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PREPARATION OF IRBESARTAN NANOPARTICLES FOR DISSOLUTION RATE ENHANCEMENT

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ABSTRACT

Nanoparticle technique offers promising methods for the formulation of poorly water soluble drugs. The objective of the present investigation was to enhance dissolution and oral bioavailability of poorly water-soluble irbesartan (IBS) by preparing stable nanoparticles. IBS nanosuspensions were produced by antisolvent precipitation under sonication. The physicochemical properties of the prepared nanoparticles were characterized by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffractometry (PXRD), transmission electron microscopy (TEM) and solubility studies, as well as measuring the particle size and in-vitro drug dissolution.

The physicochemical results indicated that the antisolvent precipitation process led to the amorphization of IBS without drug-polymer chemical interaction. IBS nanoparticles increased the saturation solubility of drug almost sixteen fold. The *in vitro* studies showed a marked increase in the drug dissolution rate After 60 min, nanoparticles were almost dissolved completely but only 53 % of unprocessed IBS and 70 % of physical mixture (PM) had dissolved owing to its crystalline nature and larger crystal size. The combining of the methods was a promising method to produce uniform nanoparticles of IBS with remarkable improvement in dissolution rate.

KEY WORDS: Antisolvent, nanosuspensions, saturation solubility, Hydroxypropyl methyl cellulose, and sodium dodecyl sulfate.

INTRODUCTION

The rate-limiting step in the absorption process for poorly water-soluble drugs is the dissolution rate of such drugs in the gastrointestinal fluids rather than the rapidity of their diffusion across the gut wall. Therefore, therapeutic effectiveness of a drug depends upon the solubility of drug molecules [1]. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation. Unfortunately, more than 90% of developed drugs suffer from poor oral bioavailability due to poor aqueous solubility and/or low dissolution rate [2,3]. The use of poorly soluble drugs has a number of drawbacks such as increasing the dosage, administration frequency and the resultant occurrence of side effects [1].

There are different techniques available to improve the dissolution rate of poorly soluble drugs [4-6]. Nanosuspensions are promising strategy for improving the dissolution rate and oral bioavailability of poorly water soluble drugs by reducing the particle size and/or transforming drugs from a crystalline to an amorphous state [7-10].

Irbesartan (IBS), figure 1. is indicated for treatment of hypertension. It belongs to class II drug according to biopharmaceutical classification system (BCS) [11] i.e. low solubility and high permeability. According to BCS drug substance is considered to be highly soluble when highest dose of drug dissolve in less than 250 ml of water. It is considered to be highly permeable when the extent of absorption in human is more than 90% of an administered dose. Theoretically IBS exhibits solubility limited bioavailability and it would be advantageous to increase the solubility of such molecule. In this study nanoparticles of IBS were prepared by antisolvent precipitation technique using low viscosity grade of hydroxypropyl methyl cellulose (HPMC) to enhance its dissolution rate and bioavailability.

MATERIALS AND METHODS

Materials

Irbesartan (IBS) was purchased from EL-Kahira Pharmaceutical Co., Egypt, Hydroxypropyl Methylcellulose (HPMC), Sodium Dodecyl Sulfate (SDS) and dimethylformamide (DMF) were obtained from Sigma-Aldrich, Co. USA, and all other chemicals, reagents and solutions used were of analytical grade.

Methodology

Preparation of Nanosuspensions

IBS suspensions were produced by antisolvent precipitation under sonication [12]. Briefly, DMF against water was used as solvent and antisolvent in a ratio of 1:20 respectively. The organic solution of IBS was prepared by dissolving IBS in 10 mL DMF. The resulted solution was injected into 200 mL 0.15% (w/v) aqueous solution (containing HPMC and SDS, 2:1, w/w) cooled to below 8°C in an ice-water bath and kept under sonication condition. The precipitation rate was controlled throughout the process by maintaining the temperature below 8°C using an ice-water bath. The particle size reduction was done with an ultrasonic probe sonicator (sonica, vibracell, USA) at ultrasonic power input of 300 W for 10 time length. The probe with a tip diameter of 10 mm was immersed 10 mm. The suspensions were kept under vacuum at room temperature for 2 h to remove the organic solvent. Then, the IBS suspensions were further homogenized by homogenization, using a homogenizer (Hielscher, Germany) for 8 minutes to obtain the final product. The homogenized suspension was evaluated for the particle size distribution.

