INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

FORMULATION AND CHARACTERISATION OF AZITHROMYCIN DIHYDRATE INCLUSION COMPLEXES USING DERIVATIVES OF β-CYCLODEXTRINS AS COMPLEXING AGENTS

Sravya Maddukuri [1]*, Radha GV [2]
[1] Department of Pharmaceutics,M L R Institute of Pharmacy, Hyderabad, Telangana, India
[2] Department of Pharmaceutics, Gitam Institute of Pharmacy, Visakhapatnam, Andhra Pradesh, India
*Corresponding Author Email: Sravya.maddukuri23@gmail.com

Article Received on: 10/05/19 Approved for publication: 12/06/19

DOI: 10.7897/2230-8407.1008249

ABSTRACT

In the present research, an attempt was made to study enhancement of solubility and bioavailability of azithromycin, a sparingly water soluble macrolide antibiotic, by use of inclusion complexation technique, using 1:1 and 1:2 w/w ratios of three complexing agents-β-cyclodextrin, epichlorohydrin-β-cyclodextrin and sulfobutylether cyclodextrin. Two methods were used - solvent evaporation and freeze drying/lyophilisation. Drug – excipients 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 content of all prepared 12 inclusion complexes ranged from 97.4 - 99.5 %. Prepared inclusion complexes were characterised using *In-Vitro* dissolution studies, kinetic modelling patterns, Scanning electron microscopy images and X Ray Diffractograms. *In Vitro* drug profiles showed 96 % drug release for inclusion complexes using Sulfobutyl ether β-cyclodextrin, prepared using freeze drying method. From the kinetics, it could be possibly stated that the release from the matrix was through diffusion. The XRD pattern depicted by optimised complexes 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, Inclusion complexation technique using freeze drying method, and newer derivatives of β-cyclodextrin as complexing agents has proven to be highly effective in improving bioavailability of poorly water-soluble drugs than β – Cyclodextrin alone.

Keywords: Azithromycin dihydrate, β-cyclodextrin, Epichlorohydrin- β-cyclodextrin, Sulfobutyl ether-β-cyclodextrin, solubility enhancement

INTRODUCTION

Recent advances in combinatorial chemistry and synthetic chemistry have led to an increased number of lipophilic drugs. Lipophilic drugs can suffer from two main problems, keeping apart the infinite problems that they encounter during the whole development process - Solubility and Permeability[1]. One of the approaches to tackle above said problems is inclusion complexation. This technique can be applied to a broader range of drugs, including the BCS class II and IV drugs, to the maximum extent with few exceptions. Polymeric solubilisers are water soluble polymers with ability to form complexes with other molecules in system.

Complexation

Complexation is the association between two or more molecules to form a non-bonded entity with a well-defined stoichiometry[2]. Complexation depends on comparatively weak forces like van der waals forces, gas bonding and hydrophobic interactions. Examples of complexing agents are; chelates- EDTA, EGTA, molecular complexes- polymers, and cyclodextrins.

Complexes are two categories:

1. Stacking complexes is driven by association of non-polar space of drug and complexes agent this leads toexclusion of the non-polar space from contact with water, thereby reducing total energy of the system. Stacking may be homogenized or mixed but results in clear solution.

2. Inclusion complexes are formed due to the ability of a compound to enclose in another complex. There are no forces involved between them and therefore there are no bond is also called as no-bond complexes[3]. Among all the solubility enhancement techniques, inclusion complex formation technique has been employed more precisely to improve the aqueous solubility, dissolution rate, and bioavailability of poorly water-soluble drugs. Inclusion complexes are formed by the insertion of the nonpolar molecule or the nonpolar region of one molecule (known as guest) into the cavity of another molecule or group of molecules (known as host) [4]. The most commonly used host molecules are cyclodextrins. The enzymatic degradation of starch by cyclodextrin glycosyl transferase (CGT) produces cyclic oligomers, Cyclodextrins (CDs). These are nonreducing, crystalline, water soluble and cyclic oligosaccharides consisting of glucose monomers arranged in a donut shaped ring having hydrophobic cavity and hydrophilic outer surface. Three naturally occurring CDs are α-Cyclodextrin, β- Cyclodextrin, and γ Cyclodextrin.

