Preparation and in vitro evaluation for amorphous solid dispersion of azithromycin

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Article

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Abstract

The present work aimed to formulate azithromycin as amorphous solid dispersion for bitter taste masking, improving stability in an acid medium, and reducing the side effects. Solid dispersion with pH-dependent polymers (Eudragit L100, Eudragit S100) were prepared by the solvent evaporation method. The influence of polymer and drug-polymer ratio on production yields and loading% were evaluated. The F2 (AZI: L100 1:4) that gave the highest yield and loading (96 ± 0.3, 92.3 ± 0.07 respectively) was examined using Scanning electron microscopy (SEM), Fourier transform infrared (FT-IR), Powder X-ray diffraction (PXRD) and Differential scanning calorimetry (DSC). Taste masking evaluation was performed in vitro by two methods (in vitro drug release at saliva pH, and comparison of bitter taste threshold with the optimal formulation). (FT-IR) study displayed that there was no interaction happen between azithromycin and Eudragit L100. DSC and PXRD emphasized the conversion of azithromycin from the crystalline to the amorphous form and entrapped inside the solid dispersion. In vitro, taste assessment detected no azithromycin release in salvia pH (6.8) within 5 min and minimal release in pH 1.2 which indicate this method might be a suitable approach to achieve taste masking of AZI and to improve stability in acid conditions.

Introduction

Taste masking is of crucial value for drugs with a high bitter taste, because of the need for raising patient compliance [1]. Taste masking is an essential issue in the development of pediatric oral dosage forms. According to a study conducted among pediatricians by the American Association of Pediatricians, the hateful or bitter taste of drugs is the most intense restriction for effective treatment of pediatric practice [2].

Another study found that the rates of children receiving treatment ranged between 11 and 93%, and the bitter taste is one of the most important influencing factors[3]. Recently, many taste-masking technologies had been developed, such as hot melt extrusion[4], solid dispersion [5], encapsulation into microspheres[6] and microparticles [7,3], complexation with ion exchanger [8,9] and Cyclodextrins [10,11], physical barriers or coating [12], granulation [13]. Among these methods, solid dispersion gets considerable attention because it cannot only mask the bitter taste but also increase the dissolution rate for water-insoluble drugs and consequently enhance oral bioavailability.

The fundamental design idea for taste-masking techniques is to suppress primary a drug's release in the saliva and get the desirable release profile in the targeted tract [14]. Regarding this concept, pH-dependent polymers have been used to avoid contact between taste buds and bitter-tasting drug. Azithromycin is a broad-spectrum azalide antibiotic. It has unique properties and extensive tissue distribution. Azithromycin is well absorbed, with a bioavailability of 37% [15]. It suppresses protein synthesis. It is effective against a variety of Gram-positive and Gram-negative bacteria but has a bitter taste [16]. This bitter taste greatly limits the more development of oral preparations of azithromycin, which led to decreasing infant and children's compliance.

Eudragit polymers (L100, S100) were chosen to prepare amorphous solid dispersion of azithromycin by using solvent evaporation; this method is simple, low cost, and available.

Materials And Methods

Materials.

Azithromycin Dihydrate was purchased for Sigma-Aldrich (Germany). Poly (meth) acrylates (Eudragit L100, S100) were obtained from Evonik Co., Ltd (Germany). All reagents used were AR grade and HPLC grade. Acetonitrile (HPLC grade-Fischer Scientific), Dipotassium hydrogen phosphate (Rankem), and Ortho Phosphoric acid (Rankem) were used for analysis. Other solvents were of analytical grade.

Methods.

Preparation of solid dispersion of AZI by solvent evaporation method.

Solid dispersion of Azithromycin Dihydrate was prepared by a solvent evaporation method. The drug and polymer were wholly dissolved in ethanol/dichloromethane (1:1, v/v). The organic solvent was evaporated at 60°C under stirring until complete evaporation and in a static oven for the next 24 hours. The prepared solid dispersion was crushed and passed through suitable sieves to harvest solid dispersion. 4 formulations were prepared with Poly (meth) acrylates (Eudragit L100, S100) (Table 1).

Table 1
Formulations for solid dispersions of AZI.

