CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

FORMULATION AND EVALUATION OF AMPICILLIN LOADED NANOPARTICLES

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Abstract:

The objective of this study was to prepare ampicillin–loaded nanoparticles for controlled delivery through the intravenous (i.v.) route to reduce the frequency of administration, increasing the bioavailability. Ampicillin nanoparticles were prepared by Desolvation method and characterized for drug content, particle size and size distribution, zeta potential, and in vitro drug-release study. In this method the Bovine Serum Albumin nanoparticles were prepared by Desolvation technique. Ampicillin Belonging to the penicillin group of beta-lactam antibiotics.It is used for the treatment of infections known to be or highly likely to be caused by bacteria. All formulations were further checked for evaluation parameters. Particle size of all formulation was found to be 200 to 500nm. Zeta potential for all formulated nanoparticles were in the range of 64.1which indicates excellent stability. The maximum percentage yield was found to be 78.66% for the formulation F4.The maximum drug content was found to be 71.90% for the formulation F1.The cumulative percentage release after 11 h was found to be 53.24 to 75.60.From results it was observed as increase polymer concentration, drug release from the nanoparticles decreases.

Keywords: *Bovine serum albumin, Nanoparticles, Ampicillin.*

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*Please cite this article in press as Pragati Lingwal et al , **Formulation and Evaluation of Ampicillin Loaded Nanoparticles,** Indo Am. J. Pharm. Sci, 2015;2(9).*

INTRODUCTION

Development of colloidal carrier systems has now been an area of great interest in the field of drug delivery. This involves multidisciplinary scientific approach, contributing to human health care. Drug delivery systems (DDS) have been developed in order to control pharmacological parameters such as bioavailability, biodistribution and pharmacokinetics of the administered substances. It can help to maintain drug levels in the therapeutically desired range by releasing the substance over a predefined period of time. [1-3]

Nanotechnology is science of matter and material that deal with the particle size in nanometers. [4]Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm.nanoparticles are made of non-biodegradable and biodegradable polymers. [5]It helps in detecting the antigen associated with diseases such as cancer, diabetes mellitus, neuro degenerative diseases, as well as detecting the microorganisms and virus associated with infections. In pharmacy size reduction has an important application as drugs in the nanometer size range enhance performance in a variety of dosage forms. [6-7]

In particular, BSA nanoparticles have been studied whether as possible carriers for controlled intravenous drug delivery or overcome the problems of administration of drugs which are unstable in the gastrointestinal tract or an inadequately absorbed.[8-9]

The present paper describes the preparation of ampicillin loaded nanoparticles. In particular, we have studied the effect of polymer on the drug release results.

MATERIALS AND METHOD

Chemicals

Albumin used as a polymer was supplied by central drug house Ltd. New Delhi. Sodium chloride, ethanol and Glutaraldehyde were also supplied by central drug house Ltd. New Delhi. Ampicillin was obtained from balaji drug Ltd. New Delhi.

Preparation of nanoparticles

Bovine Serum Albumin nanoparticles were prepared by Desolvation technique. The different amounts of bovine serum albumin were dissolved in Nacl solution, respectively, titrated to pH 8. The specified amount of drug was then added into bovine serum albumin solutions followed by the continuous addition of 8.0 ml of the desolvating agent i.e. ethanol under stirring (500 rpm) at room temperature. After the Desolvation process, few ml of 8% Glutaraldehyde in water was added to induce particle cross linking. The cross linking process was performed under stirring of the suspension over a time period of 24 h.

Preparation of Master Formula:

Table 1: Formulation Plan for Ampicillin Nanoparticles

INGREDIENTS	FORMULATION			
	F1	**F2**	**F3**	**F4**
Drug(mg)	150	150	150	150
BSA(mg)	45	90	135	225
Ethanol(ml)	8	8	8	8
Glutaraldehyde (%)	8	8	8	8

Purification of Bovine Serum Albumin Nanoparticles:

The resulting nanoparticles were purified by three cycles of differential centrifugation (10,000 rpm for10 min) and redispersion of the pellet to the original volume of 10mM Nacl at pH values of 8, respectively. Each redispersion step was performed in an ultrasonication bath over 5 min. The solvent was removed and the nanoparticles were collected and stored in a refrigerator.

