International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 04 (G); April 2019; Page No.18476-18481
DOI: http://dx.doi.org/10.24327/ijcar.2019.18481.3533

Research Article

STUDY OF ENHANCEMENT OF BIOAVAILABILITY OF AZITHROMYCIN DIHYDRATE BY SOLID DISPERSION WITH POLOXAMERS

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A R T I C L E I N F O

Article History:
Received 06th January, 2019
Received in revised form 14th February, 2019
Accepted 23rd March, 2019
Published online 28th April, 2019

Key words:

Azithromycin, Bioavailability enhancement, Poloxamers, Solid dispersions, carriers

A B S T R A C T

In the present study, an attempt was made to study enhancement of bioavailability of azithromycin, sparingly water soluble macrolide antibiotic, by use of solid dispersion technique, using 1:1 and 1:2 w/w ratios of three carriers- poloxamer 188, poloxamer 407 and polyethylene glycol 20,000. Two methods were used to prepare solid dispersions - solvent evaporation and freeze drying/lyophilisation. Drug – excipient incompatibilities were studied using Fourier transform Infra red spectroscopy and Differential scanning calorimetry. IR spectra did not show any significant changes in characteristic peaks of pure drug and excipients. DSC thermograms showed characteristic endothermic peaks at respective melting points of pure drug and excipients, thus ruling out of any undesirable interactions. Drug contentof all prepared 12 solid dispersions was found to be satisfactory. Prepared solid dispersions were characterised using In-Vitro dissolution studies, kinetic modelling patterns, Scanning electron microscopy images and X Ray Diffractograms. . From the in vitro drug release profile, it could be observed that, formulation FSD2 (prepared using freeze drying method with Poloxamer 188) drug release was 88.71%,FSD6 (prepared by freeze drying method with PEG 20,000) drug release was found to be 87.14%, FSD1 (prepared by freeze drying method with Poloxamer 188) drug release was found to be 86.87%, ESD2 (prepared by solvent evaporation method with Poloxamer 188)drug release was found to be 87.5% .From the kinetics, it could be possibly stated that the release from the matrix was through diffusion. The XRD pattern depicted by solid dispersions reveals a decrease in the number of 2θ peaks which probably represents decrease in crystallinity. SEM images also showed the nature of particles to be highly porous. Thus, it can be concluded that, solid dispersion technique using freeze drying method, and carriers such as poloxamers has proven to be highly effective to improve bioavailability of poorly water-soluble drugs.

INTRODUCTION

Solubility is defined in quantitative terms as the concentration of solute in a saturated solution at a certain temperature, and in a qualitative way, it can be defined as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. Solubility is an intrinsic material property that can be altered only by chemical modification of the molecule[1].

It is estimated that ~40% of active substances identified through combinatorial screening programs are difficult to formulate as a result of their lack of significant solubility in water. Solubility and bioavailability are the overwhelming challenges in drug development[2].One such widely and successfully employed and established technique for improvement of drug solubility is solid dispersions.

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Solid dispersions may be defined as a drug-polymer two component system in which drug is dispersed into an inert carrier at solid state, prepared by melting, solvent or melting-solvent method. Solid dispersions have been classified into:

1. First generation SD - crystalline carriers, second generation SD – Amorphous carriers, third generation SD – self emulsifying or surfactant assisted amorphous carriers (Based on carrier used.)
2. Binary SD - drug and carrier used, Ternary SD- drug, carrier and surfactant (Based on number of component used).
3. Eutectic mixtures, solid solutions, glass solutions, glass suspensions (Based on structure of solid dispersions)[3].

Successful dispersion of solid into the carrier depends on nature of solvent, carrier used, method of preparation of solid dispersions. Proper selection of carriers into which the solid has to be dispersed is an important part of the study. Various

carriers have been used to prepare solid dispersions since the invention. Carriers in solid dispersions fall into following classes: Polymers, cyclodextrins, carbohydrates, surfactants, superdisintegrants, dendrimers, hydrotopes, polyglycolised glycerides acids, and miscellaneous.

