Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(5): 384-394

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Design Development and Evaluation of Nanosuspension of Azithromycin

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ABSTRACT

The objective of this study is to prepare a formulation of Azithromycin which increase the oral bioavailability as well as increased solubility and dissolution rate. In the present study nine formulations were formulated by using the three different polymers and varying the concentration of PEG and also varying the rotation speed of the stirrer. Results of preformulation studies like melting point 117°C, UV spectroscopy and FTIR studies showed that the drug was pure. The spherical and rough surface of F9 viewed through SEM. *In-vitro* dissolution study had shown satisfactory results. On the basis of release data and graphical analysis formulation F8 and F9 showed a good controlled zero order release profile.

Keywords: Azithromycin, polyethylene glycol (PEG), nanosuspension, sustained release, Scanning electron microscopy (SEM) and agglomeration.

INTRODUCTION

Many different techniques have been developed to overcome the solubility problem of poorly soluble drugs such as nanoparticulate formulations, liposomes, micelles nanoemulsion, nanosuspension solid lipid nanoparticles, and polymeric nanoparticles. [1-3] Nanocrystallization is a technique to produce crystalline particles of poorly soluble drugs in the nanometer range (*i.e.*, nanocrystals). Due to the size and, thus, the high surface area to volume ratio, nanocrystals can increase the saturation solubility of a drug and the dissolution rate of drug particles.

A Nanosuspension is a submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid, Biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1µm, without any matrix material, stabilized by surfactants and polymers, prepared by suitable method for drug delivery applications, through various routes of administration like oral, topical, parenteral, ocular and pulmonary routes. [4]

Azithromycin is a drug which is classified as a BCS class ll substance, since it has low aqueous solubility and high permeability. [5] Low solubility contributes to high variability in absorption after oral administration. Once the solubility problem is overcome azithromycin will be absorbed immediately showing comparatively good oral bioavailability. Therefore to overcome this problem the solubility of the drug has to be enhanced because the faster the drug dissolved, faster will it absorb and hence the pharmacological action be attained. Hence to overcome this problem nanosuspension technology is used. The main challenge in nanosuspension technology is prevention of particle agglomeration or aggregation and crystal growth.

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Received: 14 May, 2015; **Accepted:** 04 July, 2015

MATERIALS AND METHOD

The detail of materials used as given in Table 1.

Preformulation Studies

Preformulation study is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms.

Organoleptic properties

The organoleptic studies like general appearance like nature, color, odor etc. were performed by visual observations.

Color: Small quantity of drug was taken in butter paper and viewed in well illuminated place.

Odor: Very less quantity of drug was smelled to get the odor.

Melting point

For determination of melting point USP method was followed. Small quantity of drug was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which drug started to melt and the temperature when the entire drug gets melted. The temperature at which the drug started to melt was noted which the melting point of the drug. [6]

Solubility Studies

The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called solubility. For solubility determination, excess of drug was added in each solvent (3.0 ml) in separate test tubes. These test tubes were occasionally stirred for 24 hours at room temperature. After 24 hours each sample was filtered and filtrates was suitably diluted and analyzed for the drug content using UV-Visible spectrophotometer. [7]

FTIR Spectroscopy

It is a useful analytical technique used to check the interaction between drug and the polymers used in formulation; FTIR spectra of pure drug and optimized formulation were obtained by Perkia-Elmer Fourier Transform Infrared spectrophotometer by making KBr pellets. The powdered mixture of sample was scanned in the wavelength region of 2.5 to 25μ in a FTIR Spectrophotometer. The IR spectrum of samples was compared with that of physical mixture to check any possible drug-excipients interaction. [8]

Formulation of Nanosuspensions of Azithromycin

Azithromycin nanosuspensions were formed by bottom-up technique. In this method the nanosuspensions were prepared by solvent-antisolvent precipitation technique. 50 mg drug was completely dissolved in 2 ml ethanol solvent which acts as an organic phase. The organic phase was injected slowly in 25 ml water/aqueous phase (speed 0.5 ml/min.) containing different concentration of polymers like (PEG, PVP, HPMC, and POLOXAMER). The solution was stirred continuously. After mixing the organic phase into the aqueous phase, the precipitation were formed which were centrifuged at 6000 rpm for 15 min. After centrifuging, the supernatant, which contains the Azithromycin Nanoparticles were carefully collected and dried at temperature 45°C for 24 hours. [9]

Table 1: Detail of materials used

Material	Grade	Suppliers
Azithromycin	API	Ranbaxy Laboratories, Gaurgaon, India
Hydroxypropylmethyl Cellulose	K5 (2910)	Cameo Healthcare Pvt. Ltd., Goa, India
Poly Ethylene Glycol	4000	Tasc Chemical Industries Pvt. Ltd., Mumbai, India
Poly vinyl pyrrolidone	K 30	R.P Gupta and Sons, Delhi, India
Ethanol	LR	SardarLamichemPvt. Ltd., Punjab, India
Acetone	LR	Chemi Inc., Delhi, India
Methanol	LR	Upasahna Enterprises, Guargaon, India

Table 2: Organoleptic properties

Drug	Test	Specification	Observation
Azithromycin	Color and odor	White crystalline and odorless	White crystalline and odorless

Table 3: Formulation of Nanosuspensions of Azithromycin

S. No	Formulation Code	Azithromycin (mg)	Conc. of different polymers used (mg)	Solvent used Ethanol (ml)	Rotation Speed (rpm)
1	F1	50	(HPMC) 125	2	500
2	F2	50	(PVP) 125	2	500
3	F3	50	(PEG) 125	2	500
4	F4	50	(HPMC) 250	2	500
5	F5	50	(PVP) 250	2	500
6	F6	50	(PEG) 250	2	500
7	F7	50	(PEG) 250	2	250
8	F8	50	(PEG) 250	2	1000
9	F9	50	(PEG) 250	2	2000

Preparation of Reagents

Phosphate Buffer Solution (pH 6.8)

Placed 50 ml of 0.2 M monobasic potassium phosphate in 200 ml volumetric flask. Then added the specified volume (24.4 ml) of 0.2 M NaOH solution and then added water to make up the volume up to 200 ml.

