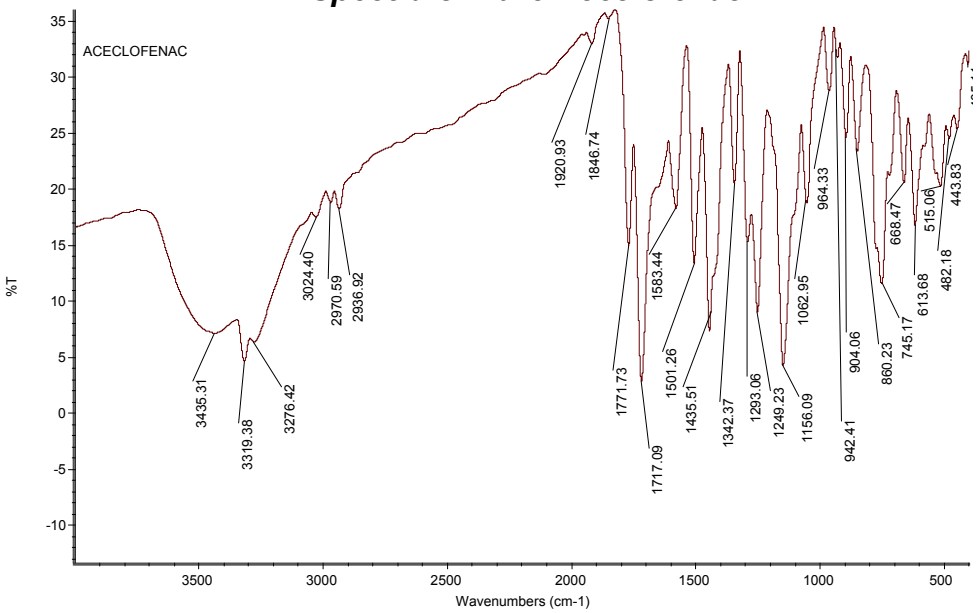
Melting point determination: The melting point of the obtained sample was found to about 151°C, which is within reported range (149°C to 153°C) that indicates the purity of sample.

Compatibility Studies: Preformulation studies were carried out to study the compatibility of pure drug Aceclofenac with bovine serum albumin prior to the preparation of microspheres of Aceclofenac.

IR spectra of pure drug Aceclofenac and bovine serum albumin and combination of Aceclofenac and bovine serum albumin were obtained which are shown in spectra no. 1, 2, and 3. All the characteristic peaks of Aceclofenac were present in combination, thus indicating compatibility between drug and excipients.