First generation carriers: Sugars, organic acid, and urea. Second generation carriers: Starch derivatives like cyclodextrins, Cellulose derivatives like ethylcellulose, hydroxyl propylcellulose, hydroxyl propylmethylcellulose and fully synthetic polymers like polyethylene glycols,povidone, polymethacrylates. Third generation carriers: These includes-Tween 80, poloxamer 408 (Kolliphor P 408), Gelucire 44/14.
Azithromycin dihydrate is a macrolide antibiotic, belonging to BCS Class II (Characterised by low solubility and high permeability), used for treatment of variety of bacterial infections.

Poloxamers are non ionic tri block co polymers composed of a central hydrophobic chain of polyoxypropylene (PPO) flanked by two hydrophobic chains of polyoxyethylene (PEO). Poloxamer is available in different grades based on the physical parameter like Molecular Weight, %Weight of oxyethylene etc. The common available grades are poloxamer (68, 88, 98, 108, 124, 188, 237, 338, and 407). Poly Ethylene glycol is a polyether compound with many pharmaceutical applications[4].

Though solid dispersions have been extensively used to enhance solubility, there are also other pharmaceutical applications of solid dispersions such as use in preparation of orodispersible tablets, taste masking, mouth dissolving tablets, and controlled release tablets. In this research work, an attempt was made to improve solubility and bioavailability of azithromycin dihydrate, a poorly soluble macrolide antibiotic, whose aqueous solubility is low and absolute bioavailability is 38 % following oral administration. Carriers such as poloxamer 188(Kolliphor P 188), Poloxamer 407(Kolliphor P 407), Poly Ethylene Glycol 20,000(PEG 20,000) were used in different concentrations to improve aqueous solubility of azithromycin dihydrate.

MATERIALS AND METHODS

Materials used for the research were of analytical grade and of highest purity. Azithromycin dihydrate was a kind gift from Aurobindo Pharma Ltd. Poloxamer 188(Pluronic®F-188), Poloxamer 407(Pluronic®F-407), Poly ethylene glycol 20,000 were obtained as gift samples from BASF India ltd., Acetone, methanol, ethanol, PVP used in this work were obtained from Qualichems, Fischer scientific, Changshu yangyuan chemical, Oxford laboratory respectively. Distilled water was used to prepare aqueous solutions and was obtained by a suitable process.

Determination of λ max: The wavelength at which the drug absorbs to its maximum is called as λ max. As a part of preliminary studies, λ max of drug was found out using stock solution of 1 mg/ml, first by dissolving drug in small quantity of methanol and diluted with 100 ml of phosphate buffer (pH 6.8). The stock solution was serially diluted to get solutions in the range of 2-12 µg/ml and λ max of the solution was found out by scanning from 200 - 400 nm in a double beam UV-Visible spectrophotometer.

Determination of Calibration Curve

Stock solution of 1 mg/ml of azithromycin dihydrate was prepared. The stock solution was serially diluted to get solutions in the range of 2-20 µg/ml. The absorbances of the different diluted solutions were measured in a double beam UV-Visible spectrophotometer at 210 nm. A calibration curve was plotted by taking concentration of solution on X axis and absorbance on Y axis and correlation coefficient 'r' was calculated.

Determination of Melting Point

Melting point of the drug was determined by taking a small amount of the drug in a capillary tube that was closed at one end. The capillary tube was placed in thermionic melting point apparatus and the temperature at which the drug melted was noted. Average of three readings was taken.

Drug Excipients Interaction study by FTIR

FTIR emission spectrometer (Shimadzu, Japan) was used to record the FTIR spectrum of the drugs from 400 to 4000 cm^{-1} to confirm compatibility between the excipients used and pure drug in the formulation. FTIR spectra of pure drug, along with physical mixture of polymers and drug were taken separately.The sample was grounded with KBr and pressed to a suitablesize disk for measurement.

Drug Excipients Interaction study by Differential Scanning Calorimetric (DSC) analysis

DSC study was used to investigate and predict any physicochemical interactions between components in a formulation. 2mg of sample was placed in a 50 µL perforated aluminium pan and sealed. Heat runs for each sample were set from 50 ^{0}C to 300 ^{0}C using nitrogen as purging gas and the samples were analyzed.

Preparation of solid Dispersions

Solvent Evaporation Method

Solid dispersions of azithromycin were prepared in drug: carrier weight ratios of 1:1 and 1:2 using solvent evaporation method. 100 mg of azithromycin dihydrate was dissolved in 10 mL of 96% ethanol and the carriers (poloxamer 188,407 and PEG 20,000) were then added separately (as per formulae showed in Table 1) and dissolved at 40 °C, following continuous mixing on a hot plate to obtain a clear solution. Afterwards, the solvent was allowed to evaporate[5]. The prepared solid dispersions were collected, sieved, and stored in desiccators until subsequent analysis. The process of evaporation was continued until a constant weight was obtained. Overall, the yield was approximately 98%.