Standard stock solution

Drug (10 mg) was dissolved in 100 ml of 0.1N HCl to make the stock solution of 100μg/ml. This solution was further diluted to obtain various concentrations.

Different concentrations of stock solution

Stock solution (0.2 ml) was taken in 10 ml volumetric flask and further diluted up to 10 ml to make concentration of 2μg/ml. similarly 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2 ml stock solution taken in 10 ml volumetric flask and diluted up to the mark to obtain 4, 6, 8, 10, 12, 14, 16, 18, 20μg/ml concentration.

Preparation of 0.1N HCl

Dissolve 8.838 ml concentrated HCl in 100 ml distilled water to prepare 0.1N HCl.

Preparation of calibration curve in 0.1 N HCl

Absorbance of concentrations was measured at λ_{max} 254 nm using Shimadzu UV-1800 UV/V double beam spectrophotometer and 0.1N HCl as reference standard

(blank). The standard curve was generated for the entire range from 2-20µg/ml.

Preparation of calibration curve in phosphate buffer solution pH 6.8

Absorbance of all prepared solutions of concentrations was measured at λ_{max} 254 nm using Shimadzu UV-1800 UV/V double beam spectrophotometer and phosphate buffer pH 6.8 as reference standard (blank). The standard curve was generated for the entire range from 2-20µg/ml.

Characterization of nanosuspensions of Azithromycin

Percentage yield

The percentage yield of the nanosupensions was determined for drug and was calculated using the following equation.

$$\% \text{ Yield} = (M/M0) \times 100$$

Where M = weight of nanoparticles; Mo = total weight of drug and polymer.

Drug entrapment efficiency [10]

The amount of drug entrapment of nanosuspension was estimated by extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance is measured by spectrophotometer against appropriate blank. The amount of drug entrapped in the nanoparticle was calculated by the following formula

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Amount of drug used}} \times 100$$

Scanning electron microscopy

The sample for the scanning electron microscopy (SEM) analysis was prepared by sprinkling the nanosuspensions one side of double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. The SEM analysis of the nanosuspensions was carried out by using JeolJSM 5300, Japan. The nanosuspensions were viewed at an accelerating voltage of 15 kV. [11]

***In vitro* release studies** [12-15]

In vitro drug release from the nanosuspensions is complicated because the nanosuspensions are adhering to the inner surfaces of dissolution basket, which leads to the non- participation of nanosuspensions or their surface in release study. The release rate of nanosuspensions were determined in a United States Pharmacopoeia (USP) XXIII basket type dissolution apparatus. A weighed amount of nanosuspensions equivalent to 200 mg drug was filled into a hard gelatin capsule (No. 0) and placed in the basket of dissolution rate apparatus. Nine hundred milliliters of the 0.1N HCl and pH 6.8 Phosphate Buffer was used as the dissolution medium. The dissolution fluid was maintained at 37 ± 1°C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. 5 ml samples were withdrawn at each 1 hr. interval in 0.1 N HCl for 2 times and buffer pH 6.8 at the interval of 2 hrs. Three times respectively, passed through a 0.25µm membrane filter (Millipore), and analysed using UV method to determine the concentration present in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal. All experiments were run in triplicate.

Comparative study of release kinetics of the formulation batch [16-20]

In order to determine the release mechanism of Azithromycin from the prepared nanosuspensions of the formulations, the results of the dissolution study were examined in accordance to the kinetic models. The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero order, first order, Higuchi and Korsmeyer-Peppas and finding the R2 value of the release profile corresponding to each model.

Zero Order Kinetics

The zero order rate describes the systems where the drug release rate is independent of its concentration. A zero-order release would be predicted by the following equation;

$$At = A0 - K0t$$

Where At is the amount of drug released in time t, A0 is the initial concentration of drug (most times, A0=0) and K0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K0.

First Order Kinetics

The first order describes the release from system where release rate is concentration dependent. A first-order release would be predicted by the following equation;

$$\text{Log } C = \text{Log } C_0 - Kt/2.303$$

Where C is the amount of drug released in time t, C0 is the initial concentration of drug and K is the first order rate constant. When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Higuchi's Model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then extended to different geometrics and porous systems. This model is based on the hypothesis that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi)

Perfect sink conditions are always attained in the release environment. Higuchi was the first to derive an equation to describe the release of a drug from an insoluble matrix as the square root of a time dependent

process based on Fickian diffusion. Simplified Higuchi equation is following;

$$Qt = KH(t)0.5$$

Where, Qt is the amount of drug released in time t and KH is the release rate constant for the Higuchi model. When the data is plotted as cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'KH'.

Korsmeyer and Peppas Model

The release rates from controlled release polymeric matrices can be described by the equation proposed by Korsmeyer *et al*.

$$Q = Ktn$$

Where, Q is the percentage of drug released at time 't', K is a kinetic constant incorporating structural and geometric characteristics of the tablets and 'n' is the diffusional exponent indicative of the release mechanism. For Fickian release, n=0.45 while for anomalous (Non-Fickian) transport, n ranges between 0.45 and 0.89 and for zero order release, n = 0.89.To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time.

RESULTS AND DISCUSSION

Preformulation Studies

The key component of a preformulation study is to characterize the chemical and physical properties of drug substance. FT-IR, UV-Visible spectrophotometry, melting point and solubility were used for identification of chemical and physical properties of Azithromycin. The first requirement of any preformulation study is the development of a simple analytical method for quantitative estimation in subsequent steps.

Most of drugs absorb light in UV range, UV-Visible spectrophotometry being a fairly accurate and simple method for estimation of drug. UV absorption maximum of Azithromycin in 0.1 N HCl was determined and exhibited characteristic absorption at 254 nm in PBS 6.8. The calibration curve of Azithromycin was linearly regressed. [21-23]

Beer's law is obeyed in concentration range of 10-40µg/ml, using regression analysis the linear equation (y = 0.0238x + 0.001), with correlation coefficient R2= 0.997 was obtained and in phosphate buffer solution pH 6.8 (y = 0.008x+0.001).