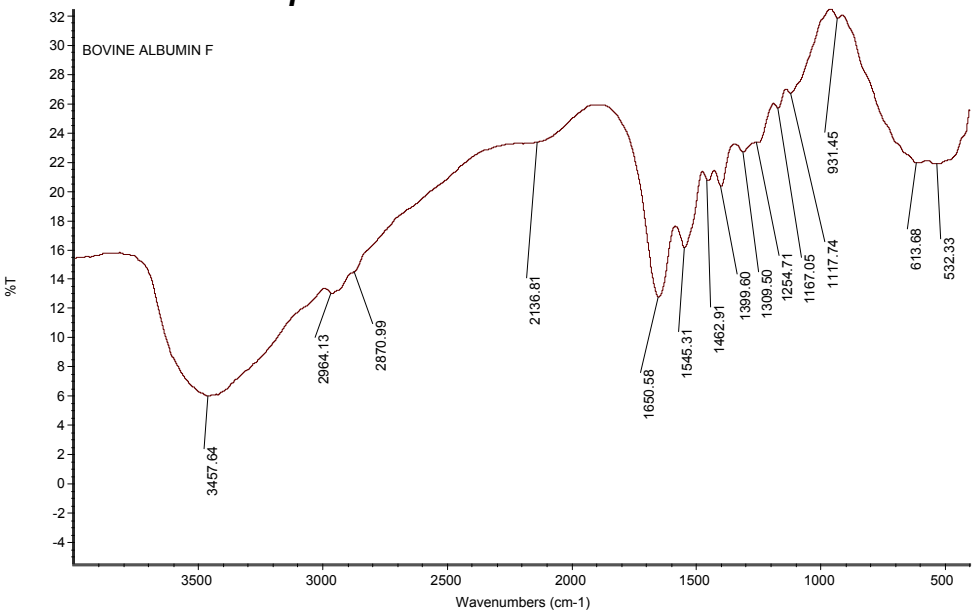
Spectra No.1

FTIR Spectra of Pure Aceclofenac



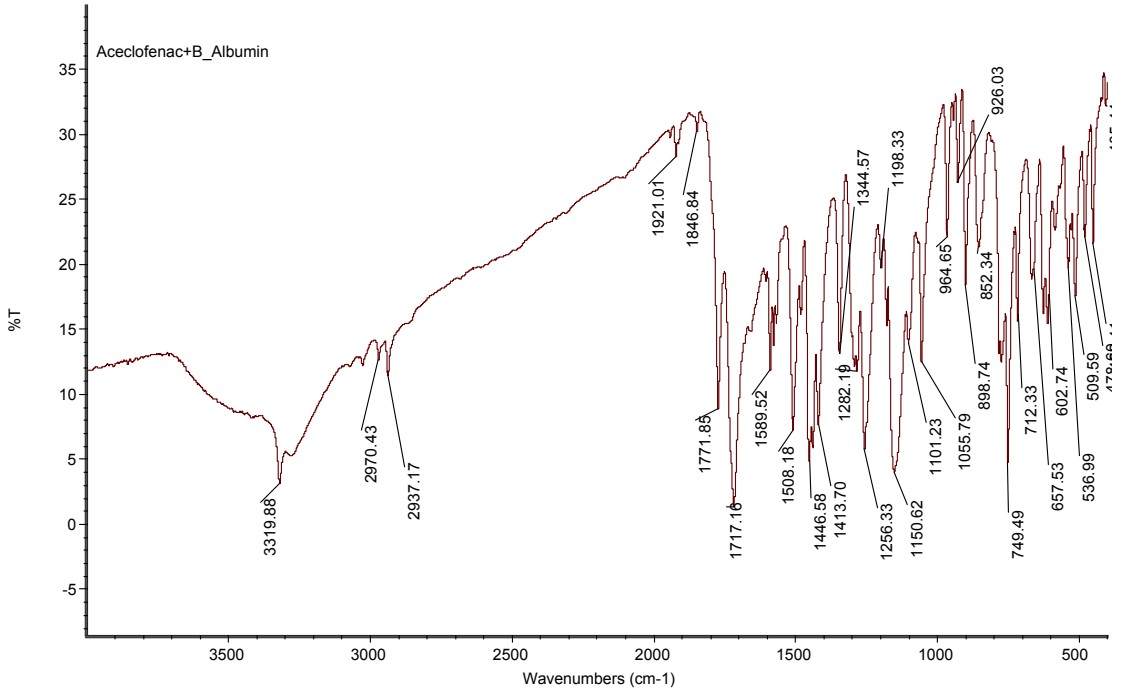
Spectra No.2

FTIR Spectra of Bovine Serum Albumin



Spectra No.3

FTIR Spectra of Aceclofenac + Bovine Serum Albumin



Standard calibration curve of Aceclofenac by UV spectrophotometry:

