Site specific delivery of anti-arthritic drug by gelatin surface modified bovine serum albumin microspheres

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ABSTRACT

Non-steroidal anti-inflammatory drugs are the most commonly used and widely prescribed drugs all over the world. With the wide advantages they are also associated with severe Gastro-Intestinal side effects. Developments of novel drug delivery systems have always been a challenge to formulation scientists because of their high instability and economic factor compared to the conventional dosage forms. Thus the main objective of the present investigation was to develop gelatin surface modified bovine serum albumin microspheres containing anti-arthritic drug and to evaluate its potential as targeted drug delivery system. Hence there is a prolonged release of the drug along with minimized side effects. A brief overview of the methods developed for the preparation of albumin microspheres and the most suitable techniques for optimum entrapment of drug is emphasized. The in-vitro evaluations are also explained. In order to appreciate the medical application possibilities of albumin microspheres in novel drug delivery, some fundamental aspects are also briefly discussed.

Keywords: Site specific drug delivery, gelatin surface modified Bovine serum albumin microspheres, Aceclofenac.

Introduction

Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of "old" drugs has been attempted using different methods such as individualizing drug therapy and therapeutic drug monitoring. Delivering drug at controlled rate, slow delivery, and targeted delivery are other very attractive methods that have been pursued very vigorously (Patel *et al*., 2009, Jain *et al.*, 2001).

For drugs with short half-lives and with a clear relationship between concentration and response, it will be necessary to dose at regular, frequent intervals in order to maintain the concentration within the therapeutic range. Higher doses at less frequent intervals will result in higher peak concentrations with the possibility of toxicity. For some drugs with wide margins of safety, this approach may be satisfactory (Tripathi *et al.*, 2003).

A trend in NSAID development has been to improve therapeutic efficacy and reduce the severity of upper GI side effects through altering dosage forms of NSAIDs by modifying release of the formulations to optimize drug delivery.

These formulations are designed to increase patient compliance through a prolonged effect and to reduce adverse effects through lowered peak plasma concentrations. Many controlled-release dosage forms are designed to release the drug at a predetermined rate, thus maintaining relatively constant drug levels in the plasma for an extended period of time.

Several benefits may result from the use of such formulations. Reduction of frequency of dosing, lowered adverse effects, and improved patient compliance are considered the primary advantages of controlled-release dosage forms (Sam *et al*., 2008).

Further, currently available slow release oral dosage forms, such as enteric coated/ double-layer tablets which release the drug for 12-24 hours still result in inefficient systemic delivery of the drug and potential gastrointestinal irritation. Therefore, currently available slow release oral dosage forms of NSAIDs induces systemic effects and the drug is not efficiently used at the site of inflammation (Lewis *et al*., 1992).

Formulations can affect the safety of preparations by controlling the rate of release of the drug at sensitive sites, by delivering drug to specific sites to minimize systemic exposure, or delivering drug in such a way so as to change the rate or extent of the formation of toxic metabolite (Lachman *et al.*, 1991).

One such formulation uses polymeric microspheres as carriers of drugs. Many authors have reported that nanoparticles and micro particles have a tendency to accumulate in the inflamed areas of the body. It has been reported that microspheres of NSAIDs reduce the GI toxic effects and exhibit sustained action, thus increasing patient and therapeutic compliance.

1.1 Microspheres

Microspheres can be described as small particles (in 1-1000 micrometer size range) for use as carriers of drugs and other therapeutic agents. The term microspheres describe a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles.

Microspheres are used as targeted drug delivery system due to; small size and relatively narrow size distribution which provide biological opportunities for site-specific drug delivery, controlled release of active drug over a long period can be achieved and surface modification can easily be accomplished and hence can be used for site specific drug delivery system (Liggins *et al*., 2004, Vyas *et al*., 2002).

The present study is an attempt to develop extended release formulation of Aceclofenac to addresses the above issues.

The objectives for the present study are –

1. To identify formulation excipients based on compatibility studies.
2. To Optimize formulation and processing parameters (Stirring speed, Viscosity of oil phase and Percentage of emulsifying agent, etc.) using optimization technique (Response Surface Methodology) to get the desired response (particle size, entrapment efficiency and drug release). To predict the optimized formulation based on the desired response obtained.
3. To prepare the Aceclofenac microspheres based on predicted optimized formulation. To evaluate the product through various in-vitro (entrapment efficiency, surface characteristics, particle size, drug release & stability) studies.

A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery, micro-carriers being one of them. These micro-carriers include microspheres, liposomes, nanoparticles, resealed erythrocytes, and micro emulsion etc.

Microspheres have large surface area on which ligands can be attached for delivering drugs to a specific local area. Modification of microsphere surface with specific ligands have been attempted to use these microsphere as targeted drug delivery system.

Gelatin has a specific interaction with fibronectin. Gelatin microspheres have been investigated and demonstrated to target S-180 mouse sarcoma cells (pathological tissue), which are known to express extra fibronectin on their surface. This results in the cell line's extra fibronectin creating an affinity between cancer cells and gelatin microspheres.

Excess fibronectin in the local tissues is associated with a number of disease states, such as inflammatory disorders, cardiovascular disease, rheumatoid arthritis, and cancer. Consequently, gelatin surface modified microspheres may be useful as a targetable controlled-release microsphere system. These microspheres are intended for localized delivery to fibronectin enriched pathological tissues.

Thus the objective of the present investigation was to develop gelatin surface modified bovine serum albumin microspheres containing anti-arthritic drug and to evaluate its potential as targeted drug delivery system in vivo.

Drug:- Aceclofenac is one of the well tolerated COX-2 inhibitor and is often the drug of choice in the treatment of osteoarthritis, rheumatic arthritis and other related conditions. However, because of its short half-life (2-4 hrs.) it requires dosing of 100 mg twice daily. Missing of dose, which is often common, would cause inconsistence in drug level in the blood, which would in turn reflect in poor therapeutic outcome.

It has been reported that more than 50% of patients fail to take medicine as advised. Extended release formulations are the tools useful in promoting medication adherence and improving therapeutic outcomes. Medication adherence in chronic conditions like arthritis improves the quality of life of the patients.

MATERIALS AND METHODS

METHOD OF PREPARATION

3.4.1 Selection of method

Following two methods were investigated for the preparation of BSA microspheres:

3.4.1.1 Emulsion chemical cross-linking

100 mg of bovine serum albumin (BSA) was dissolved in 5 ml of distilled water. Tween-80 was added at a concentration of 2% wt/wt. 20 mg of finely powdered Aceclofenac was added the above solution and sonicated to obtain a uniform dispersion. One milliliter of this dispersion was injected (drop by drop) into a mixture of 20 ml of heavy liquid paraffin and 1.0 ml of span-85, while stirring at 2000 rpm. Stirring was continued for 10 minutes to obtain a water/oil (w/o) emulsion. One milliliter of 25% w/v glutaraldehyde was added into the emulsion to cross-link the albumin present in the internal phase of the emulsion. Microspheres formed were then separated by centrifugation (8000 rpm for 15 minutes) and washed with 30 ml of petroleum ether to remove liquid paraffin. The microspheres were then suspended in 10 ml of 5% wt/vol. sodium bisulphite solution and stirred on a magnetic stirrer for 10 minutes to remove the residual glutaraldehyde. Finally, the microspheres were washed with distllled water until they were free from residual glutaraldehyde. The microspheres were dried at room temperature and stored in a dessicator (Thakkar H, *et al*., 2005)

3.4.1.2 Emulsification-heat stabilization technique

10 mg of aceclofenac was added to a 5% w/v solution of BSA containing 0.1% Tween 80 and used as the aqueous phase. The oil phase composed of 30 ml maize oil and 10 ml petroleum ether with 1% Span 80 as emulsifier were stirred for 10 min at 1000 rpm. The aqueous phase was added drop wise to the oil phase and stirred at 1000 rpm for 30 min to form the initial emulsion. This

emulsion was then added to 40 ml of maize oil preheated to 120° C and stirred at 1000 rpm for 15 min to allow the formation of microspheres. The microsphere suspension was centrifuged at 3500 rpm for 30 min and the settled microspheres were washed three times with 50 ml ether to remove traces of oil. The microspheres were dried in a desiccator overnight and stored (Tabassi SAS, *et al*., 2003).

Table 3.4 Selection of method

Batch	Method	Average Particle size (µm)	Shape
M1	Emulsion polymerization chemical cross-linking	15-30	Spherical
M2	Emulsion polymerization heat stabilization	20-40	Spherical

Emulsification polymerization technique was selected as a method of choice as it showed better formulation results when compared to heat stabilization method.

3.4.2 FORMULATION COMPOSITION

Table 3.4.2 Microspheres Formulations

Formulation	EXPERIMENTAL CONDITION					
	Stirring rate (rpm)	BSA concentration (mg)	Drug (mg)	Surfactant concentration (%)		Glutardehyde concentration
				Tween 80	Span 85	
BM-1	1500	400	100	0.5	1	1
BM-2	1500	500	100	0.75	1.25	1.5
BM-3	1500	600	100	1	1.5	2.0
BM-4	2000	400	100	0.75	1.25	2.0
BM-5	2000	500	100	1	1.5	1.0
BM-6	2000	600	100	0.5	1	1.5
BM-7	2500	400	100	1	1.5	1.5
BM-8	2500	500	100	0.5	1	2.0
BM-9	2500	600	100	0.75	1.25	1.0

3.5 GELATIN SURFACE MODIFICATION

Gelatin surface modified BSA microspheres (GBM) were prepared by the adsorption of the gelatin onto the BSA microsphere. 10 mg microspheres were dispersed in gelatin solution (2% w/v) at room temperature. After 4 hours the microspheres were collected by centrifugation at 4000 rpm for 15 minutes (Tsung MJ, *et al*., 2001).

EVALUATION OF MICROSPHERES

3.6.1 Morphology/Electron microscopy

The morphological study of microspheres was done by Scanning Electron Microscopy (SEM.) (Tuncay M, *et al*., 2000).

