Preparation and evaluation of zinc oxide (ZnO) metal nanoparticles carriers for azilsartan

Preparación y evaluación de portadores de nanopartículas metálicas deóxido de zinc (ZnO) para azilsartán

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Resumen

El presente estudio implica la utilización de nanopartículas de óxido metálico, óxido de zinc (ZnO), como módulo de administración de fármacos para el fármaco antihipertensivo azilsartán, como herramienta de reducción del tamaño de partícula. Según el sistema de clasificación biofarmacéutica (BCS), el azilsartán pertenece a la clase II con baja solubilidad y alta permeabilidad. Se sintetizaron nanopartículas de ZnO usando el método de precipitación química, y luego se prepararon tres formulaciones (FI, FII y FIII) usando relaciones molares variables de azilsartán medoxomilo / nanopartículas de ZnO. Se encontró que la Fórmula III, con una relación molar de fármaco / nanoportador de 2: 1, contenía el mayor porcentaje de fármaco cargado y, por lo tanto, se seleccionó para estudios posteriores como la formulación más óptima. La velocidad de liberación de azilsartán medoxomilo del complejo de ZnO mostró una mejora significativa ($p \leq 0,05$) que el fármaco puro con liberación completa después de 60 minutos. Los valores altos de rendimiento porcentual, eficacia de atrapamiento y carga de fármaco de FIII indican una carga satisfactoria y una formulación eficaz de FIII. El difractograma de rayos X y el termograma DSC de FIII revelan una formulación estable con una cristalinidad disminuida del fármaco cargado en la superficie. Las imágenes de AFM revelan una reducción del tamaño de partícula como se observa en la relación de área de superficie aumentada de FIII (114) en comparación con azilsartan medoxomil (2.9) en el mismo tamaño de imagen. FTIR indica una formulación estable de FIII sin interacción química. Como conclusión, azilsartan medoxomil se cargó con éxito en nanopartículas de metal ZnO sintetizado con propiedades optimizadas en términos de velocidad de disolución y cristalinidad.

Palabras clave: *Azilsartan medoxomil, Clase II BCS, Alteración de la cristalinidad, Nanopartículas de ZnO, Poca solubilidad.*

Abstract

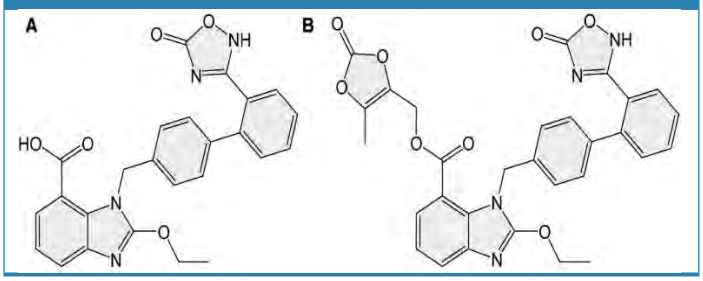
The present study involves the utilization of metal oxide nanoparticles, zinc oxide (ZnO), as a drug delivery module for the antihypertensive drug, azilsartan, as a tool of particle size reduction. According to biopharmaceutical classification system (BCS), azilsartan belong to class II with low solubility and high permeability. ZnO nanoparticles was synthesized using chemical precipitation method, and then three formulations (FI, FII, and FIII) were prepared using variable azilsartan medoxomil / ZnO nanoparticles molar ratios. Formula III, with 2:1 drug / nanocarrier molar ratio, was found to contain the higher percent of loaded drug and therefore was selected for further studies as the most optimum formulation. The release rate of azilsartan medoxomil from ZnO complex showed significant ($p \leq 0.05$) enhancement than pure drug with complete liberation after 60 minutes. High values of percent yield, entrapment efficiency and drug loading of FIII indicate successful loading and efficient FIII formulation. The X- ray diffractogram and DSC thermogram of FIII reveal stable formulation with decreased crystallinity of surface loaded drug. AFM images reveal particle size reduction as noted by the increased surface area ratio of FIII (114) in comparison to azilsartan medoxomil (2.9) at the same image size. FTIR indicate stable FIII formulation with no chemical interaction. As a conclusion, azilsartan medoxomil was loaded successfully on synthesized metal ZnO nanoparticles with optimized properties in term of dissolution rate and crystallinity.