Trial	AZI (g)	Eu L100 (g)	Eu S100 (g)	DCM:Ethanol (1:1, ml)
F1	1	2	-	28
F2	1	4	-	28
F3	1	-	2	28
F4	1	-	4	28

Characterization of the solid dispersion:

1. Production yield, and drug loading.

The production yield was defined by Eq. (1). The drug loading was determined using the HPLC method and calculated using Equations 2.[17]

Chromatographic Conditions: [18]

The HPLC instrument used was the Shimadzu LC-20AD system supplied with a photodiode array (PDA) detector.

The mobile phase was Acetonitrile, and phosphate buffer in the ratio of (35:65 v/v) and pH 6.5 adjusted with orthophosphoric acid. It was filtered through a 0.45 µ membrane filter. All determinations were conducted using C8, (250×4.6 mm, 5 µm), reverse phase column (GL Science) at ambient temperature (25°C). The column effluent was set at 200 nm. The injection volume was 20 µl with a flow rate of 1.5 ml/min.

$$\text{Production yield} = \frac{\text{totalmassofsoliddispersion}}{\text{totalmassofrawmaterials}} \times 100\% \quad (1)$$

$$\text{Loading\%} = \frac{\text{drugretainedintosoliddispertion}}{\text{Initialdrugconcentration}} \times 100\% \quad (2)$$

2. Comparison of the bitter taste threshold of AZI with the solid dispersion in vitro.

The taste-masking capacity of AZI solid dispersion was determined by in vitro drug release test because none of the polymers combined in taste-masked AZI solid dispersion had a bitter taste.

According to previous literature, the bitter taste threshold was between 25.3 and 30.4 µg/ml [19]. Solid dispersion containing about 200 mg azithromycin was put into a test tube containing 10 ml distilled water. The mixture was directly shaken for 30 s and then filtered through membranes 0.45 µm. AZI concentration in the filtrate was analyzed by high-performance liquid chromatography (HPLC) and compared to the bitter taste threshold. If the drug concentration in the filtrate was lower than the bitter taste threshold, the solid dispersion was considered to have masked the bitter taste of AZI.

3. Drug release in different media.

For the in vitro dissolution test to mimic pH situations in vivo solid dispersion containing 200 mg AZI was tested using the paddle method with a rotation speed of 100 rpm.

A volume of 900 ml for hydrochloric acid solution (pH = 1.2 to study stability formulations in acid media) or phosphate buffer (pH = 6.80 corresponding to saliva pH) was used as the dissolution media at 37 ± 0.5°C. Aliquots of 5 mL of solution samples were withdrawn for 5 min for phosphate buffer pH 6.8 and 2 hours for hydrochloric acid, and an equal volume of the fresh medium with the same temperature was added immediately. The aliquot samples were immediately filtered through a 0.22 µm membrane and quantified on a HPLC-UV system[20, 21].

4. Fourier transform infrared (FT-IR) spectroscopy.

FTIR spectroscopy was conducted using FT/IR (Shimadzu IR, Germany). Azithromycin, Eudragit L100, physical mixtures, and formulation F2 were prepared by compressing each sample with pure Potassium bromide (KBr).

5. Thermal analysis.

Azithromycin, Eudragit L100, physical mixtures, and formulation F2 were tested using DSC 131, SETARAM, France. Samples were prepared in open aluminum pans (2–5 mg). The samples were heated at 10 C/min under a nitrogen atmosphere in a temperature range between 0 and 250°C.

6. Powder X-ray diffraction (PXRD) analysis.

Azithromycin, Eudragit L100, physical mixtures, physical mixtures, and formulation F2 were tested using STOE-Stadi P diffractometer with a Cu Kα radiation source tube and 1.54 Å X-ray wavelengths. Emission filament voltage and amperage were 40 kV and 30 mA respectively. The scanning range of 5–59.98° 2θ with a step size of 0.02° and reflection mode was used.

7. Scanning electron microscopy (SEM).

The surface morphology of Formula F2 was analyzed with scanning electron microscopy (SEM). Solid dispersion was mounted on double-faced adhesive tape, coated with a thin gold-palladium layer by a sputter-coated unit, and analyzed with a scanning electron microscope (MIRA3 LMU).