Characterization of Nanoparticles

The formulated nanoparticles were evaluated for particle size and shape, zeta potential, drug content uniformity, entrapment efficacy, drug loading, in-*vitro* drug release study.

Shape and Size

The morphology and size of plain and mannose-coated nanoparticles was determined by Scanning electron microscopy (SEM).

Zeta Potential

The zeta potential and surface charge of nanoparticles was determined by the Zeta Potential Analyzers. The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles.

Drug Content Uniformity

Drug content was determined by centrifugation method. The redispersed nanoparticles suspension was centrifuged at 15,000 rpm for 40min at 25^{o}c to separate the free drug in the supernatant. Concentration of ampicillin in the supernatant was determined by UV-VIS spectrophotometrically at 270nm after suitable dilution.

Percentage Yield

It is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material.
It can be calculated using following formula:

$$\textbf{Percentage yield} = \frac{\textbf{Practical yield} \times 100}{\textbf{Theoretical yield}}$$

***In- vitro* Drug Release**

In-vitro drug release study was carried out by Modified Diffusion Apparatus. The apparatus consists of a beaker containing 50 ml of phosphate buffer pH 7.4 maintained at 37.5^{0}c under mild agitation (50 rpm) using a magnetic stirrer acts as receptor compartment. An open -ended tube acts as donor compartment and the egg membrane was tied into upper part of the donor compartment. 10 mg of nanoparticles were placed into the donor compartment over the membrane which was dipped in the receptor compartment consisting buffer. Then, the samples were taken at different time intervals from the receptor compartment and were analyzed by UV spectrometer at 270nm.

RESULTS AND DISCUSSION

Four formulations of ampicillin were formulated using different drug polymer ratios. The formulation is subjected to evaluation parameters like particle size, zeta potential, drug content uniformity, percentage yield, entrapment efficiency, drug loading efficiency, *in-vitro* drug released study.

Preformulation Studies

The results of Preformulation studies of ampicillin are described as follows:

Organoleptic Characteristics:

The color, odor and taste of the drug were characterized and recorded using descriptive terminology; the results are shown in Table No. :2

Table 2: Results of Organoleptic Properties

PROPERTIES	RESULTS
Description	Amorphous powder
Color	White or slight yellow
Odor	Characteristics

Solubility Analysis

Table 3: Results of Solubility Studies

Solvent	Descriptive term	Solubility(µg/ml)
Water	Sparingly soluble	4.167
Ethanol	Sparingly Soluble	5.261
0.1M HCL	freely soluble	3.954
7.4 PBS	Soluble	1.915

Melting Point Determination:

Table 4: Results of Melting Point determination

Observed Melting Point	**210-215**
Reported Melting Point	208-216

Identification of Drug by FTIR:

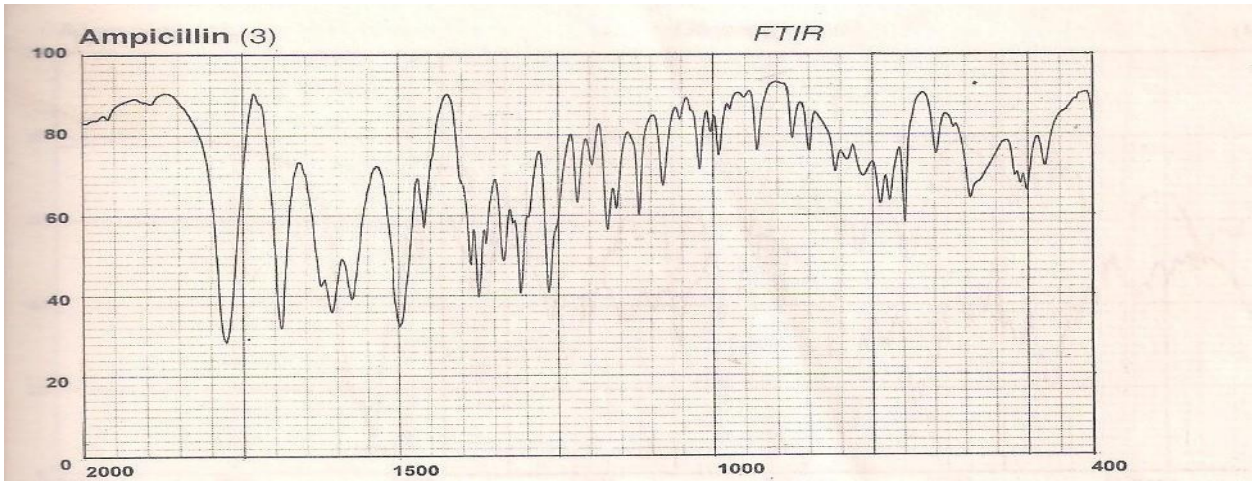

Fig 1: FTIR of Pure Drug (Ampicillin) Reference Standard (I.P. 2010)