3.6.2 Particle size

Average particle size of microspheres was measured by optical microscopy (Jahanshahi M, *et al*., 2008).

3.6.3 Drug loading and drug entrapment efficiency

20 mg of BSA microspheres (BM) were accurately weighed and transferred into a volumetric flask containing 10 ml of methanol. The dispersion was shaken for 12 hours on mechanical shaker. The resultant solution was filtered through whatman filter paper and absorbance was determined spectroscopically using UV-VIS Spectrophotometer (Shimazdu) at 277.0 nm. The concentration of drug was determined from the calibration curve (figure 3.4). The drug loading and entrapment was calculated by the following equation (Sahin S, *et al*., 2002,

$$\% \text{ Drug loading} = \frac{\text{Amount of drug in supernatant}}{\text{Amount of drug + amount of excipient}} \text{ X } 100 \ldots\ldots\ldots\ldots (1)$$

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Amount of drug added}}{\text{Amount of drug in supernatant}} \text{ X } 100 \ldots . (2)$$

3.6.4 Determination of gelatin surface modification

Gelatin surface modification was estimated by Bradford dye assay method. The Bradford dye assay is based on the equilibrium between three forms of Coomassie Blue G dye. Under strongly acid conditions, the dye is most stable as a doubly-protonated red form. Upon binding to protein, however, it is most stable as an unprotonated, blue form.

Red (470nm) <=> Green (650nm) <=> Blue (590nm) <=> Blue-Protein (590nm)

H^+ H^+

3.6.5 *In-vitro* drug release study

In-vitro drug release study of aceclofenac from BSA and gelatin surface modified BSA microspheres was carried out for up to 24 hrs using dissolution test apparatus (paddle method) containing 900 ml of phosphate buffer saline (PBS) pH 6.8 as dissolution medium. Microspheres equivalent to 20 mg aceclofenac were kept in muslin cloth and tied to the paddles, stirred at 100 rpm. At various time periods (0.25-24hrs), 5 ml of the sample solutions were withdrawn and diluted with methanol up to 10 ml. An equivalent volume of fresh buffer was added into the dissolution medium. The quantity of drug was estimated against reagent blank by UV spectroscopy at 277.0 nm (Jayaprakash S, *et al*., 2009).

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3.7 KINETIC MODELING OF DISSOLUTION PROFILES

In vitro dissolution has been recognized as an important element in drug development under certain assessment of Bioequivalence. Several theory kinetic model describe drug dissolution from immediate and modified release dosage forms. There is several models to represent the drug dissolution profile where ft is function of 't'(time) related to the amount of drug dissolved form the pharmaceutical dosage system. whenever a new solid dosage form is developed or produced, the drug release/dissolution from solid pharmaceutical dosage form is necessary to ensure that the drug dissolution occurs in an appropriate manner. Several theories/kinetics models describe drug dissolution form immediate and modified release dosage. These represent the drug dissolution profile where ft is a function of 't'(time) related to the amount of drug dissolved from the pharmaceutical dosage form. The quantitative interpretation of the value obtained from the dissolution assay by mathematical equation which translates the dissolution curve in function of some parameters related with the pharmaceutical dosage form.

In the present study, data of the *in vitro* release were fitted to different equations and kinetic models to explain the release kinetics of Aceclofenac from the microspheres. The kinetic models used were a Zero order equation, first order, Higuchi model, Hixon crowell and peppas model.

RESULT

Infra-red spectrum

IR Spectrum of the drug sample was obtained using FTIR - 80400S, Shimadzu. 1.0 mg of the drug was mixed with 100mg of KBr in a mortar by trituration and the mixture was compressed in to a pellet at 10 ton /cm^2 in a pellet maker. The sample was scanned at 4000 cm^{-1}- 400 cm^{-1}. The IR Spectrum obtained was found concordant with the I.R Spectrum of Aceclofenac reported in the official monograph (Indian Pharmacopoeia 2007, Figure 4.1.6 & 4.1.7, Table 4.1.6)

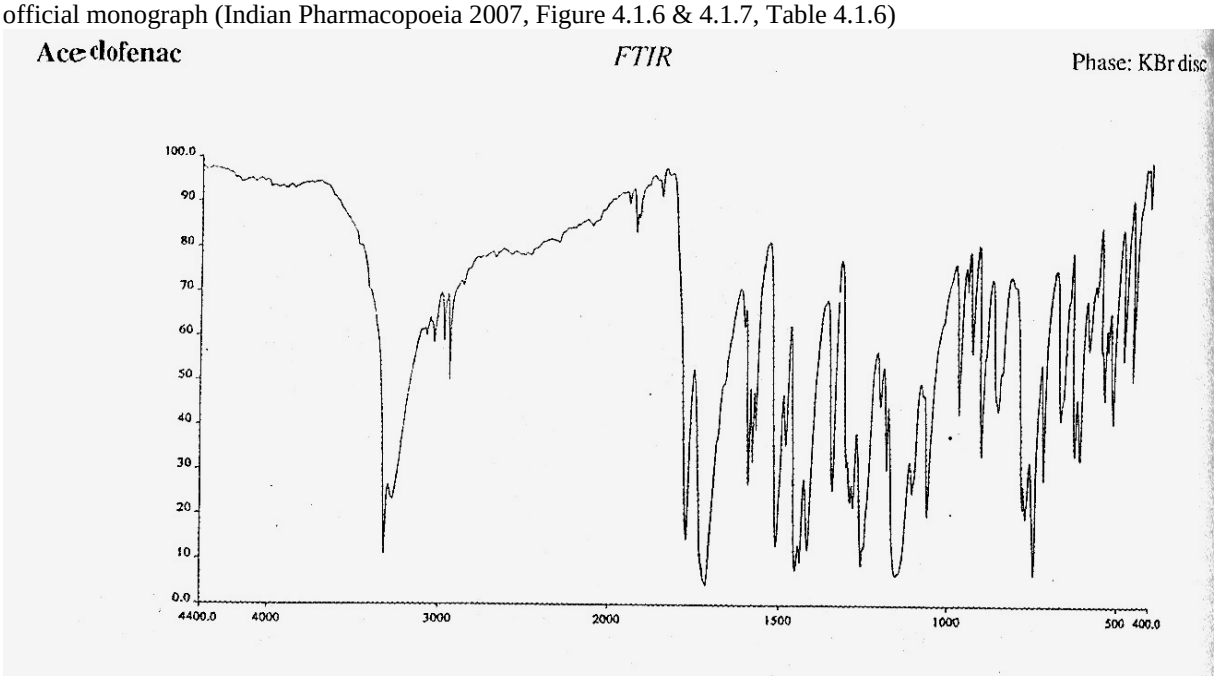


Figure 4.1.6.1: IR Spectrum of Aceclofnac (Standard)

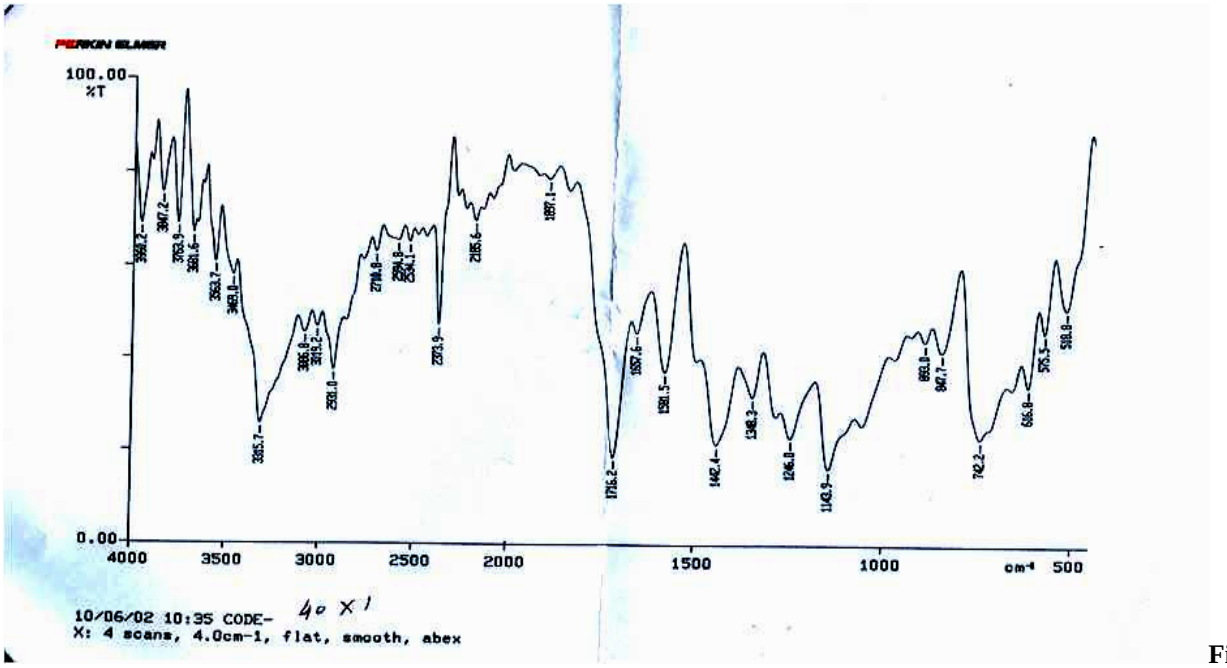


Figure 4.1.6.2: IR Spectrum of Aceclofenac (Test)