Keywords: *Azilsartan medoxomil, Class II BCS, Crystallinity alteration, ZnO nanoparticles, Poor solubility.*

Introduction

Azilsartan, is a selective AT1 receptor blocker (figure 1) that binds tightly and slowly separates[1], thus preventing the binding of angiotensin II leading to vasodilation and minimized aldosterone effects[2,3]. It was developed by Takeda Global Research Development and approved in Japan in 2012 for the management of hypertension and marketed as a prodrug, azilsartan medoxomil, which is hydrolyzed to its active moiety, azilsartan, at gastrointestinal tract. Absolute bioavailability of azilsartan is 60% due first pass metabolism by CYP2C9 and elimination half-life of 11 hours[2]. According to biopharmaceutical classification system (BCS), azilsartan belong to class II with low solubility of $4.28X10^{-3}$ mg/L in water at 25°C, (practically insoluble) and high permeability[4] which make the drug attractive for solubility enhancement techniques as its dissolution is the rate limiting step for azilsartan absorption. Several methods with different principles have been utilized for increased aqueous solubility of azilsartan such as solid dispersion[5,6], and liquisolid compact[7]. Nanocarriers are the most evolving tools for solubility enhancement, nanocarriers have been used to improve the therapeutic effect of drugs and minimize the side effect. Nanocarriers are defined as particulate dispersion or solid particles with a size in the range of 10–1000 nm in at least one dimension that act as a carrier module for other molecules such as drugs, where the drug is dissolved, entrapped, encapsulated, or attached to a nanocarrier[8,9]. Inorganic nanoparticles, including metal (gold, copper, silver, iron), metal oxide (iron oxide, zinc oxide) and quantum dots (cadmium selenide and cadmium sulfide) are among the nanoparticles of biomedical advantages including their utilization as drug delivery system[10,11]. One member of metal oxide nanoparticles family, zinc oxide (ZnO) nanoparticles, has excellent UV-radiation protecting properties, making these nanoparticles excellent sunscreen blockers[12]. Other properties have also been explored, such as their antibacterial and anticancer activity, resulting from their ability to induce ROS generation. ZnO nanoparticles are also excellent drug carrier system in addition to their inherent biomedical properties[13]. In addition, the U.S. Food and Drug Administration (FDA) has recognized ZnO as a generally accepted safe substance (GRAS), and ZnO nanoparticles larger than 100 nm are considered moderately biocompatible, which support their use as drug delivery module[13]. The objective of this work involves the synthesis and utilization of metal oxide nanoparticles, zinc oxide (ZnO), as a drug delivery module for the antihypertensive drug, azilsartan, as a tool of particle size reduction.

Fig. 1. Structure of (A) azilsartan and (B) azilsartan medoxomil(4)



Materials

Azilsartan medoxomil ($C_{30}H_{24}N_4O_8$), with M.Wt of 568.542 g/mol[14] was purchased from (Hangzhou Hyper Chemical Limited, China) while zinc nitrate hexahydrate [$Zn(NO_3)2{\cdot}6H_2O$] and sodium hydroxide (NaOH) used in this study were purchased from (Sigma-Aldrich), acetone, methanol were of analytical grade.

Methods

Synthesis of zinc oxide (ZnO) nanoparticles

Zinc oxide (ZnO) nanoparticles were synthesized using chemical precipitation method, in which 0.2 M NaOH solution was slowly titrated for 15 min onto 0.1 M $Zn(NO_3)_2$ aqueous solution with continuous stirring at room temperature, stirring process continued four hours after titration completion. Afterward, the reactant mixture was centrifuged at 8000 rpm for 15 min to harvest a white precipitate of zinc hydroxide, $Zn(OH)_2$, which then washed with deionized water and ethanol three times and heated at 140°C for 4 h. Finally, a yellow powder of ZnO nanoparticles was obtained following 24 h drying at 80°C[15,16].

