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The use of spray freeze drying for dissolution and oral bioavailability improvement of Azithromycin

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Abstract

Azithromycin is a poorly water-soluble drug with a low bioavailability. The main purpose of this investigation was to increase the solubility and dissolution of Azithromycin by the preparation of its solid dispersions, using the spray freeze drying (SFD) technique. The physicochemical properties of solid dispersions and interactions between the drug-polymer were evaluated using UV-Vis, FT-IR, DSC and PXRD. The surface morphology of the samples was observed by SEM. In order to carry out an assessment of the drug released, the dissolution test was performed. The in vitro drug released profiles were studied and it was found that the dissolution rate of the solid dispersion SFD sample (SFD-SD) was higher than the intact drug. In addition, the saturation solubility of the SFD-SD was significantly higher than the pure drug. The FT-IR spectra showed there was no chemical incompatibility between the drug and polyvinyl alcohol (PVA). The DSC thermograms revealed no possible physical interaction between the drug and the carrier. The surface morphology of samples showed the amorphous state and the distribution of the drug within the carrier particles. The dissolution of the SFD-SD formulation was increased up to 8.9 times more than pure drug utilizing the SFD technique.

Keywords: Azithromycin; Polyvinyl alcohol; Spray freeze drying; Solid dispersion; Dissolution
1. Introduction

Azithromycin is the first and most important member of a class of antibiotics known as Azalides (1). This antibiotic belonging to the Macrolides group and is a semi-synthetic antibiotic obtained from Erythromycin. Azithromycin has activity against gram-positive organisms and offers increased gram-negative coverage over Erythromycin and Clarithromycin (1, 2, 3). Azithromycin is generally used for middle ear infections, tonsillitis, laryngitis, throat infections, bronchitis, pneumonia and typhoid, etc. (1, 4).

Azithromycin dihydrate (C_{38}H_{72}N_{2}O_{12}.2H_{2}O) is a white crystalline powder, the molecular weight of the drug being 748.984 g.mol\(^{-1}\) (2, 3). Azithromycin is a BCS\(^1\) class II drug (5). The class II specification includes drugs with low solubility, low oral bioavailability and high permeability. The substances require modification to increase their solubility in the upper part of the digestive system. One of the major problems of Azithromycin is its very poor solubility in biological fluids, resulting in poor bioavailability after oral administration (6).

Poly(vinyl alcohol) (PVOH, PVA, or PVAI) is a water-soluble synthetic polymer and is used primarily in topical pharmaceutical and ophthalmic formulations. It is utilized as a stabilizing agent for emulsions as well as a viscosity enhancing agent for viscous formulations such as ophthalmic products (7). It has the idealized formula \([\text{CH}_2\text{CH(OH)}]_n\). PVA is white (colorless) and odorless. PVA was selected as model polymer because it is a commonly used pharmaceutical excipient with a simple chemical structure. Poly(vinyl alcohol) is linear, amorphous and hydrophilic. It consists of a vinyl backbone with pendant hydroxyl group at a 1,3-separation. (8). It is sometimes supplied as beads or as solutions in water (9). Polyvinyl alcohol is a synthetic alcohol with molecular weights ranging from 25,000 to 300,000. PVA is used as a binder, film former, and viscosity increasing agent. It is approved for use as an indirect food additive. A critical evaluation of the existing information on PVA supports its safety for use as a coating agent for pharmaceutical and dietary supplement products. Polyvinyl alcohol was identified as a no immunogenic polymer. Based on the available data, the CIR Expert Panel concludes Polyvinyl Alcohol to be safe as used in cosmetic formulations (10).

The clinical observations of human tissue response to PVA exposure are limited. PVA is used in ophthalmic solutions such as viscid artificial tears. A double-blind study of 5% PVA solution in the treatment of keratoconjunctivitis resulted in no adverse effects in any of 11 patients (11). There is a single report connecting exposure of PVA to human carcinogenesis. Proutt and Davis (10) report the case of a 40-year-old man with hemangiopericytoma of the

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\(^1\) Biopharmaceutics Classification System.
bladder. This man had a 2-year history of daily dermal exposure to a solution of PVA in water that did not contain solvents or plasticizers. The Cosmetic Ingredient Review Panel critically evaluated available information on PVA to determine its safety for use in cosmetic formulations. It was concluded that Polyvinyl Alcohol is safe as used in cosmetic formulations (13). Using PVA as adjuvant leads to more stable morphology, superior aqueous re-dispersibility, and higher production yield compared to the other polymers such as many polysaccharides. Also, the use of PVA as adjuvant leads to nanoparticle aggregates possess large, spherical, and porous morphologies suitable for aerosolization delivery and can effectively reconstitute into primary nanoparticles upon exposure to an aqueous environment (14).