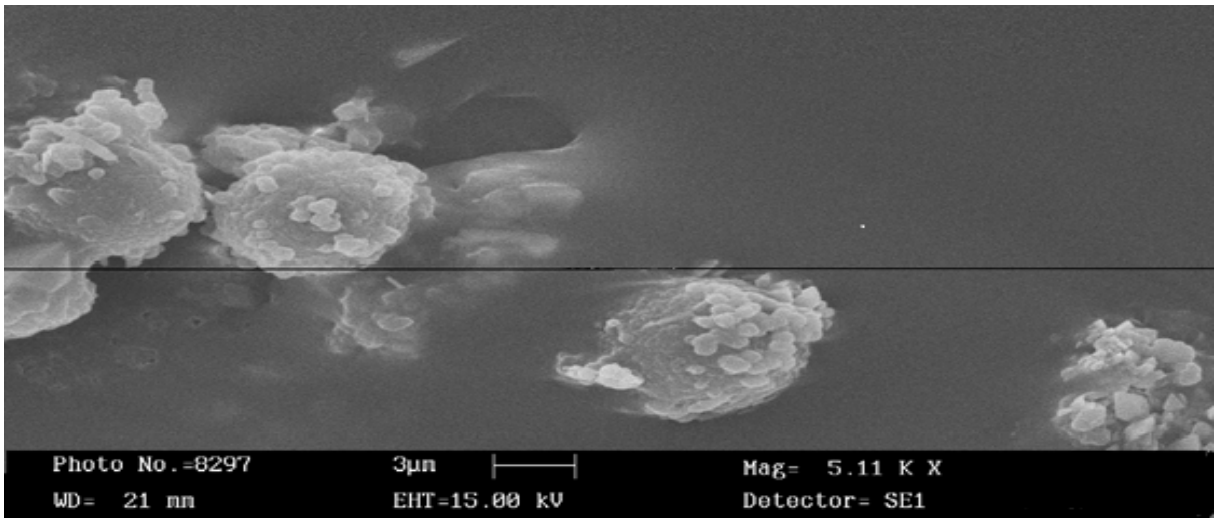
Table 4.1.6.: IR Spectrum peaks of the Aceclofenac

Groups	Standard peak (cm^{-1})	Observed Peak (cm^{-1})	Remarks
C- Cl	785-540	742.2	Stretching of halogen
=C-H-	3150-3050	2931.0	C-H stretching of CH_2 groups
$-CH_2-$	1465	1442.4	$-CH_2-$ bending
N-H	3500-3100	3086.8	Aromatic Stretching of N-H
N-H	1640-1550	1657.6, 1581.5	Bending N-H
=O	1725-1700	1716.2	Stretching of carboxylic acid
C-OH	3400-2400	2873.38	Stretching, carboxylic acid
C-O	1300-1000	1143.9	Anhydride

4.2 EVALUATION OF MICROSPHERES

4.2.1 Shape and surface morphology

Figure 4.2.1: (a) SEM of Aceclofenac loaded Bovine Serum Albumin microspheres (BM-6).



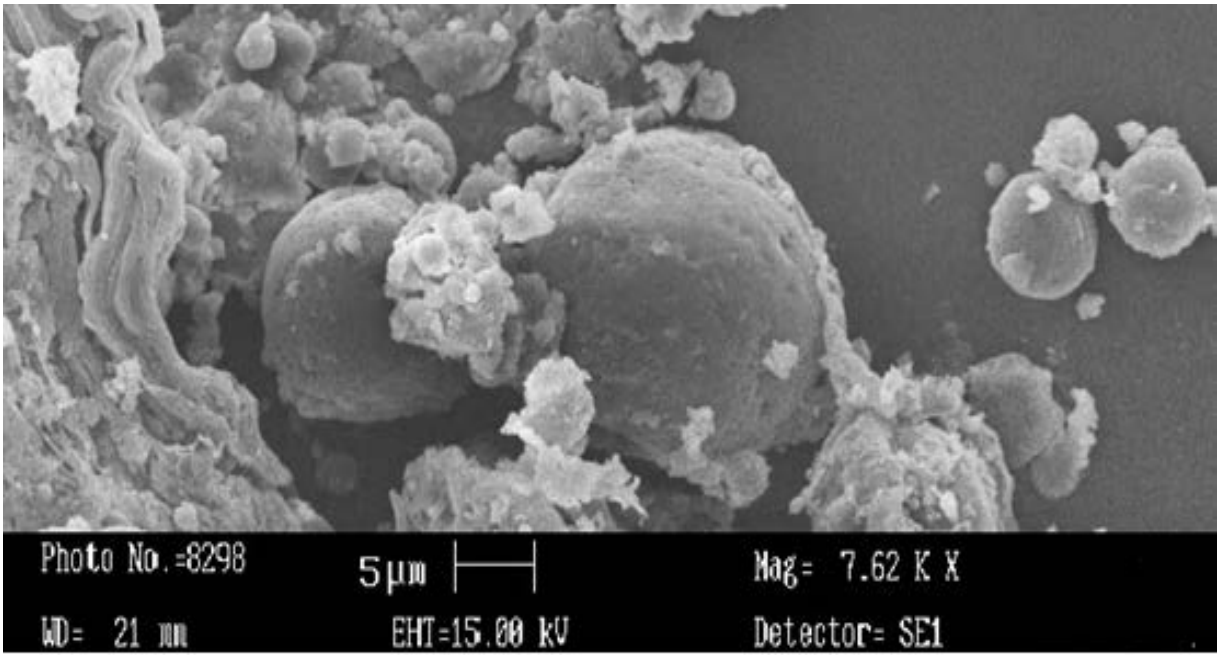


Figure 4.2.1: (b) SEM of aceclofenac loaded gelatin surface modified BSA microspheres (GBM-6).

4.2.2. Particle Size

Table 4.2.2.1: Experimental measured values for particle size of BSA microspheres (Taguchi orthogonal array table of L9).

Formulation	EXPERIMENTAL CONDITION				Average Particle size* (µm) (BSA)
	Factor A	Factor B	Factor C	Factor D	
BM-1	1	1	1	1	21.573±5.28
BM-2	1	2	2	2	19.918±3.91
BM-3	1	3	3	3	24.573±7.82
BM-4	2	1	2	3	19.466±4.78
BM-5	2	2	3	1	18.665±5.34
BM-6	2	3	1	2	20.817±3.21
BM-7	3	1	3	2	16.443±3.05
BM-8	3	2	1	3	18.573±4.57
BM-9	3	3	2	1	19.308±3.03

Values are Mean ±SD, n=10

Table 4.2.2.2: Experimental measured values for particle size of gelatin surface modified BSA microspheres (Taguchi orthogonal array table of L9).

Formulation	EXPERIMENTAL CONDITION				Average Particle size* (µm)
	Factor A	Factor B	Factor C	Factor D	
GBM-1	1	1	1	1	24.512±4.28
GBM-2	1	2	2	2	21.821±2.91
GBM-3	1	3	3	3	26.321±3.82
GBM-4	2	1	2	3	22.924±5.78
GBM-5	2	2	3	1	20.935±6.21
GBM-6	2	3	1	2	22.523±5.45
GBM-7	3	1	3	2	19.732±4.23
GBM-8	3	2	1	3	20.454±3.34
GBM-9	3	3	2	1	22.142±3.03

Values are Mean ±SD, n=10

4.2.3. Percent drug content and entrapment efficiency

Table 4.2.3.1: Percent drug content and entrapment efficiency of various formulations of bovine serum albumin microspheres. (BM-1 to BM-9)

Formulations	Drug Loading (%)	Entrapment Efficiency (%)

BM-1	15.37	32.18
BM-2	21.84	36.87
BM-3	21.81	41.12
BM-4	12.95	33.65
BM-5	13.99	43.92
BM-6	23.51	53.3
BM-7	10.498	30.52
BM-8	21.91	49.29
BM-9	7.48	47.16

Table 4.2.3.2: Experimental measured values for entrapment efficiency of BSA microspheres and S/N ratio (Taguchi orthogonal array table of L9).

Formulations	EXPERIMENTAL CONDITION				Entrapment efficiency (%)	S/N ratio
	Factor A	Factor B	Factor C	Factor D		
BM-1	1	1	1	1	32.18	30.13
BM-2	1	2	2	2	36.87	31.33
BM-3	1	3	3	3	41.12	32.28
BM-4	2	1	2	3	33.65	30.54
BM-5	2	2	3	1	43.92	32.86
BM-6	2	3	1	2	53.3	34.56
BM-7	3	1	3	2	30.52	29.65
BM-8	3	2	1	3	49.29	33.87
BM-9	3	3	2	1	47.16	33.48