Determination of absorption maxima in 0.1N HCl

For estimation of λ_{max}, 10 mg of the drug Azithromycin was dissolved in 100 ml of 0.1 N HCl and scanning was done for working stock solution of 100µg/ml from 0.1 N HCl in range 400-200 nm. An absorption maximum was found to be 254 nm and this wavelength was used for further studies.

Standard Curve of Azithromycin in 0.1 N HCl

A solution of Azithromycin in 0.1 N HCl when scanned between 400 nm to 200 nm exhibits λ_{max} at 254 nm. Absorbance of the solution was taken in triplicate and average absorbance was calculated. Calibration curve in media was prepared by plotting absorbance with respect to concentration. The regression coefficient value R2 was found to be 0.9979. The absorbance values in 0.1 N HCl was given in figure.

Table 4: Data for standard plot of Azithromycin in 0.1 N HCl

Concentration (µg/ml)	Absorbance
2	0.049
4	0.104
6	0.147
8	0.191
10	0.235
12	0.287
14	0.322
16	0.371
18	0.431
20	0.49

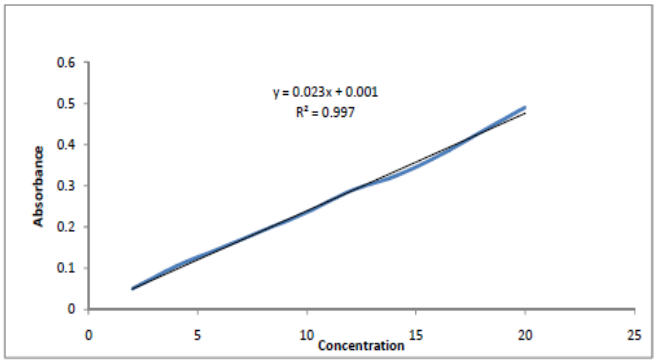

Fig. 1: Calibration curve of Azithromycin in 0.1 N HCl

The R2 value was found to be 0.9979 which is very close to 1. It has shown good linearity. So, from this result we concluded that molecular drug was in pure form.

Determination of absorption maxima in PBS 6.8

For estimation of λ_{max}, 10 mg of the drug Azithromycin was dissolved in 100 ml of 0.1N HCl and scanning was done for working stock solution of 100µg/ml from PBS pH 6.8 in range 400-200 nm. An absorption maximum was found to be 254 nm and this wavelength was used for further studies.

Table 5: Data for standard plot of Azithromycin in PBS 6.8

Concentration (µg/ml)	Absorbance
2	0.036
4	0.049
6	0.063
8	0.079
10	0.105
12	0.123
14	0.139
16	0.157
18	0.162
20	0.181

Standard Curve of Azithromycin in PBS 6.8

A solution of Azithromycin in PBS pH 6.8 when scanned between 400 nm to 200 nm exhibits λ_{max} at 254 nm. Absorbance of the solution was taken in duplicate and average absorbance was calculated. Calibration curve in media was prepared by plotting absorbance versus concentration. The regression coefficient value

R2 was found to be 0.991. The absorbance values in PBS 6.8 were given in figure.

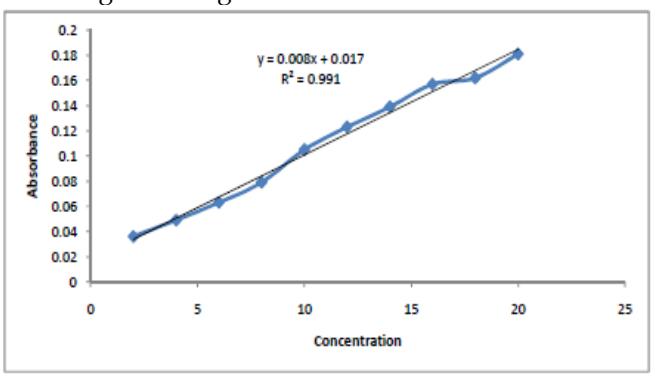

Fig. 2: Calibration curve of Azithromycin in PBS 6.8

The R2 value was found to be 0.991 which is very close to 1. It has shown good linearity. So, from result we concluded that molecular drug was in pure form.

Melting Point

Melting point determination is one of the analytical techniques applied to check the purity of pharmaceutical drugs. Capillary tube method widely used for the determination of the melting point. Melting point was found to be 116°C. Theoretical melting point of Azithromycin is 114°C. Practical melting point was found to be 116°C. Practical values were found to be near theoretical value so we can say that drug was in pure form.

Solubility

The solubility of drug is an important physicochemical property because it affects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. Solubility of the Azithromycin was determined in various solvents at room temperature. For solubility determination, excess amount of drug was added in each solvent (5.0 ml) in separate test tubes. These test tubes were occasionally stirred for 24 hours at room temperature. After24 hours each sample was filtered and filtrates was suitably diluted and analysed for the drug content using UV-Visible spectrophotometer. [24-27]

Drug is found to be freely soluble in methanol and ethanol so these solvents were used for formulation of the nanosuspensions as no single solvents can dissolve both drug and polymers. Solubility of Azithromycin in various solvents as found to be as follows

Table 6: Solubility of Azithromycin in different solvents

S. No.	Solvent Used	Inference
1	Water	Partially soluble
2	Ethanol	Freely Soluble
3	0.1 N HCl	Soluble
4	Methanol	Freely Soluble
5	Phosphate Buffer 6.8	Soluble

FTIR studies

The FT-IR spectrum of Azithromycin and interpretation of data is given in Table 7. The FT-IR spectrum of Azithromycin showed a O-H stretching vibration at 3433 cm^{-1}, C-H stretching vibration at 2968 cm^{-1}, C=O stretching at 1723 cm^{-1}, CH3-O (alkyl ether) stretching at 1458 cm^{-1}, C-O-C asymmetrical stretching (aliphatic ethers) stretching was seen at 1167 cm^{-1}. The FT-IR spectrum of drug sample was found to be concordant with the reference spectrum given in United State Pharmacopoeia. All these vibrational peaks at different wave numbers corresponding to its functional groups, confirming the purity of the drug as per established standards. From the FTIR scans it was observed that none of the polymers and drug showed any significant change in the peak of Azithromycin. In addition to it, none of the polymer tends to shift λ_{max} of Azithromycin. Azithromycin FTIR spectrum shows the peak at 3433 cm^{-1}. Rests of the major peaks are showed below in Table.