In the last 20 years, more than 41% of the failures in new drug development have been attributed to poor biopharmaceutical properties, including water insolvability. The enhancement of the oral bioavailability of poorly water-soluble drugs is one of the important and challenging aspects of drug development. Solubilization, salt formation, and particle size reduction (as common mechanisms) have been used to increase the dissolution rate and, thereby, bioavailability and oral absorption of poorly water-soluble drugs. Chemical modification, micronization, co-solvency, complexation, pH adjustment, micellar-solubilization, solid dispersion, hydrotropic solid dispersions (HSD), crystal habit modification, etc., are widely used as multiple access techniques for solubility enhancement of poorly water-soluble drugs. Among the various approaches of poorly water-soluble drug dissolution improvement, the solid dispersion of the drug in a water-soluble carrier is one of the most effective methods (15, 16, 17). The first description of solid dispersions was from Sekiguchi and Obi in 1961. Sekiguchi and Obi developed the concept of solid dispersion of water-insoluble drugs (18, 19, 20, 21). Solid dispersion is defined as a dispersion of one (more) active ingredient in an inert carrier, usually highly water-soluble compound, which could be prepared by different methods including: Melting, Fusion method, Solvent and Melting-solvent techniques, Solvent evaporation method, Extruding method, Lyophilization (freeze drying) method, Microencapsulation (22). (The findings of this technique suggest that microsphere formulation offers some new opportunities in the development of solid dispersions which normally encounter processing difficulties.), Spray drying (23, 24) and Spray freeze drying technique (3, 15, 25, 26). Increasing the wettability of the drug, solubilization of the drug and enhancing the concentration gradient of the drug are probable reasons for drug dissolution enhancement in solid dispersions (3, 16, 27). In order to obtain desirable results, a balance must be achieved between all of the above-mentioned factors.

Micronized powders can be generated with the spray-freeze-drying (SFD) technique. In general, the liquid feed is an aqueous or co-solvent solution or
suspension containing an Active Pharmaceutical Ingredient (API) and various pharmaceutical excipients. The spray freeze drying technique combines processing steps common to spray- and freeze-drying. First, atomization of a solution into liquid nitrogen generates droplets. The frozen particles are then transferred to a freeze-dryer where the particles are dried to give a flowable powder. The SFD process offers the following advantages compared with other particle formulation technologies:

1) High process yield
2) Fine control of particle parameters
3) Good compatibility with various biopharmaceuticals and excipients, and
4) Mild stresses on biopharmaceuticals (28).

The term spray-freeze-drying is a general description for different techniques and set-ups (28):

1) Atmospheric spray-freeze-drying
2) Spray-freezing into vapor (SFV) with atmospheric freeze-drying
3) Spray freezing into vapor over liquid (SFV/L)
4) Spray-freezing into liquid (SFL)
5) Spray-freezing into liquid with atmospheric freeze-drying

The ability of the spray-freeze-drying process for the production of aerosol powders was proved by Maa et al. (28). The performed formulations possessed favourable aerodynamic properties. Furthermore, SFD influenza vaccine powder samples were found to be suitable for epidermal powder immunization. Nevertheless further studies are needed to evaluate the SFD process and to characterize the resulting powders more precisely. In summary, the SFD process consists of (29, 30):

1) Atomization of liquid solutions or suspension using ultrasound, one-or two fluid nozzles or vibrating orifice droplet generators
2) Freezing of the droplets in a cryogenic liquid or cryogenic vapor
3) Ice sublimation at low temperature and pressure or alternatively atmospheric freeze drying using a cold desiccant gas stream

So far, few studies were performed about the production of pharmaceutical formulations using spray-freeze-drying (30, 31, 32), such as preparation of low water soluble drugs (33), Ciprofloxacin (34), Phenytoin (35) and Carbamazepine (CBZ) (36), that the overwhelming majority of this researches increase and improve of bioavailability of the used drug have been reported.