The natural α-, β- and γ-cyclodextrin consist of six, seven, and eight glucopyranose units, respectively. The natural cyclodextrins, in particular β-cyclodextrin, are of limited aqueous solubility meaning that complexes resulting from interaction of lipophiles with these cyclodextrin can be of limited solubility resulting in precipitation of solid cyclodextrin complexes from

water and other aqueous systems. In fact, the aqueous solubility of the natural cyclodextrins is much lower than that of comparable acyclic saccharides. This is thought to be due to relatively strong intermolecular hydrogen bonding in the crystal state. Substitution of any of the hydrogen bond forming hydroxyl groups, even by lipophilic methoxy functions, results in dramatic improvement in their aqueous solubility. Cyclodextrin derivatives of pharmaceutical interest include the hydroxyl propyl derivatives of β- and γ- cyclodextrin, the randomly methylated β-cyclodextrin, sulfobutylether β-cyclodextrin and the so-called branched cyclodextrins such as glucosyl-β- cyclodextrin[5].

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. β-cyclodextrin, epichlorohydrin waspurchased from BASF India ltd., Sulfobutyl ether β-cyclodextrin was a kind gift from cyclolab cyclodextrin research and development laboratory ltd. and captisol. 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 suitable size 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. 2 mg 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.

Synthesis of Epichlorohydrin-β-cyclodextrin

Synthesis of epichlorohydrin- β-cyclodextrin was attempted as per method reportedby R Deveswaran *et al*[7]. Approximately, 5 g of β-cyclodextrin was weighed and dissolved in 10 ml of 50% w/v sodium hydroxide solution, stirred for about 24 hrs using a magnetic stirrer at room temperature. To this mixture, 6 ml of epichlorohydrin was added rapidly and stirred continuously for 40 minutes at 400 rpm. Reaction was stopped by adding 15 ml of acetone. This mixture was set aside for 30 minutes, acetone was decanted. Solution was left at 50^{0}C overnight. After cooling, 19.9 ml of 6 N HCl was added and resulting clear solution was evaporated to dryness. To the above residue, 44 ml of ethanol was added which resulted in a white precipitate. Precipitate was dried at 50^{0}C for 24 hrs. Obtained product was pulverised, passed through sieve and stored until further use.[6]

Preparation of Inclusion complexes by solvent evaporation method

Inclusion complexes were prepared by dissolving β-Cyclodextrin, synthesized epichlorohydrin- β-cyclodextrin, Sulfobutyl ether β-Cyclodextrin w/w ratios of 1:1 (drug: complexing agent), 1:2 (drug: complexing agent) and azithromycin dihydrate in required amounts of 50% aqueous ethanol. The solution was stirred till a clear solution was observed and the obtained solution was then evaporated under vacuum at a temperature of 45°C. The solid residues were further dried completely at 45°C for 48 h, the dried complex was pulverized into a fine powder and sieved through sieve No. 100. The resulting samples were stored in a desiccator until further use.[8]

Preparation of Inclusion complexes by freeze drying/lyophilisation method

Azithromycin dihydrate and β-Cyclodextrin, synthesized epichlorohydrin- β-cyclodextrin, Sulfobutyl ether β-Cyclodextrinin w/w ratios of 1:1 and 1:2 were taken separately in 20 ml of water and mixed thoroughly using a magnetic stirrer. 25% liquid ammonia was added drop wise and stirred until a clear solution was obtained. The above step was done only if clear solution was not obtained. The resultant solution was frozen in a deep freezer at -20°C for about 12 h. The frozen mixture was then freeze dried in the freeze dryer for 8 hour at -50°C under vacuum. The resultant product was stored in a desiccator.[9]

Carrier	**Beta Cyclodextrin**				**Sulfo butyl ether β cyclodextrin**				**Epichlorohydrin Beta Cyclodextrin**			
Preparation	Kneading method		Freeze Drying method		Kneading method		Freeze Drying method		Kneading method		Freeze Drying method	
Formulation code	EIC1	EIC2	FIC1	FIC2	EIC3	EIC4	FIC3	FIC4	EIC5	EIC6	FIC5	FIC6
Drug: Carrier	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2

Formulation table of inclusion complexes

Characterization and evaluation of inclusion complexes

Drug Content

An accurately weighed quantity of drug-inclusion complex equivalent to 100 mg of azithromycin was taken into a 100 ml volumetric flask, dissolved in methanol and suitably diluted with 6.4 pH Phosphate buffer. The content of azithromycin was determined spectrophotometrically at 201 nm against suitable blank using UV-visible spectrophotometer and amount of drug in each formulation was calculated.