FTIR spectrum of Azithromycin

The infrared (FT-IR) spectra were obtained using a perkinelmer FT-IR spectrometer at resolution from 4000 to 400 cm^{-1}. The typical IR spectrum of pure Azithromycin reveals the presence of a peak at 3433 cm^{-1}, assigned to γOH stretching (broad-intermolecular hydrogen bonding) and one at 2968 cm^{-1}, corresponding γC-H (aliphatic) stretching vibration which were the identification peaks of Azithromycin. Azithromycin showed characteristics peaks at 1723 cm^{-1} which indicates C=O stretching vibration. 1458 cm^{-1} indicates that δCH3-O(alkyl ether) group is present, 1376 cm^{-1} indicates δCH2-O (alkyl ether) group is present. The other peaks and their groups shown in Table 7.

Table 7: Infrared spectral assignment of Azithromycin

S. No.	Frequency (cm^{-1})	Groups
1	3433	γOH stretching (broad-intermolecular hydrogen bonding)
2	2968	γC-H (aliphatic) stretching vibration
3	1723	γC=O Carbonyl ester stretch
4	1458	δCH3-O (alkyl ether)
5	1376	δCH2-O (alkyl ether)
6	1167	C-O-C asymmetrical stretching (aliphatic ethers)
7	1050	C-O-C symmetrical stretching (aliphatic ethers)

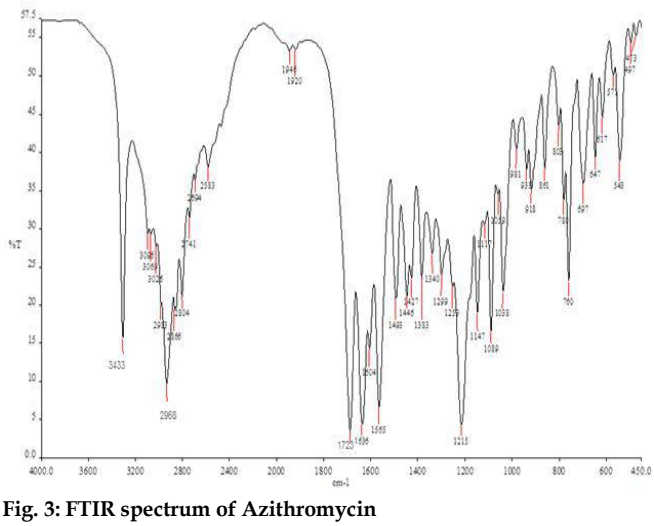

Fig. 3: FTIR spectrum of Azithromycin

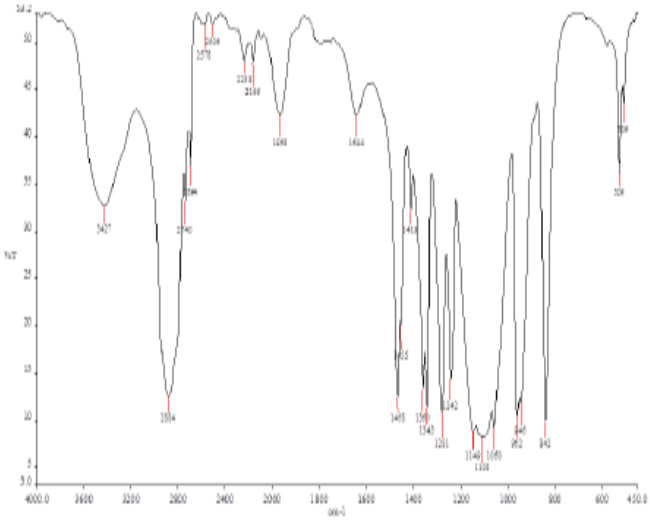

Fig. 4: FTIR spectrum of poly ethylene glycol 4000

FTIR spectra of Azithromycin + PEG 4000

FTIR spectrum of Azithromycin with polymers ethyl cellulose and PEG 4000 shows no alteration in peaks which confirms the absence of drug polymer interactions. Values of peaks with respect to their groups were shown in Table 8.

Table 8: Infrared spectral data of Azithromycin + poly ethylene glycol 4000

S. No.	Frequency (cm^{-1})	Groups
1	3433	γOH stretching (broad-intermolecular hydrogen bonding)
2	2968	γC-H (aliphatic) stretching vibration
3	1723	γC=O Carbonyl ester stretch
4	1458	δCH3-O (alkyl ether)
5	1376	δCH2-O (alkyl ether)
6	1167	C-O-C asymmetrical stretching (aliphatic ethers)
7	1050	C-O-C symmetrical stretching (aliphatic ethers)