Conversion of Liquid Nanosuspension to Dry Nanoparticles

A freeze dryer was used to convert IBS nanosuspensions into dry nanoparticles, freeze-dried overnight (Free-Zone 180, Labconco Corporation, Missouri, USA). Dried nanoparticles were stored at −20°C for furthers investigations.

Characterization of IBS Nanoparticles

Phase Solubility Studies

Solubility studies were carried out according to Higuchi and Connors reports. IBS or its equivalent as PM and nanoparticles were added in amounts beyond its solubility to 25 ml of phosphate buffer solution (pH 7.4) in stoppered flasks. The flasks were positioned in a shaker maintained at 25 °C for 72 hours to reach equilibrium. The content of each flask was passed through 0.22 µm filter unit and its concentration was determined spectrophotometricaly by measurement of UV absorption at 244 nm against a suitable blank.

Nanoparticles Size Measurements

The size of the prepared nanoparticles was determined by the laser diffraction technique using a particle size analyzer (Quantachrome CLIAS00, France). The samples were properly diluted with PBS and measured at 25°C. Nanoparticles size was expressed in terms of volume diameter and the measurements were done in triplicate and the average values were used.

Transmission Electron Microscopy (TEM)

Selected samples were examined under TEM. A drop of the nanoparticles sample was transferred into the copper mesh grids. After the sample was adsorbed (about 15~20 min), the staining dye (potassium phosphotungstate) was dripped onto the film. The staining time was about 1~2 min. After drying the copper mesh grids, the morphology of the investigated nanoparticles was visualized.

Differential Scanning Calorimetry (DSC)

The DSC studies were performed on the drug, the polymers, the drug-polymers PM and the prepared nanoparticles. The samples (3-4 mg) were inserted in aluminum pan and heated at the rate of 10ºC/min, to a temperature of 300ºC using a differential scanning calorimeter (Shimadzu DSC-50, Japan).

Fourier Transfer Infrared Spectrophotometery (FT-IR)

The FT-IR studies were carried out on the drug, the polymers, the drug-polymers PM and the prepared nanoparticles. Samples were mixed with IR grade KBr. The prepared disks were scanned over a wave number range (4000 – 400 cm^{-1}).

X-Ray Diffraction Analysis (XRD)

The X-Ray diffraction patterns were obtained using Jeol, SPX 60-PA Diffractometer. The target used was CuKα radiation operating at 35 kV and a current of 15 mA. The diffraction patterns were achieved using continues scan mode with 2θ º ranging from 8º- 80º at a rate of 2º/ min.

In-vitro Dissolution Studies

In-vitro dissolution studies were performed on the unprocessed drug, the PM and the nanoparticles using the USP type II paddle method. Powder samples equivalent to 150 mg of IBS were placed in dissolution vessels containing 500 ml 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 50 rpm. Samples were collected manually and replaced with an equivalent volume of fresh dissolution media. IBS concentration was determined spectrophotometricaly by measurement of UV absorption at 244 nm using a suitable blank .

Statistical Analysis

All values were expressed as Mean ± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA). The value of p less than 5% ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Solubility Studies

Solubility data for IBS, PM and the prepared nanoparticles are given in figure 2. It could be observed that both HPMC and SDS enhanced the solubility of IBS in the PM. This is due to surfactant and wetting property. It could be observed also from the figure that the solubility of IBS was significantly increased in case nanoparticles compared to the unprocessed drug and PM. The enhancement of solubility of IBS nanoparticles could be attributed to the reduction in the particle size and the conversion of crystalline drug to the amorphous state [7-10].

Particle Sizes

The mean volume diameter of the prepared particles composed of IBS and the surfactant and HPMC was studied. The mean diameter values of the prepared particles ranged from 420 ± 2.3 to 580 ± 4.6 nm, figure 3. The size measurements were repeatable and reproducible from batch to batch (acceptable deviations from the average values).

Transmission Electron Microscopy (TEM)

The nanostructures of the prepared particles were studied using TEM (Figure 4). The nanographs captured revealed spherical-shaped particles of smooth surfaces. However, the diameters are not correlated with those obtained from laser diffraction measurements. The steps involved in TEM are likely to promote the formation of smaller sizes and artifacts during sample preparation [13].

