Original Article

Preparation and Characterization of Azithromycin Loaded Solid Dispersion: A New Approach to Enhance *in vitro* Antibacterial Activity

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ABSTRACT

Aim: Azithromycin (AZ) possess high permeability but low solubility in gastrointestinal (GI) fluid and exhibited unpredictable dissolution profile resulting in poor oral bioavailability. Therefore, the study was aimed to enhance the dissolution rate of AZ and evaluation of its *in-vitro* antibacterial activity. **Materials and Methods:** Solid dispersions of AZ (ASDs) were prepared by solvent evaporation technique using Na-CMC alone or in combination with Carplex-80 as carrier in different ratios. Subsequently, *in-vitro* dissolution study were performed followed by physicochemical characterization using differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) and finally antibacterial activity were assessed. **Results:** The formulation ASD-6 exhibited faster (6.59% at 5 min) and higher (20.02% at 120 min) drug release which were 4.84 and 7.94 fold higher than that of pure AZ. A significant increase in relative zone of inhibition (RZOI) was observed with ASD6 ($p<0.001$) when compared to that of pure AZ against *S. aureus and E. coli* at each sampling point. **Conclusion:** The *in-vitro* dissolution study indicated that among the six formulations AZ: Carplex-80: Na-CMC (1:3:2) complexes prepared by solvent evaporation technique exhibited highest dissolution rate and which might be responsible for enhanced antibacterial efficiency.

Keywords: Solid dispersion, Azythromycin, Hydrophilic carrier, *In-vitro* dissolution, Physicochemical characterization, Antibacterial activity.

INTRODUCTION

Poor aqueous solubility of drug is one of the critical problems encountered during the formulation and development stage leading to inadequate absorption and thus, poor bioavailability.[1] About 75% of the new candidates in drug development are poorly soluble in water and of which 40% of marketed drugs experienced poor aqueous solubility and thus, low therapeutic outcomes.[2] Drugs with poor aqueous solubility create major problems in both *in-vivo* and *in-vitro* assays and impart significant burden during drug development process. Therefore, low water solubility continued to be a major problem in the formulation of new medicines.[3,4] According to the biopharmaceutics classification system (BCS) drugs belongs to BCS-II category have high permeability and low solubility; and hence, the dissolution rate becomes the main determinants of bioavailability for drugs in this category.[5,6] The enhancement of drug solubility and dissolution profiles without altering its molecular structure is a big challenge for scientists working in formulation area.[7]

Azithromycin (AZ) is a broad spectrum semisynthetic macrolide antibiotic containing a 15-membered lactone ring, which is derived from erythromycin containing nitrogen atom in the ring structure substituted with methyl group; and that makes it distinct

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from the other available macrolide antibiotics.[8,9] It is a broad spectrum antibiotic and effective against both gram positive and gram negative bacteria. AZ acts both as bacteriostatic and bactericidal substance depending on the microorganism involved and drug concentration; and exerts its mechanism of action by reversibly binding to the 50S ribosomal subunit and inhibit protein synthesis in sensitive microorganisms.[10-12] AZ is primarily used for the management of respiratory tract infections (RTI) such as pneumonia, bronchitis, pharyngitis, tonsillitis, acute watery diarrhea, gonorrhea, skin and soft tissue infections. It may also be used for the prophylactic treatment of *Mycobacterium avium* complex (MAC) infections and active against *H. influenza*. It is used for the management of trachomatis, cervicitis, Chlamydia and typhoid and also recommended for the prophylaxis of endocarditis in at-risk patients unable to take penicillin.[10,13,14]

AZ possess inadequate aqueous solubility (0.1mgm/L) which is responsible for its relatively poor oral bioavailability (only 37%).[15] AZ belongs to BCS class II drug, and hence, dissolution of drug may be a rate-limiting step during absorption process.[6,16] Various attempts have been taken to overcome this problem including development of self-emulsifying drug delivery system using spray drying process,[17-18] encapsulation of drug into various silica,[19-21] impact of fillers,[22] mesoporous silica nanoparticles used to prepare oral push-pull osmotic pump,[23] electrosprayed nanospherules,[24] nanosuspension[25] etc. However, the most popular and widely used solid dispersion technique was utilized by numerous methods such as fusion method, melting method and solvent evaporation method.[26-28] This solid dispersion technique is highly preffered due to its simple process and provides high production throughput including sufficient drug loading capacity into the carrier molecules. The method requires at least two different components, generally a hydrophobic drug and another one is hydrophilic matrix carrier in which the drug is dispersed molecularly, in amorphous particles or crystalline state.

Recent studies indicated many advantageous properties of solid dispersions in improving the solubility and dissolution rate of poorly water soluble drugs including reduction of particle size, enhancement of wettability and porosity, as well as conversion of drug crystalline state preferably into amorphous particle.[29-30] In this study attempts have been taken to enhance the dissolution of AZ by solid dispersion technique using silica (Carplex 80) and sodium carboxymethyl cellulose (Na-CMC) as carriers that are not yet used in combination with AZ based on available literatures.

MATERIALS AND METHODS

Drugs and Chemicals

AZ was a generous donation obtained from Square Pharmaceuticals Ltd., Pabna, Bangladesh. Silica (Carplex 80) was obtained from Evonik Pvt. Ltd. (Hanua, Germany). Na-CMC was procured from Qualikem Fine Chem Pvt. Ltd. (India). Acetone was purchased from Merck, Germany. Agar media purchased from HiMedia Pvt. Ltd. (India). The chemicals and solvents used were of standard grade.

Preparation of Solid Dispersions of Azithromycin

Solid dispersions of azythromycin (ASDs) were prepared by the solvent evaporation method using two different carriers e.g., silica (Carplex-80) and Na-CMC. In brief, 50 mg of AZ weighed and were dissolved in 20 ml of acetone, into which carriers were added at different ratios (Table 1) and then, dispersed by continuous stirring with a magnetic stirrer at 200 rpm allowing maximum loading of the drug into carriers. The temperature of the dispersion system was maintained at 50°C to evaporate the solvent to dryness. Finally, the magnetic stirrer was stopped and the ASDs formulattion were dried at room temperature. The dried mass was crushed in a mortar to yield fine powder. The ASDs thus obtained were kept separately depending on the type of carriers used (Table 1) and stored in a screw-cap glass vial at room temperature until further use.

Preparation of Standard and Working Solution of Azithromycin

100 mg of AZ was weighed and dissolved 20 ml of acetone in a 100 ml standard flask followed by addition of phosphate buffer (pH 6.8) to make the final volume (100 ml). From this stock solution, a diluted solution at 40µg/ml was prepared to use as a standard. The content of drug in sample solution was quantified by using standard curve (4 µg/ml, 8µg/ml, 12 µg/ml, 16 µg/ml, 20 µg/ml

Table 1: Formulations of ASDs using different drug carrier ratio.