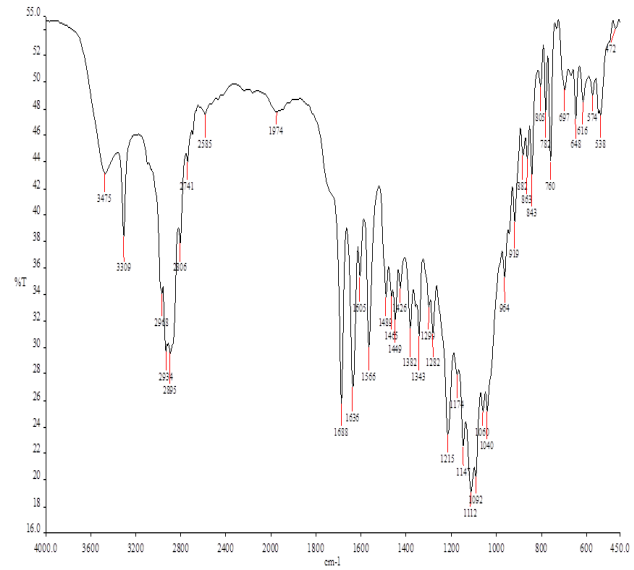

Fig. 5: FTIR spectrum of Azithromycin + Poly Ethylene Glycol 4000

In conclusion, the FT-IR spectrum, UV spectrum and melting point results suggested that Azithromycin was pure and good in quality and the estimation procedure was found to be quite reliable, accurate and suitable for formulation development.

Preparation and Optimization of Nanosuspensions [28-32]

The present invention relates to composition and method for preparing nanosuspensions or nanoparticles with controllable size and shape. These nanoparticulate systems have been used widely in various biomedical and pharmaceutical applications such as drug delivery system, targeted delivery system etc. Azithromycin is partially soluble in water. The solvent ethanol could dissolve the drug alone. For optimization of Azithromycin nanosuspensions, different formulations (F1-F9) were prepared using the various quantities of polymers and change in rotation speed. Formulation with maximum entrapment efficiency and maximum drug content considered as optimized formulation. For optimization, trial batches were formulated by keeping the drug concentration constant and varying the polymer concentrations. Formulation F3 was found to possess good entrapment efficiency and good percentage yield. Further batches were prepared by varying the parameter of rotation speed. For this purpose rotation speed was varied in between the range 250 2000. At 2000 rpm the nanosuspensions were found to possess good entrapment efficiency and percentage yield. Increase in rotation speed increases the rate of evaporation. So, the organic phase gets more evaporated, when the rotation speed was increased. This results in decrease in particle size which further leads to decrease in drug entrapment efficiency.

Scanning Electron Microscopy

The nanosuspensions of Azithromycin were spherical and having a rough surface as shown in figure. SEM of nanosuspensions had taken before dispersion in 0.1 N HCl showing no pores on the surface but SEM taken after dispersion in 0.1 N HCl because of solubilization of PEG from ethyl cellulose microspheres when they are dispersed in acidic media.

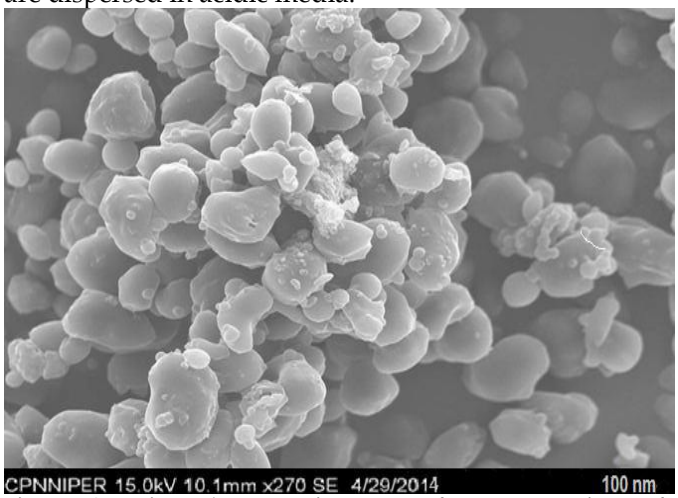

Fig. 6: Scanning Electron Microscopy of nanosuspensions of Azithromycin

Characterization of Nanosuspensions of Azithromycin

Percentage yield

From the formulations, it was observed that increase in the concentration of polymers increases the percentage yield of the nanosuspensions, as poly ethylene glycol acts as matrix forming polymer, so increase in concentration of polymers leads to increase in percentage yield. Rotation speed also has the effect on percentage yield of the nanosuspensions. Increase in rotation speed leads to decrease in particle sizes. Effect of concentration and speed is shown in Table 9.

Drug Entrapment

Increase in rotation speed leads to decrease in particle size, which directly leads to decrease in entrapment efficiency. Drug entrapment increased with increase in ethyl cellulose concentration as ethyl cellulose forms matrix in which the drug gets entrapped. Effect of polymer and rotation speed on drug entrapment shown in table.

Table 9: Percentage yield and drug entrapment of various formulations

Formulation Code	% Yield	Drug Entrapment
F1	9.98	6.5
F2	18.18	38.91
F3	42.53	63.16
F4	54.45	71.34
F5	62.77	78.49
F6	54.60	83.72
F7	54.55	61.4
F8	74.56	80.95
F9	73.72	94.90

In vitro Drug Release

In vitro release of nanosuspension is difficult as they tend to stick on the dissolution basket. *In vitro* release studies of nanosuspension was carried out in USP XII Basket. Azithromycin was found to be absorbed on both stomach and intestine, so the release studies had to be carried out at both acidic and intestinal pH. Drug was found to be absorbed in stomach for 2 hours and then get absorbed in intestine. So, the release was studied at the in acidic medium for 2 hours at the interval of 1 hour and in PBS pH 6.8 for 6 hours at the interval of 2 hours.

***In vitro* drug release of F4**

The drug showed release in a controlled manner. In first 2 hours, when given in acidic media release showed by the nanosuspension was 24.45 and 36.27% respectively. The drug release in PBS 6.8 was noted at the interval of 2 hours. Release was found to be 42.25, 46.25 and 47.98% at 4, 6 and 8 hours respectively. Release was found to be lesser than F4 as F3 contain only 125 mg poly ethylene glycol which forms the matrix, so release can be easy in F4 as compared to F5.