Differential Scanning Calorimetry (DSC)

DSC thermogram of IBS, HPMC, SDS, their PM and nanoparticles are depicted in figure 5. IBS showed an endothermic sharp peak at 185 °C which is corresponding to the melting point of the drug. Since HPMC has no exact melting point and char when heated, no sharp endothermic peaks were observed. But, a broad endothermic band was observed from 40-110°C for HPMC could be attributable to the vaporization of the moisture present in the samples. HPMC showed also an exothermic peak at 239 °C. The DSC curve of SDS showed

a broad endothermic peak due to water loss between 85°C and 110°C and another sharp endothermic peak at 198°C that is corresponding to its melting point. Melting endotherm not appreciably change in IBS either in PM and nanoparticles (figure 5D and 5E). This observation suggested the absence of chemical interaction of drug with SDS and HPMC. But, it is revealed from DSC thermogram of the nanoparticles (figure 5E) that there was a decrease in sharpness and intensity of characteristic endothermic peaks of drug which could be attributed to the conversion of most of the crystalline form of the drug to the amorphous form.

FT-IR

FT-IR spectra of IBS are depicted in figure 6. The characteristic absorption peaks of IBS was found at 3055 cm^{-1} and 3032 cm^{-1} (N–H stretch), 1731 cm^{-1} (C=O stretch), 1622 cm^{-1} (C–N stretch). Regarding the spectrum of HPMC, figure 6B, it could be easily to distinguish the broad peak at 3580-3650 cm^{-1} that is characteristic for the alcoholic (O-H). Regarding SDS figure 6C, the SO_2 assymetric vibration is located as a double band at 1219 cm^{-1} and 1249 cm^{-1} [14]. It could be observed that all the characteristic peaks of IBS were also viewed in the physical mixture and the prepared nanoparticles. This observation confirmed the compatibility and absence of chemical interaction between the drug and both HPMC and SDS.

X-Ray Diffraction Analysis (XRD)

XRD patterns of unprocessed IBS, HPMC, PM and nanoparticles are depicted in figure 7. The characteristic peaks appeared in the XRD of IBS at diffraction angles (2θ) 4.6°, 12.3°, 19.3° and 23.1 showing a typical crystalline pattern. However, all major characteristic crystalline peaks appeared in the diffractogram of PM as well as nanoparticles but of low intensity suggesting decrease in crystallinity of IBS. XRD of IBS showed sharp and intense characteristic peaks at different angles suggesting crystalline nature of IBS. However, decrease in intensity of characteristic peaks of IBS in case of nanoparticles indicated conversion of some of crystalline IBS to amorphous form. The XRD result of PM and nanoparticles suggest more amorphous nature of IBS in case of nanoparticles than in PM. Thus, results of XRD support the findings of the DSC study.

In-vitro Dissolution Studies

The dissolution rates of raw IBS, PM and nanoparticles were studied to confirm the extent of IBS solubility. Nano-sized IBS displayed an increase in the rate and extent of dissolution in comparison with unprocessed IBS especially during the initial stage (first 10 min). Nanoparticles exhibited 53.8 % drug dissolution within 10 min whereas only 24.5% of unprocessed IBS and 27 % of IBS in PM was dissolved during the same period. After 60 min, nanoparticles were almost dissolved completely but only 53 % of unprocessed IBS and 70 % of PM had dissolved owing to its crystalline nature and larger crystal size.

The *in vitro* dissolution of nano-sized IBS was excellent in comparison with that of unprocessed IBS (Figure 8). According to Noyes-Whitney equation, the solid dissolution rate is directly proportional to its surface area exposed to the dissolution medium. The increased dissolution rate of nanoparticles could be attributed to the combination of effects like amorphization and particle size which was reduced to nano-scale, greatly increasing the specific surface area and decreasing diffusion layer thickness [15].

Statistical studies showed that there were insignificant differences in both the rate and the extent of dissolution between the unprocessed drug and the drug present in the PM. While the differences became significant upon comparing the release rate of the nano-sized IBS with both unprocessed IBS and PM.