Results And Discussion

Characterization of solid dispersions:

1. Evaluation of the formulated solid dispersion production yield (PY %), and drug loading%.

Table 2
Results of azithromycin solid dispersion PY% and loading%.

formula	PY % ± SD	Drug loading % ± SD
F1	97 ± 0.22	75.4 ± 0.03
F2	98.4 ± 0.18	92.3 ± 0.07
F3	96 ± 0.3	60.8 ± 0.04
F4	98 ± 0.25	80.2 ± 0.02

The production yields and loading of AZI solid dispersion formulations are given in Table 2 and Fig. 1. All formulations revealed high loading% and PY%. Table 2 shows that the production yields and loading of solid dispersions significantly increased by increasing the amount of the polymer. Production Yields ranged from 96 ± 0.3 to 98.4 ± 0.18%. Drug loading was between 60.8 ± 0.04 and 92.3 ± 0.07%. The highest loading% reached 92.3% in the F2 formulation that contains Eu L100. We observed that Eu L100 gave Drug loading % higher than Eu S100. As we know, Eu L100 is an anionic copolymer based on

methacrylic acid and methacrylic acid esters. The ratio of carboxyl groups to ester units is about 1:1. Many carboxyl groups are free in the molecules. AZI is an alkaline drug. So, it is assumed that some unknown reactions may happen between them.

2. Estimation of the bitter taste of solid dispersion in vitro.

When comparing the bitter taste threshold of azithromycin with the F2 formulation that gave the highest load and yield, the results did not show any release of azithromycin in vitro, indicating that it is below the bitter taste threshold.

3. In vitro release results.

In vitro release at pH 1.2:

AZI has a high possibility to be degraded in an acidic medium. The stability kinetics of AZI and formulations were studied at pH 1.2. the results are shown in (Table 3, Fig. 2.). In F1, and F3 formulations which contained the ratio of (drug-to-polymer ratio 1:2), the release reached 36.12% in F1, and 42.36% in F3, this may be attributed to free AZI, we noticed that the release reached only 4.28% in F3 and 6.26% in F4 formula, which indicates that the ratio of drug-to-polymer ratio 1:4 improved the stability of azithromycin at acid pH, reduced the undesirable effects of AZI due to minimizing exposure of free drug in the upper GI tract.

Table 3
Dissolution %Release of AZI at pH 1.2.

Time (min)	AZI %Release at pH 1.2, n = 3 ± standard deviation			
	F1	F2	F3	F4
2.00	6.14 ± 0.16	0.00	8.57 ± 1.05	0 ± 0
5.00	15.20 ± 1.54	0.00	19.25 ± 2.14	0 ± 0
15.00	36.12 ± 2.89	4.28 ± 0.84	42.63 ± 4.21	6.62 ± 0.22
30.00	22.75 ± 1.30	3.90 ± 0.27	25.70 ± 5.30	4.00 ± 0.17
60.00	5.52 ± 0.75	3.10 ± 0.14	7.62 ± 2.37	3.70 ± 0.19
90.00	3.68 ± 0.87	2.50 ± 0.25	4.00 ± 3.95	2.10 ± 0.14
120.00	2.90 ± 0.95	2.00 ± 0.10	3.80 ± 1.80	1.47 ± 0.20

In vitro release at pH 6.8:

The release of AZI in vitro at saliva pH was esteemed to know the release of AZI into a salivary fluid. The test is based on the released amount of AZI in Phosphate buffer with pH = 6.8) and the results are shown in (Table 4, Fig. 3.). The higher release rate of the drug into the artificial saliva resulted in a greater amount of AZI in the saliva and in a bitter sensation in the mouth. In F1, and F3 formulations (drug-to-polymer ratio 1:2), the released drug amount after 5 min in artificial saliva was 33.16% in F1, 44.28% in F3, and a tendency of decrease to 0% in F2, F4 (drug-to-polymer ratio 1:4) which indicate that this ratio is the best for taste masking. The release in F2, F4 formulations started after 30 min.

Table 4
Dissolution %Release of AZI at pH 6.8.

Time (min)	AZI %Release at pH 6.8, n = 3 ± standard deviation			
	F1	F2	F3	F4
1.00	6 ± 3.54	0 ± 0	8.00 ± 1.30	0 ± 0
2.00	11.81 ± 2.48	0 ± 0	15.88 ± 1.98	0 ± 0
3.00	18.19 ± 2.12	0 ± 0	23.17 ± 1.18	0 ± 0
4.00	23.02 ± 2.17	0 ± 0	30.54 ± 1.13	0 ± 0
5.00	32.16 ± 1.17	0 ± 0	40.28 ± 2.80	0 ± 0

4. Selection of optimized formulations.

F2 formula that masked the bitter taste in vitro, improved the stability of azithromycin at acid pH and gave maximum loading and PY%, considered it as an optimized formulation and evaluated its structure.