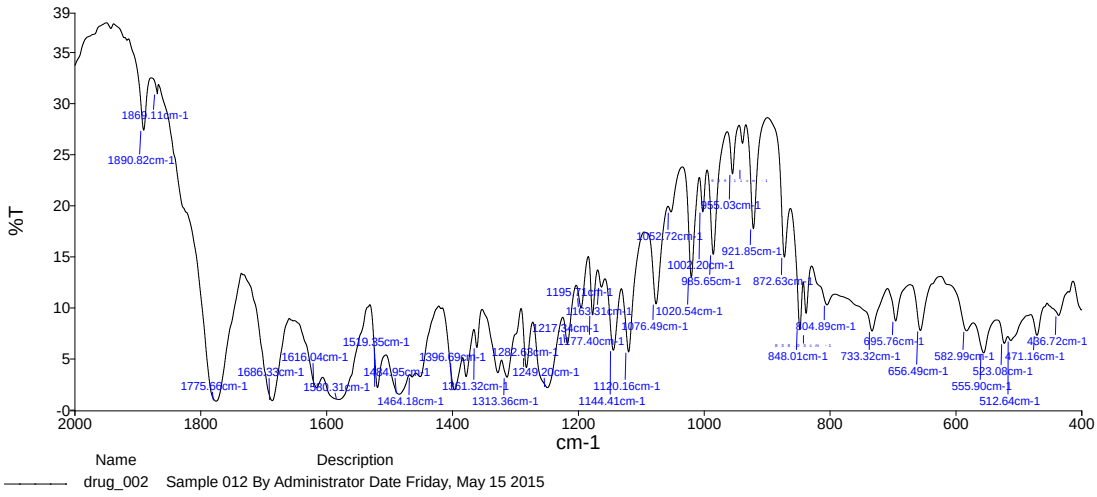

Fig 2: Spectra of Ampicillin

Calibration Curve of Ampicillin with pH 7.4

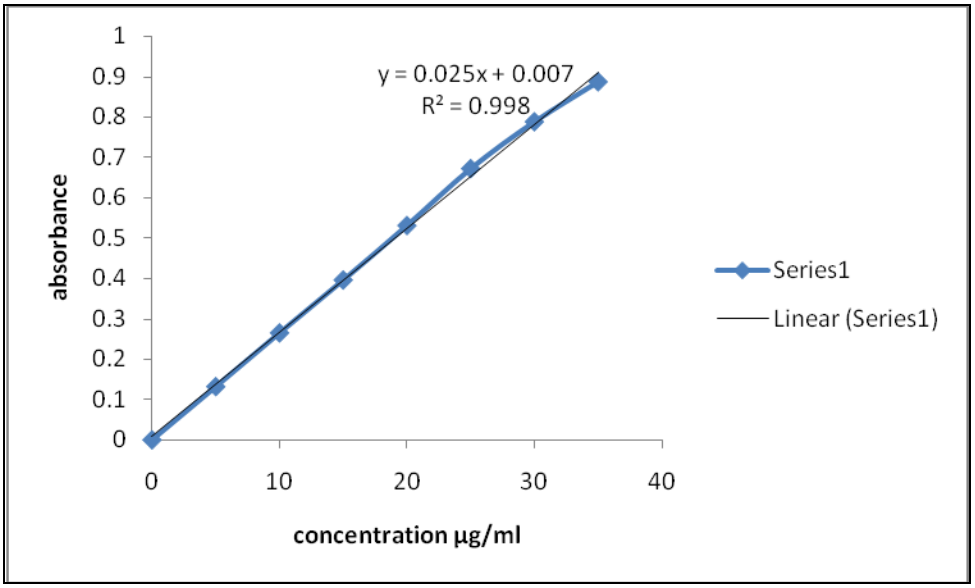

Fig 3: Calibration curve of ampicillin with pH7.4

Characterization of Nanoparticles

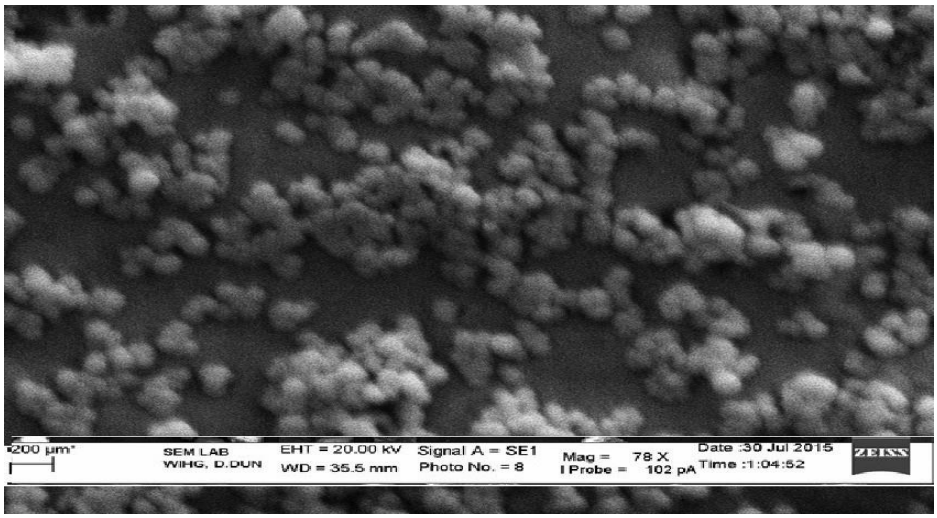

Fig4: Particle size Analysis

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Zeta Potential

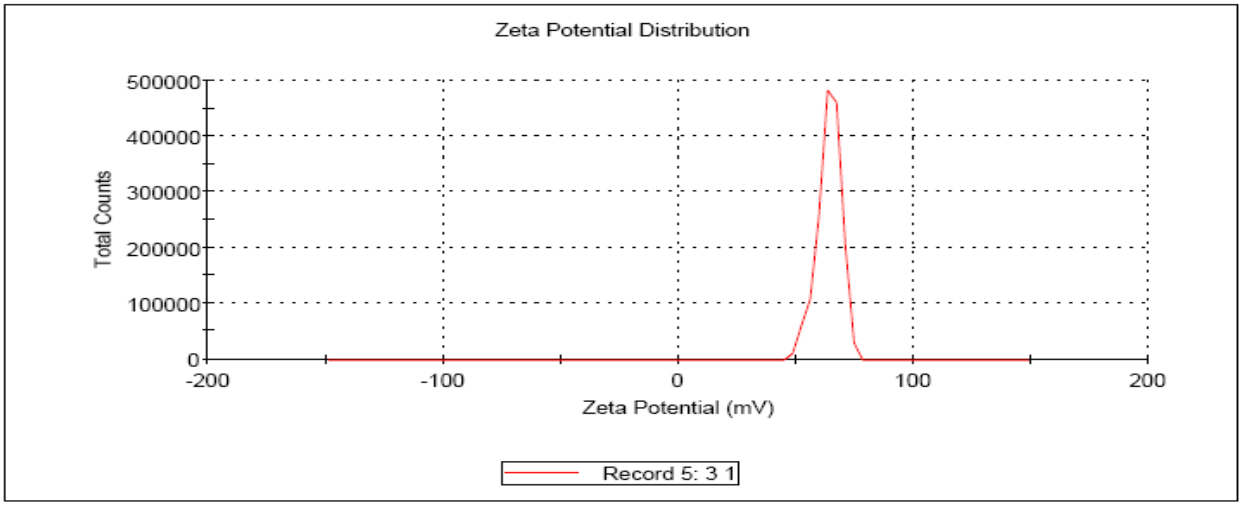

Fig 5: Zeta Potential of Albumin Nanoparticles