In the present study, the probable mechanism might be an increase in surface of the area in the free-flowing system by amorphous phase formation of the drug-carrier by spray-freeze-drying. Finally, the SFD-SD preparations for this investigation can be used for the formulated drug nano-suspension, needle-free powder or dry powder inhalation (inhaled aerosol drug delivery).
2. Experimental procedures

2.1. Materials

Azithromycin dihydrate was purchased from Fluka, USA (Pharmacopeia grade). Polyvinyl alcohol (PVA) was purchased from Sigma-Aldrich, USA (United States Pharmacopeia (USP), Reference Standard Pharmaceutical grade). Sodium hydrogen phosphate and Sodium hydroxide (Analytical grade), Ethanol 96% and Methanol 96% (Analytical grade) were purchased from Merck, USA. All other chemical reagents were Analytical grade. Nitrogen gas, with standard purity of 99.99% with less than 1 ppm water content, was purchased from Aran Gas Esfahan Co. (Esfahan, Iran).

2.2. Preparation of SFD solid dispersions (SFD-SDs) using SFV/L method

In this investigation, the used method, for preparation of SFD-SD is SFV/L. The SFV/L is characterized by atomization of the liquid solution or suspension into the cold gas phase of the evaporation cryogen. Possible set-ups used for spray-freezing into vapour over a liquid cryogen:

1) The porous flow chamber is cooled down to the desired temperature (< -50°C) using refrigerated gases or liquid nitrogen  
2) Liquid feed is atomized into the cryogenic gas within the flow chamber  
3) Frozen droplets are collected on an exit filter at the bottom

In spray freezing into vapor over liquid (SFV/L), the liquid feed is nebulized through a nozzle positioned at a distance above the boiling refrigerant. Two-fluid nozzles and ultrasonic nozzles are used for the atomization step (23). Solidification of the droplets starts while passing through the refrigerant vapor above the liquid surface (37). The particles then freeze “completely” as contact is made with the boiling refrigerant liquid.

The principle of SFV/L spray-freeze-drying is as follows: the liquid feed is sprayed into a fluidized bed containing dry ice and dry air or, alternatively, only pre-dried cryogenic air (38). Figure 1 shows the spray freeze drying apparatus. A two-fluid nozzle, with heating facilities, nebulizes the solutions or dispersions into the stream of cryogenic air (-60°C; top spray). As a result, frozen droplets are formed. The cryogenic air stream then sublimes the frozen solvent(s). A filter maintains the fine product within the drying chamber. The water vapor is driven to the cooling system where it condenses on the refrigerated surfaces (39). To lower the relative humidity and to deliver sufficient energy for sublimation, the cryogenic air passes through a heater. The resulting drying air temperature must, however, be kept below the eutectic temperature, $T_e$, or the glass transition temperature, $T_g$, of the frozen product. Further sublimation
takes place when the recycled air reenters the drying chamber. A laboratory scale SFD apparatus is illustrated in Figure 1. This SFD-device contains two cooling systems working alternately: while one is cooling, the other is de-icing. A bypass connecting tube makes it possible to open the drying chamber without effecting the drying air’s temperature or humidity. To protect sensitive drug formulations from oxidation, nitrogen as an inert drying medium can be used. Aggregation of the individual particles of the frozen powder during the drying step is prevented by the bed fluidization. The particle agitation also increases the sublimation rate, giving drying times that are below those required for standard lyophilization.

All excipients and reagents were used for preparation of the spray freeze-dried powders. The first part of this investigation dealt with the preparation of the SFD-SD sample. Nano-spheres of the drug and polymer were prepared in the drug: carrier ratios of 1:1 (w/w) using SFD technique. A laboratory scale SFD apparatus was used as illustrated in Figure 1. An ultrasonic nozzle (Sono-Tek, 60 kHz or 120 kHz) was suspended 5 cm above a circular stainless steel bowl of 16 cm diameter and 6 cm height. The ultrasonic nozzles were operated at a generator power of 7.0 W, unless otherwise stated. The bowl rested on a magnetic stirrer. A liquid nitrogen (LN$_2$) source was contained in a 50 liter sealed Dewar (CMR-Direct, USA). The bowl was filled with LN$_2$ and, after a short pause; the solution was sprayed into the bowl using a peristaltic pump (Pharmacia, P1; tubing 1.0 mm id.) with a feed rate of 2 mL/min, unless otherwise stated.

The solutions of Azithromycin with polyvinyl alcohol (PVA) were prepared in methanol and distilled water and (volume ratio=1:4) the final solution concentration obtained was 5% w/v. 10 mL of final solution were sprayed. The LN$_2$ was continually stirred with a stir bar during the process. On completion of spraying, the bowl was topped up with LN$_2$ and immediately placed on the shelves of a pre-cooled (-45°C) freeze-dryer (Martin Christ Freeze Dryers, Christ Delta 1-24 KD, Osterode, Germany). The cryogenic liquid was allowed to evaporate before the start of the primary drying step. The available shelf area was sufficient for six bowls, allowing up to six formulations to be prepared in one batch.