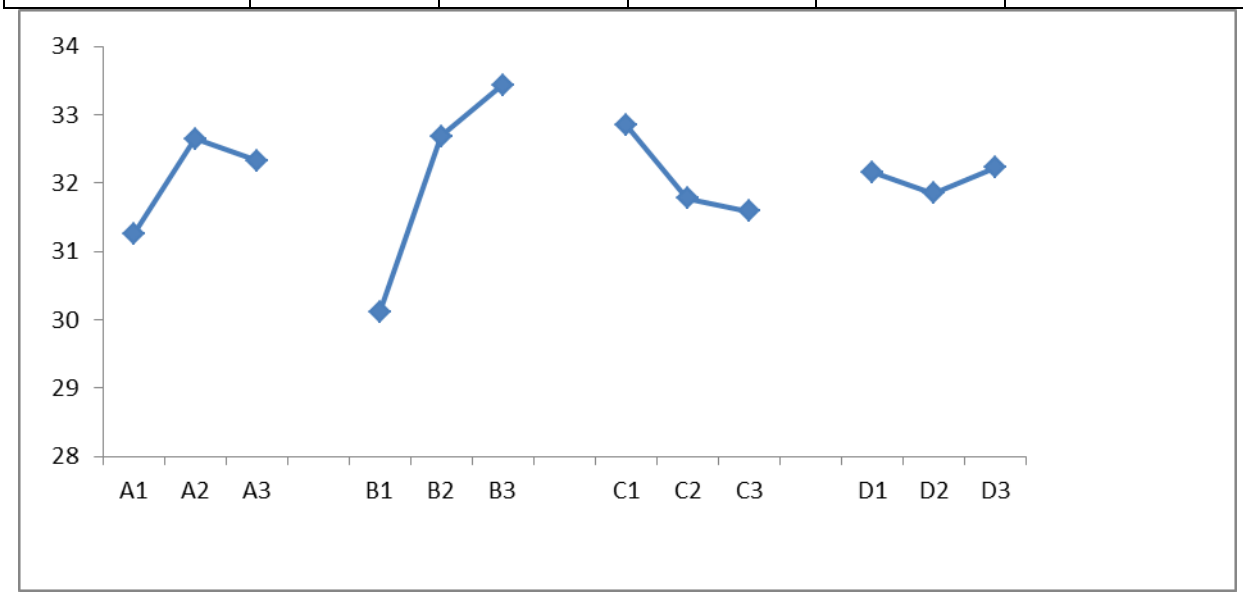


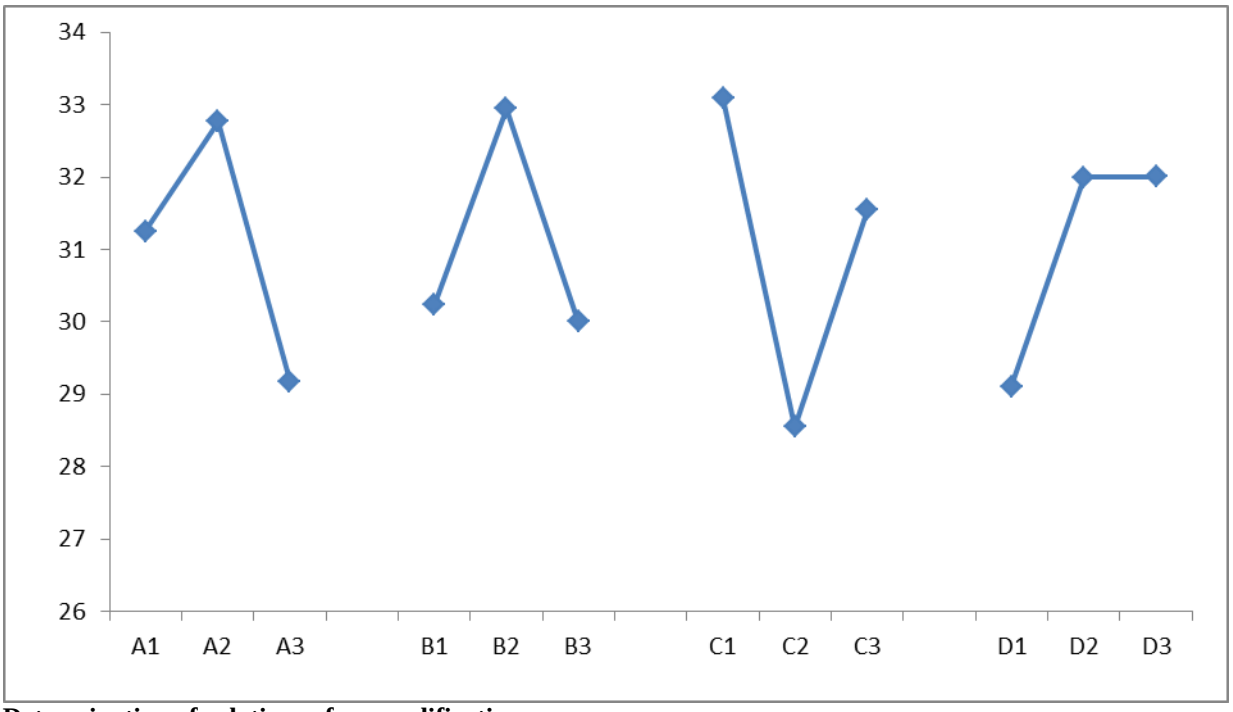
Table 4.2.3.4: Percent drug content and entrapment efficiency of various formulations of gelatin surface modified bovine serum albumin microspheres. (GBM-1 to GBM-9)

Formulations	Drug Loading (%)	Entrapment Efficiency (%)
GBM-1	16.24	33.83
GBM-2	23.21	37.86
GBM-3	24.34	41.86

GBM-4	14.72	33.07
GBM-5	14.86	46.12
GBM-6	24.23	54.18
GBM-7	11.17	30.86
GBM-8	22.83	50.37
GBM-9	8.87	48.12

Table 4.2.3.5: Experimental measured values for entrapment efficiency of gelatin surface modified BSA microspheres and S/N ratio (Taguchi orthogonal array table of L9).

Formulations	EXPERIMENTAL CONDITION				Entrapment efficiency (%)	S/N ratio
	Factor A	Factor B	Factor C	Factor D		
BM-1	1	1	1	1	33.83	30.58
BM-2	1	2	2	2	37.86	31.56
BM-3	1	3	3	3	41.86	31.62
BM-4	2	1	2	3	33.07	30.38
BM-5	2	2	3	1	46.12	33.27
BM-6	2	3	1	2	54.18	34.67
BM-7	3	1	3	2	30.86	29.78
BM-8	3	2	1	3	50.37	34.04
BM-9	3	3	2	1	48.12	33.48



Determination of gelatin surface modification

Determination of gelatin surface modification by Bradford protein assay procedure:-

4.2.4.1 Preparation of dye stock solution

100 mg of Coomassie Blue G was dissolved in 50 ml of methanol. To this solution 100 ml of phosphoric acid (85%w/v) was added and the resulting solution was diluted to 200 ml with water to give dark red colored solution. The solution is stable indefinitely in dark bottle at 4^0C.

4.2.4.2 Preparation of assay reagent

The assay reagent was prepared by diluting one volume of dye stock solution with four volumes of distill water. The solution is brown in color with a pH of 1.1. It is stable for weeks in dark bottle at 4^0C.

4.2.4.3 Preparation of Standard curve of gelatin

Accurately weighed 100 mg of gelatin was dissolved in 100 ml of PBS pH (7.4) to give a solution of 1000 µg/ml concentration. This solution served as standard stock solution (I). From this solution 10 ml was taken and diluted to 100 ml using PBS pH (7.4) to get 100 µg/ml concentrations. 1 ml, 2 ml, 3 ml and 4 ml samples were taken and diluted up to 10 ml with PBS pH (7.4) to get concentration of 10, 20, 30, and 40µg/ml respectively. 2 ml of the assay reagent was added into each test tube and incubated at 37 ^{0}C for 1 hrs. The resultant color intensity was measured by colorimetry at the wavelength 590 nm.

Table 4.2.4.3: Standard curve of gelatin using Bradford Dye Assay at 590nm

S.N.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	100	0.127
3	200	0.182
4	300	0.219
5	400	0.251

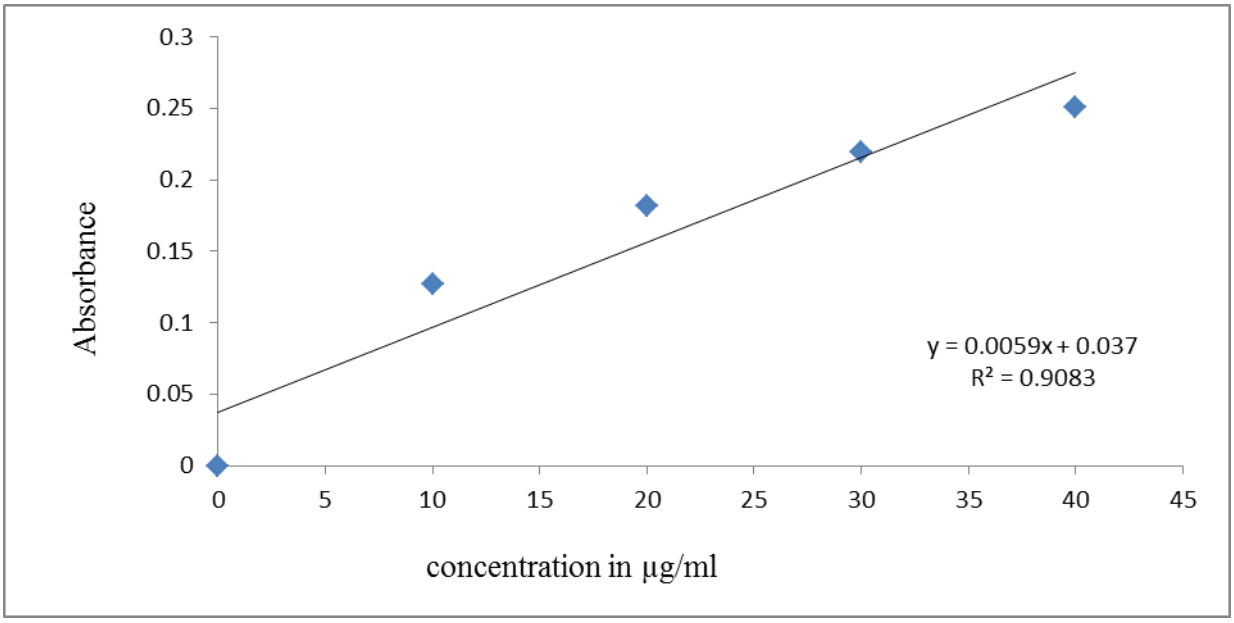


Figure 4.2.4.3: Regression curve of gelatin in phosphate buffer saline pH 7.4

4.2.4.4 Protein Assay Procedure

Accurately weighed 10 mg of gelatin adsorbed BSA microspheres of aceclofenac (GBM-6) were taken and mixed with 10 ml PBS pH (7.4). 2.0 ml assay reagent was added and the solution was shaken for 10 minutes. Gelatin content of the microspheres was determined by measuring the optical density (OD) of the solution at 590 nm (Bradford M, 1976).

Total protein bound to the microspheres was measured by Bradford dye assay method. The concentration of gelatin adsorbed on to the surface of BSA microspheres of the optimized batch (GBM-6) was found to be 19.425µg/ml.