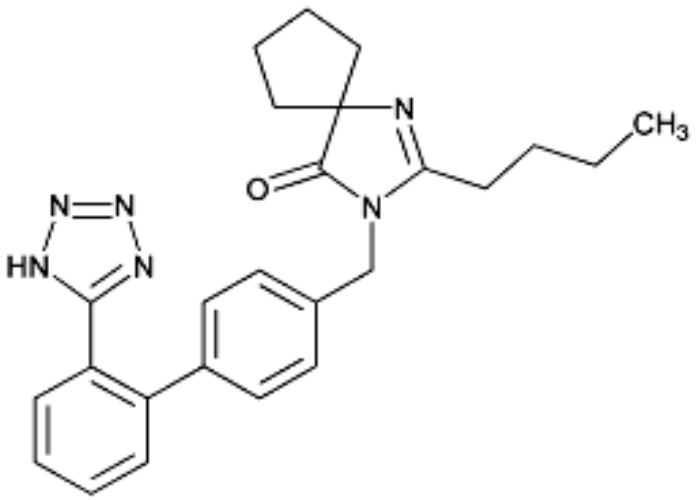

Figure 1: Chemical structure of irbesatran

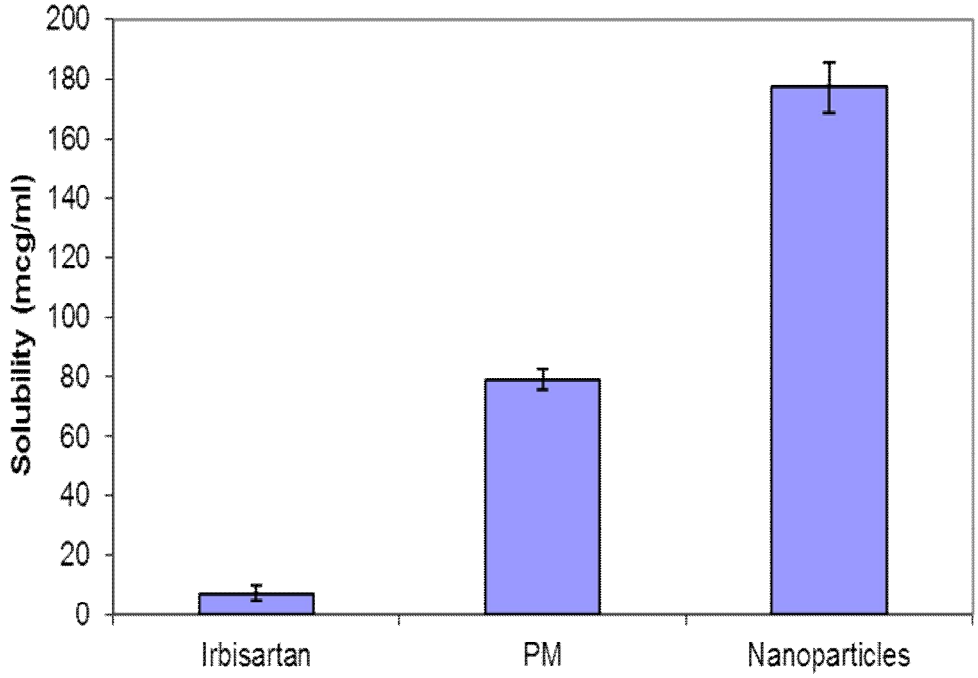

Figure 2: Solubility of Irbesartan, PM and nanoparticles