At the end of the pre-programmed drying cycle, the lyophilizer was ventilated with dry air (relative humidity; RH ≤ 10% at room temperature). The powders were removed from the bowls and immediately transferred to closed glass containers. A dry-air purged glove box was used for the powder collection before powder physicochemical parameters were tested (39).

2.3. Preparation of physical mixtures
The physical mixtures were prepared as controls. The appropriate amount of pure Azithromycin and PVA was mixed using a mortar and pestle for 5 min to obtain the physical mixture.

2.4. Determination of drug content and saturation solubility

The second part of this work was performed in order to evaluate the SFD-SD. In the classic saturation solubility method, the concentrations of saturated solutions were determined using UV spectrophotometry. Therefore, the specific absorptivity of the compounds on the exact pH in the buffer solution must be determined earlier. This was measured using two or more dilution series, then the calibration curve was calculated using Lambert-Beer law. The slope of the curve gave specific absorptivity. In this study, 100 mg of the solid dispersions or physical mixtures were accurately weighed and dissolved in 100 mL of phosphate buffer pH=6.0. The samples were added to glass flasks including phosphate buffer pH=6.0 and then they were mixed for 24 hours at a temperature equal to 25°C with a fixed speed of 300 RPM (VARIOMAG® Poly 15, Multipoint Stirrer, Germany). The solutions were filtered and then centrifuged with a fixed speed of 10000 RPM for 10 min in two sessions. Then Crystallization took place. Moreover, the samples were spectrophotometrically measured by UV SHIMADZU Spectrophotometer (SHIMADZU UVmini 1240 Spectrophotometer, Japan) at 215 nm (40). The standard curve for the estimation was prepared in a concentration range of 5-40 μg/mL. In this concentration range, good linearity was observed with the correlation coefficient ($R^2=0.9995$). The graph followed the Beer-Lambert’s law in the selected concentration range. This test was repeated three times for each of the samples and their means were recorded. Data were analyzed by analysis of variance (ANOVA) and T-test.

2.5. Dissolution studies

*In vitro* release studies of Azithromycin and its solid dispersions and physical mixtures were performed in USP Apparatus II and by a dissolution test apparatus (Erweka DT6R, Germany) at 37.0±0.5°C and 100 RPM and 900 mL of phosphate buffer pH=6.0 (41). Approximately 100 mg of samples (pure drug, solid dispersions and physical mixture) were analyzed for the dissolution studies. Aliquots of 5 mL were withdrawn at a specified time at intervals of 5, 10, 15, 20, 30, 45 and 60 min and replaced with fresh media. The samples were filtered and analyzed by a spectrophotometer at 215 nm for the dissolved drug. The dissolution studies were carried out in triplicate (Tables 1 and 2).
2.6. Dissolution efficiency

Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time "t" and expressed as a percentage of the area of the rectangle, described by 100% dissolution at the same time (the following equation). Using this method has several advantages; for example, it can describe all points on the dissolution rate of curve (42).

\[
\text{Dissolution Efficiency (DE\%)} = \left( \frac{\int_0^t y \, dt}{y_t \times 100} \right) \times 100
\]

Where: "y" is the percent drug dissolved at the time “t”.

2.7. Fourier Transform Infra-Red spectroscopy

The Fourier Transform Infra-Red (FT-IR) spectra of pure drug, carrier, solid dispersions and physical mixtures were obtained using a Perkin-Elmer IR spectrometer 843 System (Shelton, USA). Approximately 2-3 mg of the sample was mixed with dry KBr and the spectra scanned over the wavenumber range of 4000-200 cm\(^{-1}\). An average of 20 scans was taken.

2.8. Differential scanning calorimetry

The differential scanning calorimetry (DSC) is widely used in pharmaceutical testing. The DSC thermograms of Azithromycin, PVA and all preparations were performed using (SHIMADZU DSC-60, Japan). The weighed (6.00 mg) samples were crimped in Aluminum pans and heated from 20.0°C to 250.0°C at a heating rate of 10°C/min in a Nitrogen atmosphere. The calorimeter was calibrated using Indium as standard in 156.6±1°C. An empty sealed Aluminum pan was used as a reference.