Solubility Studies

Solubility studies were carried out according to the method reported by Higuchi and Connors. Excess (usually more than 1 mg/ml concentration) of drug were added to 25 ml of distilled water, each containing variable amount of β-CD, Epichlorohydrin- β-CD and SBECD such as 0, 2, 4, 6, 8, and 10 milimoles/liter, taken in stoppered conical flasks and mixtures were shaken for 24 hrs in rotary flask shaker. After shaking to achieve equilibrium, 2 ml of aliquots were withdrawn at 1 hr intervals and filtered through Whatman filter paper. The filtrate was diluted if necessary and analyzed by UV-spectrophotometer at 201 nm. Shaking was continued until three constitutive readings were same.[10,11]

The apparent stability constants (1:1) were calculated from the phase solubility diagrams, according to the following equation:

$$K_c = \frac{Slope}{S_0(1 - Slope)}$$

Where, Kc = apparent stability constant, S_0 = Intercept

***In Vitro* Dissolution Studies of Inclusion Complexes**

Quantity of inclusion complexes equivalent to 20 mg of azithromycin was filled in hard gelatin capsule by hand filling method. The dissolution study of capsules was conducted using dissolution testing USP apparatus I (basket method) in 900 ml of 6.4 pH Phosphate buffer at 37 ± 0.5ºC and at a speed of 50 rpm. Aliquot of 5 ml 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 201 nm against suitable blank using UV-visible spectrophotometer.

The amount of azithromycin dihydrate released from each inclusion complex was calculated and plotted against time and compared with pure drug.

Kinetics of *In Vitro* Drug Release

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.

Powder X-Ray Diffractometry (PXRD)

The powder X-RD patterns of optimized inclusion complexes were recorded by using Philips Holland -PW 1710 scanner with filter Cu radiation over the interval 5- 60°/2θ. The operation data were as follows: voltage 35 kV, current 20 mA, filter Cu and scanning speed 1° / min 52. 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 (Philips Holland -PW 1710 scanner) 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 40° in increments of 0.12°/s every 0.02°. The samples used for this study were preserved in a desiccator before using.

Scanning Electron Microscopy (SEM)

The morphology of the inclusion complexes by kneading method, and lyophilisation method was studied using a scanning electron microscope.

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

RESULTS

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

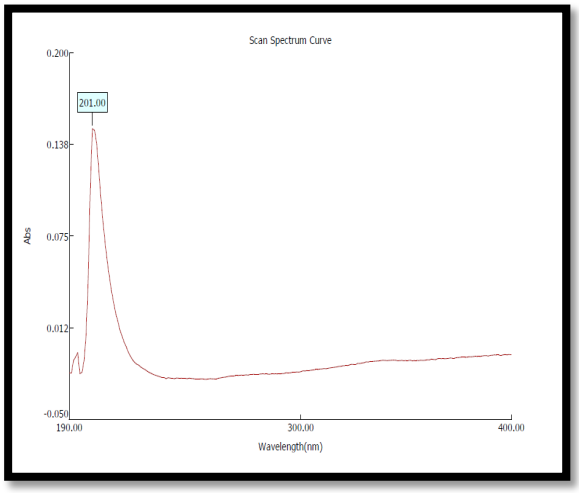
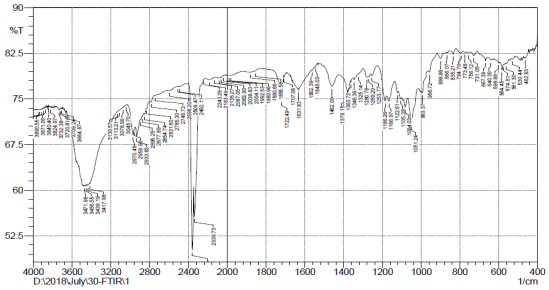


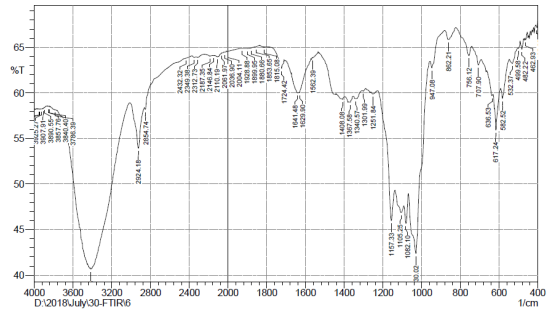
Figure 1: UV spectrum scan of Azithromycin dehydrate

Table 2: Melting point determination of azithromycin dihydrate

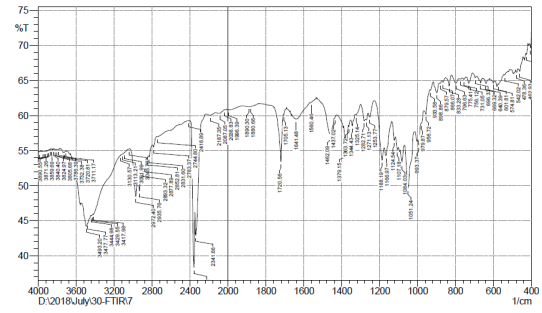
Trial number	Melting point (^{0}C)	Average of three readings (^{0}C)
1	112	114
2	114	
3	118	