***In vitro* dug release of F5**

Release of the drug loaded nanosuspension in acidic medium was 24.45 and 39.15 respectively. In PBS 6.8 the drug shows release in a controlled manner. Release was found to be 42.25, 46.52 and 50.18% at 4, 6 and 8 hours respectively in PBS 6.8. Release in F5 was found to be almost similar with F4 in acidic medium but the release is increased in PBS pH 6.8. Release was greater as it was formulated with 250 mg HPMC concentration.

Table 10: Release data for F4

Time (h)	√T	Log T	Absorbance	Conc. (µg/ml)	Amt.(mg/ml) in 900 ml	% Drug Release	Cumulative % release
0	0	0	0	0	0	0	0
1	1	0	0.006	0.217	0.978	0.978	24.45
2	1.414	0.301	0.009	0.304	1.369	1.370	36.27
4	2	0.602	0.014	0.375	1.68	1.690	42.25
6	2.449	0.778	0.0145	0.412	1.85	1.860	46.25
8	2.82	0.903	0.0146	0.45	2.02	2.03	47.98

Table 11: Release data for F5

Time (h)	√T	Log T	Absorbance	Conc. (µg/ml)	Amt. (mg/ml)in 900 ml.	% Drug Release	Cumulative % release
0	0	0	0	0	0	0	0
1	1	0	0.006	0.217	0.978	0.978	24.45
2	1.414	0.301	0.009	0.347	1.565	1.566	39.15
4	2	0.602	0.0141	0.375	1.680	1.690	42.25
6	2.449	0.778	0.0143	0.412	1.856	1.860	46.52
8	2.82	0.903	0.0144	0.425	1.912	1.919	50.18

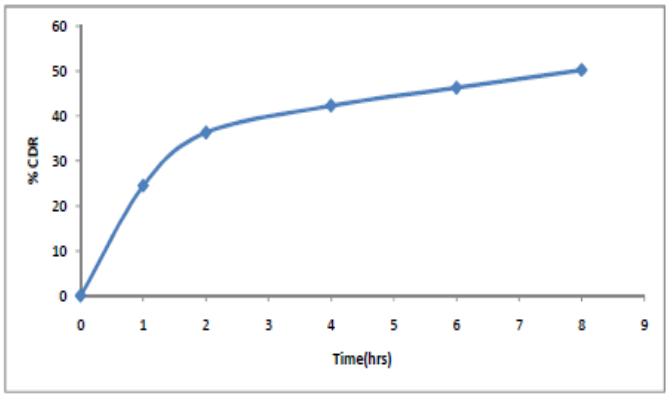

Fig. 7: *In vitro* drug release of F4

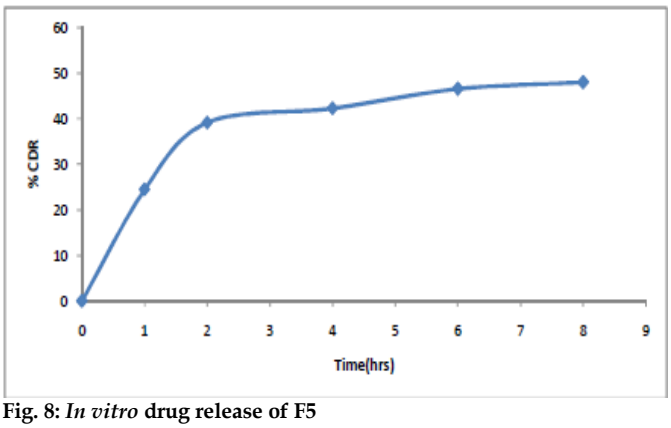

Fig. 8: *In vitro* drug release of F5

In vitro drug release of F6

The formulation optimized using 250 mg poly ethylene glycol and 500 rpm shows good release. In acidic medium release was noted at interval of 1 hour release was found to be 24.45 and 36.85% at 1 and 2hrs respectively. In PBS 6.8 release noted at intervals of 2 hours. Release was found to be 47.98, 57.75 and 66.26 in 4, 6 and 8 hours respectively in PBS pH 6.8. Release of the formulation *i.e* F6 was more as it was formulated with more rotation speed i.e. 1500 rpm which leads to decrease in particle size due to increase in kinetic velocity which further leads to lesser entrapment so the drug is released at much faster rate.

Table 12: Release Data for F6

Time (h)	√T	Log T	Absorbance	Conc. (µg/ml)	Amt.(mg/ml) in 900 ml.	% Release	Cumulative % release
0	0	0	0	0	0	0	0
1	1	0	0.006	0.260	1.17	1.173	24.45
2	1.414	0.301	0.008	0.347	1.56	1.566	36.85
4	2	0.602	0.014	0.45	2.02	2.028	47.98
6	2.449	0.778	0.015	0.525	2.362	12.367	57.75
8	2.82	0.903	0.0157	0.587	2.64	2.65	66.26

Table 13: Release data for F9

Time (h)	√T	Log T	Absorbance	Conc. (µg/ml)	Amt.(mg/ml) in 900 ml.	% Release	Cumulative % release
0	0	0	0	0	0	0	0
1	1	0	0.011	0.434	1.95	1.95	48.91
2	1.414	0.301	0.013	0.521	2.34	2.35	58.75
4	2	0.602	0.015	0.6	2.7	2.70	67.61
6	2.449	0.778	0..016	0.675	3.03	3.04	76.11
8	2.82	0.903	0.017	0.762	3.43	3.44	86.06