Freeze drying/Lyophilisation method[6]

Solid dispersions of azithromycin and carriers (Poloxamer 188, 407 and PEG 20,000) were prepared in drug:carrier ratios of 1:1 and 1:2 using lyophilisation method. Pure drug was weighed and dispersed into carrier solution. Dispersion was continuously stirred using magnetic stirrer at 70-100 rpm until slightly turbid solution was obtained. To the above solution being stirred, few drops of 25 % liquid ammonia were added until a clear solution was obtained. Clear solution was frozen to a temperature of -45˚C (Unicryo freezer) lyophilized in a freeze dryer (Lark, Penguin classic plus, 6 kg, Freeze dryer) at a temperature of -40^{0}C and vacuum of 60x10^{-3} Mbar. The

freeze dried mass was then sifted through 60 mesh sieve and stored in dessicators until further use.

Characterisation and Evaluation of solid dispersions[7,8]

Drug Content: An accurately weighed quantity of solid dispersion equivalent to 100mg of azithromycin was taken into a 100ml volumetric flask, dissolved in methanol and suitably diluted with 6.4 pH Phosphate buffer. The content of azithromycin was determined spectrophotometrically at 210 nm against suitable blank using Double beam UV-visible spectrophotometer (UV 1700, Shimadzu, Japan) and amount of drug in each formulation was calculated.

In-vitro dissolution studies

Quantity of solid dispersion equivalent to 20mg of azithromycin was filled in hard gelatin capsule by hand filling method. The dissolution study of capsules was conducted using dissolution testing USP apparatus 1 (basket method) in 900 ml of 6.4 pH Phosphate buffer at 37±0.5ºC and at a speed of 50 rpm. Aliquot of 5ml was withdrawn at predetermined time interval and equivalent amount of fresh medium was replaced to maintain a constant volume after each sampling and analyzed spectrophotometrically at 210 nm against suitable blank using UV-visible spectrophotometer.The amount of azithromycin dihydrate released fromeach solid dispersion was calculated and plotted against time and compared with pure drug.

Kinetics of in vitro drug release[9]

To study the release kinetics of in vitro drug release, data obtained from in vitro release study were plotted in various kinetic models:
Zero order as % drug released versus time, First order as log % drug retained versus time, Higuchi as % drug released versus √time, Korsmeyer-Peppas as log % drug released versus log time.

Tokyo, Japan).Scanning Electron Microscopy (SEM) SEM studies was used to reveal the surface morphological properties of the solid dispersion indicating whether the solid dispersion was in amorphous state or crystalline state.

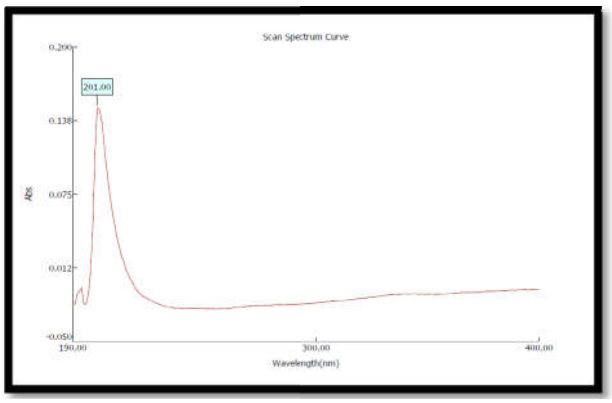

Figure 1 UV spectrum scan of Azithromycin dehydrate

Table 1 Standard curve in pH 6.8 phosphate buffer

S.No.	Concentration(µg/ml)	Absorbance
1	4	0.148
2	8	0.379
3	12	0.582
4	16	0.797
5	20	0.951

Table 2 Melting point determination of azoithromycin dihydrate

Trial number	Melting point(^{0}C)	Average of three readings (^{0}C)
1	112	
2	114	114
3	118	

Table 3 Formulation table of solid dispersions of azithromycin dihydrate with carriers