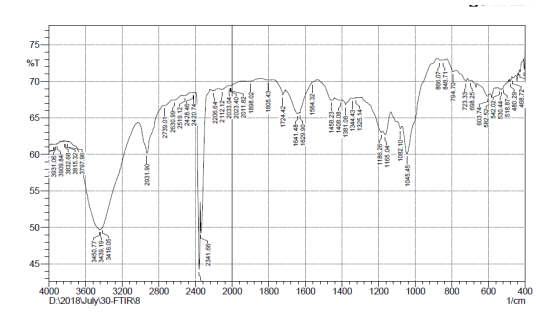
FTIR Spectrum of Pure drug



FTIR Spectrum of β-cyclodextrin



FT-IR Spectrum of Pure drug + Epichlorohydrin β-cyclodextrin



FT-IR Spectrum of Pure drug + Sulfobutyl ether cyclodextrin (SBECD)

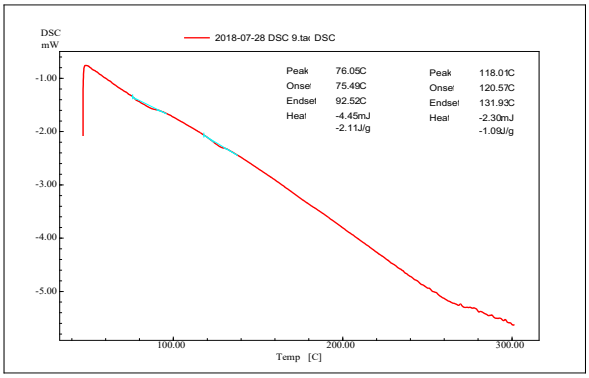
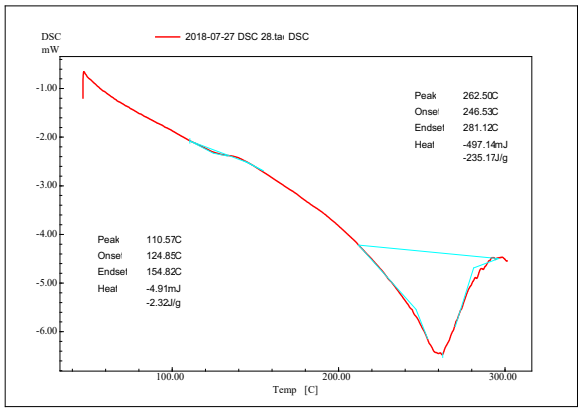
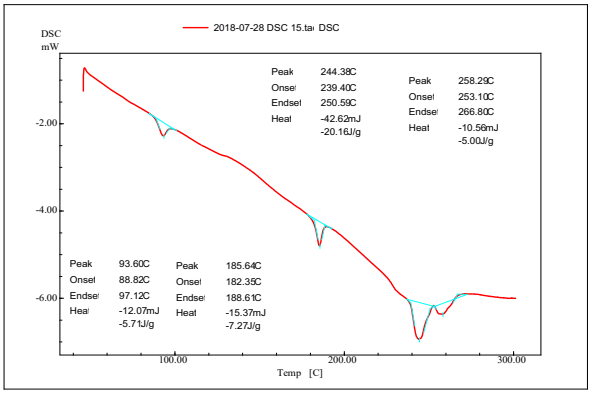


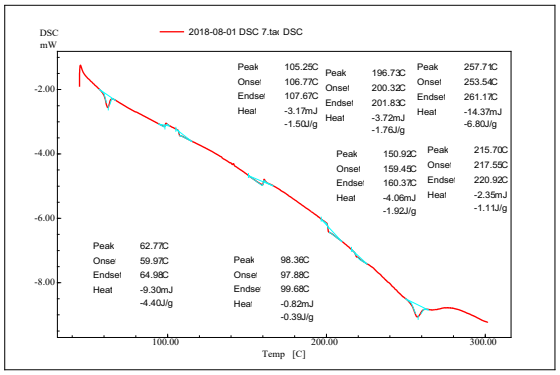
Figure 2: FT-IR spectra overlay of pure drug and complexing agents