Preparation of azilsartan medoxomil loaded ZnO nanoparticles

For loading process, solvent evaporation precipitation technique was utilized by using three variable molar ratios of azilsartan medoxomil and ZnO nanoparticles, 1:1, 2:1 and 1:2. (FI, FII, and FIII respectively). Where 50 mL of different concentrations of azilsartan medoxomil solutions were prepared separately, using acetone as a solvent, and then solid ZnO nanoparticles with specified weights were added slowly under specific condition (stirring at 1500 rpm and temperature of 50°C) using hot plate magnetic stirrer (Dragon Lab, USA). After completion of ZnO nanoparticles addition, stirring was continued for 2 hours, followed by filtration, and washing thrice with deionized water to discard any solvent remaining. Finally, the prepared azilsartan loaded ZnO nanoparticles was placed in silica gel containing desiccator for 3-5 days to ensure complete drying and solvent evaporation, to be collected for further characterization[9,17].

Calibration curve

To detect maximum absorbance peak, λ max, of azilsartan medoxomil, 10 mg of drug was dispersed in 100 mL phosphate buffer solution of pH 7.4 to prepare 0.1 mg/mL stock solution, from which a dilute solution of 10 μg/mL was prepared and scanned spectrophotometrically by UV-Visible spectrophotometer (Shimadzu, Japan) at UV range of 200-400 nm. Using the stock solution of 0.1 mg/mL of azilsartan medoxomil, serial dilutions with phosphate buffer pH 7.4 were prepared and scanned at the detected λ max to draw the calibration curve of the drug by plotting absorbance vs. concentration. The same procedure was followed for calibration curve of azilsartan medoxomil in methanol. Each experiment was performed in triplicate[18,19].

Drug content

To determine the azilsartan medoxomil loaded on ZnO nanoparticles, take 10 mg of final product of loading process

and dissolve in 100 mL methanol with sonication until a nearly clear solution was obtained. Then, 2 mL sample was taken using filter syringe of 0.45 μg pore size and scanned spectrophotometrically at the detected λ max of the drug using calibration curve equation of azilsartan medoxomil in methanol[18,20]. Each experiment was performed in triplicate. This test was used as the basis of selection criteria, the formula with the higher drug content was used for further study.

Saturation solubility of the selected formula

Saturation solubility of azilsartan medoxomil was performed before and after loading process with ZnO nanoparticles using the shake flask method. Where excess amount of pure azilsartan medoxomil and azilsartan medoxomil loaded ZnO nanoparticles added separately in phosphate buffer solution of pH 7.4 as a medium for dissolution and kept in an incubating shaker at 25°C for 48 hours. After equilibrium, samples withdrawal done using 0.45 μm pored size filter syringe and then scanned spectrophotometrically at the detected λ max of the drug to determine the solubility of each sample within concentration range of calibration curve[5,18,19]. Each experiment was performed in triplicate.

***In Vitro* Drug Release Study of the selected formula**

The *in vitro* drug release profile of azilsartan medoxomil from loading with ZnO nanoparticles was obtained by plotting percent cumulative drug release versus time using USP type II paddle apparatus (Copley, UK) at 37°C ± 0.5°C in 900 mL phosphate buffer pH 7.4 as a dissolution medium and rotating speed of 50 rpm. 5 mL samples were withdrawn at programmed time intervals, 5, 10, 15, 20, 30, 45 and 60 min with 0.45μm Millipore filter syringe and replaced with the same volume of fresh media for dissolution. Samples then analyzed using a spectrophotometer at specified λ max of azilsartan medoxomil. The same experiment under similar conditions was performed for unloaded drug for comparison as a control[7,21]. Experiments were performed in triplicate.