Group	Formulations	Drug: Carrier	Ratio
I	ASD-1	AZ : Na-CMC	1:1
	ASD -2	AZ : Na-CMC	1:1.5
	ASD -3	AZ : Na-CMC	1:2
	ASD -4	AZ : Na-CMC	1:3
II	ASD -3	AZ : Na-CMC	1:2
	ASD-5	AZ : Na-CMC: carplex-80	1:2:3
	ASD-6	AZ : carplex-80: Na-CMC	1:3:2

and 40 µg/ml of AZ). The solutions were assayed by UV spectrophotometer (UV mini-1240, Shimadzu Co., Japan) at 262 nm and results were recorded.

Determination of Drug Content

The amount of AZ in ASDs was quantified by UV-spectrophotometric method. Briefly, ASD equivalent to 25 mg of AZ was accuartely weighed and dispersed in 25 ml of acetone. The dispersion was filtered and 1 ml of filtrate was added into a volumetric flasks and diluted to 25 ml with phosphate buffer (pH 6.8). The drug content was analyzed by using the calibration curve as described earlier.

In-vitro Dissolution Study

Dissolution of both pure AZ and prepared ASD formulations were performed using dialysis system in a dissolution tester (Tianjin Guoming Medicinal Equipment Co., Ltd.) and demineralized (DM) water was used as dissolution medium. Fisherbrand regenrated cellulose dialysis tubing (MW: 12,000-14,000; width: 45 mm; wall thickness: 20 µm; dry cylinder diameter: 28.6 mm; volume/cm: 6.42) was loaded with ASDs equivalent to 90 mg of AZ and soaked in 900 ml of water contained in dissolution vessel. The paddle speed and temperature in dissolution apparatus were maintained at 50±2 rpm and 37.0±0.5°C, respectively. An aliquots of 10ml each were withdrawn at 5, 15, 30, 60, 90 and 120 min, which was replaced with an equal volume of water. The sample withdrawn was transferred into a volumetric flask and diluted with phosphate buffer (pH 6.8) to 25 ml and thus the concentration of AZ at each time point was analyzed. *In-vitro* dissolution study were repeated three times and mean values from three replicates were calculated. Thus, the mean concentration of AZ was plotted against time to derive the dissolution profile.

Solid-state Characterization of ASD

a) Differential Scanning Calorimetry (DSC)

Thermogram of samples (AZ, Na CMC, Carplex-80, ASD3, ASD5, and ASD6) were obtained from DSC (Exstar, SII DSC7020). Briefly, 3-5 mg of samples were placed in sealed standard aluminium pans and heated from 0°C to 300°C (at a scanning rate of 10°C/min), under nitrogen purge, and an empty aluminium pan was used as a reference.

b) Powder X-Ray Diffractometer (PXRD)

The powder diffraction studies of AZ, Na CMC, Carplex-80, ASD3, ASD5, and ASD6 were performed by an X-ray diffractometer (RAD-C, Rigaku Denki Co., Ltd.). The samples were exposed to CuKα radiation (30 kV, 50 mA) and scanned from 2°C to 40°C, 2θ at a scanning rate of 5°C /min.

c) Fourier Transform Infrared Spectroscopy (FTIR)

The samples were analyzed by the diffuse reflection method using an FTIR spectrometer (IR-Prestige 21, Shimadzu Co.) to study any incompatibility between AZ, Na-CMC, Carplex-80, ASD3, ASD5, and ASD6. Additionally, differences in the spectra of powder sample and subtractions of the spectrum of Na-CMC, Carplex-80 was determined.

d) Scanning Electron Microscopy (SEM)

Scanning electron microscope (SSX-500, Shimadzu Co., Japan) imaging was used to determine the shape, surface and cross-sectional morphology of AZ, Na-CMC, Carplex-80, ASD3, ASD5, and ASD6. An accelerating voltage of 15 kV was used with gold/palladium coating in a vacuum beforehand.

Evaluation of Antimicrobial Activity of ASD6

Source of Bacteria

American type culture collection (ATCC) bacterial strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were collected from the Microbiology Department, Rajshahi Medical College and Hospital (RMCH), Rajshahi, Bangladesh and used to perform antibacterial activity of optimized formulation against the bacterial strains.

Preparation of Antimicrobial Disks

During dossolution study an aliquet (10 ml each) of ASD6 formulations were taken from vessel at 5, 15, 30, 60, 90 and 120 min respectively. The samples were extracted with 5 ml of chloroform, the process was repeated three times and extracted samples were combined in vials. The combined samples were allowed to dry and after complete drying, 300 µl of acetone were added into each vial and the vials were shaken vigorously to ensure complete dissolution of extracts in acetone. A blank disk (Bio Maxima S.A.) was kept on a sterilized Petri dish, and sample was applied into a sterilized disk, so that the concentration of AZ was 30µg/disk. The experiments were repeated three times with each sample and mean values from three replicates were calculated. For antibacterial activity testing the disks were made readily available after being dried as a result of complete evaporation of acetone. Kirby–Bauer disk diffusion method was followed to determine the antibacterial activity of formulation ASD6 and pure AZ.[31]

Antibacterial Activity Study

For antibacterial activity testing, inoculum were prepared by adding fresh colonies of both *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) into 5 ml of saline (0.9% solution) and turbidity of the resulting solution was compared with McFarland standard.[31] The Mueller-Hinton agar plate was inoculated by spreading 100 µl of inoculum onto the freshly prepared agar medium with a sterilized swab. Precautions were taken so that there was no moisture accumulated on the lid or surface of the agar. For inoculation bacterial culture at a concentration of 10^7 colony-forming units (CFU)/mL were prepared from each strain. To perform the activity test, 5 µL aliquots of each culture were placed on the agar plates. As a control bacterial inoculums were added on each agar plates without AZ sample to confirm bacterial growth and sterility of the medium. Sample disks from different formulations and time point were prepared and placed onto the agar plate according to Kirby-Bauer disk diffusion susceptibility test protocol. The plate was kept into refrigerator for at least 3 hr to ensure proper diffusion. As a reference, standard disk from pure drug (AZ) was prepared and placed in the middle of the medium. Finally, the plate was incubated at 37°C for overnight and ZOI of each disk was measured (in mm).

Determination of Relative Zone of Inhibition (RZOI)

The RZOI was determined by calculating the diameters of each ZOI of samples (in mm) divided by diameter of control disk (in mm) on the same Petri dish according to the following equation:

$$RZOI = \frac{ZOIs}{ZOIc}$$

Where, RZOI = Relative zone of inhibition; ZOIs = ZOI of sample in mm; ZOIc= ZOI of control in mm

Statistical Analysis

Date were presented as the mean ± standard deviation (SD). Differences between groups were determined by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A value of $p<0.05$ were considered as statistically significant.

RESULTS

Dissolution Study

Dissolution tests were performed to understand the release pattern of AZ from ASDs formulation. Thus, newly prepared ASDs were subjected to dissolution study and their dissolution profile were constructed and compared with that of pure AZ.