Nanoparticles positively charged and thus the nanoparticles show the excellent stability.

Table 5: Drug Content of Ampicillin Nanoparticles

Drug content (%) S.D.	
Formulation Code	**Nanoparticles**
F1	**71.90±0.02**
F2	**67.56±0.03**
F3	**65.23±0.05**
F4	**50.45±0.02**

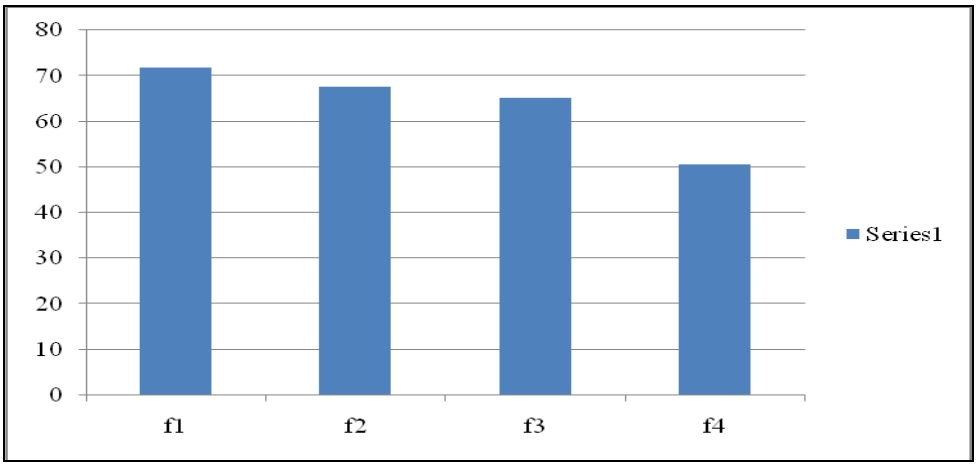

Fig 6: Drug Content Comparison of Different Formulation

Table 6: Percentage Yield of Ampicillin Nanoparticles

Formulation code	Total amount of ingredients(mg)	Percentage Yield (%)
F1	195	51.28
F2	240	62.53
F3	285	73.68
F4	375	78.66