5. Fourier transform infrared (FT-IR) spectroscopy results

The IR spectra of AZI powder, Eudragit L100, PM, and F2 formulation are as shown in (Fig. 4.) respectively. AZI spectrum shows peaks at the following locations: at 3393.36 cm^{-1} due to O–H stretching in the sugar group, at 2971.22 cm^{-1} due to C–H stretching of the alkyl group, at 1719 cm^{-1} related to C = O stretching of the carbonyl group, and C-O-C ether stretching at 1187 cm^{-1}. The IR spectrum of formulation F2 is totally different from azithromycin powder. It is more similar to EuL100 spectra which might be due to the encapsulation of AZI inside the solid dispersion. In the infrared spectra formulation F2, no additional peak was observed, which emphasized the absence of any possible interaction between AZI and Eu L100.

6. Differential scanning colorimetry

DSC thermogram (Fig. 5.) show that AZI showed a sharp endothermic peak of 125°C corresponding to its melting point. While Eu L100 displayed a wide endothermic peak from 46°C to 95°C due to moisture content. The physical mixture (PM) of AZI and Eudragit L100 showed a peak at about the melting point

of the AZI. The intensity of this peak was decreased in the thermogram of PM due to the dilution effect of the polymer matrix. In the F2 formulation, the endothermic peak of AZI disappeared which indicated that AZI has been converted from crystalline phase to amorphous phase and entrapped into solid dispersion.

7. Powder X-ray diffraction (PXRD) results.

PXRD analysis of AZI, Eu L100, PM, and F2 formulation are shown in Fig. 6. AZI is observed to be crystalline by showing several sharp peaks ranging from 8.06, 9.8, 11.2, 12.7, 13.08, 14.02, 15.5, 16.4, 17.6, 18.8, 19.8, 20.9 2 respectively, while EuL100 is amorphous with no peaks. The P-XRD patterns of PM showed the same peaks as AZI showing there was no complex formation; The F2 formulation did not show diffraction peaks of AZI concluding that the physical state of AZI converted from crystalline form to amorphous form during the formation of solid dispersion. This finding confirms that entrapped AZI is dispersed on the F2 matrix.

8. Scanning electron microscopy (SEM) results

The optimized F2 formulation was then subjected to SEM analysis to assess the morphology and surface topography. The captured images of SEM analysis were represented in Fig. 7. SEM image showed the solid dispersion was an irregular shape, where no drug crystals on the surface could be observed, indicating an encapsulation of AZI inside the polymeric matrix.

Conclusion

Taste masking of a bitter drug, Azithromycin, was successfully prepared using the solvent evaporation method and solid dispersion method. In vitro taste masking studies showed AZI: EuL100 (1:4) has good taste masking properties, and high drug loading%. FT-IR spectra confirmed the possible interaction between the drug and EuL100. PXRD and DSC confirmed that the drug was in an amorphous state in the Solid dispersion. This method might be a suitable approach to achieve taste masking of AZI and to improve stability in acid conditions.

Abbreviations

AZI
Azithromycin
SEM
Scanning electron microscopy
FT-IR
Fourier transform infrared
PXRD
Powder X-ray diffraction
DSC
Differential scanning calorimetry
Eu L100

Eudragit L100
Eu S100
Eudragit S100
DCM
Dichloromethan
PY
Production yield

Declarations

Availability of data and material

Correspondence and requests for materials should be addressed to R.A.-S.

Competing interests

The authors declare no competing interests.

Author contributions

All authors have contributed to the work and reviewed the manuscript. Specifically, R.A.-S. Conducted the experiments, analyzed the results and wrote the main manuscript text and A.L. supervised the project and the interpretation of the results.

Additional information

Correspondence and requests for materials should be addressed to R.A.-S.

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Figures

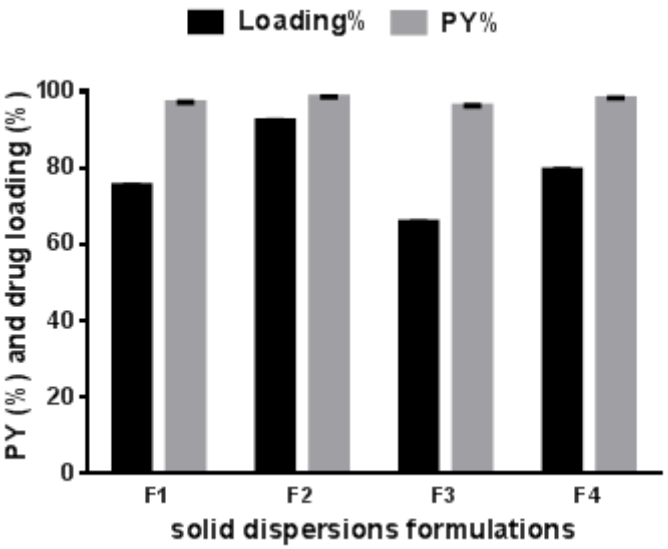

Figure 1

Graph Showing PY (%) and loading (%). Data are expressed as mean ± SD (n = 3).