Dissolution Profile of Formulations Containing Na-CMC (Group-I)

Dissolution profiles of various ASD formulations with Na-CMC are depicted in Table 2 and Figure 1(a). Maximum drug release from pure AZ was observed as 2.52% at 120 min, whereas all ASD containing Na-CMC showed faster drug release than that of pure AZ at each time point. At 30 min, drug release was 2.74%, 5.48%, 7.64% and 6.30%, from ASD1, ASD2, ASD3, and ASD4 respectively. Among these, ASD3 showed significantly higher drug release than pure AZ, ASD1, ASD2 and ASD4 (Table 2). At 30 min drug release from ASD3 was 4.47 fold greater when compared to that of pure AZ.

Dissolution Profile of Formulations Containing Na-CMC and Carplex-80 (Group-II)

Dissolution profile of ASD containing carplex-80 in combination with Na-CMC showed in Table 3 and Figure 1(b). In this group of formulation ASD3, ASD5, ASD6 showed drug release at 30 min as 7.64%, 13.13% and 14.41%, respectively. ASD5 and ASD6 showed significantly higher drug release (Table 3) than pure AZ and ASD3. Both ASD5 and ASD6 showed better drug release of which ASD6 is the most promising. Drug release from ASD6 at 30 min is 1.72 fold higher than ASD3 and 8.24 fold greater than that of pure AZ. Maximum drug release from ASD6 was found at 120 min.

Solid State Characterization of ASDs

Thermal Analysis by DSC

Thermal analysis was used to measure the thermal stability of ASD formulations and also suggested the existence of melting and re-crystalline forms of crystalline materials. DSC is a fundamental tool to understand the crystalline properties of ASDs. The thermodynamic properties of AZ and various ASD formulations are shown in Figure 2. Pure AZ generated a sharp endothermic peak at 132.62°C, which implies the crystalline nature of AZ. The carrier Na-CMC and carplex-80 showed broad peak at 107.68 and 95.48°C respectively. In addtion, the thermogram of ASD3, ASD5 and ASD6 showed broad peak with reduced intensity at 95.86, 98.93 and 98.98°C respectively. The shift of melting point towards lower temperature value and broader endothermic peak suggested that the drug AZ might be converted into the amorphous state in the form of ASD. Finally, the results demonstrated the possibility of amorphization of crystalline drug in solid dispersions prepared by solvent evaporation technique.

Table 2: Percentage of drug release from pure AZ, formulations ASD1, ASD2, ASD3 and ASD4.

Time(min)	AZ	ASD1	ASD2	ASD3	ASD4
5	1.36 ± 0.17	2.13±0.027*	4.75±0.11***a	5.20±0.06***a	5.11±0.07***a
15	1.46 ±0.27	2.50±0.16*	5.18±0.16***a	6.64±0.10***an	5.88±0.24***a
30	1.71 ±0.27	2.74±0.11	5.48±0.42***a	7.64±0.51***an	6.30±0.12***a
60	2.15±0.10	2.86±0.16	5.85±0.32**c	7.92±0.24***a	6.58±1.24***b
90	2.32±0.18	3.11±0.11	6.15±0.34***a	8.03±0.20***am	7.49±0.25***ao
120	2.52±0.05	3.29±0.11*	6.89±0.22***a	8.31±0.10***am	7.70±0.19***ao

Results are presented as the mean ± S.E.M. $p< 0.001$, ***, $p< 0.01$,**,$p< 0.05$,* vs AZ; $p< 0.001$, [a], $p< 0.01$,[b],$p< 0.05$,[c]vs ASD-1; $p< 0.001$, [m], $p< 0.01$,[n],$p< 0.05$,[o]vs ASD-2; $p< 0.001$, [x], $p< 0.01$,[y],$p< 0.05$,[z]vs ASD-3.

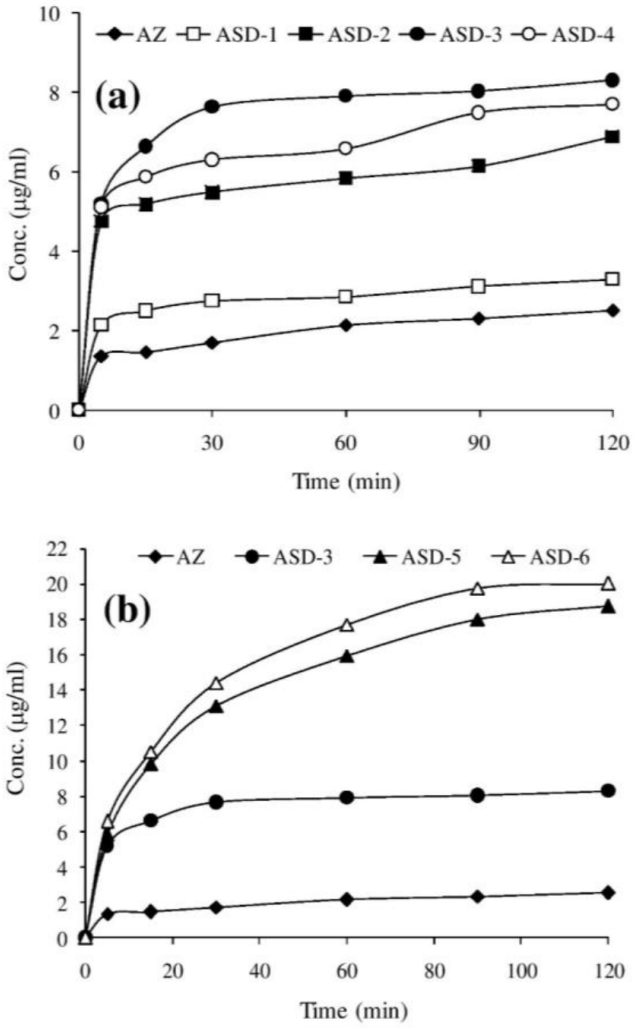

Figure 1: (a) Drug dissolution from AZ, ASD1, ASD2, ASD3 and ASD4; (b) Drug dissolution from AZ, ASD3,ASD5 and ASD6.

Table 3: Percentage of drug release from pure AZ, formulations ASD3, ASD5 and ASD6.

Time (min)	AZ	ASD3	ASD5	ASD6
5	1.36 ± 0.17	5.20±0.06*	5.86±0.28*	6.59±0.21*q
15	1.46 ±0.27	6.64±0.10*	9.83±0.32*p	10.50±0.16*p
30	1.71 ±0.27	7.64±0.51*	13.13±0.06*p	14.41±0.06*pz
60	2.15±0.10	7.92±0.24*	15.93±0.28*p	17.70±0.32*py
90	2.32±0.18	8.03±0.20*	18.01±0.16*p	19.78±0.21*px
120	2.52±0.05	8.31±0.10*	18.74±0.22*p	20.02±0.32*py

Results are presented as the mean ± S.E.M. $p< 0.001$, * vs AZ; $p< 0.001$, [p], $p< 0.01$,[q],$p< 0.05$,[r]vs ASD-3; $p < 0.001$, [x], $p< 0.01$,[y],$p< 0.05$,[z]vs ASD-5.