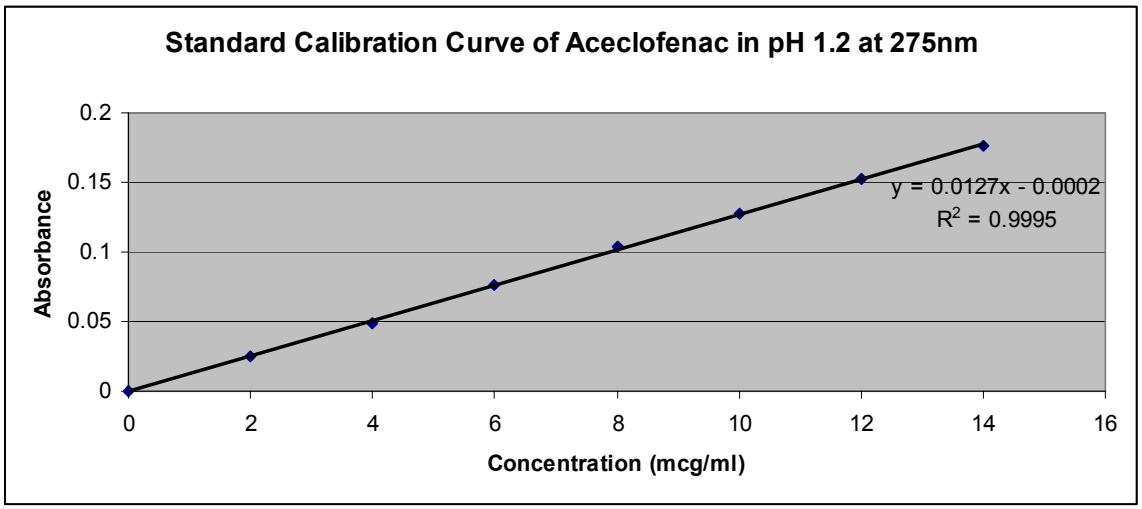
A standard curve from the stock solution was obtained in the range of 2-14 μg/ml concentration using pH 1.2 (acid buffer) and pH 7.4 (phosphate buffer) by measuring absorbance at 275 nm. The absorbance values are given in table no. 1 and standard plots of Aceclofenac are shown in graph 1.

Table 1

Standard Calibration Curve of Aceclofenac

S. No.	Concentration (μg/ml)	Absorbance at 275 nm	
		pH 1.2	pH 7.4
1.	2	0.025	0.053
2.	4	0.049	0.115
3.	6	0.076	0.164
4.	8	0.104	0.220
5.	10	0.128	0.280
6.	12	0.153	0.335
7.	14	0.176	0.384

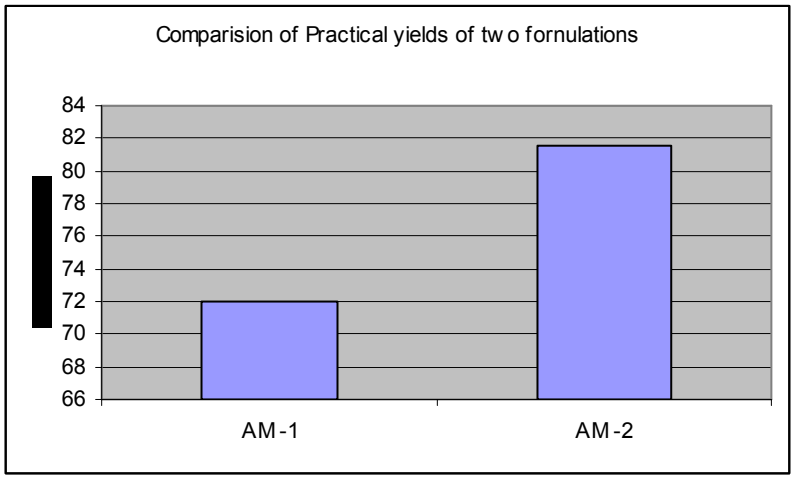
Graph 1
Standard Calibration curve of Aceclofenac in pH 1.2



% Practical Yield:

The results of % practical yield studies are shown in table no. 2and comparison of % practical yield of different formulations of Aceclofenac loaded microspheres is shown in figure 1.

Figure 1
Comparison of Practical yields of two formulations



The % practical yield increased as the amount of the polymer added to each formulation.