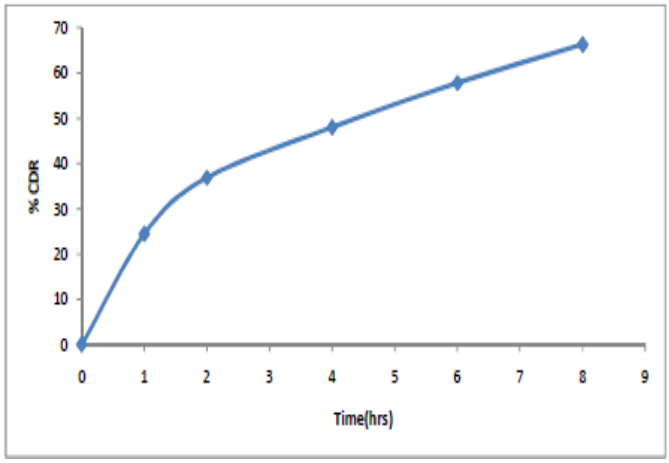

Fig. 9: *In vitro* **drug release of F6**

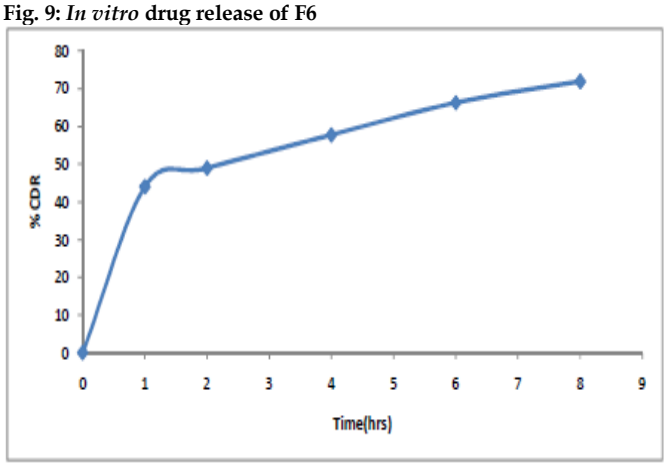

Fig. 10: *In vitro* **drug release of F8**

In vitro drug release of F8

The formulation optimized using 250 mg PEG and 1000 rpm shows good release. In acidic medium release was noted at interval of 1 hour release was found to be 44.02 and 48.96 at 1 and 2hrs respectively. In PBS 6.8 release noted at intervals of 2 hours release was found to be 57.75, 66.26 and 71.95 in 4, 6 and 8 hours respectively. Release was greater than F6 as it was formulated with more rotation speed this results in decreased particle size and quick drug release.

In vitro drug release of F9

The formulation optimized using 250 mg poly ethylene glycol and 2000 rpm shows good release. In acidic medium release was noted at interval of 1 hour release was found to be 48.91 and 58.75% at 1 and 2 hours respectively. In PBS 6.8 release noted at intervals of 2 hours. Release was found to be 67.61, 76.11 and 86.06 in 4, 6 and 8 hours respectively.

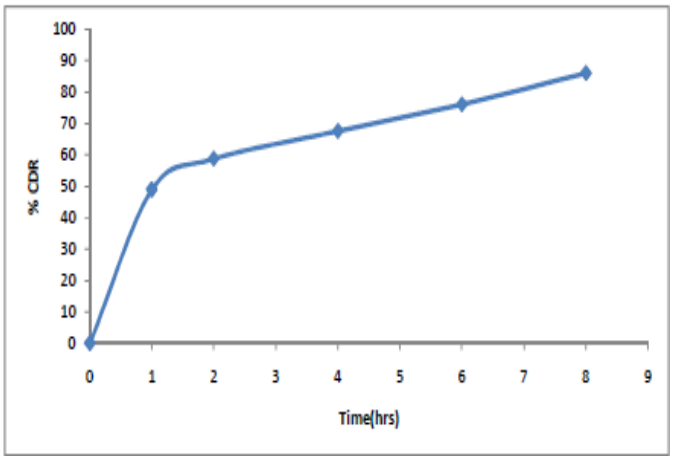

Fig. 11: *In vitro* **drug release of F9**

Comparison of release of various formulations

F9 was found to have excellent release with good gastric retention. This shows that increase in poly ethylene glycol concentration leads to increase in drug release and increase in rotation speed helps to enhance the drug release. This is because of the fact that PEG forms core matrix which slows the release of the drug but increase in rotation speed leads to decrease in particle size which can diffuse through the pores present in the surface of nanosuspension.

Table 14: Comparison of release of various formulations

Time (Hours)	% Cumulative Drug Release				
	F4	F5	F6	F8	F9
0	0	0	0	0	0
1	24.45	24.45	29.34	44.02	48.91
2	34.26	39.15	39.16	48.02	58.75
4	42.25	42.25	50.7	57.75	67.61
6	46.25	46.52	59.19	66.26	76.13
8	47.98	50.18	66.29	71.95	86.06

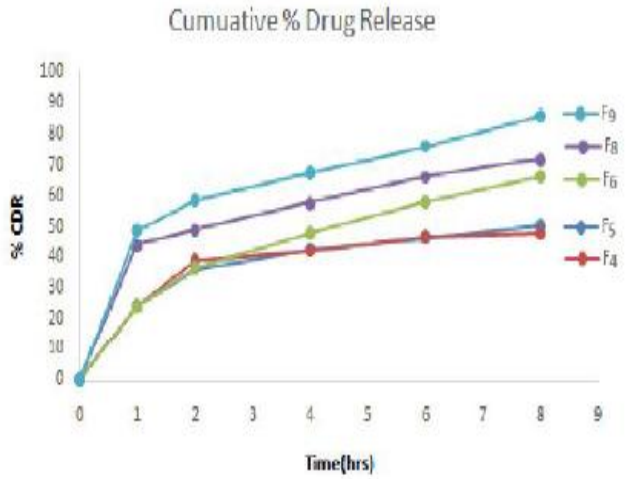

Fig. 12: Comparative *in vitro* release study of Azithromycin nanosuspensions

Higuchi model

Higuchi model was plotted between % cumulative drug release and square root of time. When the data is plotted as cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism.

