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Research Article

Solubility Enhancement of Azithromycin by Solid Dispersion Technique Using Mannitol and β-Cyclodextrin

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Abstract

Background: Azithromycin is a poorly water soluble drug having low solubility. Dissolution is the rate-limiting step in absorption of such drugs. Therefore, solubility of such drugs need to be enhanced in order to have a stable and effective dosage form having greater bioavailability. Solid dispersion technique is one of the effective methods to enhance the solubility of poorly soluble drugs.

Aim and Objectives: The present study aimed at enhancing the solubility of biopharmaceutical classification system Class II Drug, i.e. Azithromycin using Mannitol and β-Cyclodextrin as a carrier.

Method: Solid dispersions of Azithromycin with Mannitol and β-Cyclodextrin were prepared by Melting, Kneading and Solvent Evaporation method. The solubility of these prepared dispersions was evaluated.

Results: Solubility of prepared dispersions of Azithromycin were reported in µg/mL. The solubility of solid dispersion which was prepared using the Drug: Mannitol mixture in 1:4 ratio was found to be maximum, i.e. reported solubility of 7.8 µg/mL. The solubility of solid dispersion which was prepared using Drug: β-Cyclodextrin mixture in 1:1.5 ratio was found to be maximum, i.e. reported solubility of 9.52 µg/mL. The solubility of those dispersions that were prepared using Melting and Kneading Method were found to be maximum.

Conclusion: Drug having less aqueous solubility can have an enhanced rate of dissolution by using solid dispersion technique.

Keywords: Azithromycin; Solubility; Solid Dispersion; BCS (Biopharmaceutical Classification System); Absorbance

Abbreviations

BCS: Biopharmaceutical Classification System; HCL: Hydrochloric Acid.

Introduction

Azithromycin, a macrolide antibiotic of the azalide subclass, exerts its antibacterial action by binding to the 50s ribosomal subunits of susceptible bacteria and suppressing protein synthesis; however, it differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring. Azithromycin is a white crystalline powder with a molecular formula of $C_{38}H_{72}N_2O_{12}$ and a molecular weight of 749.0 gm/mol. Azithromycin is a broad spectrum antimicrobial agent with oral

bioavailability about 37%. The drug has pKa values around 8.74 and is sparingly soluble in water (~ 2.37 mg/L) and soluble in ethanol [1,2].

Sr. No.	BCS Class	Solubility	Permeability	Example
1	Class I	High	High	Metoprolol, Verapamil
2	Class II	Low	High	Gilbenclamide, Phenytoin
3	Class III	High	Low	Cimetidine, Captopril
4	Class IV	Low	Low	Cholorthiazide, Taxol

Table 1: The Biopharmaceutical Classification System.

The Biopharmaceutical Classification System (BCS) has been developed to provide a scientific approach for classification of drugs based on solubility in relation to dose and the permeability in combination with the dissolution properties of the dosage form [3]. According to the BCS, Azithromycin can be classified as a Class II drug; therefore the drug dissolution may be the rate-limiting step in the drug absorption process. It shows erratic dissolution problem in gastric and intestinal fluid due to its poor water solubility. Rate of absorption and/or extent of bioavailability for such insoluble drugs are controlled by rate of dissolution in gastrointestinal fluids. The effort to improve the dissolution and solubility of a poorly water-soluble drug remains one of the most challenging tasks in drug development. Several methods have been introduced to overcome this problem like micronization, solubilization, salt formation, complexation with polymers, change in physical form, solid dispersions, complexation and the use of hydrophilic carriers [1,4].

Solid dispersions

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles. Transformation of crystalline drug to amorphous drug upon solid dispersion formulation increases the dissolution rate. Solid dispersion techniques have been used to increase the solubility of a poorly water soluble drug. Solid dispersion is a viable and economic method to enhance bioavailability of poorly water soluble drug [4,5].

Methods of preparing solid dispersions

Various methods for preparation of Solid Dispersions are as shown in the figure 1.

Figure 1: Types of methods for preparation of Solid Dispersion.

Kneading technique

In this method, carrier is permeated with water and transformed to paste. Drug is then added and kneaded for particular time. The kneaded mixture is then dried and passed through sieve if necessary.

Advantages

- No organic solvents are required in this method.
- Uniform sized kneads are formed which ensures uniformly fine sized powder formation of the dispersion mixture.