Table 2
% Practical Yield of Different Formulations of Aceclofenac Loaded Microspheres

Formulation code	Total amount of ingredients (mg)	Practical yield (mg)	Percentage yield (%)
AM -1	400	288	72.0
AM -2	600	489	81.5

% Yield = Practical yield / Theoretical yield

Particle size analysis:

Particle size distribution of microspheres as determined by optical microscopy by using stage micrometer and ocular micrometer are shown in tables 3 and 4. Average particle size of all the formulation is shown in table 5 and figure 2. 100 microspheres of each batch were sized and the average percentage frequency was plotted against size range.

The mean size range of all eight batches of microspheres was estimated between 50 to 500 μm. Microspheres size can be affected by the polymer concentration, temperature, viscosity, the stirring rate in the second emulsion step, and the amount of emulsifier employed.

Figure 2
Comparison of Mean particle size

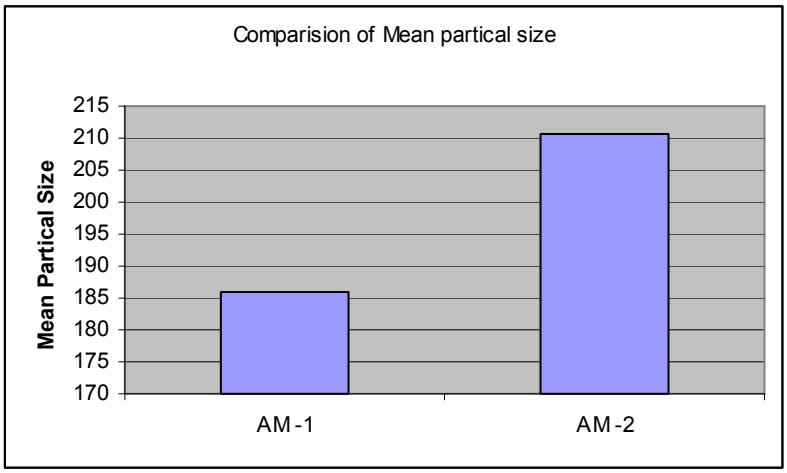


Table 3
Particle Size Data of AM--1

S. No.	Particle size range in μm	Midpoint size Range (d)	Frequency (n)	Nd	Average Particle size (μm)
1	50-100	75	10	750	186
2	100-150	125	25	3125	
3	150-200	175	34	5950	
4	200-250	225	12	2700	
5	250-300	275	07	1925	
6	300-350	325	07	2275	
7	350-400	375	05	1875	
			Σn = 100	Σnd = 18600	

$$\text{Average Particle Size} = \frac{\Sigma nd}{\Sigma n} = \frac{18600}{100} = 186$$

Table 4
Particle Size Data of AM-2

S. No.	Particle size range in μm	Midpoint size Range (d)	Frequency (n)	Nd	Average Particle size (μm)
1	50-100	75	09	675	210.5
2	100-150	125	12	1500	
3	150-200	175	29	5075	
4	200-250	225	18	4050	
5	250-300	275	18	4950	
6	300-350	325	09	2925	
7	350-400	375	05	1875	
			Σn = 100	Σnd = 21050	

$$\text{Average Particle Size} = \frac{\Sigma nd}{\Sigma n} = \frac{21050}{100} = 210.5$$

Table 5
Average Particle Size of Different Formulation of Aceclofenac Loaded Microspheres

S. No.	Formulation code	Average Particle Size (μm)
1	AM -1	186.00
2	AM -2	210.50

Flow Property:

Flow properties were characterized by measuring the angle of repose. All the formulation showed improved flow properties, as compared to pure drug showed in table no. 6

Table 6
Flow Properties of Different Formulation of Aceclofenac Loaded Microspheres

S. No.	Formulation code	Angle of Repose
1	AM -1	33°52”
2	AM -2	31°05”

The value of θ between 20-40° indicates reasonable flow and all the batches were found to fit in respect of flowability.

Shape and surface morphology:

The determination of shape and surface morphology was done by scanning electron microscope. SEM analysis of the samples revealed that all microspheres prepared were spherical in shape.

Plate 1 represents the surface morphology of AM-1, which shows un-uniform and rough surface of the microspheres. Plate 2 represents the morphology of AM-2, which shows slightly smooth surface of microspheres.