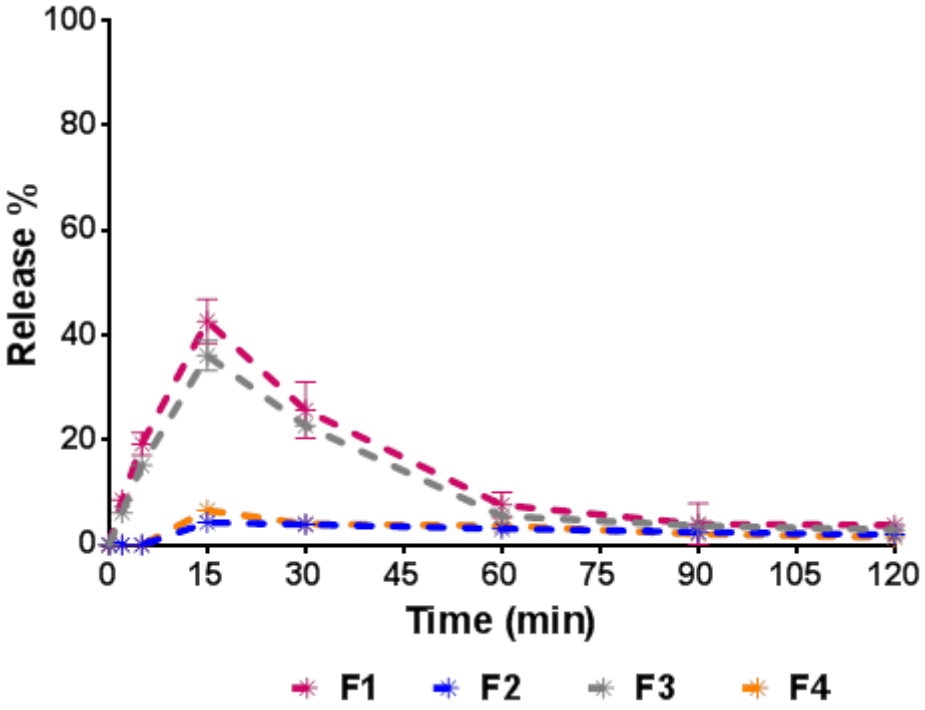

Figure 2

Percentage drug release of AZI at pH 1.2 from prepared formulations against time (min). Data are expressed as mean ± SD (n = 3).