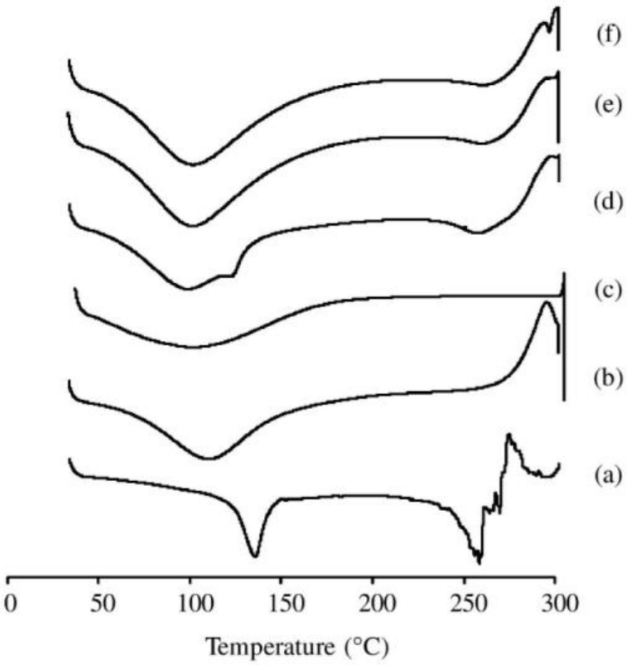

Figure 2: DSC thermograms of (a) AZ, (b) Na-CMC, (c) Carplex80, (d) ASD3, (e) ASD5 and (f) ASD6.

Characterization of Crystalline Behavior by PXRD

Figure 3 shows, the X-ray diffraction patterns of AZ, Na-CMC, carplex-80 and ASDs. The PXRD pattern of pure AZ exhibits the presence of large number of sharp peaks as observed at the 2θ scattered angles 8.13, 10.04,

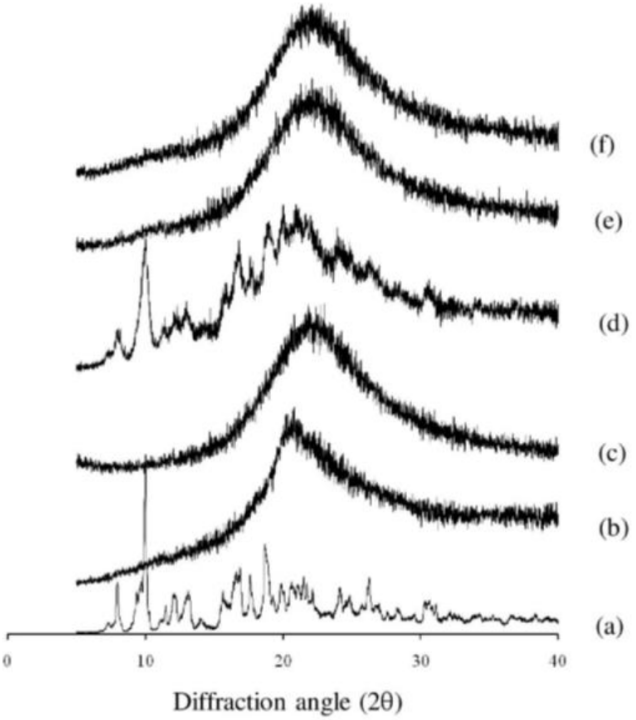

Figure 3: Diffraction studies by PXRD of (a) AZ, (b) Na-CMC, (c) Carplex80, (d) ASD3, (e) ASD5 and (f) ASD6.

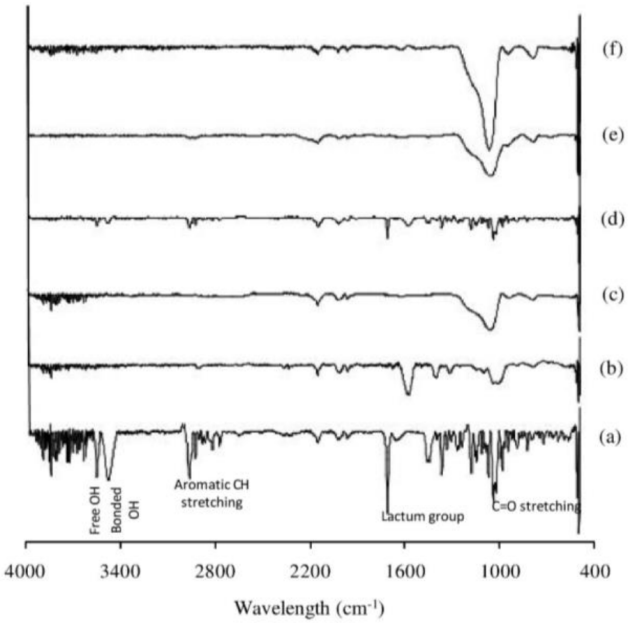

Figure 4: FTIR spectra of (a) AZ, (b) Na-CMC, (c) Carplex80, (d) ASD3, (e) ASD5 and (f) ASD6.

13.17, 17.65, 18.75, 26.30°C. The distinctive diffraction peaks of AZ persisted in the formulation ASD3 were at 10.04, 16.79 and 19.07°C with decreased intensity as compared to that of pure AZ. These peaks might be due to the formation of weak bond like hydrogen bond, dispersion forces, and dipole-dipole interactions between AZ with Na-CMC and Carplex-80. But no sharp peak was observed for Carplex 80 and Na-CMC due to its large surface area and smaller particle size resulting in amorphous formulations. Also absence of sharp peak in formulation ASD5 and ASD6 suggested that the degree of crystallizations of AZ in ASD5 and ASD6 is almost abolished.

Interaction Study by FTIR

The compatibility between the drugs and additives were studied by FTIR spectroscopic approach. The optimized ASDs were subjected to FTIR spectral analysis to know the possible molecular interactions between AZ and carrier (Na-CMC and Carplex-80) during the process of solid dispersion. The FTIR spectral data of AZ, Na-CMC, Carplex-80 and ASDs are presented in Figure 4.

In FTIR spectrum, AZ was featured by number of peaks at 3556 cm^{-1} (for free OH group), 3477 cm^{-1} (for bonded OH group), 2937 cm^{-1} (for C-H stretching group), 1718.57 cm^{-1} (for C=O stretching in the lactone ring) 1052 cm^{-1} (symmetrical aliphatic ether), 1721 cm^{-1} (C=O stretching of the ester form).[8,16] However, the intensity of band for stretching of C-O-C, O-H and C-H was decreased in FTIR spectra of ASD3, ASD5, and ASD6. The bands due to lactam group, aromatic C-H stretching, bonded hydroxyl group were disappeared in all the formulations. Furthermore, the evidence suggested a possibility of chemisorption of the AZ on carriers (Na-CMC and Carplex-80) that might have occurred by weak hydrogen bonding, dispersion forces and dipole-dipole interactions.