Plate No.1

SEM Photograph of Aceclofenac-loaded Microspheres containing Albumin (AM-1)

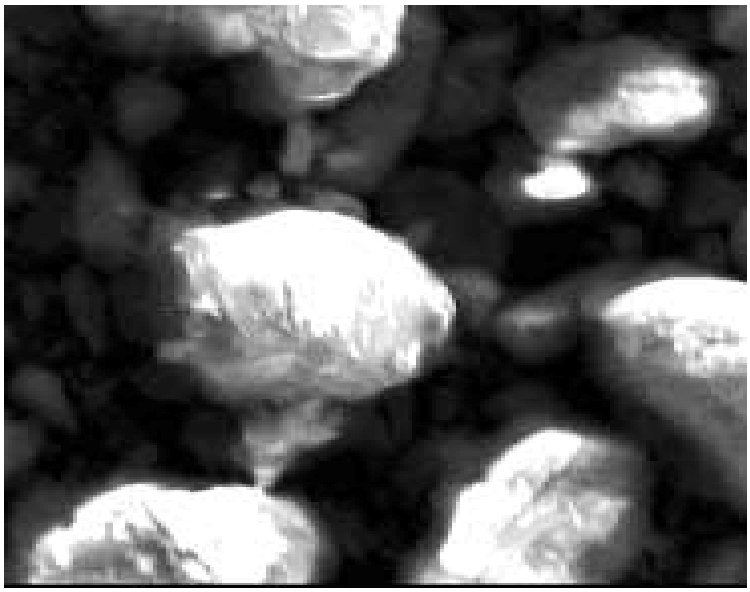
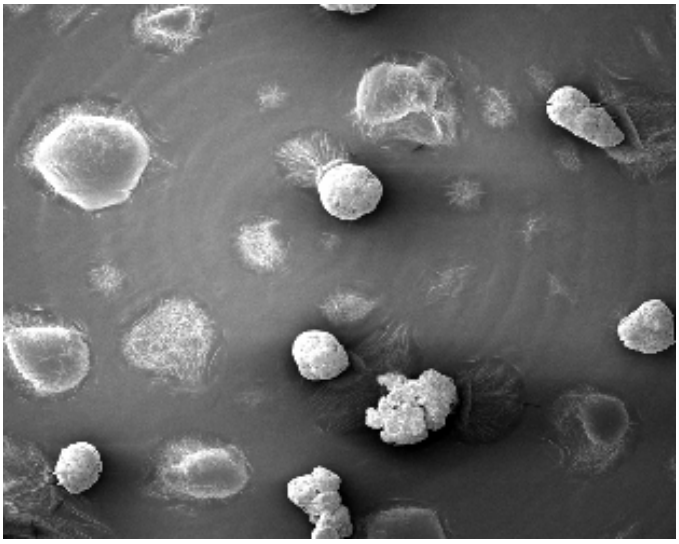


Plate No.2

SEM Photograph of Aceclofenac-loaded Microspheres containing Albumin (AM-2)



Drug content and encapsulation efficiency:

Actual drug content and encapsulation efficiency of the two formulations are shown in table no.7 and figure no.3. Many factors affect the entrapment efficiency of the drugs in microspheres. E.g. Nature of the drug, polymer concentration, drug – polymer ratio and stirring speed, etc. Generally, a low concentration of polymer shows low encapsulation efficiency.