Calculations of % yield, % entrapment efficiency, and % drug loading of the selected formula

The percentage of azilsartan medoxomil yield in the drug-ZnO nanoparticles compound was calculated according to equation below [17]:

$$\%\ \text{Yield} = \frac{\text{weight of nanoparticles after drug incorporation (actual)}}{\text{weight of nanoparticles and drug before incorporation (theoretical)}} \times 100\%$$

Eq1

The percentage of drug entrapped efficiency within ZnO nanoparticles (% EE) was calculated using the following equation [22]:

$$\%EE = \frac{\text{weight of drug in nanoparticles after incorporation (actual)}}{\text{weight of drug fed initially before incorporation (theoretical)}} \times 100\%$$

Eq2

The percentage of drug loaded (DL) by ZnO nanoparticles was calculated [23] as follows:

$$\%DL = \frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles loaded with the drug}} \times 100\%$$

Eq3

Characterization techniques of the selected formula

Powder X-ray diffraction (PXRD)

To evaluate crystallinity of azilsartan medoxomil after loading with ZnO nanoparticles, PXRD was done for the drug before and after loading process with metal oxide nanoparticles, in addition to blank ZnO nanoparticles alone. Samples analyzed in special conditions (scanning speed 5°/min, and θ-2θ axis range of 5 to 60 degrees) using PXRD instrument (Shimadzu, Japan) equipped with Cu-Ka radiation λ = 1.54060 Å, voltage 40 Kv and current 30 mA[24].

Differential scanning calorimetric (DSC)

Thermal behavior of azilsartan medoxomil after incorporation with ZnO nanoparticles was analyzed using differential scanning calorimetric (Linseis, Germany), where 10 mg of pure drug, blank ZnO nanoparticles and azilsartan loaded ZnO nanoparticles placed separately in sample cell within aluminum pan of device. The sample cell was heated from 25°C to 300 °C, at 10°C/min heating rate and 10 mL/min supply of dry nitrogen gas as a carrier[25].

Atomic force microscopy (AFM)

The morphology, particle size and size distribution of these particles were evaluated using atomic force microscope (Augestrom Advance Inc., USA). Two- and three-dimensional images taken for pure azilsartan medoxomil, drug loaded ZnO nanoparticles, blank ZnO nanoparticles. Samples were dissolved in methanol and a few drops were placed from each sample on a glassed silica plate and allowed to dry at room temperature for deposition on the plate. The film was then scanned by the atomic microscope instrument[19].

Fourier Transform Infra-red spectroscopy (FTIR)

FTIR instrument (Shimadzu Japan), using potassium bromide (KBr) disc and frequency 4000-500 cm^{-1}, was utilized to check compatibility and nature of loading process on azilsartan medoxomil functional groups, whether chemical or physical. FTIR spectroscopy was done for azilsartan medoxomil before and after loading process as well as for blank ZnO nanoparticles[16].

Statistical analysis

All experiments were conducted in triplicates, Student T-test was used for quantitative comparison of *in vitro* release study for azilsartan medoxomil from ZnO nanoparticles as well as for dissolution of free unloaded drug, the results were expressed as mean ± standard deviation. Statistical calculations were performed using the statistical package SPSS for windows (version 13, SPSS Inc., Chicago, IL, USA) considering the statistical significance for each test a *P* value of less than 0.05 was adopted.

Results and Discussion

Calibration curve

Maximum absorbance (λ max) of azilsartan medoxomil was detected at 249 nm. Calibration curves in phosphate buffer pH 7.4 and in methanol gave rise to straight line in the concentration range of Beers Lamberts with high regression values as shown in Fig. 1 and Fig. 2 respectively.

Fig. 2. Calibration curve of azilsartan medoxomil in phosphate buffer pH 7.4

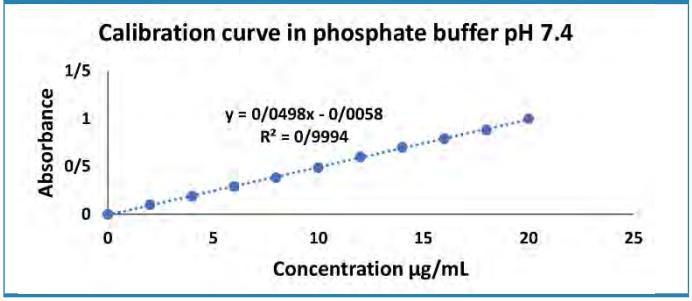
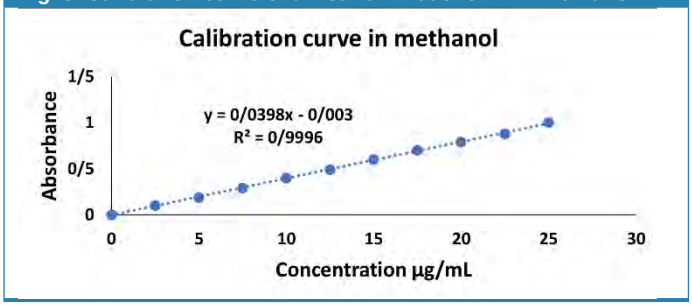