Morphological Study by SEM

The SEM images of AZ, Na-CMC, carplex-80 and ASDs are shown in Figure 5. In micrographs, the pure AZ appeared as non-uniform cubic-shaped crystals with sharp and elongated edges and agglomerates seriously whereas in ASD3, crystal structure of AZ was reduced than that of pure AZ. But in the micrographs of ASD5 and ASD6 crystal structure of AZ is much more reduced when compared to that of pure AZ and ASD3. Thus, the alterations in crystalline structure of AZ might have occurred due to the adsorption of AZ molecules on the surfaces of Na-CMC and carplex-80 which inturn resulted due to the presence of Vander Waal's forces, hydrogen bonding, dispersion forces, and dipole-dipole interactions between drug with carriers (Na-CMC and Carplex-80) as evidenced by FTIR spectral data.

The results from DSC, PXRD, and SEM images revealed that the crystalline nature of AZ was altered to various extent while using different ratios of carriers

(Na-CMC and Carplex-80) in ASDs. The possibility of this conversion might be occured due to the chemisorption of AZ on the surface of carrier forming weak H-bonding between –OH groups of carriers and the functional groups displayed by the drug molecules. The improved and difference in drug release profile exhibited by ASDs might be the result of varying degree of interaction mediated chemisorption. Among the six ASDs, ASD-6 can be considered as an optimum formulation depending on its dissolution behavior and solid state characteristics.

Evaluation of *in-vitro* Antibacterial Activity of Pure AZ and ASD6

In-vitro antibacterial activity of the optimized ASD6 and pure AZ was performed according to the Kirby-Bauer standard disk diffusion method against both *Gram positive* (*S. aureus*, ATCC 25923) and *Gram negative* (*E. coli*, ATCC 25922) bacteria.

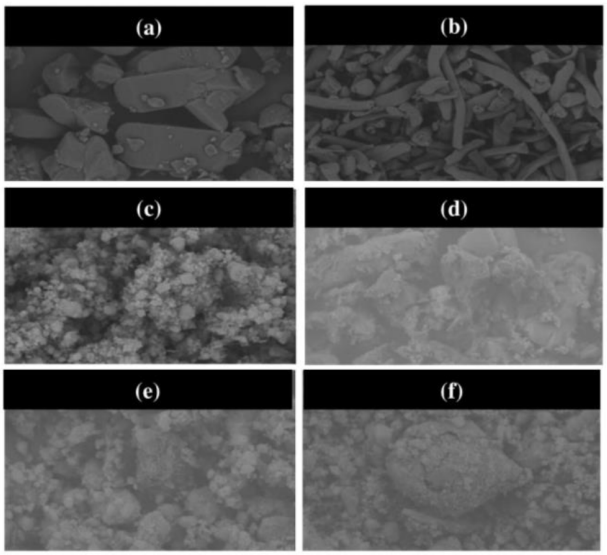

Figure 5: Micrographs by SEM of (a) AZ , (b) Na-CMC, (c) Carplex80, (d) ASD3, (e) ASD5 and (f) ASD6.

Relative Zone of Inhibition of AZ and ASD6 against *S. aureus* and *E. coli*

In antibacterial activity study a significant increase in RZOI was observed with ASD6 than pure AZ ($p<0.001$) at each time point (5, 15, 30, 60, 90 and 120 min) and the effect of ASD6 was more prominent against *S. aureus* than that of pure AZ (Table 4 and Figure 6a).

ASD6 also showed greater RZOI against *E. coli* than that of pure AZ with time, and the effect of ASD6

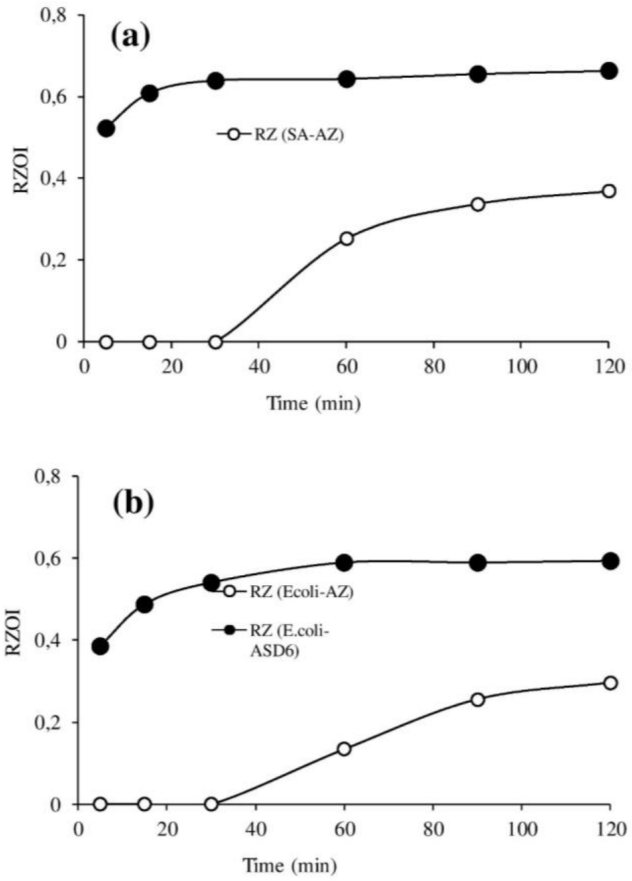

Figure 6: (a) Relative zone of inhibition of pure AZ, and ASD6 against *S. aureus*; (b) Relative zone of inhibition of pure AZ and ASD6 against E. coli.

Table 4: Relative zone of inhibition (RZOI) of pure AZ and ASD6 against *S. aureus*.

Against *S. aureus*	Zone of inhibition (mm) of sample		Zone of inhibition (mm) of control		Relative zone of inhibition	
Time(min)	AZ	ASD-6	AZ	ASD-6	AZ	ASD-6
5	0	22.5	43	43	0.00±0.00	0.52±0.50***
15	0	26.2			0.00±0.00	0.61±0.29***
30	0	27.5			0.00±0.00	0.64±0.50***
60	11	27.7			0.25±0.29	0.64±0.29***
90	14.5	28.2			0.34±0.50	0.66±0.29***
120	15.5	28.5			0.37±0.29	0.66±0.50***

Results are presented as mean ± S.D. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 5: Relative zone of inhibition (RZOI) of pure AZ and ASD6 against *E. coli.*

Against *E coli*	Zone of inhibition (mm) of sample		Zone of inhibition (mm) of control		Relative zone of inhibition	
Time(min)	AZ	ASD-6	AZ	ASD-6	AZ	ASD-6
5	0	15.8	41	41	0.00±0.00	0.39±0.76***
15	0	20.0			0.00±0.00	0.49±0.50***
30	0	22.2			0.00±0.00	0.54±0.29***
60	5.5	24.2			0.13±0.50	0.59±0.29***
90	10.5	24.2			0.26±0.50	0.59±0.29***
120	12.5	24.3			0.30±0.29	0.59±0.29***

Results are presented as mean ± S.D. *p<0.05, **p<0.01, ***p<0.001.

on RZOI was significant up to 120 min in comparison to pure AZ (p<0.001). The ability of ASD6 to inhibit growth of *E. coli* was far better than pure AZ. The increased RZOI confirmed the superiority of ASD6 over pure AZ against *E. coli* (Table 5 and Figure 6b).