Formulation code	Solvent Evaporation Method						Freeze Drying Method					
(All Ingredients in mg)	ESD1	ESD2	ESD3	ESD4	ESD5	ESD6	FSD1	FSD2	FSD3	FSD4	FSD5	FSD6
Azithromycin dihydrate	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Poloxamer 188	1000	2000	--	--	--	--	1000	2000	--	--	--	--
Poloxamer 407	--	--	1000	2000	--	--	--	--	1000	2000	--	--
Polyethylene glycol 20,000	--	--	--	--	1000	2000	--	--	--	--	1000	2000

X ray Diffraction Study

Vacuum grease was applied over a glass slide to adhere the sample. About 100 mg of sample was sprinkled over it to make a layer with a thickness of 0.5 mm. All the experiments were performed on an XRD instrument (Japan Science D/max 2500) with a sensitivity of 0.001. The samples were exposed to CuKα radiation under 40 kV and 40 mA over the 2θ range from 5° to 90° in increments of 0.12°/s every 0.02°. The samples used for this study were freshly prepared (48 h prior) and preserved in a desiccator before sending to the facility[10].

Scanning Electron Microscopy Study[11]

Prior to imaging, samples were mounted onto aluminum stages using double-sided carbon tape and sputter-coated using an electron microscopy sputter coater equipped with an Au source. Samples were exposed to the Au for 2.5 min and then examined using a Hitachi S-4800 field emission scanning electron microscope (Hitachi High-Technologies Corp.;

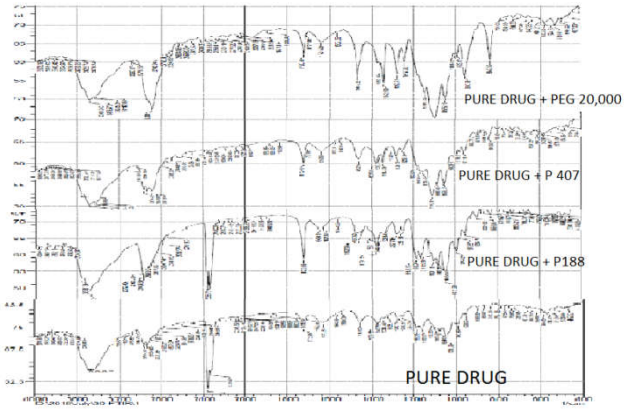

Figure 2 Drug Interaction study by FTIR

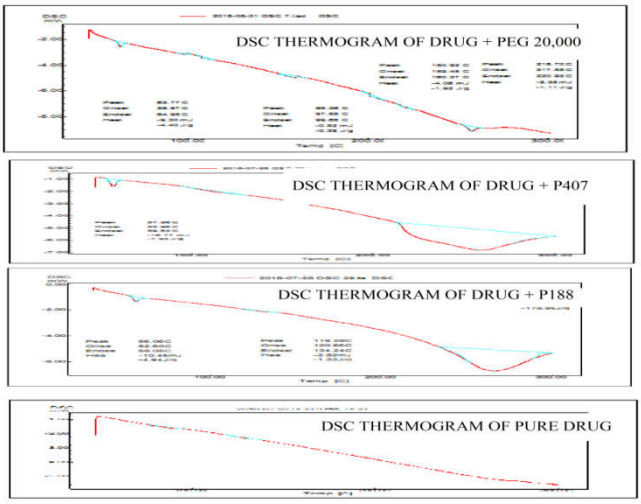

Figure 3 DSC thermograms of pure drug and carriers

In Vitro Dissolution Profile

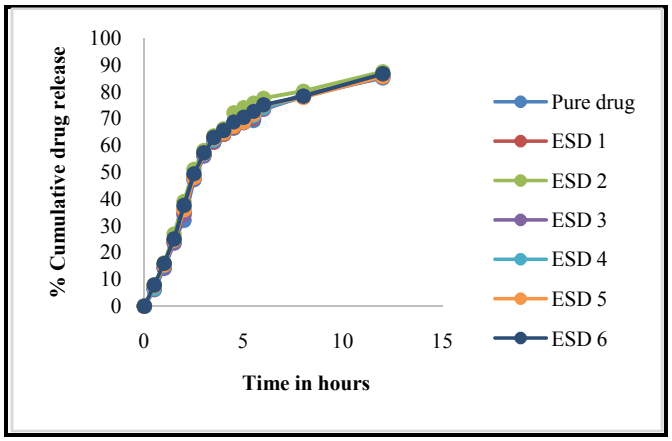

Figure 4 In vitro dissolution profile of solid dispersions by solvent evaporation method