4.2.5 *In-vitro* drug release study

Table 4.2.5.1: Percent cumulative drug release of aceclofenac from bovine serum albumin microspheres

Time	BM-1*	BM- 2*	BM-3*	BM- 4*	BM-5*	BM-6*	BM-7*	BM-8*	BM-9*
15 min	18.41±0.28	21.15±0.24	11.42±0.32	8.97±1.05	20.26±0.81	22.54±0.49	16.01±0.54	14.09±0.65	23.65±0.82
30 min	23.71±0.50	26.57±0.41	17.53±0.42	12.23±0.76	25.70±0.42	28.12±0.64	22.66±0.39	19.08±0.82	28.91±0.36
1 hrs.	27.16±0.16	27.89±0.24	22.47±0.31	18.38±0.96	29.89±1.02	37.61±0.31	28.71±0.49	24.15±0.40	37.08±0.99
2 hrs.	36.45±0.51	36.13±0.40	28.05±0.39	24.40±0.32	34.86±0.40	44.14±0.67	31.03±0.57	28.40±0.43	43.43±0.58
3 hrs.	44.21±0.37	43.53±0.42	37.75±0.37	37.09±0.41	38.72±0.42	49.81±0.41	38.24±0.88	32.86±0.94	47.89±1.04
4 hrs.	58.55±0.25	57.26±0.19	47.24±0.40	46.53±0.57	45.11±1.05	54.77±0.35	46.77±0.33	37.16±0.48	54.85±0.22
6 hrs.	66.62±0.39	62.5±0.37	57.87±0.39	56.17±0.86	53.28±0.63	62.97±0.66	53.86±0.51	40.05±1.29	67.48±0.63
12 hrs.	75.17±0.22	72.7±0.42	64.96±0.24	63.61±0.38	74.27±0.57	68.54±0.42	71.84±0.67	67.64±1.19	76.68±0.99
24 hrs.	80.06±0.46	77.42±0.32	71.60±0.51	68.85±0.94	84.94±0.22	81.93±0.84	79.09±0.89	75.03±1.01	82.44±0.92

*Values are Mean ±SD

Table 4.2.5.2: Experimental measured values of %cumulative release after 24 hrs. of BSA microspheres and S/N ratio (Taguchi orthogonal array Table of L9)

Formulations	EXPERIMENTAL CONDITION				Dissolution data after 24 hrs.	S/N ratio
	Factor A	factor B	Factor C	Factor D		
BM-1	1	1	1	1	80.06	38.13
BM-2	1	2	2	2	77.42	37.78
BM-3	1	3	3	3	71.60	37.10
BM-4	2	1	2	3	68.85	36.76
BM-5	2	2	3	1	84.94	38.58
BM-6	2	3	1	2	81.93	38.27
BM-7	3	1	3	2	79.09	37.96
BM-8	3	2	1	3	75.03	37.50
BM-9	3	3	2	1	82.44	38.32

4.2.6 In-vitro drug release

Table 4.2.6: Percent cumulative drug release of aceclofenac from gelatin surface modified BSA microspheres

Time	GBM-1*	GBM- 2*	GBM-3*	GBM-4*	GBM-5*	GBM-6*	GBM-7*	GBM- 8*	GBM-9*
15 min	15.51±0.69	19.58±0.71	12.54±0.71	7.80±0.84	18.65±0.57	20.82±1.09	14.61±0.92	9.88±0.61	22.46±0.60
30 min	20.69±0.35	23.06±0.79	17.68±0.36	10.67±0.69	24.53±0.73	26.59±0.78	20.85±0.60	15.53±0.90	27.42±0.50
1 hrs.	23.28±0.51	26.91±0.99	23.35±0.77	17.00±0.72	28.28±0.28	36.00±0.56	27.78±0.48	20.97±0.58	36.25±0.92
2 hrs.	33.52±0.84	34.59±0.67	27.33±0.49	22.90±0.49	33.09±0.75	43.89±0.73	29.77±0.64	27.42±.72	41.94±0.66
3 hrs.	40.20±0.69	42.05±0.55	31.56±0.94	35.50±0.75	37.28±0.42	48.29±0.24	36.55±0.92	36.23±0.85	45.93±1..02
4 hrs.	51.87±0.48	55.59±0.43	36.34±0.78	44.96±0.34	43.86±0.34	53.39±0.52	45.13±1.29	45.75±0.70	53.32±0.42
6 hrs.	59.36±1.01	61.18±0.85	48.90±0.36	54.72±0.51	51.92±0.82	61.46±0.96	51.70±0.45	56.34±0.45	65.61±0.81
12 hrs.	66.74±0.47	70.96±0.99	66.72±0.92	62.11±0.81	74.22±0.42	67.34±0.59	70.73±1.09	63.38±0.56	75.20±0.38
24 hrs.	72.10±0.73	76.01±0.43	72.17±0.67	67.44±0.45	83.85±1.10	80.32±0.86	77.78±0.88	71.09±1.21	81.08±0.73

*Values are Mean ±SD

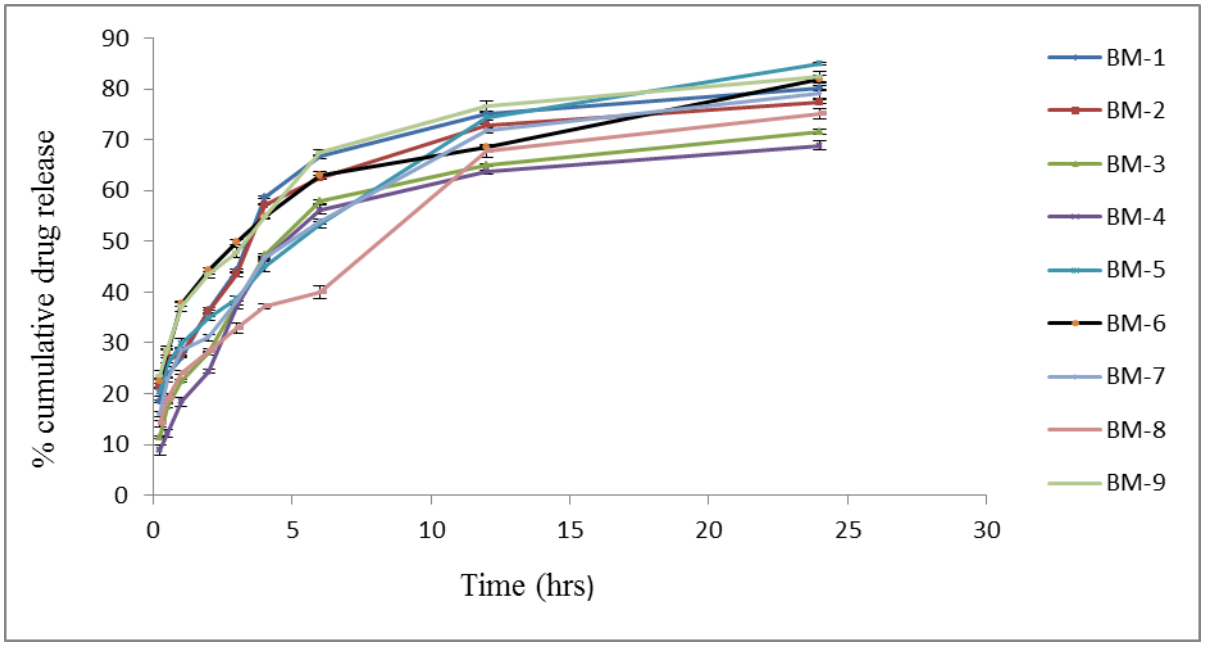


Figure 4.2.6.1: Percent cumulative drug release of Aceclofenac from bovine serum albumin microsphere in PBS (6.8)

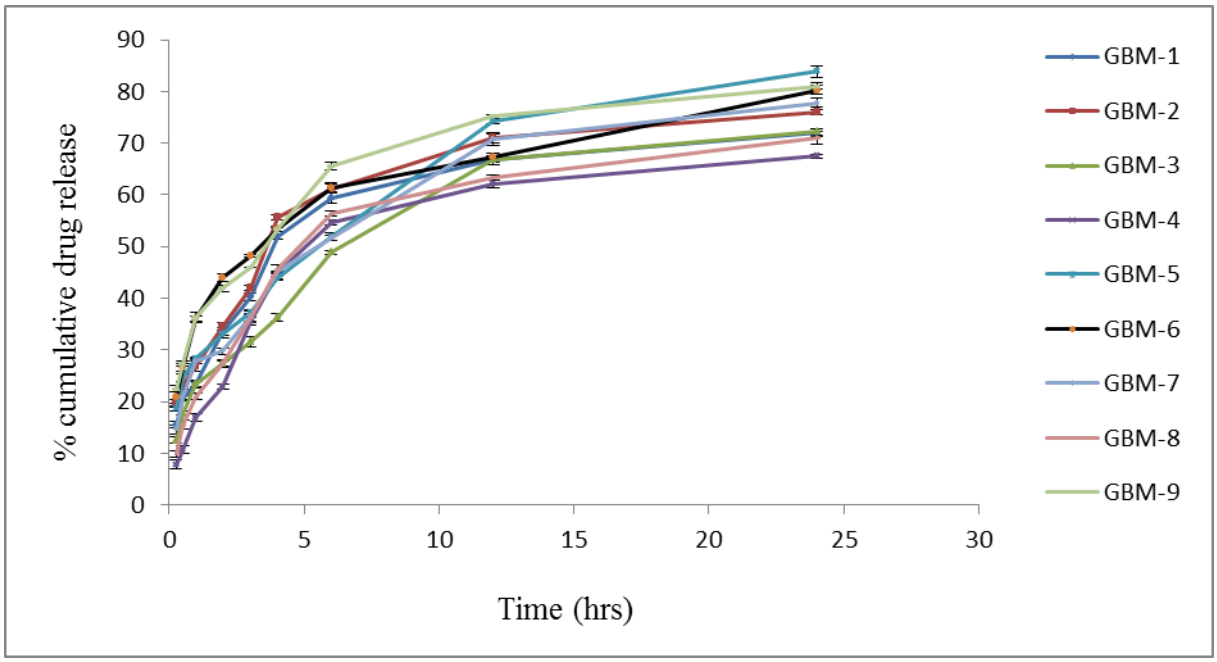


Figure 4.2.6.2: Percent cumulative drug release of aceclofenac from gelatin surface modified bovine serum albumin microsphere in PBS (6.8)

4.2.7 Drug Release Kinetics

To examine the drug release kinetics, the release data was fitted into models representing zero order, first order, and Higuchi's square root of time kinetics. The coefficient of determination (R^2) values were calculated from the plots of *Q* vs *t* for zero order, log(*Qo*-*Q*) vs t for first order and *Q* vs $\sqrt{t}$ for Higuchi model, and log cumulative % drug release vs. log time for korsmeyer model, where *Q* is the amount of drug released at time *t*, (Q_0-*Q*) is the amount of drug remaining after time t.