DISCUSSION

AZ belongs to BCS class II drugs that displays poor water solubility, thus limiting the rate of dissolution and hence poor bioavailability. However, it has high permeability so that it achieves significant intracellular penetration, extensive body distribution, and thus, tissue levels of AZ are significantly higher than plasma levels.[32] Therefore, the study was designed to develop solid dispersions of AZ with increased solubility and hence, bioavailability of the hydrophobic drug after oral administration. For this purpose, various formulations of AZ were prepared using Na-CMC and Carplex-80 as carrier in different ratios. The ASD formulations thus prepared were subjected to *in-vitro* dissolution studies, solid state characterization and finally, tested for *in-vitro* antibacterial activity.

In dissolution study of group I, at 30 min the concentration of drug released from formulation ASD3 (7.64 µg/ml) was 4.47 fold higher than pure AZ and in group II ASD6 (14.41 µg/ml) it was 8.24 fold higher when compared to that of pure AZ (Table 2 and 3, Figure 1). Thus, the preparation of solid dispersions of AZ contributed to the improved dissolution of drug in aqueous medium as a result of decreased hydrophobicity of AZ. Alterations of drug release profile mainly attributed to the chemisorption of AZ on the surface of Na-CMC and Carplex-80 and also increased wettability of the carriers which was further confirmed by studying physicochemical properties. Similar observations were obtained by Sathe *et al.* (2015) who reported that the dissolution of AZ was increased with increasing proportion of carriers and which might be due to improved wettability of the drug caused by the interaction with carrier, reduction of drug particle size and crystal polymorphism. Further, *in-vitro* study of the above AZ formulation with polyvenylpyrolidone (PVP) K30 in 1:6 ratio showed a maximum 92.69% release of drug at 90 min.[33]

In solid state characterization, DSC thermograms of ASDs (Figure 2) confirmed the transformation of crystalline solid drug to amorphous state which was further evidenced by the PXRD spectrum (Figure 3). Also, FTIR spectral analysis indicated the molecular interactions between the carriers and drug which inturn could improve the wettability of drug particles and thus better dissolution rates of ASDs (Figure 4). SEM images were used to characterize particle size distribution, shape and surface morphology of ASDs (Figure 5). The SEM analysis also showed that AZ molecules were adsorbed on the surfaces of carriers that might be responsible for the improved dissolution of AZ in ASDs (ASD3, ASD5 and ASD6). Earlier research showed that a combination of ball milling and hot-melt extrusion were used for dissolution enhancement of AZ, whereby formulation using aerosil 200 played a potential role of enhancing the solubility and dissolution rate of drug. The spectral data indicated the loss of crystallization water and the presence of intermolecular hydrogen bonding between aerosil 200 and AZ in solid dispersion formulations.[34] Before that Arora *et al.* (2010) reported that preparation of solid dispersion of AZ with urea by solvent evaporation method increased drug solubility and the drug release profile of formulation was higher than those of pure AZ. Further, no chemical incompatibility between drug and urea was observed as evidenced by FTIR spectral data.[35] Wadhwa *et al.* (2016) also showed that an improvement in aqueous solubility and dissolution rate of AZ can be achieved by making a physical mixture and preparation of solid dispersions using PEG 6000 and β-cyclodextrin.[15] They also found

that 50% of the drug was released from most of the formulation within 30 min.

Solid dispersions of AZ were successfully prepared by solvent evaporation method using poloxamer 188 and 407 which was highly water soluble and stable against enzymatic degradation.[36] Zhang *et al.* (2007) formulated a nanosuspension of AZ by high pressure homogenization and further *in-vitro* release studies showed that the dissolution rate of nanosuspension was increased in comparison to that of micronized AZ powder.[10] Thus, the ASDs developed by solvent evaporation technique using Na-CMC and Carplex-80 in different ratios enhanced the solubility, dissolution rate and thereby the bioavailability of AZ and the results of this study were found to be consistent with the previous studies.[15,33,35]

In-vitro antibacterial activity tests indicated that ASD6 has significantly higher ZOI than pure AZ and there was no ZOI of pure AZ against both *S. aureus* and *E. coli* until 30 min. AT 60 min, the RZOI of ASD6 formulation was 2.51 and 4.4 fold higher than pure AZ against *S. aureus* and *E. coli*, respectively (Figure 6). A significant differences in RZOI were observed between ASD6 and pure AZ ($p < 0.001$). The formulation ASD6 represented better antibacterial activity which can be explained by the enhanced solubility and greater diffusion achieved through ASDs formulation resulting in significant difference in RZOI. This findings were in accordance with Khan *et al.* (2016) who found that, AZ nanoparticles displayed greater antibacterial activity against *S. aureus* and *E. coli* than that of pure AZ while tested by agar diffusion method.[37] In earlier study, AZ–PLGA nanoparticles demonstrated higher antibacterial efficacy than pure AZ against *S. typhi* as reported by Mohammadi *et al.* (2010).[38] Further, colloidal nanoparticles of AZ lowered the MIC values 8 times against both gram-negative and gram-positive bacteria than pure AZ.[39] Moreover, Vanic *et al.* (2019) developed AZ-loaded liposomes - a novel approch for localized therapy in cervicovaginal bacterial infections and found that the delivery systems potentially eradicated the biofilms of *E. coli* strains than pure AZ.[40] Different ASDs were formulated utilizing dispersion techniques with a higher solubility rate and hence, the formulation ASD6 possessed superior antibacterial activity against both *S. aureus and E. coli* than pure AZ, as evidenced by above experimentation.