2.9. Powder X-Ray diffraction

The powder x-ray diffraction (PXRD) spectra (Diffractogram) of Azithromycin, PVA, solid dispersions and physical mixtures were recorded utilizing a powder X-ray diffractometer, PHILIPS PW 1800 X-Ray Diffraction Machine (Almelo, The Netherlands) with a copper tube anode on the interval 5-
90° 2θ°. The other operational parameters were as follows: Scan step time was 9 sec° and scan step size was 0.008° (2θ); Generator tension (voltage) was 45 kV; Generator current was 40 mA.

2.10. Scanning electron microscopy

The scanning electron microscopy (SEM) analysis was performed for surface morphology evaluation of samples. The surface morphology of the drug, carrier and selected sample was determined utilizing an analytical scanning electron microscope VEGA TESCAN-LMU (Czech Republic), equipped with a thermo-emission cathode (Balzers Union Ltd, Balzers, Lichtenstein). The samples were monitored, then an image was generated using a 30 kV electron beam. The samples were placed on a sample disc carrier carbon stub (10 mm diameter, 3 mm height) and sputter-coated with gold under vacuum (0.25 Torr).

2.11. Stability studies

The prepared formulations using ICH² guidelines (for a period of 60 days) were studied for accelerated stability. These studies were carried out at 40±2°C and 75±5% RH (relative humidity) for a period of 60 days by an environmental test chamber (Cooper Group 450/ME/-40, Albert Court, United Kingdom). The samples were kept in glass vials sealed with rubber plugs. Approximately 10 mg of samples were taken out in 0, 30 and 60 days; they were then analyzed for drug content and physical changes (Table 3).

2.12. Statistical analysis

All data using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA) were analyzed by ANOVA with Tuckey’s multiple comparison test. This test was used as the criteria to assess statistical significance (43, 44).

3. Results and discussion

3.1. Dissolution studies

Table 1 presents the results of the drug content and saturation solubility. The percentage of drug content was in the range of 98.48±0.07% to 99.92±1.27%. Maximum saturation solubility of Azithromycin belonged to SFD-SD with 759±0.48 μg/mL (~ 9 times more than pure drug). The saturation

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² International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.
solubility of the physical mixtures of the samples were prepared and measured. Among the prepared samples, the PM sample with weight ratios of 1:1 (drug:carrier; 151±1.49 μg/mL) has the minimum aqueous saturation solubility and shows the slight solubility and dissolution rate. The best results of the samples belonged to SFD-SD. The in vitro dissolution rate of pure Azithromycin was very poor during 60 min; the maximum drug release was ~ 22% (Table 1 and Figure 2). The maximum drug released is related to SFD-SD with weight ratios of 1:1 (Azithromycin:PVA). For SFD-SD, the drug released at 60th min was 87.67%. After a period of 60 days, based on stability studies, there were no significant changes in the dissolution rate and drug content of the samples (Table 3).

In this study, the solubility of Azithromycin was increased up to 8.9 times by an appropriate carrier such as PVA, a high performance and affordable method. Consequently, the main reason for the solubility enhancement of the prepared samples is the amorphous state formation of Azithromycin (3). The further studies were performed on SFD-SD. The results of SFD-SD were satisfying (see DE parameters of SFD-SD). The probable reasons for the dissolution improvement are: the amorphous phase formation of SFD-SD and the local solubilizing action by carrier (19, 21, 45).

Overall, in the dissolution studies, the amorphous state formation can increase the solubility to ~ 9, and the dissolution rate (under in vitro conditions) up to ~ 4 times more than the intact drug. SFD-SD was selected for further studies and other characterization tests were performed on this sample.

3.2. FT-IR studies

An FT-IR spectroscopy analysis was carried out for the evaluation of any possible interaction between the drug and carrier (Figures 3C and 3D). Figure 3 shows the FT-IR spectra of pure Azithromycin, PVA and selected samples. The spectrum of pure Azithromycin (Figure 3A) shows characteristic peaks at 1724 cm⁻¹ (C=O stretch), 1190 cm⁻¹ (C-O-C asymmetrical stretching) and 1068 cm⁻¹ (C-O-C symmetrical stretching). Also, FT-IR spectra of the PVA (Figure 3B) show the characteristic peaks at 1333 cm⁻¹ (symmetric stretching vibration -CH₃ tertiary amide), at about 1082 and 1435.5 cm⁻¹ for the -C-O group and band located at 2943 cm⁻¹ (asymmetric stretching vibration C-H), and 3000 cm⁻¹ to 3750 cm⁻¹ for (stretching vibration O-H).