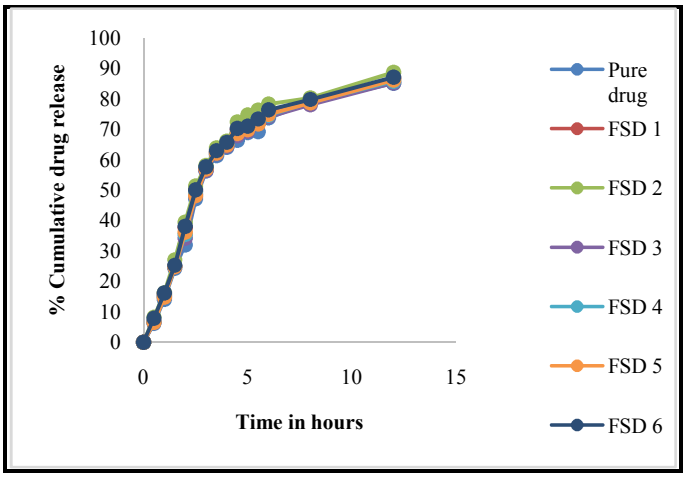

Figure 5 In vitro dissolution profile of solid dispersions by freeze-drying method

Table 4 Release order kinetics with optimized correlation coefficient

Formulation	Zero order	First order	Higuchi	Korsmeyer–Peppas	n value
FSD2	0.733	0.843	0.981	0.858	0.668
FSD6	0.811	0.848	0.960	0.869	0.676
FSD1	0.739	0.848	0.945	0.860	0.679
ESD2	0.727	0.844	0.920	0.857	0.670