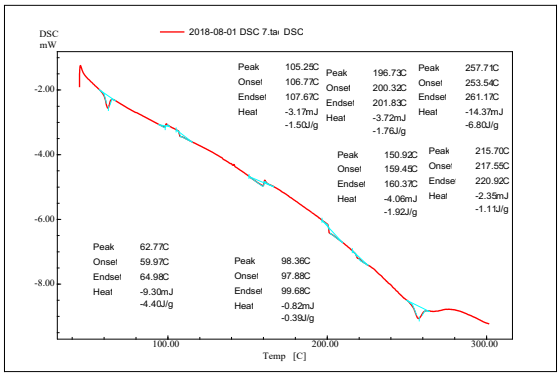
DSC thermogram of Pure drug



DSC thermogram of of Pure drug + Beta cyclodextrin



DSC thermogram of of Pure drug + Epichlorohydrin - Beta cyclodextrin



DSC thermogram of of Pure drug + sulfobutylether cyclodextrin

Figure 4: DSC thermograms of pure drug in combination with complexing agents

Table 3: Phase solubility studies of pure drug along with complexing agents

S. No	Concentration of Sulfobutyl ether β CD/Epichlorohydrin β CD/ β CD (% w/v)	Concentration of Azithromycin dihydrate in sulfobutyl ether β CD solution	Concentration of Azithromycin dihydrate in Epichlorohydrin β CD solution	Concentration of Azithromycin dihydrate in β CD solution
1	0	0.085	0.085	0.085
2	2	0.27	0.25	0.26
3	4	0.316	0.31	0.3
4	6	0.398	0.36	0.38
5	8	0.5	0.45	0.42
6	10	0.58	0.55	0.56

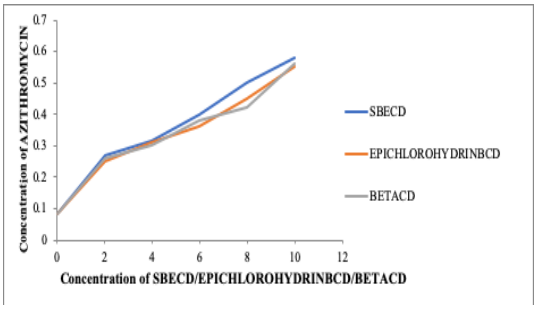


Figure 5: Phase solubility studies

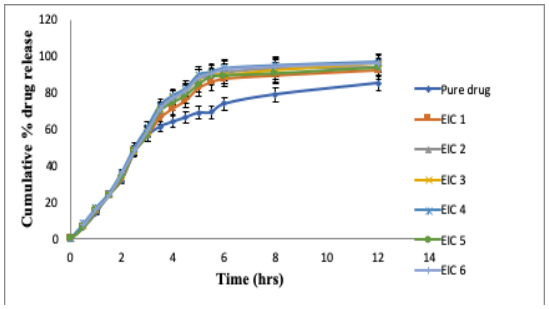


Figure 6: *In Vitro* dissolution profile of inclusion complexes prepared by solvent evaporation

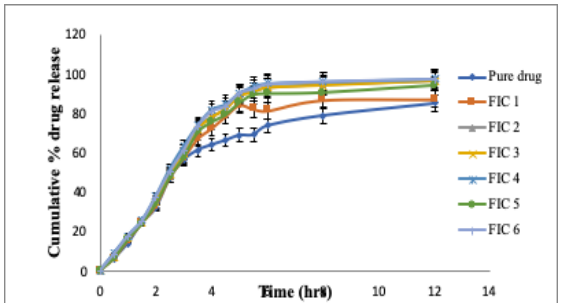


Figure 7: *In Vitro* dissolution profile of inclusion complexes prepared by freeze drying

Mathematical modellingkinetics of Inclusion complexes

Table 4: R^2 Values obtained for kinetic modelling curves of 4 optimised inclusion complexes

Formulation	Zero order	First order	Higuchi	Korsmeyer–Peppas	n value
FIC4	0.703	0.990	0.874	0.854	0.752
FIC6	0.710	0.970	0.877	0.864	0.761
EIC4	0.716	0.975	0.879	0.863	0.774
EIC6	0.722	0.954	0.881	0.871	0.784