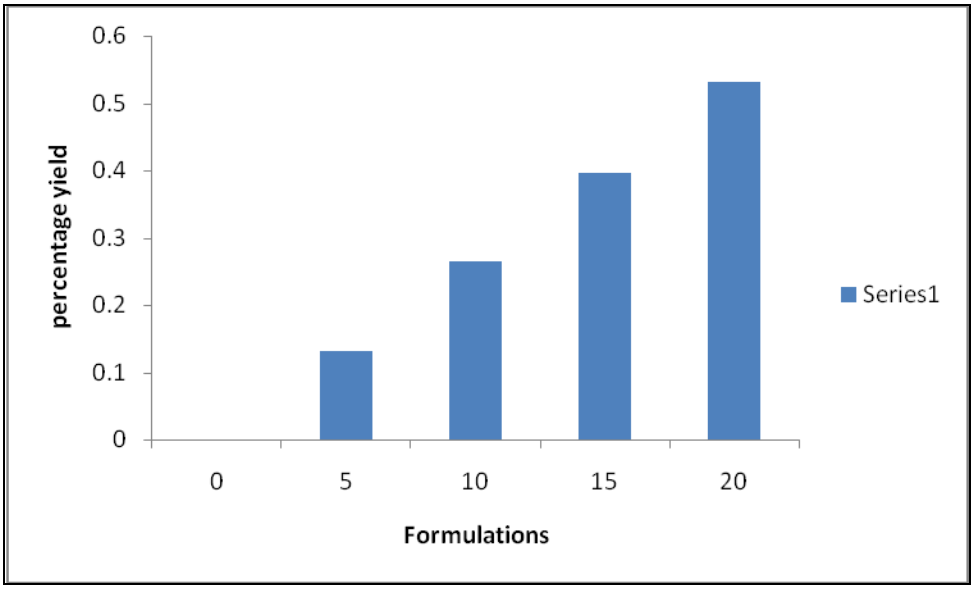

Fig 7: Percentage Yield Comparison of Different Formulation

***In-vitro* Drug Release:**

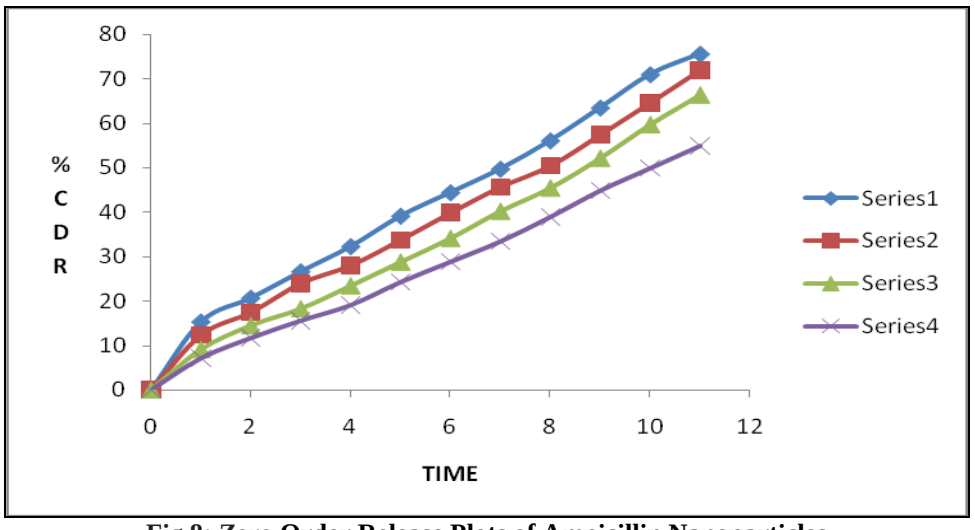

Fig 8: Zero Order Release Plots of Ampicillin Nanoparticles

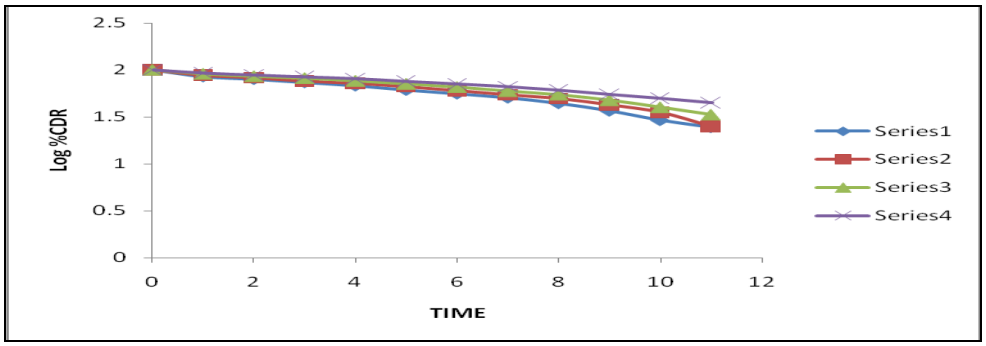

Fig 9: First Order Release Plot of Ampicillin Nanoparticles

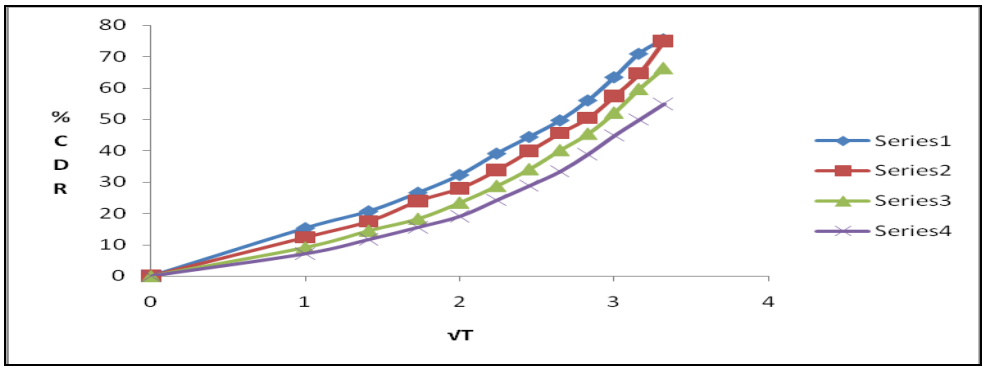

Fig 10: Higuchi Plot of Ampicillin nanoparticles

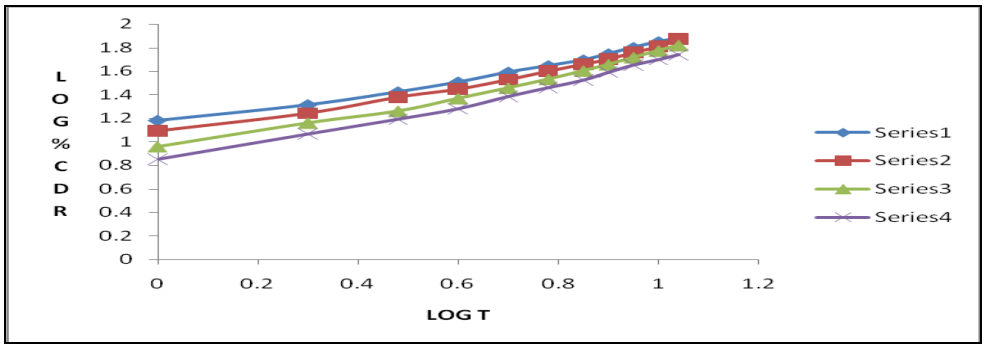

Fig 11: Korsmeyer Peppas Plot of Ampicillin nanoparticles

Table 7: Model fitting release profile of Formulations F1 to F4

Formulation code	Regression Coefficient(R^2)			Slope (n)value	Best fit model
	Zero order	First order	Higuchi's	Korsmeyer-Peppas	
F1	0.990	0.966	0.952	0.982	Zero order
F2	0.993	0.937	0.928	0.981	Zero order
F3	0.995	0.951	0.912	0.984	Zero order
F4	0.996	0.978	0.914	0.988	Zero order

Mathematical modeling-it was found that all formulations follow the zero order kinetic. The Regression Coefficient F1 to F4 of zero order was found to be almost linear. The linearity suggests that the release of ampicillin nanoparticles was controlled release mechanism.

CONCLUSION

The present paper describes the experimental conditions for preparing ampicillin loaded nanoparticles. It was observed that particle size of nanoparticles varied somewhat among the formulation due to variation in the composition of formulation. Nanoparticles were successfully prepared by Desolvation method. Bovine serum albumin is a biocompatible polymer for preparing ampicillin nanoparticles.

FTIR studies were carries out to find out possible interaction between the selected drug and polymer. The FTIR studies revealed that there was no interaction between the selected drug and polymer. The in vitro dissolution studies performed at pH 7.4 revealed that as we increased polymeric concentration, drug release from the nanoparticles decreases.

Desolvation method was found to be very simple and reproducible. The constant release of ampicillin from the nanoparticle maintain constant drug plasma concentration thereby increasing therapeutic efficacy. The developed formulation overcome and alleviates the drawbacks and limitation of ampicillin sustained release formulation so that providing controlled drug delivery system in this era of patenting of novel and controlled drug delivery system.

ACKNOWLEDGEMENT

The authors are grateful to the balaji drug Ltd for ampicillin as a gift sample, and Shri guru ram rai institute of technology and sciences, IIT roorkey, wadia institute of geological research and centre for its help in characterization studies.

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