CONCLUSION

The main objective of the current investigation was to develop suitable formulations of AZ with increased antimicrobial activity. ASDs were formulated by solvent evaporation method using silica and Na-CMC at various ratios. The *in-vitro* release of AZ from ASDs were significantly ($p < 0.05$) higher than that of pure AZ. Among the formulations ASD5 and ASD6, the later showed the highest drug release and the increased dissolution was due to conversion of AZ in ASD6 from crystal to amorphous as confirmed by DSC, PXRD, FTIR and SEM analysis. ASD6 showed greater RZOI than pure AZ against both *S. aureus* and *E. coli*. The possible mechanism of maximum dissolution of ASD-6 was mainly attributed to the weak van der Waals forces exists between drug molecules and the carriers (Na-CMC and carplex-80). ASD6 was found to be the formulation of choice. Therefore, the clinical effectiveness of the lifesaving antibiotics AZ might be enhanced if AZ is loaded in solid dispersions and marketed as commercial dosage forms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AZ: Azithromycin; **ASDs:** Solid dispersions of AZ; **ATCC:** American type culture collection; **BCS:** Biopharmaceutical classification system; **CFU:** Colony forming unit; **DSC:** Differential scanning calorimetry; **E. coli:** *Escherichia coli;* **FT-IR:** Fourier transform infrared spectroscopy; **GI:** Gastrointestinal; **MAC:** *Mycobacterium avium* complex; **PEG:** Polyethylene glycol; **PVP:** Polyvinylpyrrolidone; **PXRD:** Powder x-ray diffraction; **RTI:** Respiratory tract infections; **RZOI:** Relative zone of inhibition; **Na-CMC:** Sodium carboxymethyl cellulose; **S aureus:** *Staphylococcus aureus;* **SD:** Solid dispersion; **SEM:** Scanning electron microscopy; **ZOI:** Zone of inhibition.

Authors' Contribution

Monalisa Monwar: conducted the whole study, responsible for acquisition and analysis of experimental data and drafting the manuscript. Ranjan Kumar Barman: formulation development and statistical analysis of

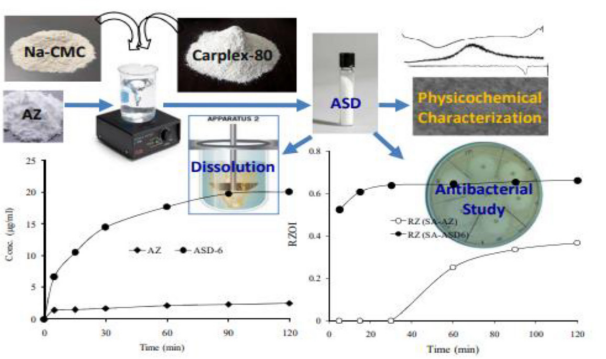
data and made critical revision of the manuscript. Bytul Mokaddesur Rahman: microbiological study and acquisition of data, critical revision of the manuscript for intellectual content.Yasunori Iwao: instrumental spectral data analysis and critical revision of the manuscripts Mir Imam Ibne Wahed: concept and design present hypothesis, analysis of experimental data, and made critical revision of the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Kumar P, Singh C. A study on solubility enhancement methods for poorly water soluble drugs. Am J Pharmacol Sci. 2013;1(4):67-73. doi: 10.12691/ajps-1-4-5.
2. Krishnamoorthy V, Nagalingam A, Priya Ranjan Prasad VP, Parameshwaran S, George N, Kaliyan P. Characterization of olanzapine-solid dispersions. Iran J Pharm Res. 2011;10(1):13-24.
3. Di L, Kerns EH, Carter GT. Drug-like property concepts in pharmaceutical design. Curr Pharm Des. 2009;15(19):2184-94. doi: 10.2174/138161209788682479, PMID 19601822.
4. Fridgeirsdottir GA, Harris R, Fischer PM, Roberts CJ. Support tools in formulation development for poorly soluble drugs. J Pharm Sci. 2016;105(8):2260-9. doi: 10.1016/j.xphs.2016.05.024, PMID 27368122.
5. Sachan NK, Bhattacharya A, Pushkar S, Mishra A. Biopharmaceutical classification system: A strategic tool for oral drug delivery technology. Asian J Pharm. 2009;3(2). doi: 10.4103/0973-8398.55042.
6. Löbenberg R, Amidon GL. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur J Pharm Biopharm. 2000;50(1):3-12. doi: 10.1016/s0939-6411(00)00091-6, PMID 10840189.
7. Wairkar SM, Gaud RS. Solid dispersions: Solubility enhancement technique for poorly soluble drugs. Int J Res Pharm Biomed Sci. 2013;4(3):847.
8. Robaina NF, De Paula CER, Brum DM, De la Guardia M, Garrigues S, Cassella RJ. Novel approach for the determination of azithromycin in pharmaceutical formulations by Fourier transform infrared spectroscopy in film-through transmission mode. Microchem J. 2013;110:301-7. doi: 10.1016/j.microc.2013.04.015.
9. Adeli E. Preparation and evaluation of azithromycin binary solid dispersions using various polyethylene glycols for the improvement of the drug solubility and dissolution rate. Braz J Pharm Sci. 2016;52(1):1-13. doi: 10.1590/S1984-82502016000100002.
10. Zhang D, Tan T, Gao L, Zhao W, Wang P. Preparation of azithromycin nanosuspensions by high pressure homogenization and its physicochemical characteristics studies. Drug Dev Ind Pharm. 2007;33(5):569-75. doi: 10.1080/03639040600975147, PMID 17520449.
11. Adeli E. A comparative evaluation between utilizing SAS supercritical fluid technique and solvent evaporation method of Azithromycin solid dispersions for dissolution rate enhancement. J Supercrit Fluids. 2014;87:9-21.
12. [cited 20.7.20]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Azithromycin.
13. Sweetman SC. Martindale: The complete drug reference. Pharmaceutical press; 2009.
14. Majed R. *In vitro* evaluation of antimicrobial activity of two Marketed Brands (zomax and Zetron) in Kingdom of Saudi Arabia. Int J Pharm Sci Rev Res. 2017;43(1):70-4.
15. Aucamp M, Odendaal R, Liebenberg W, Hamman J. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. Drug Dev Ind Pharm. 2015;41(7):1100-8. doi: 10.3109/03639045.2014.931967, PMID 24980913.
16. Wadhwa S, Singhl S, Rawat S. *In vitro* dissolution enhancement of azithromycin in solid dispersion with PEG 6000 and β-CD. J Pharm Biomed Sci. 2016;6:551-6.
17. Cho YD, Park YJ. *In vitro* and *in vivo* evaluation of a self-microemulsifying drug delivery system for the poorly soluble drug fenofibrate. Arch Pharm Res. 2014;37(2):193-203. doi: 10.1007/s12272-013-0169-4, PMID 23754165.
18. Kim GG, Poudel BK, Marasini N, Lee DW, Hiep TT, Yang KY, *et al*. Enhancement of oral bioavailability of fenofibrate by solid self-microemulsifying drug delivery systems. Drug Dev Ind Pharm. 2013;39(9):1431-8. doi: 10.3109/03639045.2012.719903, PMID 23046292.
19. Van Speybroeck M, Mellaerts R, Mols R, Thi TD, Martens JA, Van Humbeeck J, *et al*. Enhanced absorption of the poorly soluble drug fenofibrate by tuning its release rate from ordered mesoporous silica. Eur J Pharm Sci. 2010;41(5):623-30. doi: 10.1016/j.ejps.2010.09.002, PMID 20850527.
20. Azad M, Moreno J, Davé R. Stable and Fast-Dissolving Amorphous Drug Composites Preparation via Impregnation of Neusilin® UFL2. J Pharm Sci. 2018;107(1):170-82. doi: 10.1016/j.xphs.2017.10.007, PMID 29031953.
21. Bouledjouidja A, Masmoudi Y, Van Speybroeck M, Schueller L, Badens E. Impregnation of fenofibrate on mesoporous silica using supercritical carbon dioxide. Int J Pharm. 2016;499(1-2):1-9. doi: 10.1016/j.ijpharm.2015.12.049, PMID 26732521.
22. Lenz E, Sprunk A, Kleinebudde P, Page S. Impact of fillers on dissolution kinetic of fenofibrate dry foams. Pharm Dev Technol. 2015;20(5):570-8. doi: 10.3109/10837450.2014.908301, PMID 24901031.
23. Zhao Z, Wu C, Zhao Y, Hao Y, Liu Y, Zhao W. Development of an oral push–pull osmotic pump of fenofibrate-loaded mesoporous silica nanoparticles. Int J Nanomedicine. 2015;10:1691-701. doi: 10.2147/IJN.S76755, PMID 25784799.
24. Yousaf AM, Mustapha O, Kim DW, Kim DS, Kim KS, Jin SG, *et al*. Novel electrosprayed nanospherules for enhanced aqueous solubility and oral bioavailability of poorly water-soluble fenofibrate. Int J Nanomedicine. 2016;11:213-21. doi: 10.2147/IJN.S97496, PMID 26834471.
25. Dong Y, Ng WK, Hu J, Shen S, Tan RBH. Clay as a matrix former for spray drying of drug nanosuspensions. International Journal of Pharmaceutics. 2014;465(1-2):83-9. doi: 10.1016/j.ijpharm.2014.02.025.
26. Karolewicz B, Gajda M, Pluta J, Górniak A. Dissolution study and thermal analysis of fenofibrate-pluronic F127 solid dispersions. J Therm Anal Calorim. 2016;125(2):751-7. doi: 10.1007/s10973-015-5013-2.
27. Singh A, Worku ZA, Van den Mooter GV. Oral formulation strategies to improve solubility of poorly water-soluble drugs. Expert Opin Drug Deliv. 2011;8(10):1361-78. doi: 10.1517/17425247.2011.606808, PMID 21810062.
28. Irwan AW, Berania JE, Liu X. A comparative study on the effects of amphiphilic and hydrophilic polymers on the release profiles of a poorly water-soluble drug. Pharm Dev Technol. 2016;21(2):231-8. doi: 10.3109/10837450.2014.991877, PMID 25496001.
29. Kalia A, Poddar M. Solid dispersions: An approach towards enhancing dissolution rate. Int J Pharm Pharm Sci. 2011;3(4):9-19.
30. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today. 2007;12(23-24):1068-75. doi: 10.1016/j.drudis.2007.09.005, PMID 18061887.
31. Bauer AT. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1966;45:149-58.
32. Idkaidek M NM, Najib N. Salem I, Jilani J. Physiologically-based IVIVC of azithromycin. Am J Pharmacol Sci. 2014;2(6):100-2.
33. Sathe S, Surwase D, Kore KJ, Bagade MY, Shete RV. Dissolution enhancement of poorly soluble azithromycin using solid dispersion method. Eur J Pharm Res. 2015;2:314-23.
34. Li X, Peng H, Tian B, Gou J, Yao Q, Tao X, *et al*. Preparation and characterization of azithromycin--Aerosil 200 solid dispersions with enhanced physical stability. Int J Pharm. 2015;486(1-2):175-84. doi: 10.1016/j.ijpharm.2015.03.029, PMID 25794608.
35. Arora SC, Sharma PK, Irchhaiya R, Khatkar A, Singh N, Gagoria J. Development, characterization and solubility study of solid dispersions of azithromycin dihydrate by solvent evaporation method. J Adv Pharm Technol Res. 2010;1(2):221-8. PMID 22247849.
36. Raja ST. Deepthi Appikatla Kannan R, Ganapathi T, Kasirao R. Formulation and evaluation of azithromycin solid dispersion using poloxamer. World J Pharm Res. 2014;6(3):879-89.