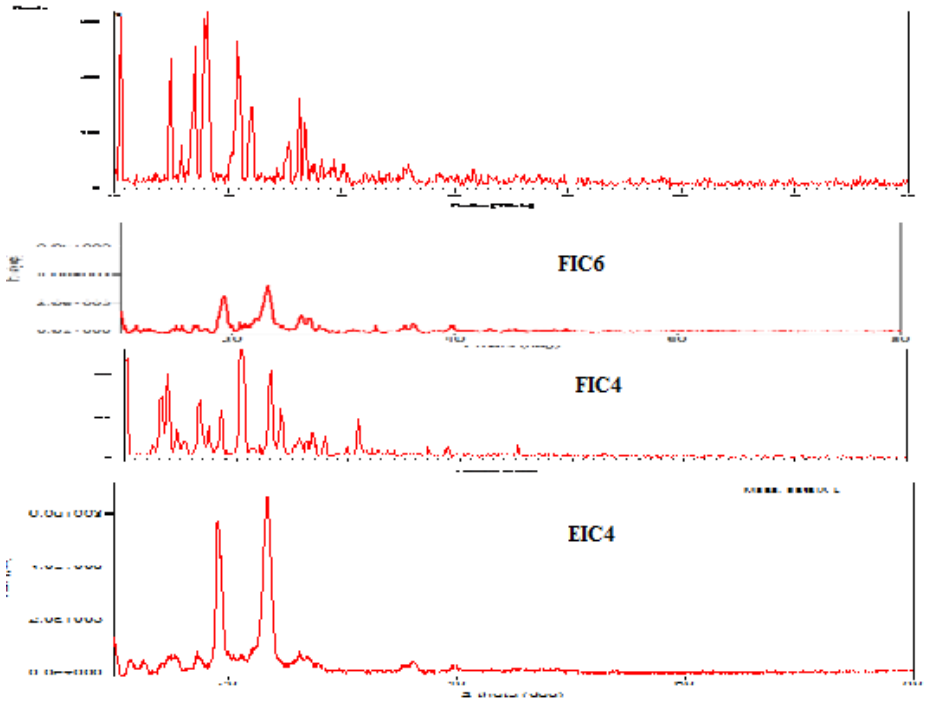


Figure 8: X ray Diffractograms of optimised formulations

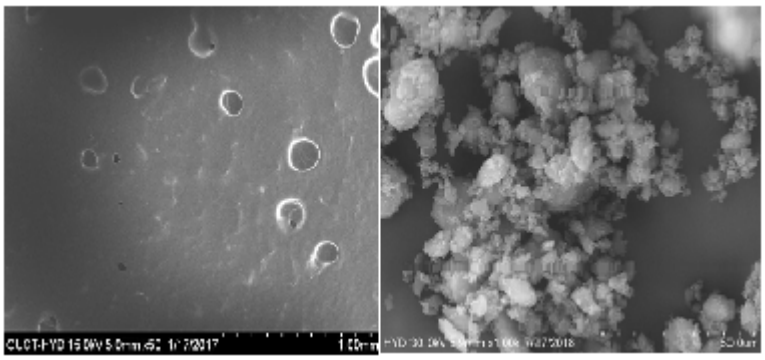


Figure 9: SEM images of optimised formulation FIC6

DISCUSSION

UV Spectrum of Azithromycin Dihydrate

From the stock solution – 1000 µg/ml azithromycin dihydrate solution, suitable dilutions were made to obtain 12 µg/ml solution of azithromycin dihydrate. This solution was scanned for maximum absorption wavelength using UV-spectrophotometer in the range of 200-400 nm. The absorption maxima for Azithromycin dihydrate were found to be 201 nm and hence the same was used as λmax for estimation of azithromycin dihydrate 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 buffer and absorbance was measured at 201 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 4-20 µg/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

Fourier Transform Infrared spectroscopic studies

FT-IR spectra were performed for pure drug as well as physical mixture of pure drug and complexing agents such as β-Cyclodextrin, Epichlorohydrin β-Cyclodextrin, Sulfobutyl ether β-cyclodextrin. The FTIR spectra of pure Azithromycin dihydrate (Figure) showed characteristic peaks at 1368.72 cm^{-1} (C-N-stretching), 2958.90 cm^{-1} (C-H-stretching), 1379.15 cm^{-1} (C-HO-stretching alcoholic group), 1545.03 cm^{-1} (C=O-stretching amidic group), 3471.98 cm^{-1} (N-H-stretching), 1707.06 cm^{-1} (C=C-bending), 794.70 cm^{-1} (C-F-stretching), 1122.61 cm^{-1} (O-H-bending).

The FTIR spectra of all other mixtures of pure drug along with complexing agents are shown in Figure 3. The FTIR spectra of Azithromycin dihydrate in combination with complexing agents were having similar fundamental peaks and pattern when compared with the pure drug. Thus there are no significant interactions among the drug and excipients.

Drug-excipients interactions by Differential Scanning Calorimetry

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, Beta Cyclodextrin Epichlorohydrin-Beta Cyclodextrin, Sulfobutylether Cyclodextrin, are shown in Figure 4. DSC thermograms showed endothermic peaks for pure drug, excipient and drug –excipient mixtures at their respective melting points. Thermogram of azithromycin dihydrate exhibited short and blunt characteristic endothermic peak at 118 °C, confirming the identity of the pure drug. There were no appreciable changes in peak value of drug in the DSC thermogram of drug- excipient mixtures from that pure drug. Hence it may be concluded that slight changes observed in melting endothermic peaks of drug were likely due to presence of excipients and not due to any significant interactions between drug and polymer.