Upon comparing the FT-IR spectra of the samples with that of the pure drug, it was noticed that the characteristic peaks of Azithromycin were also present in the sample spectra. For SFD-SD, only one stretching vibration peak was observed at approximately 2850 cm⁻¹ in the FT-IR spectrum (Figure 3C). The disruption of hydrogen bonds between the crystalline water and Azithromycin
can lead to a blue shift of the stretching vibration peak (at approximately 3400 cm\(^{-1}\)). The stretching vibration peak of Azithromycin may be concealed by the stretching vibration peak of PVA. Also, the illustrated peaks of Figure 3D could be seen clearly in curves, indicating that no interaction occurred between the different components in the physical mixture (46).

3.3. DSC studies

The DSC thermogram shows a sharp endothermic peak at 109.45°C to 127.77°C, which corresponds to the melting point of pure Azithromycin (Figure 4A). This melting point peak is also found in all of the samples. The pure PVA (Figure 4B) displays two endothermic peaks. The first peak at 88.2°C is assigned as a thermal effect due to moisture evaporation from the sample. It may also be due to a glass transition with an enthalpy 130.9 J/g and a sharp endothermic melting transition at 202.8°C with an enthalpy 67.4 J/g. The heat, required for melting of 100% crystalline PVA, is 138.60 J/g. The PM sample peaks were observed at 18°C, 64°C, 105°C and 149°C, respectively (Figure 4D). These peaks related to (I) 74°C: melting peak drug-carrier (drug-carrier fusion with a shifting); (II) 105°C: the melting peak of the drug; and (III) 149°C: the endothermic peak of Azithromycin.

Based on the DSC results, there was no appreciable change in the melting endotherms of the physical mixture. The difference in the PVA peak area of the samples correlates with the different amounts of PVA in the samples. This conclusion is in accordance with the PXRD results (47, 48, 49). The thermograms of SFD-SD did not show any distinct peaks of Azithromycin. In addition, the thermal data show a clear (sharp) melting endothermic peak (Figure 4C) between 60°C and 80°C with a shoulder for SFD-SD (a glass transition is found, approximately \(T_g=88°C\)). This must, in some way, be a melting transition of the amorphous solid solution of the drug/carrier. The thermograms did not contain any new thermal effects. However, the DSC thermogram of SFD-SD showed no endotherm around the melting point of Azithromycin, indicating that Azithromycin is available in its amorphous state.

3.4. PXRD studies

The PXRD diffraction spectra of Azithromycin, PVA, SFD-SD and PM (as related physical mixture sample) were illustrated in Figures 5A-5D, respectively. Several distinct and sharp peaks were observed at 2\(\theta\) diffraction angles of 9.56°, 7.72°, 20.67°, 19.59°, 23.80°, 17.31°, 28.68° and 26.55° for pure Azithromycin. Also, the PXRD pattern of PVA exhibits broad diffraction peaks at 2\(\theta = 11.211\), 12.687, 15.280, 19.450, 20.654, 23.520, 25.196, 28.148 and 30.741, that 2\(\theta =11°\) and 2\(\theta =19°\), which are typical fingerprints of semi-crystalline PVA and which indicated their crystalline properties.
The PXRD diffractograms of the SFD-SD show no signs of crystallinity (Figures 5C and 5D). In the SFD-SD, the peaks corresponding to Azithromycin crystals disappeared completely, suggesting that an amorphous phase was formed and so the crystallization of Azithromycin was decreased by the PVA. The main PXRD peaks of untreated Azithromycin appeared with very low intensity in SFD-SD or PM at the same 2θ, which confirmed the crystalline (crystallinity index) of Azithromycin reduced in solid dispersion, physical mixture and crystallinity changes occurred during the preparation process. Based on this result, the enhancement of Azithromycin dissolution for SFD-SD was related to the crystalline changes (with a significant reduction of crystallinity index). However, the conclusion of the PXRD patterns is that the drug is dispersed in the amorphous phase.

3.5. SEM studies

SEM with different magnifications was used for investigating the morphological differences of Azithromycin and SFD-SD. Figure 6 shows that Azithromycin is irregularly shaped (Figures 6A and 6B). The particles of SFD-SD have a general spherical morphology. The spray freeze-dried particles all showed a spherical shape, although sometimes slightly aggregated (Figures 6C and 6D). The SEM images show that the surface area of the Azithromycin reduced during the preparation of solid dispersion samples as a result of the SFD process, resulting in the dispersion of Azithromycin molecules within the medium carrier. The dry powder particles of the SFD samples (Figures 6C and 6D) have a greater and smoother surface compared to the particles from pure Azithromycin and PM. However, most of the particles show diameters which lie at the lower end of this range. Some degree of aggregation is evident. Most of the SFD particles show a crinkled morphology, as illustrated in Figure 6D. A few particles have a smoother, but porous, surface (Figure 6C), beneath which a high internal porosity is evident. The reason for the presence of these two, distinct, different morphologies is unknown.