Fig. 3. Calibration curve of azilsartan medoxomil in methanol



Drug content

The content of azilsartan medoxomil in azilsartan – ZnO nanoparticles complex was detected spectrophotometrically at λ max of the drug for the prepared formulations of three different weight ratios as summarized in the following table (Table 1). Formula III was found to contain the higher percent of loaded drug in ZnO nanoparticles and therefore was selected for further studies as the most optimum formulation.

Table 1. Azilsartan medoxomil content % in the prepared formulations I, II and III.

Formula Code	Azilsartan medoxomil: ZnO weight ratio	Drug content as percent
Formula I	1:1	48.53 % ± 1.72
Formula II	1:2	21.65 % ± 1.03
Formula III	2:1	78.07 % ± 1.91

Saturation solubility

The solubility of Azilsartan medoxomil incorporated in zinc oxide nanoparticles was 1.8 times more soluble than Azilsartan medoxomil as seen in table 2 due the reduction of the particle size of the drug.

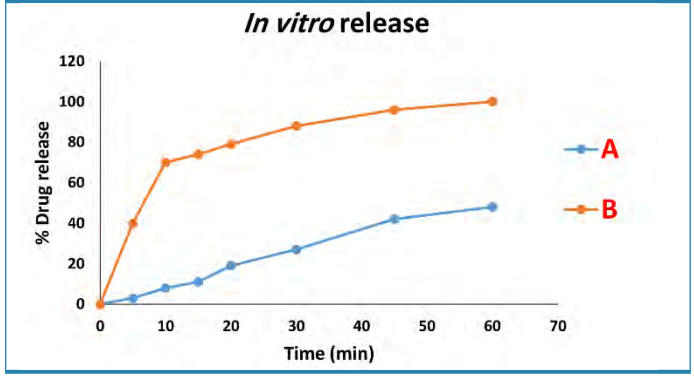
Table 2. Saturated solubility of pure azilsartan medoxomil before and after loading process with ZnO nanoparticles in phosphate buffer media at pH 7.4.

Compound	Saturated solubility (mg/mL)
Pure azilsartan medoxomil	0.92 ± 0.03
Azilsartan medoxomil: ZnO (2:1 w/w)	1.67 ± 0.07

In Vitro Drug Release Study

Azilsartan release from ZnO nanoparticles was significantly ($p \leq 0.05$) higher than from the pure drug as seen in figure 2, furthermore FIII showed first order release kinetics (R^2 = 0.9787) that attributed to solubility rate enhancement of FIII. The drug is within nanosized range and therefore the dissolution rate is enhanced.

Fig. 4. *In vitro* release study of (A) azilsartan medoxomil (B) azilsartan medoxomil : ZnO nanoparticles FIII



Calculations of % yield, % entrapment efficiency, and % drug loading

The percent yield, entrapment efficiency and drug loading capacity of FIII are shown in table 3, which are very encouraging, that has to say the preparation method of FIII was efficient.

Table 3. % yield, % entrapment efficiency, and % drug loading of FIII

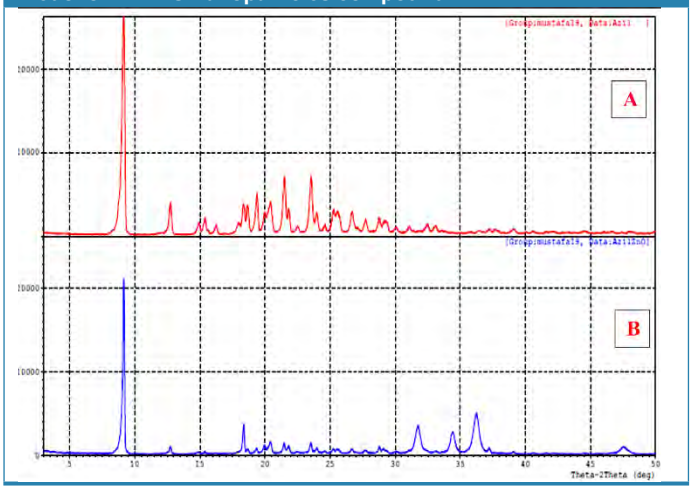
Formula FIII	% Yield	% Entrapment Efficiency	% Drug Loading
Azilsartan medoxomil: ZnO (2:1 w/w)	74.58	98.28	91.76