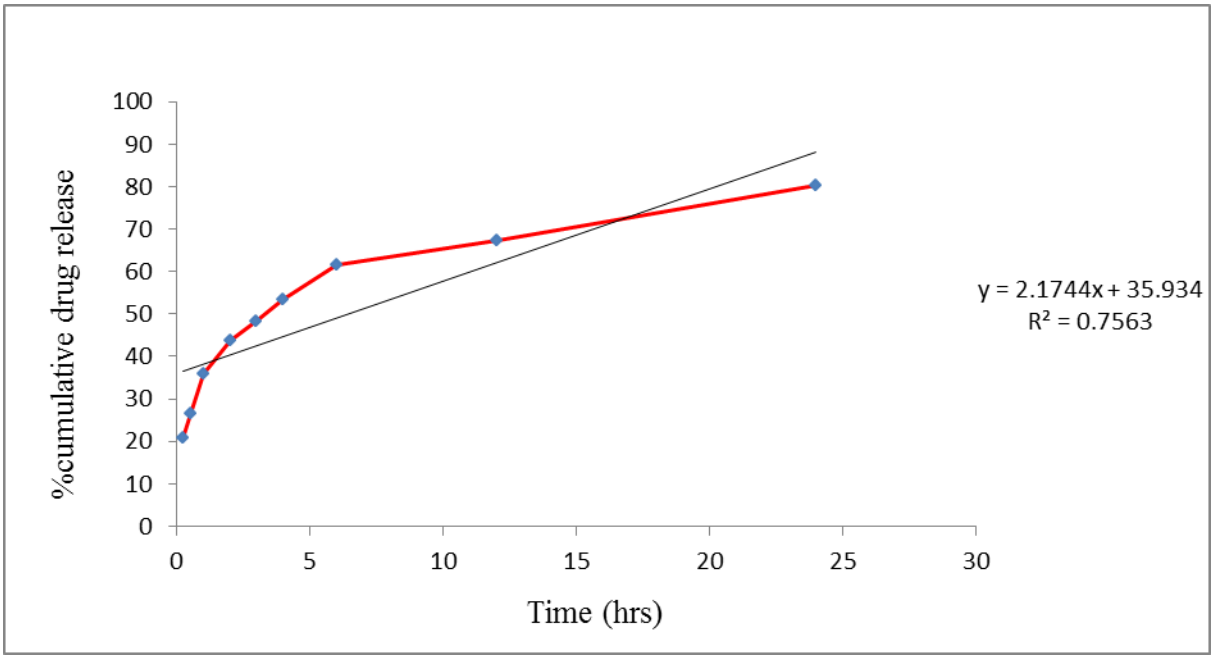


Figure 4.2.7.1: Zero order plot of GBM-6

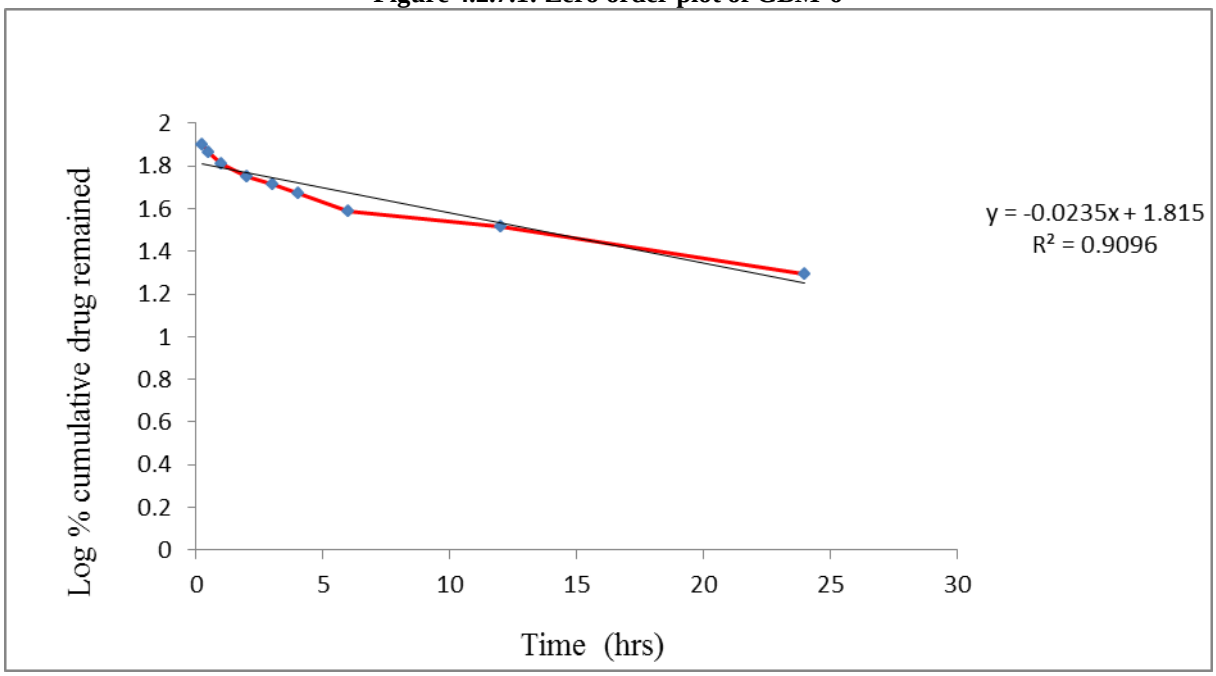


Figure 4.2.7.2: First order plot of GBM-6

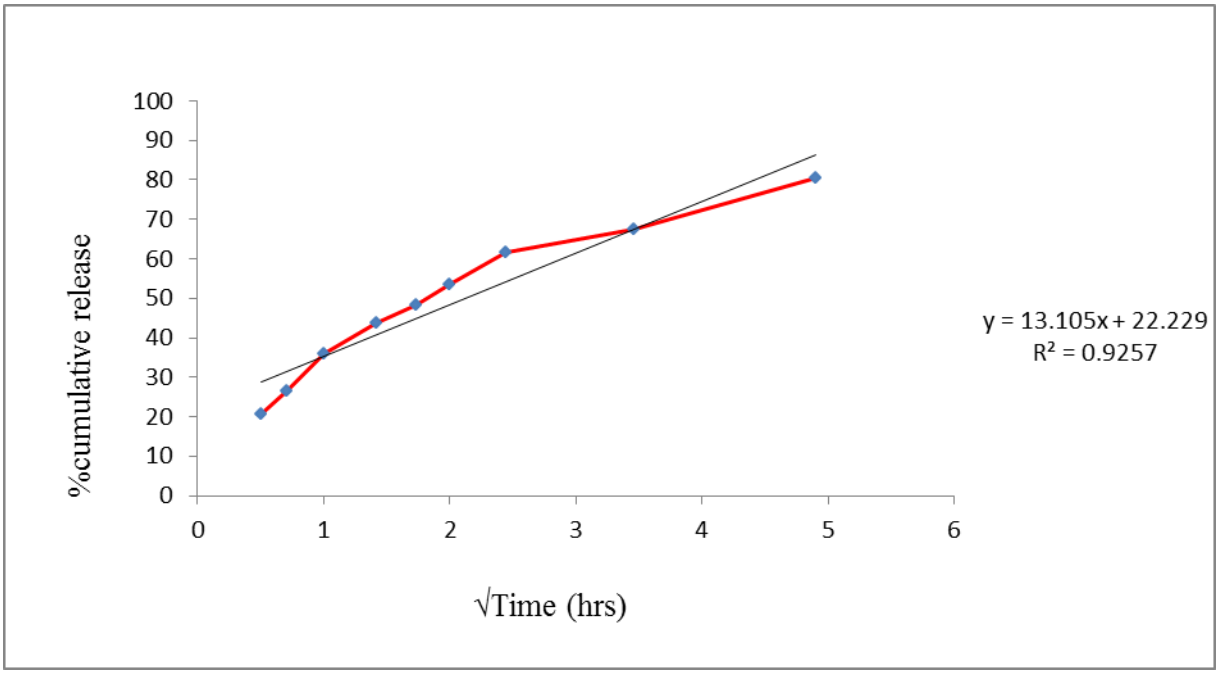


Figure 4.2.7.3: Higuchi Plot of GBM-6

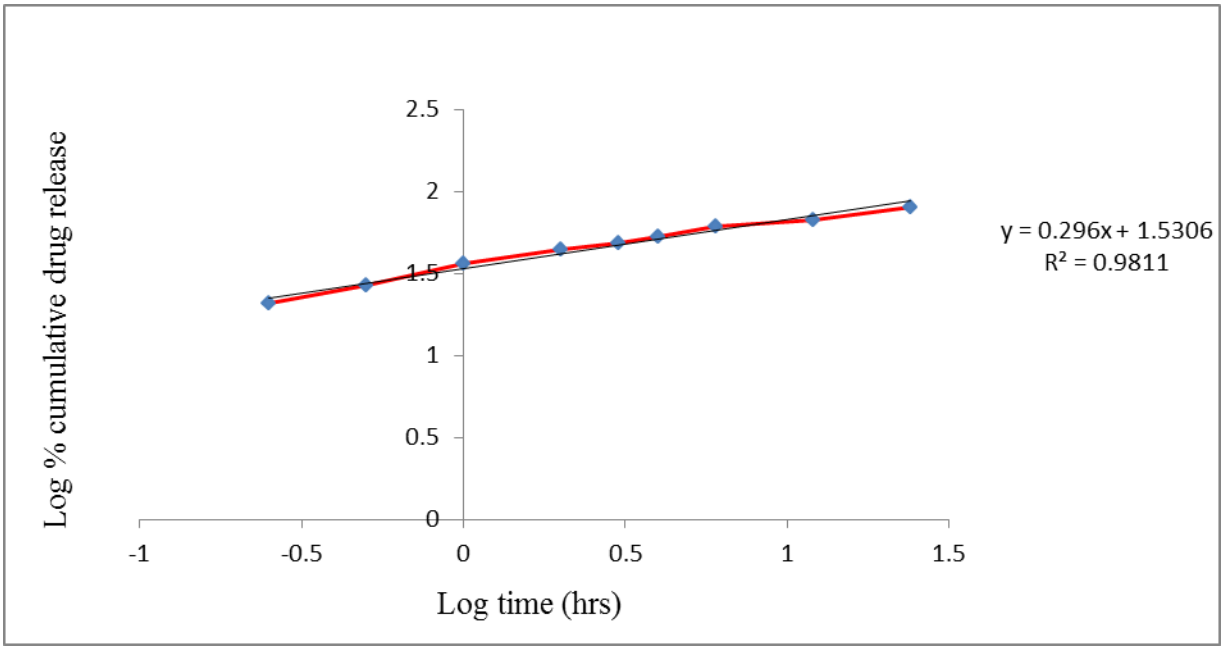


Figure 4.2.7.4: Korsmeyer's Peppas Plot of GBM-6

Formulations	Zero order	First order	Higuchi model	Korsmeyer model
	r^2	r^2	r^2	r^2
GBM-1	0.691	0.797	0.881	0.961

GBM-2	0.710	0.822	0.891	0.961
GBM-3	0.828	0.913	0.954	0.983
GBM-4	0.686	0.784	0.877	0.952
GBM-5	0.878	0.964	0.975	0.967
GBM-6	0.756	0.909	0.925	0.981
GBM-7	0.823	0.920	0.957	0.982
GBM-8	0.724	0.834	0.904	0.966
GBM-9	0.746	0.873	0.919	0.982

Table 4.2.7: Release Kinetics of Aceclofenac from gelatin surface modified BSA microspheres

4.3 STABILITY STUDIES

Ability of a formulation to retain its properties in specified limits throughout its shelf life is referred to as stability. Stability of a pharmaceutical product may be defined as capability of a particular formulation, in a specific container, to retain its physical, chemical, microbiological, therapeutic, and toxicological specification.

The objective of stability study is to determine the shelf life, namely the time period of storage at a specified condition within which the drug product still meets its established specifications.

The stability of finished pharmaceutical products depends on several factors. On one hand it depends on environmental factors such as ambient temperature, humidity, and light. On the other hand, it depends on the product related factors such as physical and chemical properties of active substance and pharmaceutical excipients, dosage form and its composition, manufacturing process, nature of container, closure system, and properties of packaging materials.