The SFD-SD has fine powder fractions, indicating a reduced mechanical strength of the powder particles. The SEM show that SFD-SD consists of discrete, spherical particles with clearly visible smooth structures on their surfaces (Figures 6C and 6D). This typical appearance of SFD-SD particles is confirmed by the results of Maa et al. and Sonner et al. (50). The appearance of the powder particles from the selected samples is strongly influenced by the mass ratio of the polymer. Higher amounts of polymer in the excipient mixture lead to smoother particles.
3.6. Particle size distributions

The particle size distributions of the SFD-SD powder were performed by a laser particle size analyzer (HORIBA, LA-960 Laser Particle Size Analyzer, Kyoto, Japan). The particle size distributions of SFD-SD were (Figure 7) in the range of approximately 10 to 100 μm, while the average of the particle size was 54.44 μm.

4. Conclusions

The present work shows that the dissolution rate of Azithromycin from SFD solid dispersions with PVA improved more than 890% compared to the pure drug. The first part of the work dealt with the preparation of SFD-SD. The SFD-SD of the drug-excipient complex was carried out to develop basic samples. The second part of this work was performed in order to evaluate the SFD-SD. Further, the SFD solid dispersion preliminary solubility analysis was carried out for the characterization and evaluation of the SFD-SD. Also, the saturation solubility of the drug, which formulated into the SFD-based solid dispersion with the PVA, was higher than that of the solubility achieved in the presence of the polymer. The amorphous nature of the drug upon solid dispersion was confirmed by the reduction of enthalpy of the drug melting in solid dispersion compared to the pure drug (according to DSC studies). PXRD analysis indicated a reduction of drug crystallinity in solid dispersion. Spray freeze drying showed its ability in producing solid powders. It can be concluded that solid dispersion is one of the best methods to increase the solubility and dissolution rate of Azithromycin.

In comparing the two methods of SFD process and normal spray drying; an increase in specific surface area and a reduction of density to approximately one-ninth for SFD powder compared to SD powder was observed by Maa et al. (49). Hollow dimpled spherical nano-aggregates are produced by normal spray drying, whereas SFD produces large spherical porous nano-aggregates. For both spray drying and SFD, PVA has been found to be effective in facilitating the nano-aggregate reconstitution. In addition, the inclusion of PVA prepared by SFD is crucial to achieve effective aerosolization of the nano-aggregates. Overall, nano-aggregates produced by SFD exhibit superior characteristics compared to those produced by SD in terms of size, yield, flowability, aqueous reconstitutibility, and aerosolization efficiency.

The results of this study perfectly are similar to other studies that where is used SFD methods and the same excipient (PVA) as an adjuvant (7). The SFD process produced a powder with an improved mass median aerodynamic diameter and fine particle fraction compared to a previously studies, similar
ciprofloxacin (34) powder. Also increasing the dissolution rate is better than of similar studies (37, 45).

The SFD solid dispersion was analyzed via an *in vitro* dissolution test; a solid dispersion of the drug with PVA had shown enhanced solubility with an improved dissolution rate (51, 52). Furthermore, the influence of the SFD process on stability of additional differing drugs must be examined to improve stabilization efforts.

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Nomenclature

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<th>°C</th>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>DE</td>
<td>Dissolution Efficiency</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform Infra-Red</td>
</tr>
<tr>
<td>HSD</td>
<td>Hydrotropic Solid Dispersions</td>
</tr>
<tr>
<td>KBr</td>
<td>Potassium Bromide</td>
</tr>
<tr>
<td>LN$_2$</td>
<td>Liquid Nitrogen</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>pH</td>
<td>Potential of Hydrogen</td>
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<tr>
<td>PM</td>
<td>Physical Mixture</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl Alcohol</td>
</tr>
<tr>
<td>PXRD</td>
<td>Powder X-Ray Diffraction</td>
</tr>
<tr>
<td>R$^2$</td>
<td>Correlation Coefficient</td>
</tr>
<tr>
<td>RH</td>
<td>Relative Humidity</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions Per Minute</td>
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<tr>
<td>Sd</td>
<td>Standard Deviation</td>
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<tr>
<td>SD</td>
<td>Solid Dispersion</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<tr>
<td>SFD</td>
<td>Spray Freeze Drying</td>
</tr>
<tr>
<td>Te</td>
<td>Eutectic Temperature</td>
</tr>
<tr>
<td>Tg</td>
<td>Transition Temperature</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet–Visible</td>
</tr>
<tr>
<td>v</td>
<td>Volume</td>
</tr>
<tr>
<td>w</td>
<td>Weight</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometer</td>
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</table>
Figure and Table Legends