Characterization techniques of Azilsartan medoxomil

Powder X-ray diffraction (PXRD)

The X-ray diffraction of powdered azilsartan medoxomil reveal its crystalline nature with the prominent sharp peak intensity at 2θ of 8.3°, in addition to other intensity peaks between 20-25° as seen in figure 5(A) [26]. While the diffractogram of FIII (figure 5 B) showed intensity peaks above 30° that belong to the crystalline ZnO nanoparticles [27, 28], as well as display the same characteristic peaks of azilsartan at 2θ 8.3° and between 20-25° but at much lower intensity, indicating amorphous nature or a less crystalline structure of loaded drug [29]. The peak at 8.3° related to the drug adsorbed on the surface of the ZnO nanoparticles.

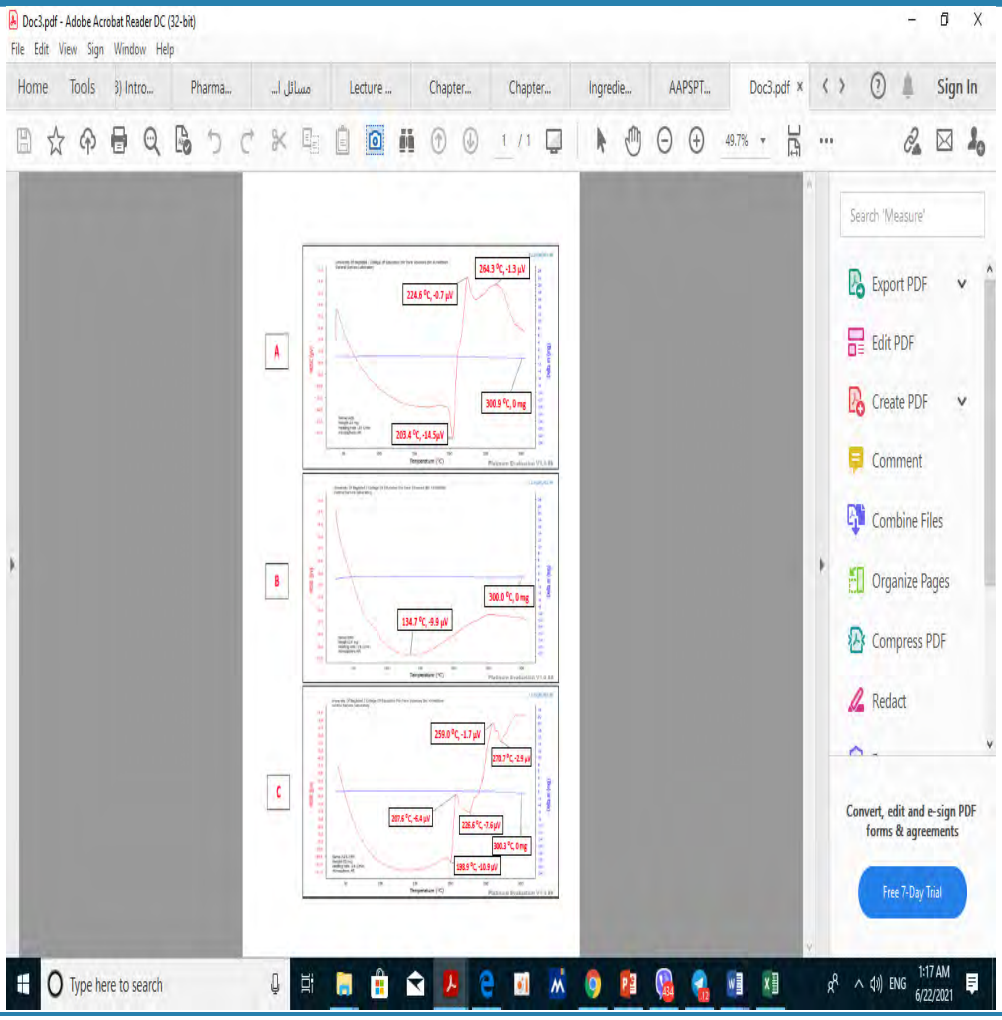
Fig. 5. PXRD diagram of (A) azilsartan medoxomil (B) azilsartan medoxomil: ZnO nanoparticles compound.