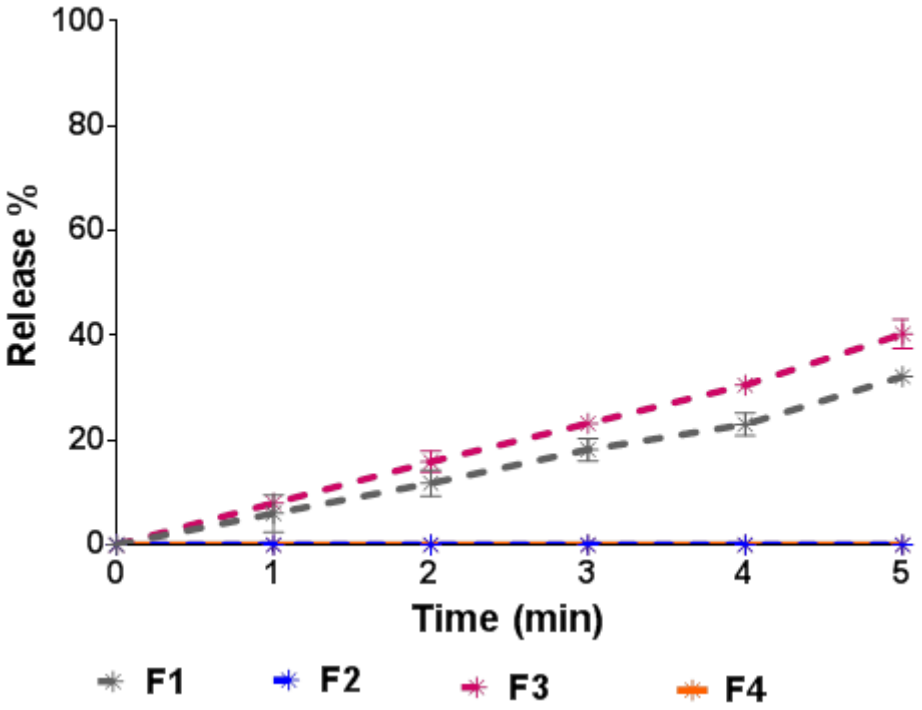

Figure 3

Percentage drug release of AZI at pH 6.8 from prepared formulations against time (min) at pH 6.8. Data are expressed as mean ± SD (n = 3).