Table 15: Higuchi data for various formulations

√T	Cumulative % release				
	F4	F5	F6	F8	F9
1	24.45	24.45	29.34	44.02	48.91
1.414	36.27	39.15	36. 85	48.96	58.75
2	42.25	42.25	47.98	57.75	67.61
2.449	46.25	46. 52	57.75	66.26	76. 11
2.82	47.98	50.18	66.29	71.95	86.06

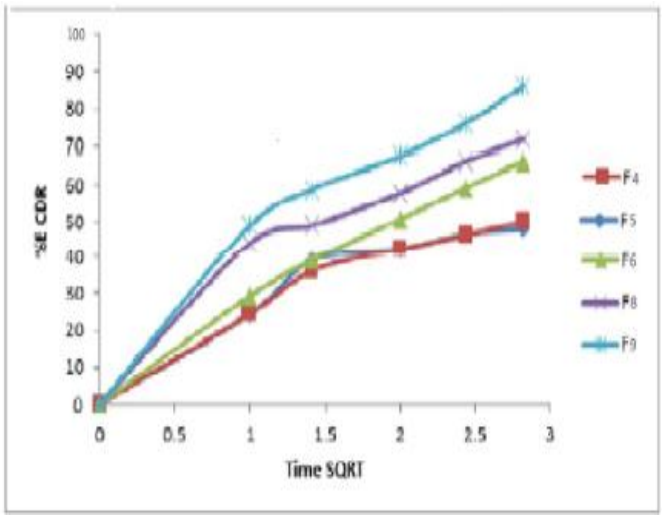

Fig. 13: Higuchi modeling of various formulations

First Order Release

Table 16: First order release data for various formulations

Log T	Log % cumulative amount remained to be released				
	F4	F5	F6	F8	F9
0	2	2	2	2	2
0.301	1.804	1.784	1.784	1.7	1.615
0.601	1.761	1.761	1.692	1.62	1.575
0.778	1.728	1.728	1.61	1.528	1.378
0.903	1.604	1.716	1.527	1.44	1.391

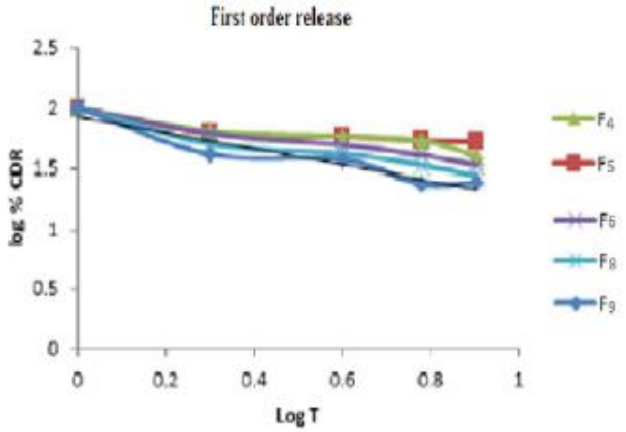

Fig. 14: First order release of various formulations

Korsmeyer Peppas Model

Table 17: Data for Korsmeyer Peppas model

Log	T Log % cumulative release				
	F4	F5	F6	F8	F9
0	0	0	0	0	0
0	1.3882	1.3882	1.4674	1.6436	1.6893
0.301	1.5595	1.5927	1.5928	1.6898	1.7690
0.602	1.6258	1.6258	1.7050	1.7615	1.8300
0.778	1.6651	1.6676	1.7722	1.8212	1.8814
0.903	1.7005	1.6810	1.8214	1.8570	1.9297

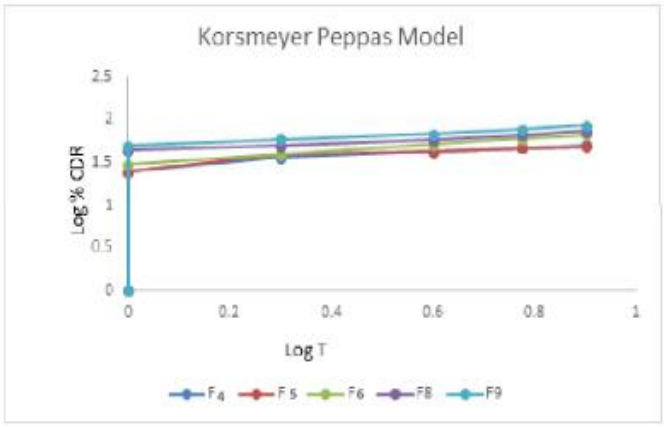

Fig. 15: Korsmeyer Peppas modeling of various formulations

Regression coefficients values of Azithromycin in various release models

Regression coefficient value is nearly one in case of zero order, so the release is likely to follow zero order, which shows that the nanosuspensions of Azithromycin showed controlled release and the nanosuspension release was concentration independent. The above result reveals the possibility of development of nano drug delivery system using EC/PEG polymer blend for sustained and local delivery to the stomach.

Table 18: Regression coefficient values (R2) values of Azithromycin Nanosuspensions

Formulation Code	R^2 values			
	Higuchi Model	Peppas Model	Zero order	First order
F4	0.944	0.449	0.905	0.774
F5	0.903	0.435	0.749	0.944
F6	0.987	0.471	0.971	0.952
F8	0.922	0.396	0.991	0.950
F9	0.953	0.399	0.985	0.810

The results of the present investigation showed the problems associated with the oral bioavailability of Azithromycin could be overcome by incorporating it into a novel formulation i.e. nanosuspensions of Azithromycin.

Oral controlled release of Azithromycin can be achieved by solvent antisolvent precipitation technique using poly ethylene glycol as polymers.

IR spectra revealed that, there was no interaction between drug and polymers. So, the drug was found to be compatible with polymers.

Increase in rotation speed leads to decrease in particle size, which directly leads to decrease in entrapment efficiency. Drug entrapment increased with increase in poly ethylene glycol concentration in which the drug gets entrapped.

Study shows that the drug release was decreased when the concentration of poly ethylene glycol was increased. Prepared nanosuspensions showed zero order release which suggests that the drug release was in controlled manner.

It can be concluded from the result obtained that the formulations developed for gastro retentive drug delivery of Azithromycin possessed better bioavailability, better stability, and higher entrapment efficiency suggested that gastro retentive formulation provided a better mode of systemic delivery of Azithromycin.

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Source of Support: Nil, Conflict of Interest: None declared.