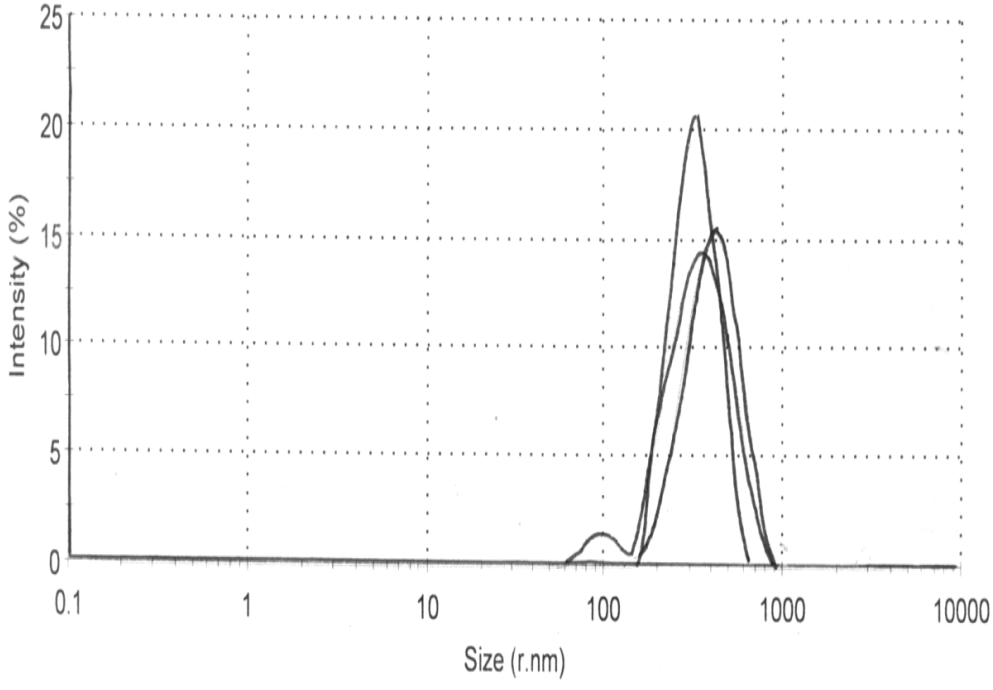

Figure 3: Particle size determination of prepared nanoparticles

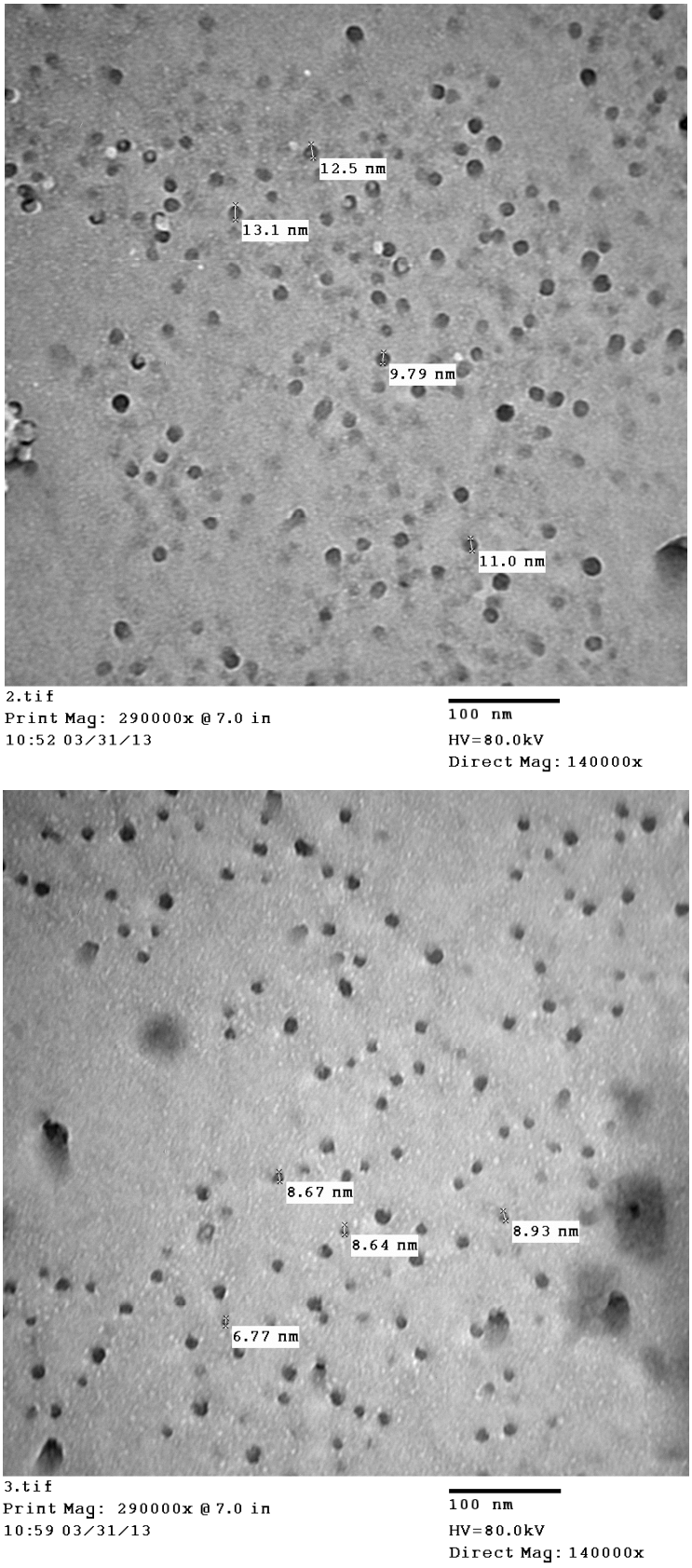