Legend to Figures:

Figure 1. Schematic construction of spray-freeze-drying (SFD) apparatus

Figure 2. (A) *In vitro* dissolution drug release profiles of pure Azithromycin, SFD-SD and the physical mixture (*n* = 3, mean ± Sd), (B) Column charts of *in vitro* dissolution drug release of pure Azithromycin, SFD-SD and the physical mixture for compare values across categories (*n* = 3, mean±Sd)

Figure 3. FT-IR spectra of: (A) pure Azithromycin, (B) PVA, (C) SFD-SD and (D) PM

Figure 4. DSC thermograms of: (A) pure Azithromycin, (B) PVA, (C) SFD-SD and (D) PM

Figure 5. PXRD Diffractograms of: (A) pure Azithromycin, (B) PVA, (C) SFD-SD and (D) PM

Figure 6. SEM Microphotograph of: (A) pure Azithromycin; (magnification: 500X), (B) pure Azithromycin; (magnification: 2500X), (C) and (D) SFD-SD; (magnification: 500X)

Figure 7. Particle size distribution (number) of SFD-SD after stirring at 3000 RPM for 5 minutes

Legend to Tables:

Table 1. Drug content and solubility data of pure Azithromycin, prepared SFD-SD and the related physical mixture (*n*=3, mean±Sd)

Table 2. Dissolution parameters of pure Azithromycin, prepared SFD-SD and the related physical mixture (*n*=3, mean±Sd)

Table 3. Drug content of SFD-SD after stability studies according to ICH guidelines (*n*=3, mean±Sd; storage conditions: 40±2°C, 75±5%RH)
Fig. 1

1) Peristaltic pump
2) Magnetic stirrer
3) Power generator
4) Stainless steel container
5) Ultrasonic nozzle
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
$d(n, 0.5) = 54.44 \, \mu m$

Fig. 7
Table 1

<table>
<thead>
<tr>
<th>Batch</th>
<th>Drug : Carrier (w/w)</th>
<th>Theoretical Drug Content Amount (mg)</th>
<th>Theoretical Drug Content Expressed (%)</th>
<th>Assayed Drug Content Amount (mg)</th>
<th>Assayed Drug Content Expressed (%)</th>
<th>Saturation Solubility (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>1:0</td>
<td>100</td>
<td>100</td>
<td>99.92</td>
<td>99.92±1.27</td>
<td>85±1.08</td>
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<td>SFD-SD</td>
<td>1:1</td>
<td>50</td>
<td>100</td>
<td>49.24</td>
<td>98.48±0.07</td>
<td>759±0.48</td>
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<tr>
<td>PM</td>
<td>1:1</td>
<td>50</td>
<td>100</td>
<td>49.85</td>
<td>99.70±0.38</td>
<td>151±1.49</td>
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Table 2

<table>
<thead>
<tr>
<th>Batch</th>
<th>Drug : Carrier (w/w)</th>
<th>DE_{30} (%)</th>
<th>DE_{30} (%)</th>
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</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>1:0</td>
<td>4.50±1.12</td>
<td>14.32±0.99</td>
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<tr>
<td>SFD-SD</td>
<td>1:1</td>
<td>46.11±0.66</td>
<td>80.40±1.83</td>
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<tr>
<td>PM</td>
<td>1:1</td>
<td>7.19±1.18</td>
<td>17.88±1.39</td>
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</table>

Table 3

<table>
<thead>
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<th>Time (day)</th>
<th>Pure Azithromycin</th>
<th>SFD-SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>99.92±1.27</td>
<td>98.48±0.07</td>
</tr>
<tr>
<td>30</td>
<td>99.90±0.14</td>
<td>98.31±2.25</td>
</tr>
<tr>
<td>60</td>
<td>99.88±0.78</td>
<td>97.35±1.55</td>
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</tbody>
</table>
Graphical abstract
Highlights

- Utilizing spray-freeze-drying, solid dispersions of Azithromycin-PVA were prepared.
- The samples were characterized by the dissolution tests, FT-IR, DSC, PXRD and SEM.
- The dissolution and solubility of SFD-SD samples were significantly increased.
- The prepared samples can be used for the drug Nano-suspensions or inhaled aerosols.