Differential scanning calorimetric (DSC)

DSC thermogram support the PXRD results, in which the crystalline nature of azilsartan medoxomil (fig. 6 A) showing an endothermic peak at 203.4°C and an exothermic peak at 224.6°C, 264.3°C[29]. Thermogram of ZnO nanoparticles (fig. 6 B) show a broad endothermic peak of the glass transition temperature (Tg) at 134.7°C due to increase mobility at the surface of ZnO nanoparticles, since the Tg depends on the size and surface of the phase been tested[25]. While FIII (fig 6 C) showed small shifting of the endothermic peaks to 198.9°C and 226.6°C for azilsartan and shifting of the exothermic peaks to 207.6°C and 259°C. The appearance of same peaks of drug with small shifting in melting point indicates thermal stability of FIII[30], decreased crystallinity within prepared formulation [31], and surface loading of azilsartan onto metal ZnO nanoparticles.

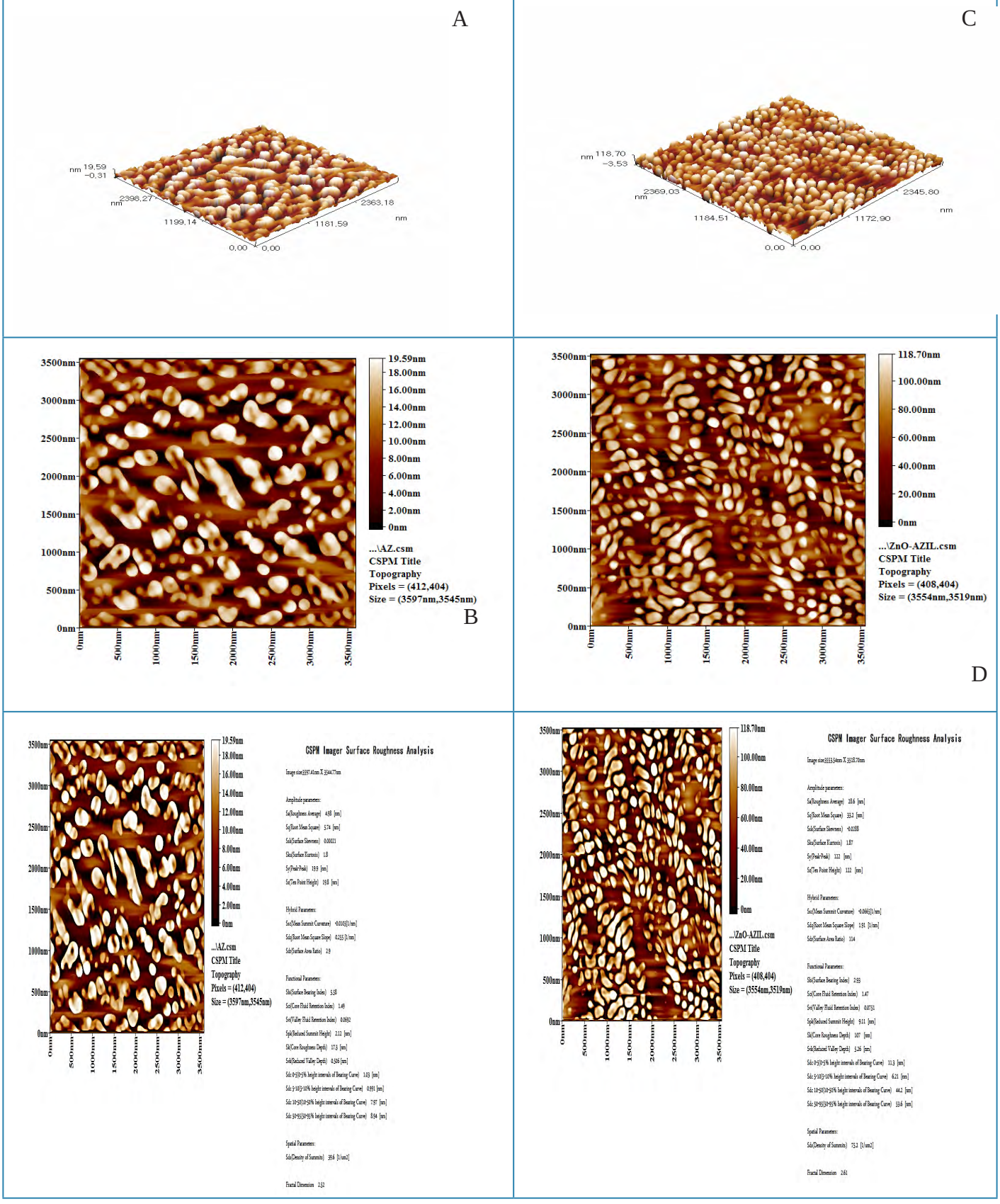
Figure 6. DSC thermogram of Azilsartan Medoxomil (A), ZnO nanoparticles (B), Azilsartan Medoxomil: ZnO (2:1) w/w% (C).