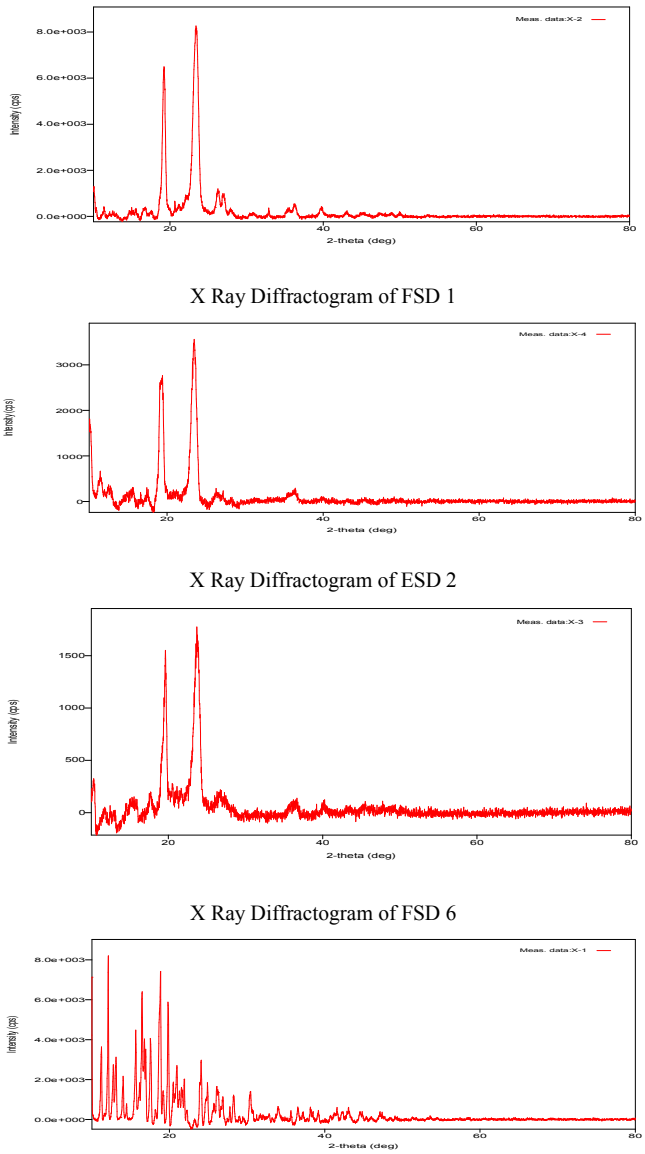

Figure 6 X Ray Diffractograms of optimised formulations

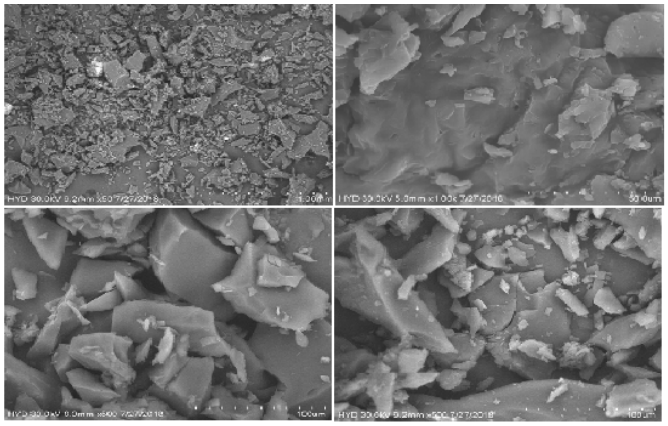

Figure 7 SEM Photographs of optimised formulations

RESULTS AND DISCUSSION

Uv Spectrum of Azithromycin Dihydrate

From the stock solution I, suitable dilutions were made to obtain 12µg/ml solution of azithromycin dihydrate. This solution was scanned for maximum absorption wavelength

using UV-spectrophotometer in the range of 200-400nm. The absorption maxima for azithromycin dihydrate were found to be 210nm and this was used as λmax in this work. The standard graph and whole analysis was performed in pH 6.8 phosphate buffer.

Calibration Curve of Azithromycin Dihydrate

The standard concentrations of Azithromycin dihydrate were prepared in pH 6.8 phosphate bufferand absorbance was measured at 210 nm. The observations are tabulated. The standard graph of azithromycin dihydrate in pH 6.8 phosphate buffer showed good linearity with R^2 value 0.9994 in the concentration range of 4-20µg/ml.

Melting Point Determination

Melting point of Azithromycin dihydrate was determined by capillary method and found to be 114 ^{0}C which correlates with standard melting point value of azithromycin dihydrate indicating purity of the drug sample.

Compatibility Studies- FTIR

FT-IR spectra were performed for pure drug as well as physical mixture of pure drug and carriers such as poloxamer 188, poloxamer 407, and polyethylene glycol 20,000.The IR spectra of pure drug showed characteristic peaks at 1740 cm^{-1} due to asymmetric C-O-C stretching, strong peak at 1710 cm^{-1} due to c=o carbonyl stretch, a medium intensity peak at 2250 cm^{-1} due to CN stretch, a strong broad intensity peak at 3400 cm^{-1} due to OH functional group.

IR spectra of pure drug along with excipients also showed characteristic peaks of functional groups of drug more or less at same frequencies, thus showing no incompatibility between pure drug and excipients.

Compatibility Studies-DSC

In this study, DSC was performed for one of its classical applications – investigating possible interactions between a drug and excipients used. The DSC thermograms of pure drug azithromycin dihydrate and carriers-Poloxamer 188, Poloxamer 407, PEG 20,000have shown endothermic peaks forpure drug, excipient and drug–excipient mixtures at their respective melting point with no much deviation. Hence it may be concluded that slight changes observed in melting endoderm of drug were likely due to presences of excipients and not due to any significant interactions between drug and polymer.

In – Vitro Dissolution Studies

In vitro studies reveal that there was a significant increase in the dissolution rate of azithromycin dihydrate in all the solid dispersion formulations when compared to pure drug (85.15%) after 12 hrs. From the in vitro drug release profile, it could be observed that, formulation FSD2 (prepared using freeze drying method with ratio 1:2 w/w of drug with Poloxamer 188) drug release was 88.71%,FSD6 (prepared by freeze drying method ratio 1:2 w/w of drug with PEG 20,000) drug release was found to be 87.14%, FSD1 (prepared by freeze drying method in ratio 1:1 w/w of drug with Poloxamer 188) drug release was found to be 86.87%, ESD2 (prepared by solvent evaporation method in 1:2 w/wratio of drug with Poloxamer 188)drug release was found to be 87.5% .