Figure 4: TEM of prepared nanoparticles

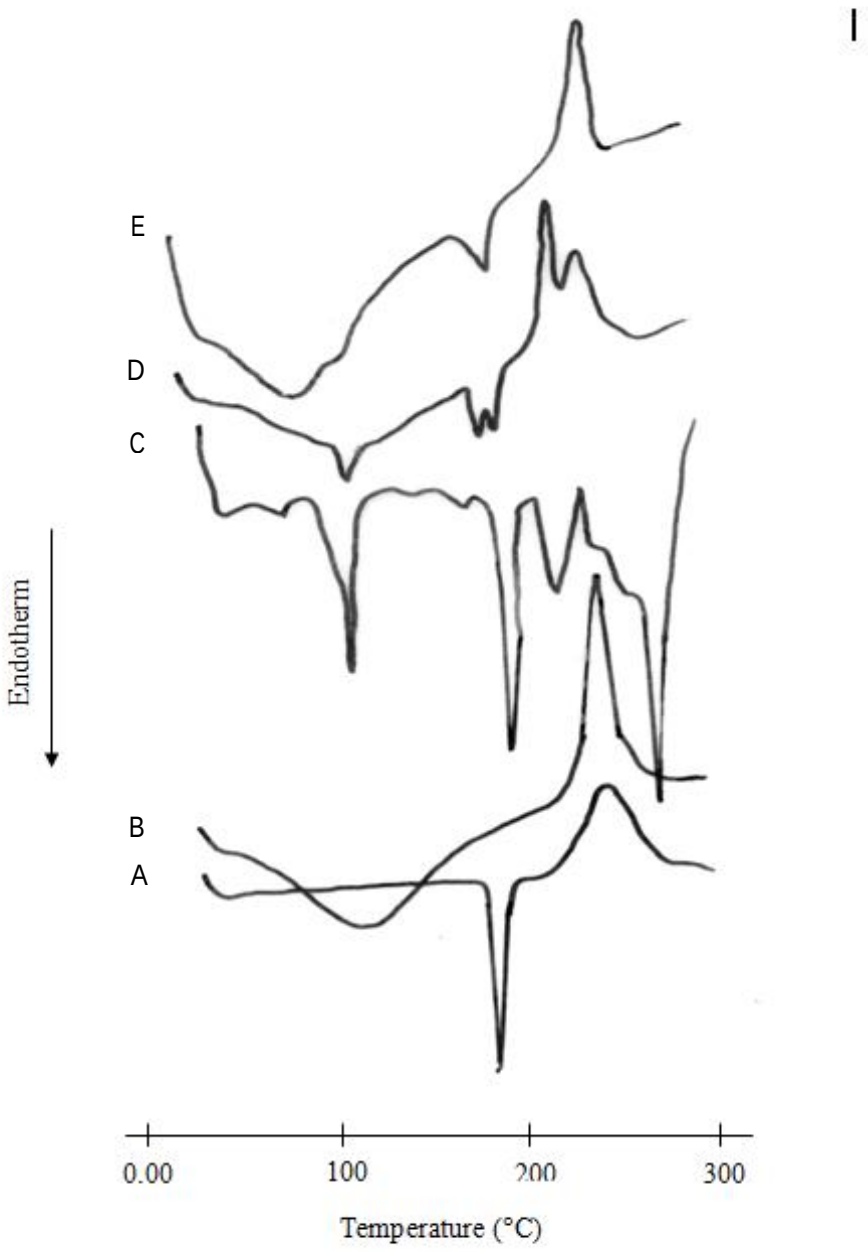

Figure 5: DSC Thermograms of (A) Irbesartan, (B) HPMC, (C) SDS, (D) PM and (E) Nanoparticles