Preparation of azithromycin dihydrate inclusion complexes

Inclusion complexes of azithromycin dihydrate were prepared by two methods co-evaporation technique and freeze-drying technique using complexing agents such as β-Cyclodextrin, Sulfo butyl ether β -cyclodextrin, Epichlorohydrin β-Cyclodextrin in ratios of 1:1 and 1:2 w/w. Assay for drug content in solid dispersions was conducted and the percentage drug content values were found to range from 95.14 - 99.55 %.

Phase Solubility Studies

As a preliminary study for formation of inclusion complexes between azithromycin dihydrate and β-Cyclodextrin/ Epichlorohydrin β-Cyclodextrin/ Sulfo butyl ether β – cyclodextrin, Phase solubility analysis was carried out as per method reported by Higuchi and Connors. Pure drug solubility was found to be 0.085 mg/ml. (Table) From these phase solubility studies carried out over a period of 24 -72 hours, Physical mixtures of drug and Sulfobutyl ether β CD has shown highest drug solubility, when compared to the mixtures of pure drug and Epichlorohydrin β CD and β Cyclodextrin. The filtrate was diluted if necessary and analyzed by UV-spectrophotometer at 201 nm. Shaking was continued until three constitutive readings were same. The apparent stability constants were calculated from the phase solubility diagrams plotted and substituting values into the equation.

Correlation coefficients (R^2) were 0.971, 0.969, 0.952 for phase solubility diagrams of pure drug along with sulfobutyl ether β CD, Epichlorohydrin β CD, β CD respectively. Solubility of pure drug was found linearly increasing with increasing concentration of complexing agents, hence solubility curve was assumed to follow AL type of diagram.

These complexing agents can be ranked according to the effect of carriers on solubility of pure drug as:

Sulfobutyl ether β CD>Epichlorohydrin β CD>β Cyclodextrin

The apparent stability constants (1:1) were calculated from the phase solubility diagrams, according to the following equation:

$$K_c = \frac{\text{Slope}}{S_0(1 - \text{Slope})}$$

Value of KC is highly sensitive towards small changes in S_0 and for poorly-soluble drugs it can be complicated to obtain accurate S_0 values

Also, self-association of lipophilic drug molecules in aqueous media can lead to erroneous results. Hence, it is more accurate to determine the complexation efficacy (CE):

$$C.E = K_c.\ S_0 = \frac{\text{Slope}}{(1 - \text{Slope})}$$

Apparent stability constants for Sulfobutyl ether β CD, Epichlorohydrin β CD, β Cyclodextrin was found to be $48*10^{-3}$, $43*10^{-3}$, $42*10^{-3}$ M^{-1} respectively

The results are tabulated in Table 3 and graphical representation was shown in Figure 5.

***In Vitro* Dissolution Studies for Inclusion Complexes**

The *in vitro* drug release test was performed for all the inclusion complexes prepared using both methods co-evaporation and freezing drying method (EIC1-EIC6 and FIC1-FIC6). *In vitro* drug release data for all the above formulations were tabulated in Table. Graph was plotted using time in minutes on x axis and percentage cumulative drug release on y-axis, which shows the cumulative percent drug released as a function of time for all formulations. The cumulative percent drug released after 12 hrs was also shown in table. *In vitro* studies reveal that there is marked increase in the dissolution rate of all the inclusion complexes of azithromycin dihydrate when compared to pure drug (85.15%) after 12 h. From the *in vitro* drug release profile, it can be seen that formulation FIC4 (prepared using freeze drying method with ratio1:2 w/w of drug and Sulfo butyl ether Cyclodextrin) was found to be 97.7 %, FIC6 (prepared by freeze drying method using ratio 1:2 w/w of drug and Epichlorohydrin β – Cyclodextrin) was found to be 97.11%, EIC4 (prepared by co-evaporation method in ratio 1:2 w/w of drug and Sulfo butyl ether Cyclodextrin) was found to be 96.68%, EIC6 (prepared by co-evaporation method in ratio 1:2 w/w of drug and Epichlorohydrin β – Cyclodextrin) was found to be 96.1%. This may be attributed to the formation of a complex between pure hydrophobic drug and complexing agents used and solubilization of the drug due to complexing agents. The graphical representation of inclusion complexes with pure drug was depicted in Figures 6 and 7.