Solvent evaporation method

In this method, both drug and carrier are dissolved in organic solvent. After entire dissolution, the solvent is evaporated. The evaporated film is formed which is then removed using spatula for getting a powder mixture.

Advantages

- Simple and easy method.
- Does not cause degradation of the drug as dissolution of the drug into organic solvent occurs at low temperature.

Co-precipitation method

Required amount of drug is added to the solution of carrier. The system is kept under magnetic agitation and protected from the light. The formed precipitate is separated by vacuum filtration and dried at room temperature in order to avoid the loss of water from the inclusion complex.

Melting method

Drug and carrier are mixed using mortar and pestle. To accomplish a homogenous dispersion the mixture is heated at or above the melting point of all the components. It is then cooled to acquire a congealed mass. It is crushed and sieved.

Advantages

- Simple and cost-effective.
- Does not require any sophisticated instrumentation.

Co-grinding method

Physical mixture of drug and carrier is mixed for some time employing a blender at a particular speed. The mixture is then charged into the chamber of a vibration ball mill steel balls are added. The powder mixture is pulverized. Then the sample is collected and kept at room temperature in a screw capped glass vial until use.

Gel entrapment technique

Hydroxyl propyl methyl cellulose is dissolved in organic solvent to form a clear and transparent gel. Then drug for example is dissolved in gel by sonication for few minutes. Organic solvent is evaporated under vacuum. Solid dispersions are reduced in size by mortar and sieved.

Spray-drying method

Drug is dissolved in suitable solvent and the required amount of carrier is dissolved in water. Solutions are then mixed by sonication or other suitable method to produce a clear solution, which is then spray dried using spray dryer.

Lyophilization technique

Freeze-drying involves transfer of heat and mass to and from the product under preparation. This technique was proposed as an alternative method to solvent evaporation. Lyophilization has been thought of a molecular mixing technique where the drug and carrier are co dissolved in a common solvent, frozen and sublimed to obtain a lyophilized molecular dispersion [6].

This study aimed at enhancing solubility of BCS Class II Drug, i.e. Azithromycin. In present study attempts were made to enhance the dissolution of Azithromycin using Solid Dispersion technique. Solid Dispersions of Azithromycin with Mannitol and β-Cyclodextrin and using appropriate solvent, i.e. Chloroform. The dispersions were prepared in different ratios using the Melting method, Kneading method and Solvent Evaporation method and those prepared formulations were evaluated. The most suitable formulation having maximum enhanced solubility was examined and the most suitable method of preparation from the selected methods of preparing the solid dispersions was evaluated and inferred.

Materials and Methods

Materials

Azithromycin was obtained as gift sample from Chemdyes Corporation, Rajkot, Gujarat. Mannitol (Suvidhinath), β-Cyclodextrin (Himedia Laboratories), Chloroform (Loba Chemi Pvt. Ltd.), Hydrochloric Acid (Fischer Scientific), Sodium Hydroxide (Atur Instru Chem), Potassium Dihydrogen Phosphate (Chemdyes) were provided by Parul Institute of Pharmacy and were of pharmaceutical and analytical Grade. Instruments like Weighing Balance (Scale Tec), UV Spectrophotometer (Shimadzu – 1700), Melting Point Apparatus (AnuLab) and Tray Dryer (Das Lab Instrument) were utilized for conducting the research work.

Methods

Characterization and identification of drug

Physical characterization of drug

The drug was evaluated for physical characteristics. The drug was kept on a clean surface and observed by naked eye and the observations were noted down.

Determination of melting point

- Melting Point Apparatus was utilized for the determination of melting point of Azithromycin.

- A few crystals of the drug were placed into a one-end sealed thin-walled capillary tube which was then placed along with the Thermometer into Melting Point Apparatus.
- The Sample was subjected to appropriate hearting for proper melting. The temperature at which Azithromycin started to melt was noted down.

Preparation of calibration curve

Preparation of standard stock solution and working standard solution

10 mg of Azithromycin was accurately weighed and transferred to 10 mL of volumetric flask and volume was made up with 0.1 N HCl (Hydrochloric Acid) to get standard stock solution of concentration 1000 µg/mL.