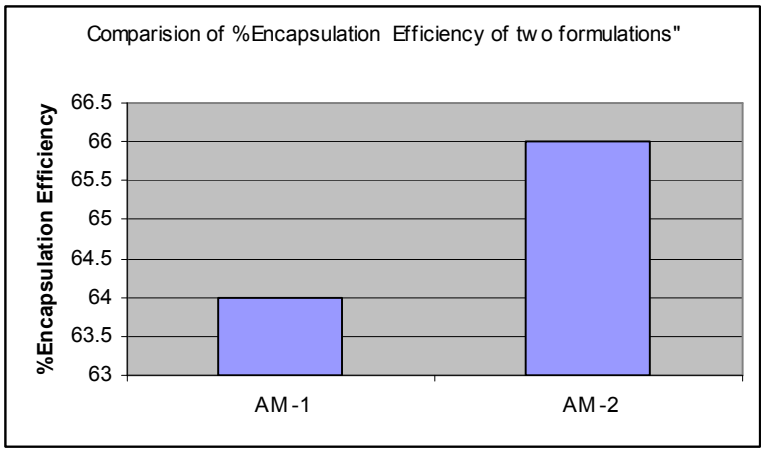
Table 7

Actual Drug Content and Encapsulation Efficiency of Aceclofenac Loaded Microspheres

Sr. No.	Formulation code	% Drug Content	% Encapsulation Efficiency
1	AM -1	32.00	64.00
2	AM -2	22.00	66.00

Figure 3

Comparison of %encapsulation efficiency of two formulations



In-vitro dissolution study:

The release pattern of all the formulations decreases with increase in the amount of polymer added to each formulation. The release showed a biphasic release with an initial burst effect. In the first 1 hr drug release was 29.70% and 29.50% for AM-1 and AM-2 respectively as shown in table no 8 and 9. The release of the drug is dependent on the microsphere size, as expected. Drug release is faster from spheres of smaller size owing to the decreased diffusional path length and the increased surface area in contact with the dissolution medium.

Table 8

In vitro Release Profile of Aceclofenac from Formulation AM -1 [Drug : Albumin (1 : 1)]

Time in hrs	Drug Released	Cum. Drug Released	% Cum. Drug Released	% Cum. Drug Retained
0.5	8.25	8.25	16.50	83.50
1	5.01	13.26	26.52	73.48
2	7.49	20.75	41.50	58.50
3	5.35	26.10	52.20	47.80
4	3.88	29.98	59.96	40.04
5	3.82	33.80	67.60	32.40
6	3.25	37.05	74.10	25.90
7	2.05	39.10	78.20	21.80
8	2.70	41.80	83.16	16.84

Table 9

In vitro Release Profile of Aceclofenac from Formulation AM - 2 [Drug : Albumin (1 : 2)]

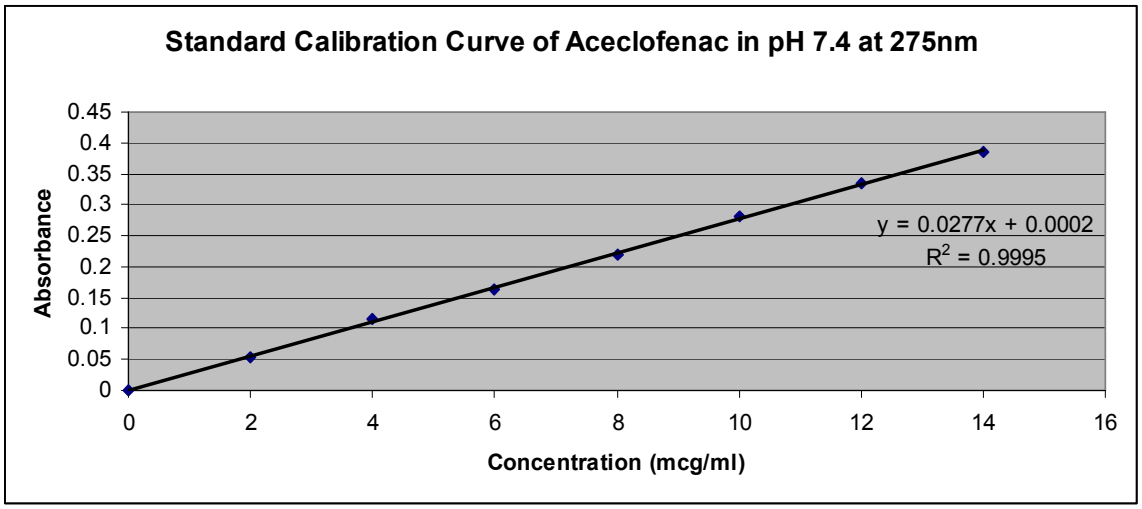
Time in hrs	Drug Released	Cum. Drug Released	% Cum. Drug Released	% Cum. Drug Retained
0.5	8.05	8.05	16.10	83.90
1	4.91	12.96	25.82	74.08
2	7.62	20.58	41.16	58.84
3	4.70	25.28	50.56	49.44
4	3.71	28.99	57.98	42.02
5	3.90	32.89	65.78	34.22
6	1.31	34.20	68.40	31.60
7	1.90	36.10	72.20	27.80
8	1.48	37.58	75.16	24.84