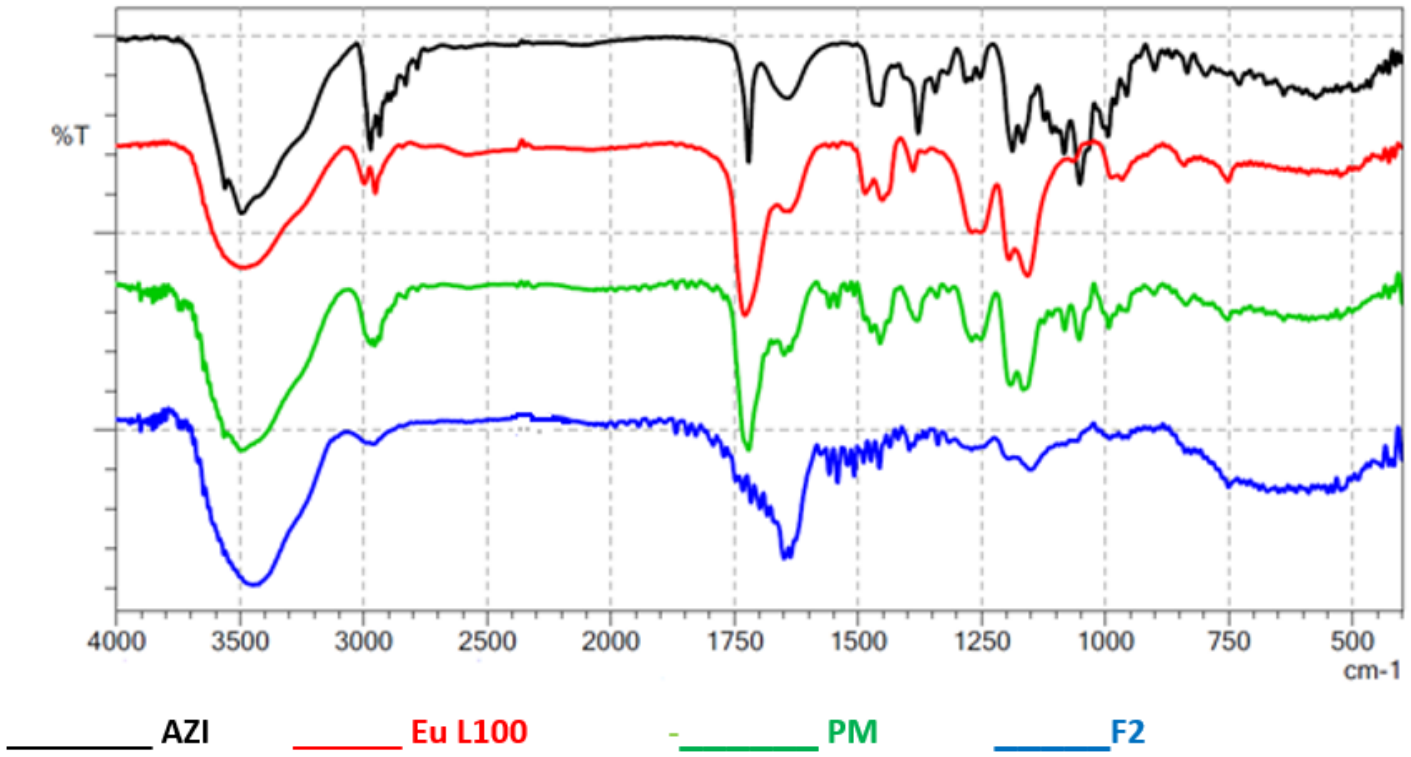

Figure 4

IR spectra of AZI, Eu L100, 1:1 PM, F2 formula

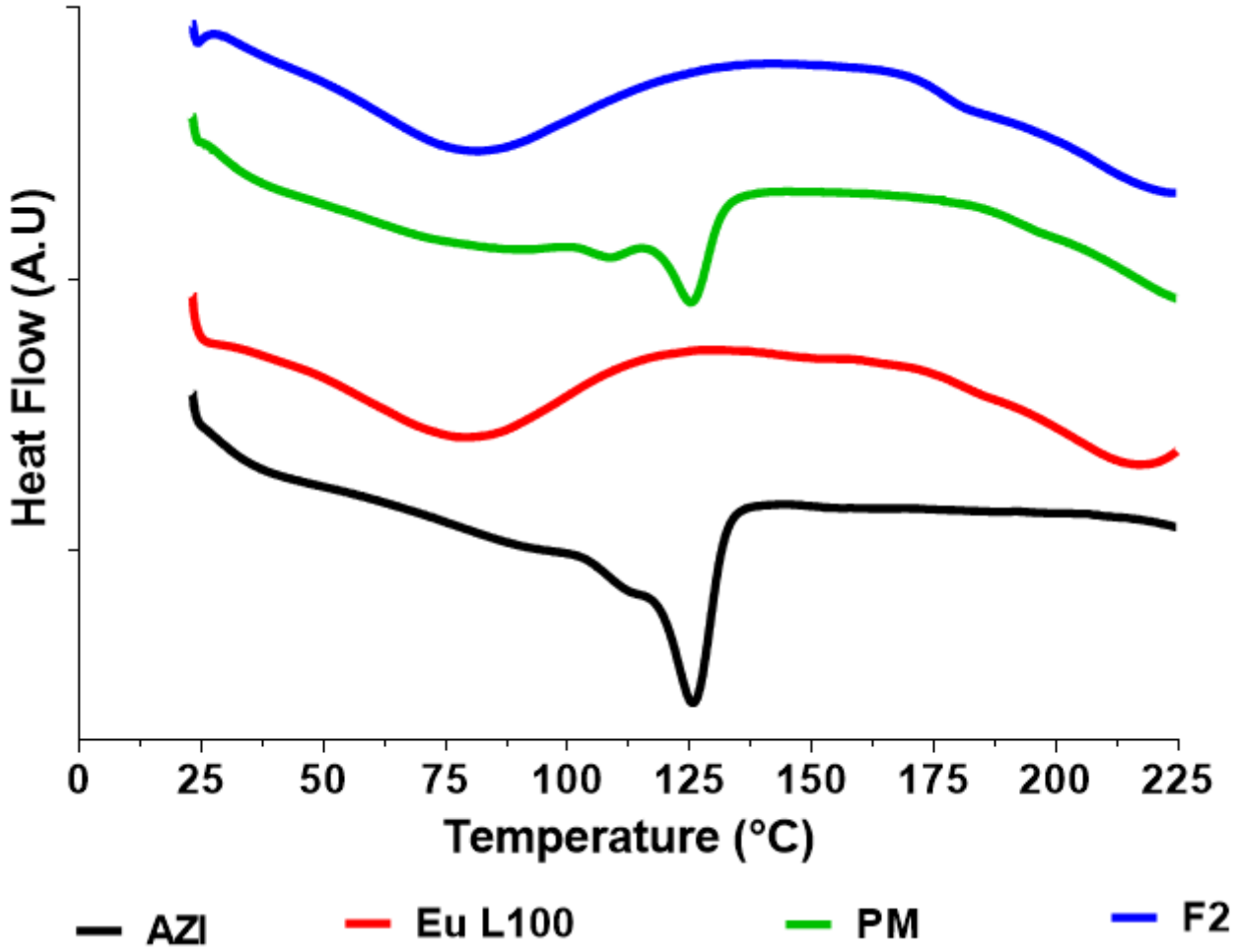

Figure 5

DSC of AZI, Eu L100, 1:1 PM, F2 formula.