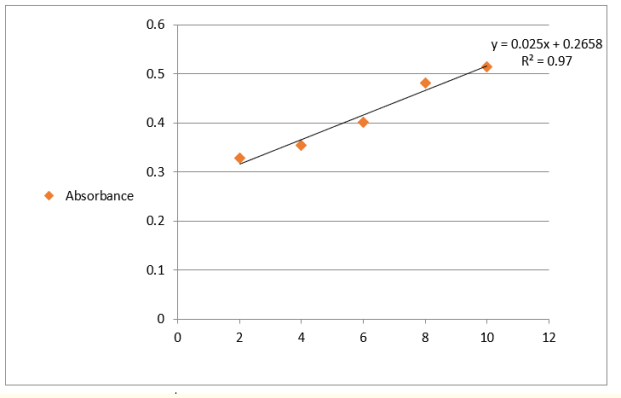

Figure 2: Calibration Curve.

From the standard stock solution, 1 mL was drawn out and diluted with pH 6.8 buffer up to 10 mL to get working standard solution having concentration of 100 µg / mL and then further dilutions were made to obtain the concentration range of 20 µg/mL to 100 µg/mL using pH 6.8 Phosphate Buffer as a solvent.

Method of preparation of solid dispersion

The solid dispersions of Azithromycin and Mannitol (carrier) in various drug-to-carrier weight ratios were prepared by melting method, kneading method and solvent evaporation method. Similarly solid dispersions of Azithromycin and β-Cyclodextrin (carrier) in various drug-to-carrier weight ratios (similar as for Mannitol) were prepared by melting method, kneading method and solvent evaporation method.

Sr. No.	Azithromycin	Mannitol	Drug: Carrier Ratio
1.	100 mg	100 mg	1: 1
2.	100 mg	200 mg	1 2
3.	100 mg	300 mg	1: 3
4.	100 mg	400 mg	1: 4
5.	100 mg	500 mg	1: 5

Table 2: Formulation Composition of Azithromycin: Mannitol.

Sr. No.	Azithromycin	β-Cyclodextrin	Drug: Carrier Ratio
1.	100 mg	50 mg	1: 0.5
2.	100 mg	100 mg	1: 1
3.	100 mg	150 mg	1: 1.5

Table 3: Formulation Composition of Azithromycin: β-Cyclodextrin.

Preparation of solid dispersions

Melting method

- 0.05 gm, 0.100 gm and 0.150 gm of β-Cyclodextrin was weighed and similarly 0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm of Mannitol was weighed accurately and was subjected to heating along with the Drug (Azithromycin) and mixed thoroughly.
- The hot drug-carrier mixture was immediately cooled.
- The formed solid cake was then collected, crushed and grinded.
- The UV Spectroscopy of the collected powder was taken and then the solubility was reported.

Kneading method

- 0.05 gm, 0.100 gm and 0.150 gm of β-Cyclodextrin was weighed and similarly 0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm of Mannitol was weighed and were taken into Mortar Pestles.
- To the above weighed Carriers, Water was added until paste like consistency of Mannitol and β-cyclodextrin was obtained.
- Azithromycin was added to the individual pastes respectively and mixed thoroughly.

- The mixture was dried well and sifted and the powder was collected.
- The UV-absorbance of the powder mixture was then measured. The solubility was then reported.

Solvent evaporation method

- grams of drug (Azithromycin) was weighed 6 times and was placed into 8 different Beakers.
- 0.05 gm, 0.100 gm and 0.150 gm of β-Cyclodextrin was weighed and added to 3 of the above mentioned Beakers and were marked with the drug: carrier ratio.
- gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm of Mannitol was weighed and added into remaining 5 different Beakers.
- The solid mixture was thoroughly mixed and to the solid mixture, Chloroform was added and the mixtures were mixed thoroughly with a glass stirrer.
- All these 8 different mixtures of different concentrations were poured into 8 different petri-dishes respectively and the solvent was allowed to evaporate.
- After proper evaporation of solvent, the dried solid dispersions were collected and were crushed, pulverized and sifted through 80# mesh sieve.
- The fine powder was then subjected to measurement of absorbance of the solid dispersion by UV Spectrophotometer (Shimadzu UV-1700). Then, the solubility was reported [5,7,8].

Qualitative identification of azithromycin and it's solid dispersions

UV spectroscopy of solid dispersions

For Qualitative identification of Drug, 25 mg of Azithromycin was weighed and added into 25 mL of Volumetric Flask and the volume was made up to the mark with distilled water.

Solubility of powdered solid dispersions

25 mg of powdered dispersion (obtained by all the three methods) was weighed and added into 25 mL of Volumetric Flask and the volume was made up to the mark with distilled water. The solution mixture was then filtered and the absorbance was taken. The solubility was then calculated using the calibration curve.