With increased load of the drug in the microspheres matrix, there is an increased release. At higher loadings, drug diffusion from the matrix produces more pores and channels through which the release occurs at a faster rate.

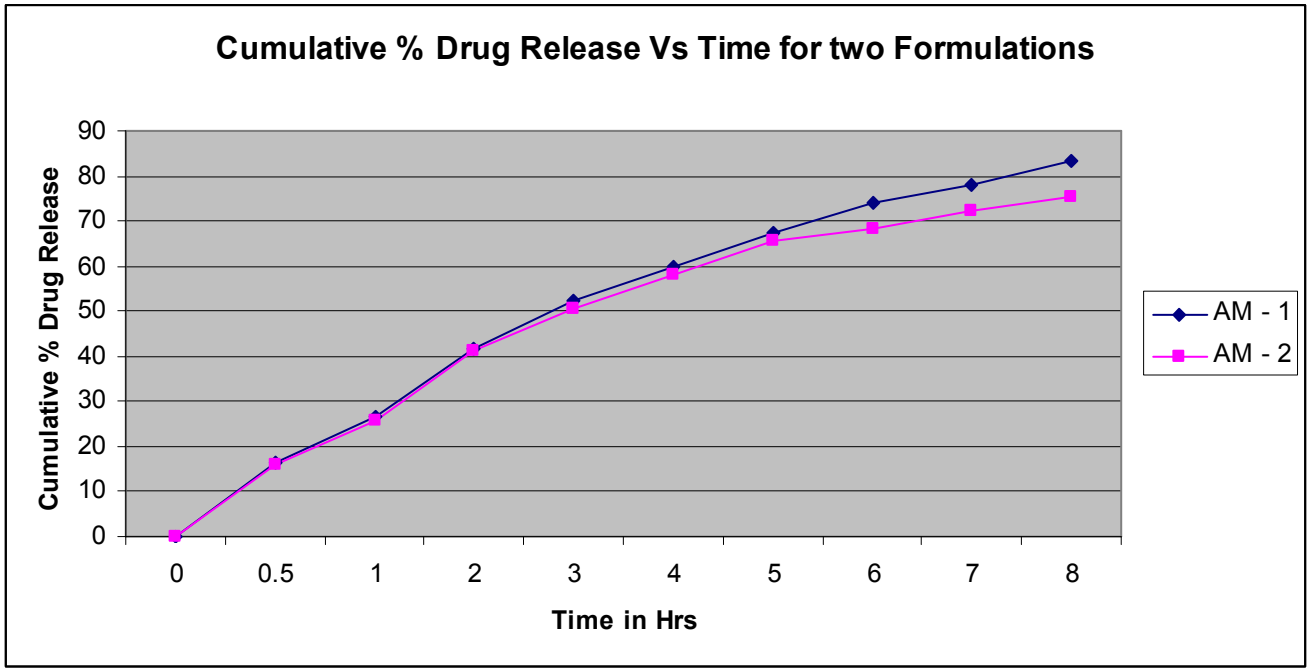
To obtain the values of the release constant and to understand the release mechanism the release data can be fitted to various mathematical models such as Peppas, Higuchi Matrix and Hixson Crowell etc.