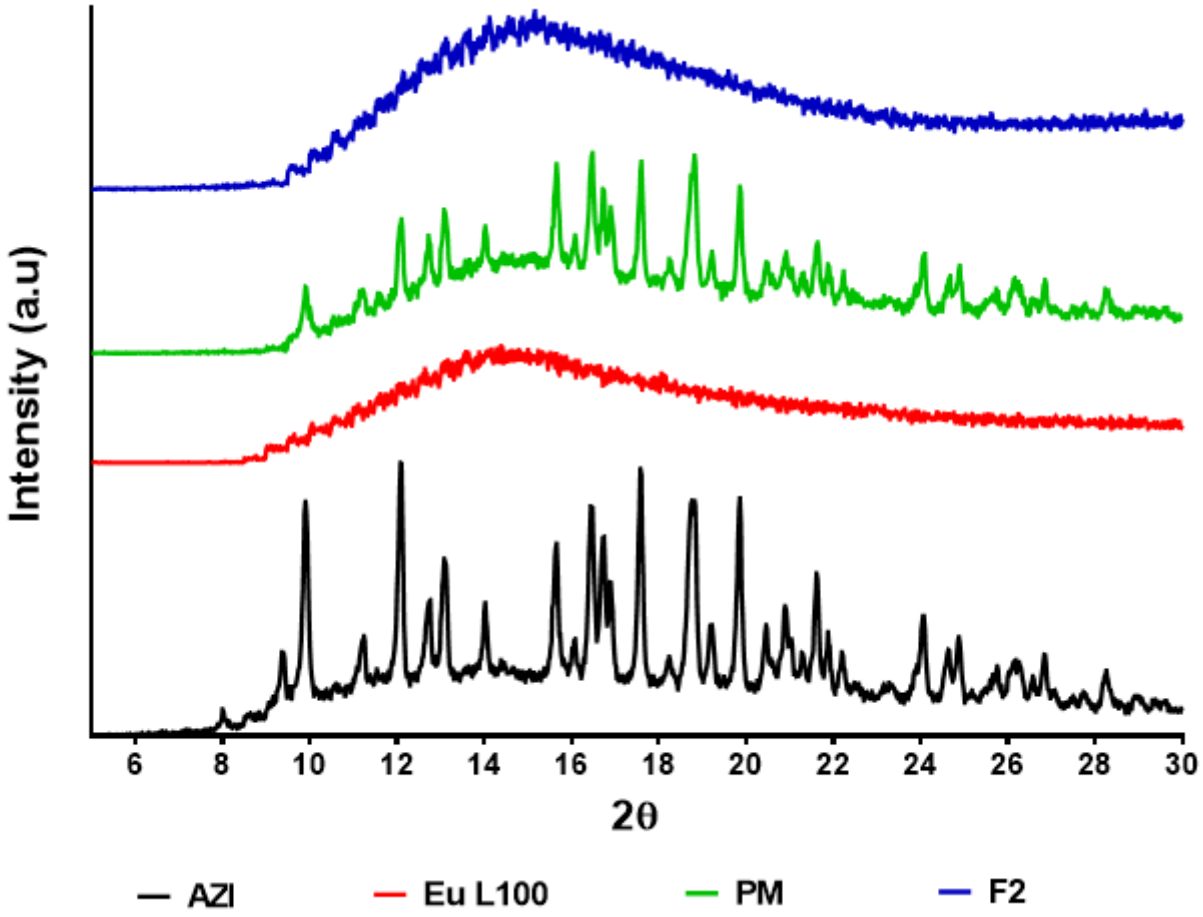

Figure 6

PXRD of AZI, EuL100, 1:1 PM, F2 formula

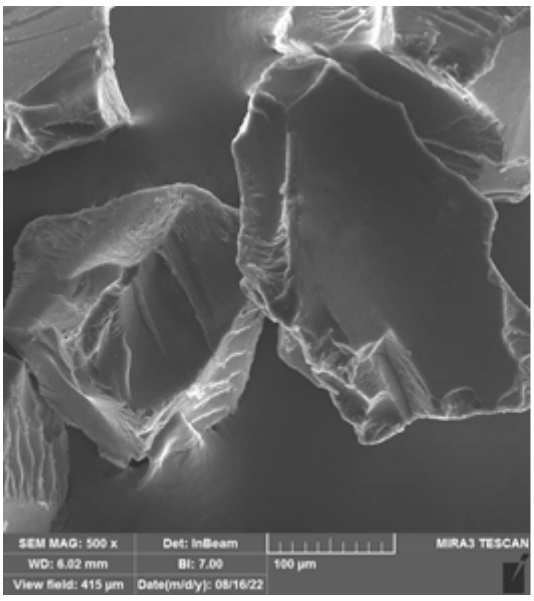

Figure 7

SEM of F2 formula