The chemical stability of drug is of great importance since it becomes less effective as it undergoes degradation. Also drug decomposition may yield toxic by products that are harmful to the patient. Microbiological instability of a sterile drug product could also be hazardous.

Stability of a formulated product on shelf becomes an important factor in successful development of any dosage form. A study of stability of a pharmaceutical product is essential for three main reasons; safety of the patient, legal requirements concerned with the identity, strength, purity and quality of product and to prevent the economic repercussion of marketing an unsuitable product.

A well designed stability testing plan is essential and pertinent part of the quality assurance program.

4.3.1 Storage Condition

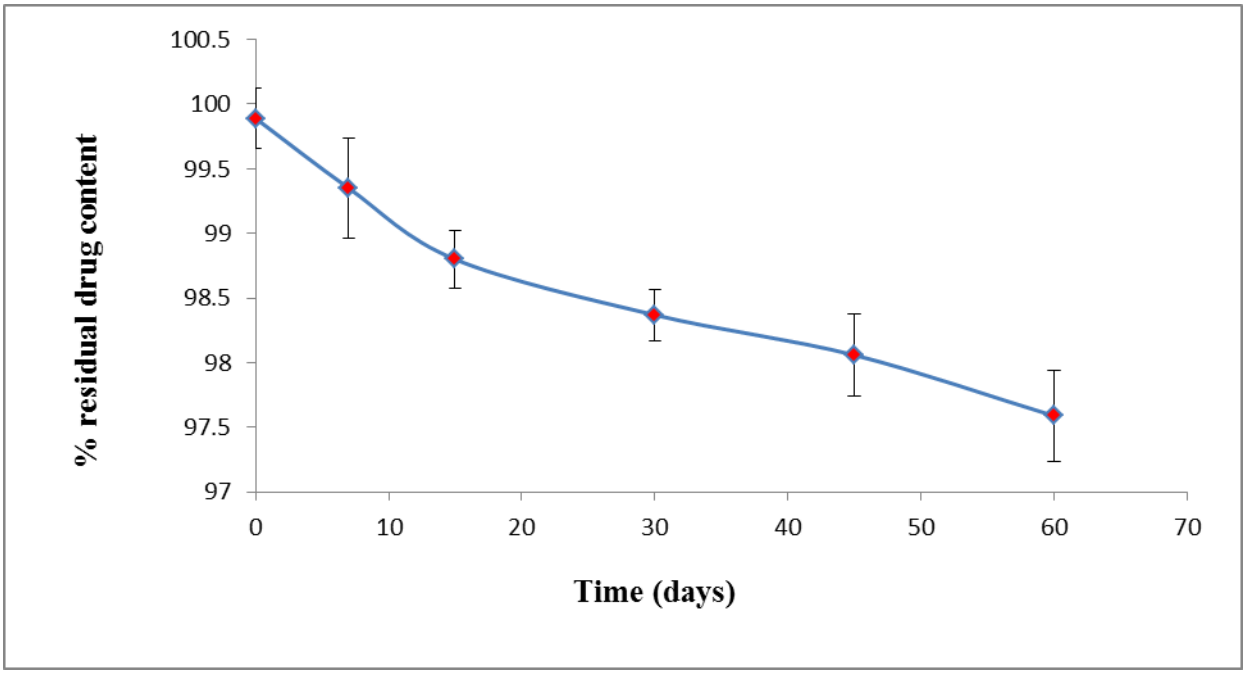
Prepared formulation (GBM-6) was stored in screw capped glass bottle at refrigerated temperature (2-8^{0}C) and room temperature (20-25^{0}C). Samples were analyzed for residual drug content after a period of 7, 15, 30 45 and 60 days. Initial drug content was taken as 100% for each formulation.(Patil SS et al),(Amirijahed AK et al).

Table 4.3.1: Effect of aging on residual drug content at refrigerated temperature

Formulation	% Residual drug contents at refrigerated temperature (2-8 ^{0}C) Mean ± S.D., n = 3					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
GBM-6	99.88±0.23	99.35±0.39	98.80±0.22	98.57±0.19	98.07±0.32	97.59±0.35

Table 4.3.2: Effect of aging on residual drug content at room temperature

Formulation	% Residual drug contents at room temperature (20-25^{0}C) Mean ± S.D., n = 3					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
GBM-6	99.85±0.21	98.61±0.63	97.09±0.52	96.23±0.53	95.52±0.27	94.88±0.26