Results and Discussion

Characterization and identification of drug

Physical characterization of drug

Following were the observations on physical identification of the drug which are:

- **Physical Sate:** Crystalline
- **Color:** White
- **Taste:** Bitter

Determination of melting point

Name of the Drug	Observed Melting Point	Standard Melting Point
Azithromycin	110 ℃ - 116 ℃	113 ℃ - 115 ℃

Table 4: Observation for Determination of Melting Point.

Absorbance of drug

Standard curve for the estimation was prepared in phosphate buffer pH 6.8 in concentration range of 2-10 µg/ml. In this concentration range good linearity was observed with the correlation coefficient (R^2) 0.97. The absorbance of pure drug was found to be 0.408 nm.

Absorbance of prepared dispersions

Method of Preparation	Absorbance of Azithromycin: Mannitol (nm)				
	1: 1	1: 2	1: 3	1: 4	1: 5
Kneading Method	0.104	0.124	0.272	0.320	0.268
Melting Method	0.108	0.157	0.268	0.460	0.391
Solvent Evaporation Method	0.137	0.186	0.270	0.348	0.332

Table 5: Absorbance of Solid Dispersion of Azithromycin: Mannitol.

Method of Preparation	Absorbance of Azithromycin: β-Cyclodextrin (nm)		
	1: 0.5	1: 1.0	1: 1.5
Kneading Method	0.365	0.425	0.503
Melting Method	0.279	0.449	0.478
Solvent Evaporation Method	0.280	0.344	0.362

Table 6: Absorbance of Solid Dispersions of Azithromycin: β-Cyclodextrin.

Solubility of prepared dispersions of azithromycin

From the obtained standard linear equation for calibration curve, i.e. y = 0.025x + 0.02658 (y = mx + c), using the noted observations of absorbance for individual Drug: Carrier Dispersions, following solubility for both the type of dispersion mixtures were reported.

Method of Preparation	Solubility of Azithromycin: Mannitol (µg/mL)				
	1: 1	1: 2	1: 3	1: 4	1: 5
Kneading Method	-6.44	-5.64	0.28	2.2	0.12
Melting Method	-6.28	-4.32	0.12	7.8	5.04
Solvent Evaporation Method	-5.12	-3.16	0.2	5.72	2.79

Table 7: Solubility of Solid Dispersion of Azithromycin: Mannitol.

Method of Preparation	Solubility of Azithromycin: β-Cyclodextrin (µg/mL)		
	1: 0.5	1: 1.0	1: 1.5
Kneading Method	4.0	6.4	9.52
Melting Method	0.56	7.36	8.52
Solvent Evaporation Method	0.6	3.16	3.8

Table 8: Solubility of Solid Dispersions of Azithromycin: β-Cyclodextrin.

The solubility of Azithromycin: Mannitol dispersion was found to be maximum (7.8 µg/mL) in the Drug: Carrier ratio of 1:4 which was prepared utilizing the melting method. Likewise, the solubility of Azithromycin:β-Cyclodextrin was found to be maximum (9.52 µg/mL) in the Drug: β-Cyclodextrin ratio of 1:1.5 which was prepared utilizing the kneading method.

Conclusion

Solid Dispersions is one of the methods utilized for enhancing the solubility of poorly soluble drugs. For poorly aqueous soluble drugs, solid dispersion method is proved to be effective for establishing dissolution enhancement. In present study, Azithromycin – a BCS Class II drug was identified using physical characterization and melting point determination. The solid dispersions of Azithromycin were prepared using melting, kneading and solvent evaporation method. The absorbance of prepared dispersions were reported using UV Spectrophotometer and solubility of individual Drug: Carrier mixture was reported in µg/mL. The solubility of Azithromycin: Mannitol mixture (7.8 µg/mL) and Azithromycin:β-Cyclodextrin mixture (9.52 µg/mL) was found to be maximum. Both these dispersions having maximum solubility were prepared by using melting method and kneading method. From the observations of the study, it can be concluded that melting method and kneading method were effective in enhancing the solubility of the prepared solid dispersions of Azithromycin. Thus, solubility of poorly soluble drugs can be enhanced by using solid dispersion technique.

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Conflicts of Interest

The authors declare that there are no potential conflicts of interest in relation with this article.

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