37. Khan FA, Zahoor M, Islam NU, Hameed R. Synthesis of cefixime and azithromycin nanoparticles: An attempt to enhance their antimicrobial activity and dissolution rate. J Nanomater. 2016;2016:1-9. doi: 10.1155/2016/6909085.
38. Mohammadi G, Valizadeh H, Barzegar-Jalali M, Lotfipour F, Adibkia K, Milani M, *et al*. Development of azithromycin–PLGA nanoparticles: Physicochemical characterization and antibacterial effect against *Salmonella typhi*. Colloids Surf B Biointerfaces. 2010;80(1):34-9. doi: 10.1016/j.colsurfb.2010.05.027, PMID 20558048.
39. Azhdarzadeh M, Lotfipour F, Zakeri-Milani P, Mohammadi G, Valizadeh H. Anti-bacterial performance of azithromycin nanoparticles as colloidal drug delivery system against different gram-negative and gram-positive bacteria. Adv Pharm Bull. 2012;2(1):17-24. doi: 10.5681/apb.2012.003, PMID 24312766.
40. Vanić Ž, Rukavina Z, Manner S, Fallarero A, Uzelac L, Kralj M, *et al*. Azithromycin-liposomes as a novel approach for localized therapy of cervicovaginal bacterial infections. Int J Nanomedicine. 2019;14:5957-76. doi: 10.2147/IJN.S211691, PMID 31440052.

PICTORIAL ABSTRACT



SUMMARY

Azithromycin (AZ) belongs to BCS-II class with low water solubility and high permeability; and possesses poor dissolution rate and erratic absorption from GIT. Solid dispersions of AZ (ASDs) were formulated using silica and Na-CMC at different ratios to enhance the solubility of AZ. ASDs was prepared by solvent evaporation technique and followed by *in-vitro* dissolution test. The solid state characterization of ASDs were performed by Differential Scanning Calorimetry (DSC) thermogram, Powder X-ray Diffractometer (PXRD), Fourier Transform Infrared (FTIR) analysis and Scanning Electron Microscopy (SEM). *In-vitro* antibacterial activity of newly formulated ASDs was tested against both *gram negative* and *gram positive* bacteria. Among the formulations ASD-6 exhibited significantly highest drug release than that of pure AZ. The enhanced dissolution profile exhibited by ASD-6 was due to the conversion of AZ in ASD-6 from crystalline into amorphous state evidenced by DSC, PXRD, FTIR and SEM. Further, ASD6 showed greater RZOI than pure AZ against both *S. aureus* and *E. coli*. Our study demonstrates that the solubility of AZ was greatly enhanced when loaded in solid dispersions which might be responsible for the improvement of its bioavailability in aqueous environment and hence, increased antibacterial activity.

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