- Cum % drug retained Vs. Time [First order]
- Cum % release Vs. Root 'T' [Higuchi's Classical diffusion equation]
- Log of cum % drug released Vs. log time [Peppas]
- (% retained)1/3 vs. Time [Hixson Crowell's]

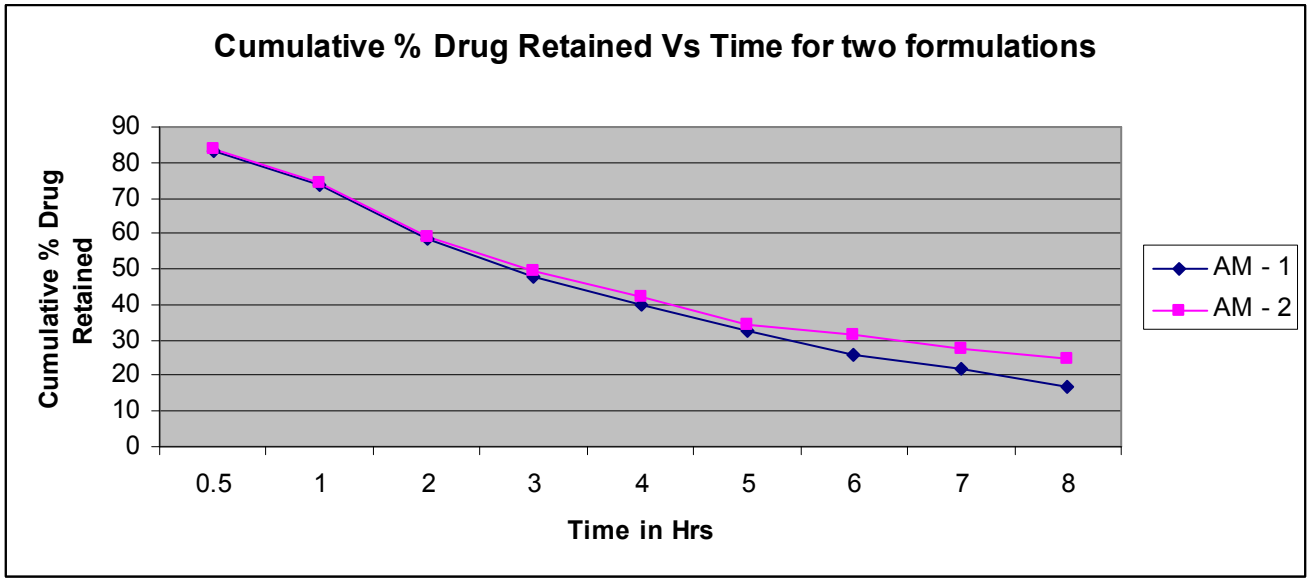
Graph 2
Standaard Calibration Curve of Aceclofenac in pH 7.4



Graph 3
Cumulative % Drug release Vs Time



Graph 4
Cumulative % Drug retained Vs time



CONCLUSION

In the present study, it was aimed to prepare microsphere formulation of Aceclofenac using a natural biodegradable polymer as a carrier for oral administration to extend the period of the drug release from the dosage form. By studying all the experimental results of prepared microspheres, the results suggest that microspheres containing NSAID's like Aceclofenac were successfully formulated by an suspension cross-linking method using biodegradable polymer albumin.

The I.R. spectra revealed that there was no interaction between polymer and drug. The prepared microspheres were spherical with narrow size distribution, with high yield, and good entrapment efficiencies. With increase in the percentage of polymer concentration, there is a significant effect on the size, drug content, entrapment efficiencies, swelling ratio and in vitro release of Aceclofenac from microperes.

In vitro release studies showed biphasic release pattern for all formulations, with initial burst effect, which may be attributed to drug, adhered to the surface of microspheres. Further detailed investigations are required to establish efficacy of these formulations.

- The *in-vitro* release data obtained is subjected to kinetic treatment to obtain the order of release and release mechanism.
- *In-vivo* investigation is required to correlate *in-vitro* release studies
- The stability studies of formulations at suitable temperature for storage of albumin microspheres of Aceclofenac
- Bioavailability study in human volunteers is necessary to establish drug product.

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