Release Order Kinetics of optimised azithromycin dihydrate Inclusion Complexes

From the kinetic graphs plotted, it could be noted that the regression coefficient value was closer to unity in case of first order plot i.e. 0.990, 0.970, 0.975, 0.954 indicating that the drug release follows a first order mechanism (Figure). 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 Korsmeyer peppas and Zero order plots. The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value possibly stating that the release from the matrix was through diffusion (Figure). Further the n value obtained from the Korsmeyer plots i.e. 0.752, 0.761, 0.774, 0.783 suggests that the drug release from inclusion complexes was anomalous Non fickian diffusion (Table 4).

X-Ray Diffractograms for Inclusion Complexes

The optimized Azithromycin Inclusion complex was analyzed to find out whether the Inclusion complexes of various drug polymer ratios are crystalline or amorphous. The presence of numerous distinct peaks in the XRD spectrum indicates that Azithromycin was present as a crystalline material. The XRD pattern depicted by physical mixture reveals a decrease in the number of peaks which probably represents decrease in crystallinity. On the other hand, the spectrum of optimized formulation FIC4 (Azithromycin inclusion complex with Sulfobutyl ether cyclodextrin), FIC6 (Azithromycin inclusion complex with Epichlorohydrin β-cyclodextrin), EIC4 (Azithromycin inclusion complex with Sulfobutyl ether cyclodextrin), EIC6 (Azithromycin inclusion complex with β-cyclodextrin) of Inclusion complex was characterized by the decrease in intensity and quantity of diffraction peaks, which is characteristic of an amorphous compound (Figure 8). The enhancement in the dissolution rate of the drug from the optimized Azithromycin Inclusion complex is ascribed to the marked reduction in the crystallinity of the drug.

Scanning Electron Microscopy for Inclusion Complexes

SEM photographs for azithromycin pure drug and optimized formulation FIC4 (azithromycin inclusion complex with Sulfobutyl ether cyclodextrin are shown in Figure 9. The drug crystals seemed to be smooth-surfaced, irregular in shape and size. In case of inclusion complexes, it was difficult to distinguish the presence of drug crystals. Inclusion complex appeared as uniform and homogeneously mixed mass with wrinkled surface. The results could be attributed to complex of the drug in the mass of the complexing agent.

CONCLUSION

In the present research work, derivatives of β-cyclodextrin like Epichlorohydrin- β-cyclodextrin, Sulfobutyl ether cyclodextrin (SBECD) have been employed to investigate solubilising effect of cyclodextrins on poorly soluble drugs. Model drug used was azithromycin dihydrate, a poorly soluble macrolide antibiotic. β-cyclodextrin was treated with epichlorohydrin to synthesis epichlorohydrin- β-cyclodextrin, which was stored until further use. β-cyclodextrin, Epichlorohydrin- β-cyclodextrin, Sulfobutylether cyclodextrin were used to complex azithromycin dihydrate in 1:1 and 1:2 w/w ratios. 2 methods were employed to prepare inclusion complexes- Solvent evaporation and lyophilisation method. Drug excipients 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 complexing agents used. Prepared inclusion complexes (EIC1-EIC6-inclusion complexes were prepared by solvent evaporation technique and FIC1-FIC6 - inclusion complexes prepared by freeze drying technique) were characterised and evaluated. SEM images of inclusion complexes seemed to be wrinkle-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 inclusion complexes showed freeze dried inclusion complexes having better drug release when compared to solvent evaporated inclusion complexes. Release order kinetics predicted drug release followed first order and drug release from the matrix of solid dispersion was most probably through diffusion. In addition to the above findings, Inclusion complexes prepared using Sulfobutyl ether cyclodextrin and epichlorohydrin- β-cyclodextrin were found to possess improved solubility, when compared to β-cyclodextrin.

Hence, Research findings conclude that Inclusion complexation technique, a well known technique to improve solubility for poorly water soluble drugs, can be put to better use, with newer and efficient complexing agents such as β-cyclodextrin, Epichlorohydrin- β-cyclodextrin, Sulfobutyl ether cyclodextrin.

ACKNOWLEDGEMENTS

We sincerely acknowledge Aurobindo Pharma pvt. Ltd. for providing gift sample of the pure drug. We would also like to thank BASF India, Cyclolab, Budapest, Hungary; Captisol, San diego, CA; for providing gift samples of complexing agents. 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 University for their contribution.

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Cite this article as:

Sravya Maddukuri and Radha GV. Formulation and Characterisation of azithromycin dihydrate inclusion complexes using derivatives of β-cyclodextrins as Complexing agents. Int. Res. J. Pharm. 2019;10(8):77-85 http://dx.doi.org/10.7897/2230-8407.1008249

Source of support: Nil, Conflict of interest: None Declared