This may be attributed to the increase in drug wettability, conversion to amorphous form and solubilization of the drug due to hydrophilic carrier. The graphical representation of solid dispersions with pure drug is depicted in Figures 4 and 5.

Release Order Kinetics Ofazithromycin Solid Dispersion For Fsd2, Fsd6, Fsd1, Esd2

Four formulations - FSD2, FSD6, FSD1, and ESD2 were selected to be better amongst all the 12 formulations based on results from i*n-vitro* drug release data and were subjected to kinetic studies. From the kinetic plots, it is apparent; that the regression coefficient value was closer to unity in case of Higuchi order plot i.e.0.981, 0.960, 0.945, 0.920 indicates that the drug release follows a Higuchi order mechanism. Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modelling such as Zero order and first order plots. The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value. From the kinetics, it could be possibly stated that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e. 0.668, 0.676, 0.679, 0.670 suggesting that the drug release from solid dispersions was anomalous Non fickian diffusion (Table 4).

X-Ray Diffractograms

The optimized azithromycin solid dispersions were examinedto find out whether the solid dispersions of various drug polymer ratios are crystalline or amorphous. The presence of numerous distinct 2θ peaks in the XRD spectrum of pure drug indicates that azithromycinwas present as a crystalline material. The XRD pattern depicted by solid dispersions reveals a decrease in the number of 2θ peaks which probably represents decrease in crystallinity(**Figure 6**). The enhancement in the dissolution rate of the drug from the optimized Azithromycinsolid dispersion is ascribed to the marked reduction in the crystallinity of the drug.

Scanning Electron Microscopy

SEM photographs for optimisedformulationsare shown in **Figure 7**. The drug crystals seemed to be smooth-surfaced, irregular in shape and size. In case of Solid dispersions, it was difficult to distinguish the presence of drug crystals. The drug surface in solid dispersion seems to be more porous in nature.

CONCLUSION

In the current research work, capability of solubility improvement by use of poloxamers and high molecular weight polyethylene glycols have been investigated. Model drug used was azithromycin dihydrate, a poorly soluble macrolide antibiotic. Solid dispersions were prepared using poloxamer 188, poloxamer 407, polyethylene glycol 20,000 by solvent evaporation method and freeze-drying method in drug:carrier ratios of 1:1 and 1:2. Drug excipient compatibility studies were studied by FTIR spectra and DSC thermograms. FTIR spectra showed characteristic peaks of azithromycin dihydrate with no much significant changes in physical mixtures of drug and carriers.DSC thermograms also showed no interactions or incompatibilities between pure drug and carriers used. Prepared solid dispersions were subjected to SEM analysis, XRD scans, In Vitro drug release studies in pH 6.4 phosphate buffer, kinetic modelling studies. SEM images of solid

dispersions seemed to be smooth-surfaced, irregular in shape and size. X Ray diffractograms showed the slight modification of crystalline drug forms to an amorphous nature, by loss of characteristic 2θ peaks of azithromycin dihydrate. In vitro drug release studies of solid dispersions showed freeze dried solid dispersions showed better drug release when compared to solvent evaporated solid dispersions. Release order kinetics predicted drug release followed higuchi order and drug release from the matrix of solid dispersion was most probably through diffusion. In addition to the above finding, Solid dispersions prepared using poloxamer 188 were found to possess improved solubility, when compared to polyethylene glycol 20,000 and poloxamer 407.

Hence, Research findings conclude that solid dispersion technique, using poloxamer 188, polyethylene glycol 20,000 and poloxamer 407 can be used as an efficient alternative to improve the solubility of poorly soluble drugs such as azithromycin dihydrate.

Acknowledgements

We sincerely acknowledge Aurobindo Pharma pvt. Ltd. for providing gift sample of the pure drug. We would also like to thank BASF India for providing gift samples of carriers. Authors wish to thank management of MLR Institute of pharmacy for providing necessary facilities for carrying out research work. Also, we are thankful to SURA Labs, Hyderabad, Osmania universityfor their contribution.

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How to cite this article:

Sravya Maddukuri and Radha GV (2019) 'Study of enhancement of Bioavailability of Azithromycin Dihydrate By solid Dispersion with Poloxamers', *International Journal of Current Advanced Research,* 08(04), pp. 18476-18481.
DOI: http://dx.doi.org/10.24327/ijcar.2019.18481.3533

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