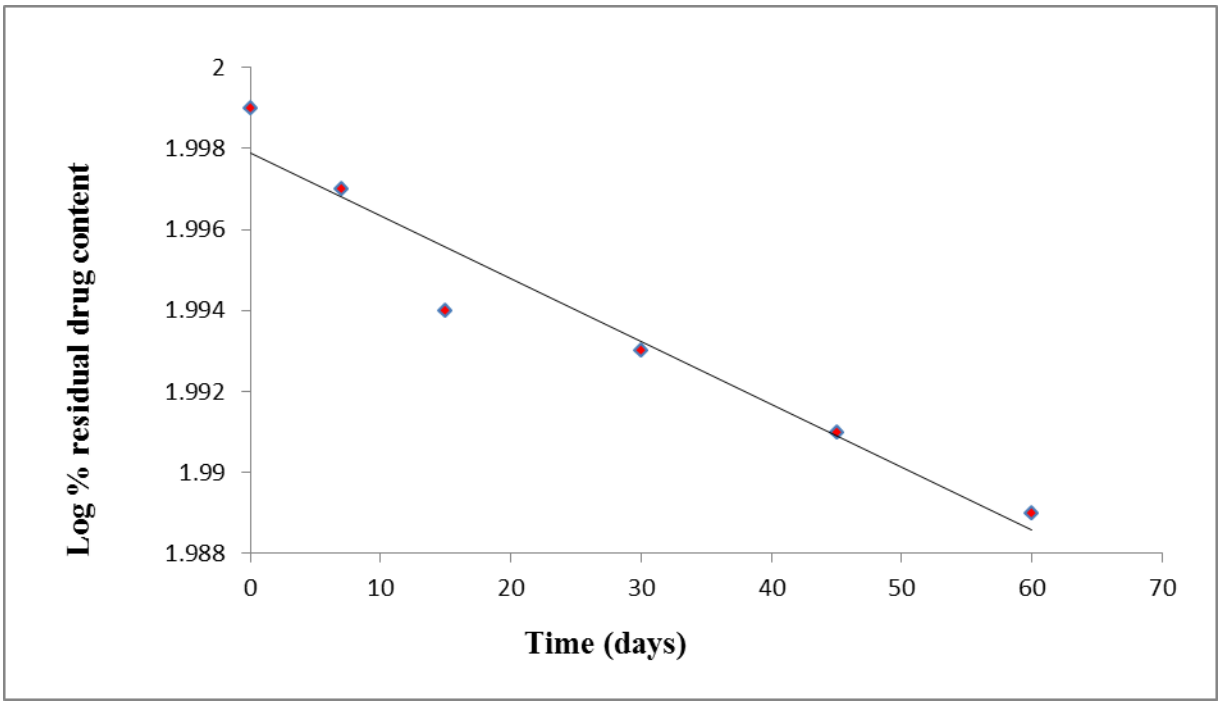


Figure 4.3.1: Residual drug content of GBM-6 at refrigerated temperature

Figure 4.3.2: Log % residual drug content of GBM-6 at refrigerated temperature

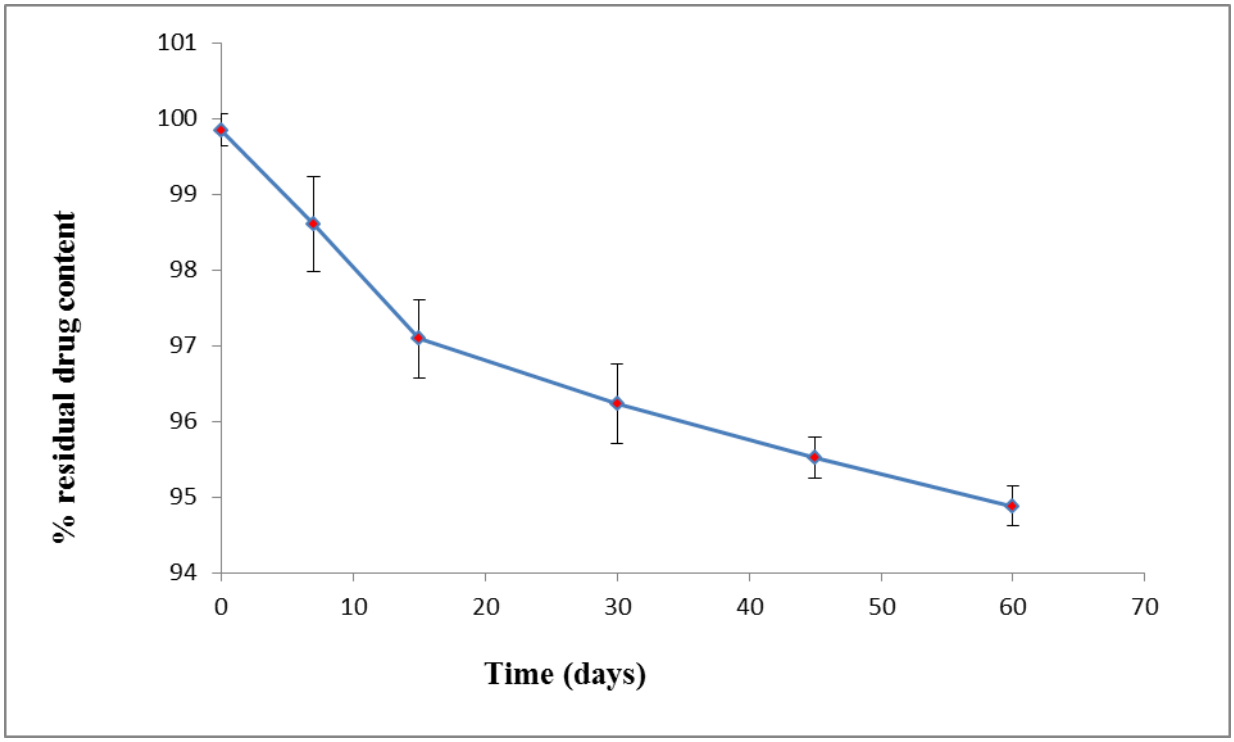


Figure 4.3.3: Residual drug content of GBM-6 at room temperature

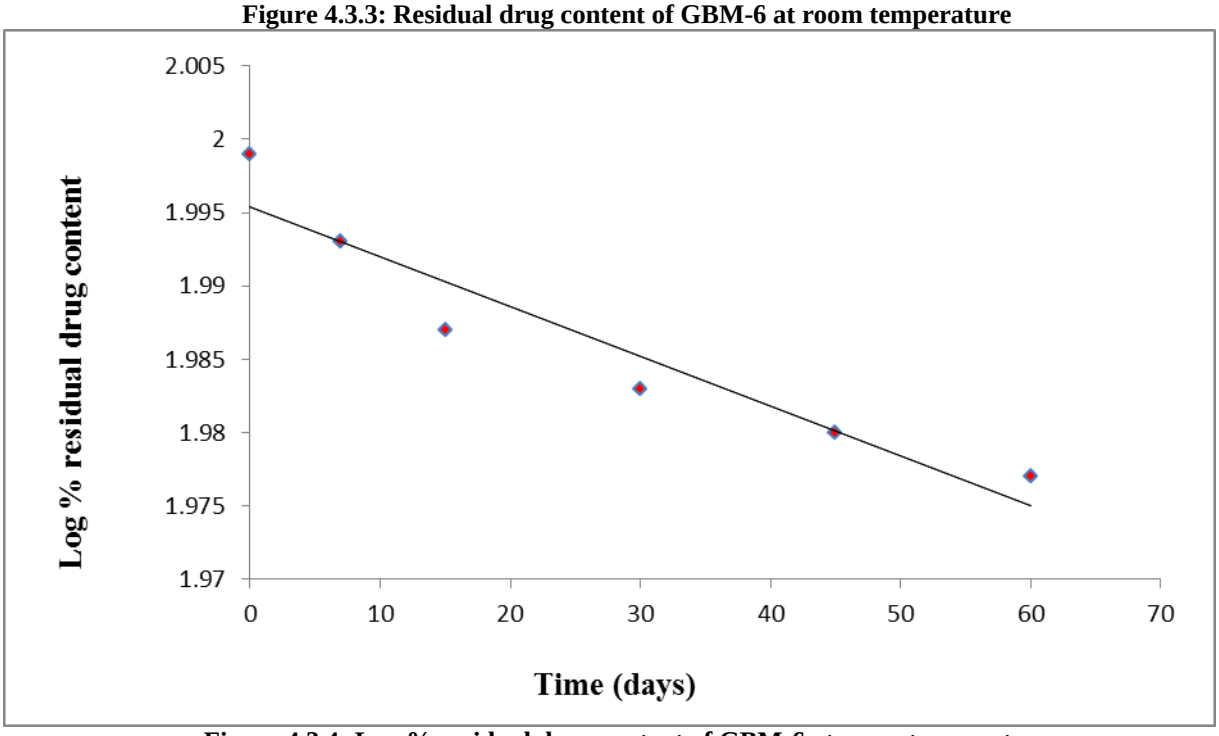


Figure 4.3.4: Log % residual drug content of GBM-6 at room temperature

4.3.2 Results and Discussion of stability

The optimized formulation GBM-6 was stored at two different temperature conditions refrigerated temperature and room temperature. To obtain an optimal storage condition, percent residual drug content and log % residual drug content was calculated at different time interval (0, 7, 15, 30, 45, and 60 days) and graphs were plotted between percent residual drug content vs time, and log % residual drug content vs time. [Figure 4.3.1, 4.3.2, 4.3.3, 4.3.4].

Figure 4.3.2 and 4.3.4, reflect an almost linear relationship. Degradation rate constant 'k' was calculated from the log % residual drug content vs time plot. By putting the value of 'k' in equation (8) and (9) values of $T_{10\%}$ and $t_{1/2}$ were calculated.

Table 4.3.3: Shelf life and half-life of optimized formulation (GBM-6)

S.N.	Parameters	Storage condition	
		Refrigerated temperature (2-8 ^{0}C)	Room temperature (20-25 ^{0}C)
1.	K (days)	$3.29x10^{-4}$	$9.87x10^{-4}$
2	$T_{10\%}$ (days)	316	105
3.	$t_{1/2}$ (days)	2079	691

CONCLUSION

Shape and surface morphology of prepared BSA/GBSA microspheres was evaluated by SEM. The study revealed that most of the microspheres were fairly spherical in shape.

The geometric mean diameter of the bovine serum albumin microspheres was found to be 19.926 ± 2.271 µm. Mean particle size of the gelatin surface modified BSA microspheres was found to be 22.523 ± 5.45 µm. The particle size was found to be mainly dependent on the albumin concentration, surfactant concentration, and stirring speed. An increase in the albumin concentration from 4:1 to 6:1 led to a significant increase in the particle size. An increase in the surfactant concentration led to decrease in the particle size. There was a decrease in the particle size with an increase in the stirring speed from 1500 rpm to 2500 rpm.

Drug entrapment efficiency was mainly dependent on the drug polymer ratio and concentration of surfactant. Entrapment efficiency varied from 30.52% to 53.3%. An increase in the concentration of albumin from 4:1 to 6:1 led to a significant increase in the entrapment efficiency. There was a significant decrease in the entrapment efficiency with an increase in the concentration of surfactant.

All the batches showed an initial burst release ranging from 8.97% (BM-4) to 23.65% (BM-6). Initial burst release was followed by sustained release varying from 71.60 % (BM-3) to 84.94 % (BM-5). Percent cumulative drug release for the optimized formulation GBM-6 was found to be 80.32±0.86 at the end of 24 hours. This could be accounted for sustained release of the drug from gelatin surface modified BSA microspheres. Percent cumulative drug release decreased with an increase in the concentration of glutaraldehyde. The decrease in the drug release with an increase in the concentration of the glutaraldehyde is because of the formation of dense polymer cross-links leading to an increase in the diffusional path length that the drug molecules have to traverse.

The formulated gelatin surface modified BSA microspheres showed good entrapment and encapsulation efficiency with spherical geometry. The *in vitro* release profile revealed the ability of microspheres to prolong the drug release for more than 24 hrs.

It is possible to prepare gelatin surface modified BSA microsphere by emulsification chemical cross linking method which may be a suitable tool for site specific delivery of aceclofenac to the arthritic joints.

The development of a system whereby drugs could be administered in a single dose that maintains active levels of the drug for a prolonged period would be the ideal system. Such a targeted and sustained release system has been developed in this study in the form of gelatin surface modified BSA microsphere containing aceclofenac.

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