Atomic force microscopy (AFM)

Atomic force microscopy (AFM) technique provides a real picture of ultra-high resolution in particle size measurement, which is performed without any specific treatment[11]. The atomic force images are shown in figure 7, the particle size decrease is noted as the surface area ratio is increased in FIII (114) in contrast to azilsartan medoxomil (2.9) at the same image size.

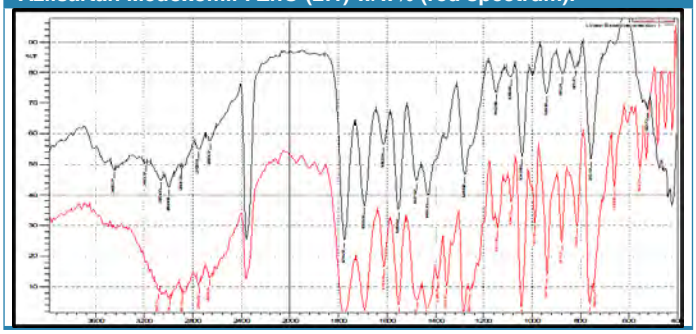
Figure 7. Topography of Azilsartan Medoxomil (A), (B) and Azilsartan Medoxomil: ZnO (2:1) nanoparticles (C) and (D) 2D and 3D images



Fourier Transform Infra - Red spectroscopy (FTIR)

FTIR spectrum of pure azilsartan medoxomil, the black spectrum in figure 8, display a characteristic absorbance band at 1691.63 cm^{-1} and 1774.57 cm^{-1} due to C=O stretching vibrations of carboxyl functional groups. While C–O stretching bands appeared at 1276.92 and 1429.3 cm^{-1}, C-O-C stretching vibration band at 1085.96 cm^{-1}, N-H bending of amine group appeared at 1477.52 cm^{-1}, and C=N stretching at 1691.63 cm^{-1} [5,32]. Absorbance spectrum of loaded azilsartan on ZnO nanoparticle (FIII) display same characteristic peaks, indicating no chemical modification of drug after loading process, as well as surface loading of azilsartan on metal ZnO nanoparticles[33].

Figure 8. FTIR of Azilsartan Medoxomil (black spectrum), and Azilsartan Medoxomil : ZnO (2:1) w/w% (red spectrum).



Conclusion

Azilsartan medoxomil was successfully loaded with ZnO nanoparticles with improved pharmaceutical properties of enhanced solubility, as revealed by higher saturated solubility and faster dissolution rate and altered lattice of less crystalline structure as indicated by XRD and DSC. This improvement could attributed to the drug size reduction into nano-scale with optimized properties.

Acknowledgement

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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