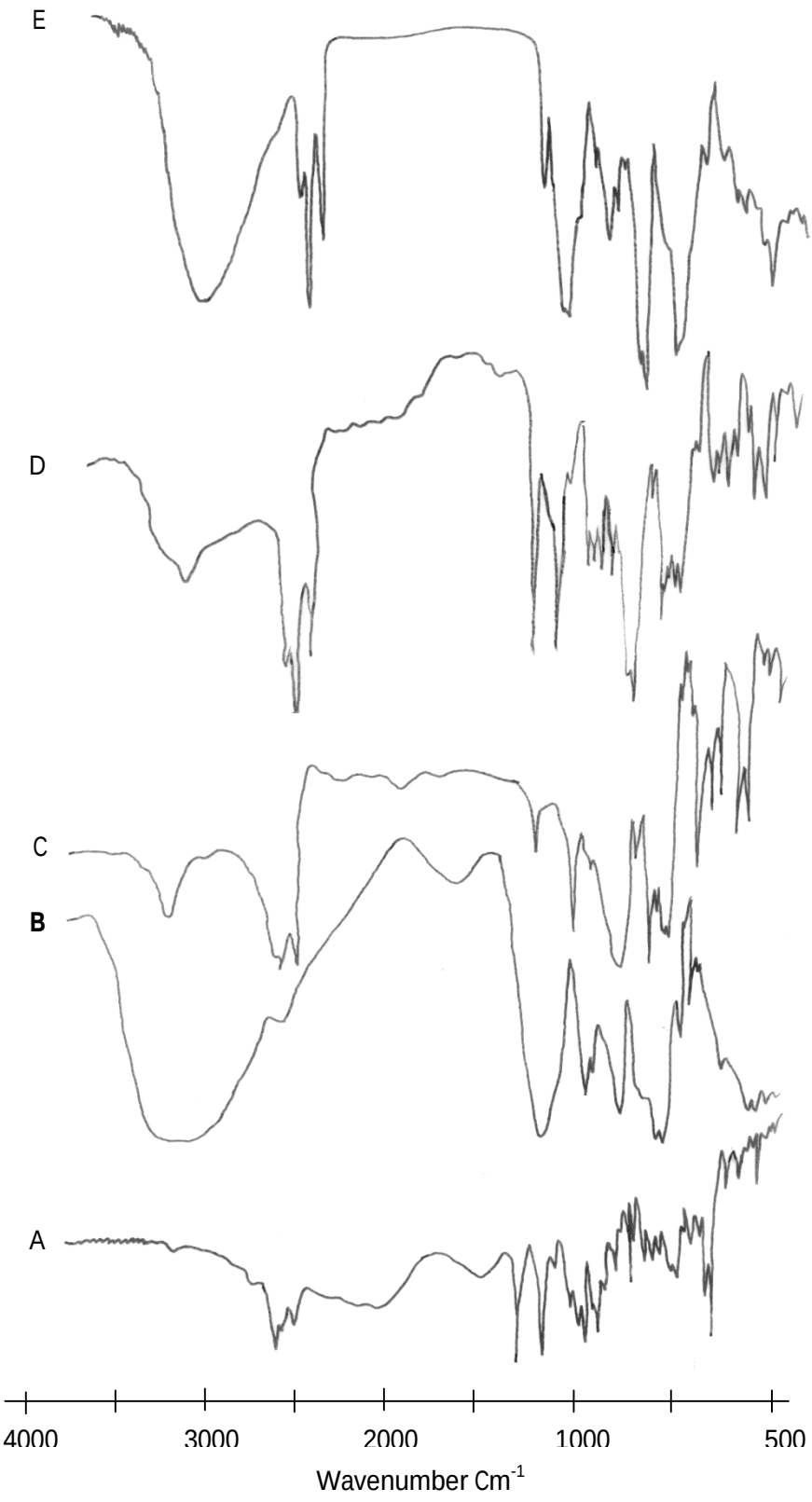

Figure 6: IR-Spectra of (A) Irbesartan, (B) HPMC, (C) SDS, (D) PM and (E) Nanoparticles

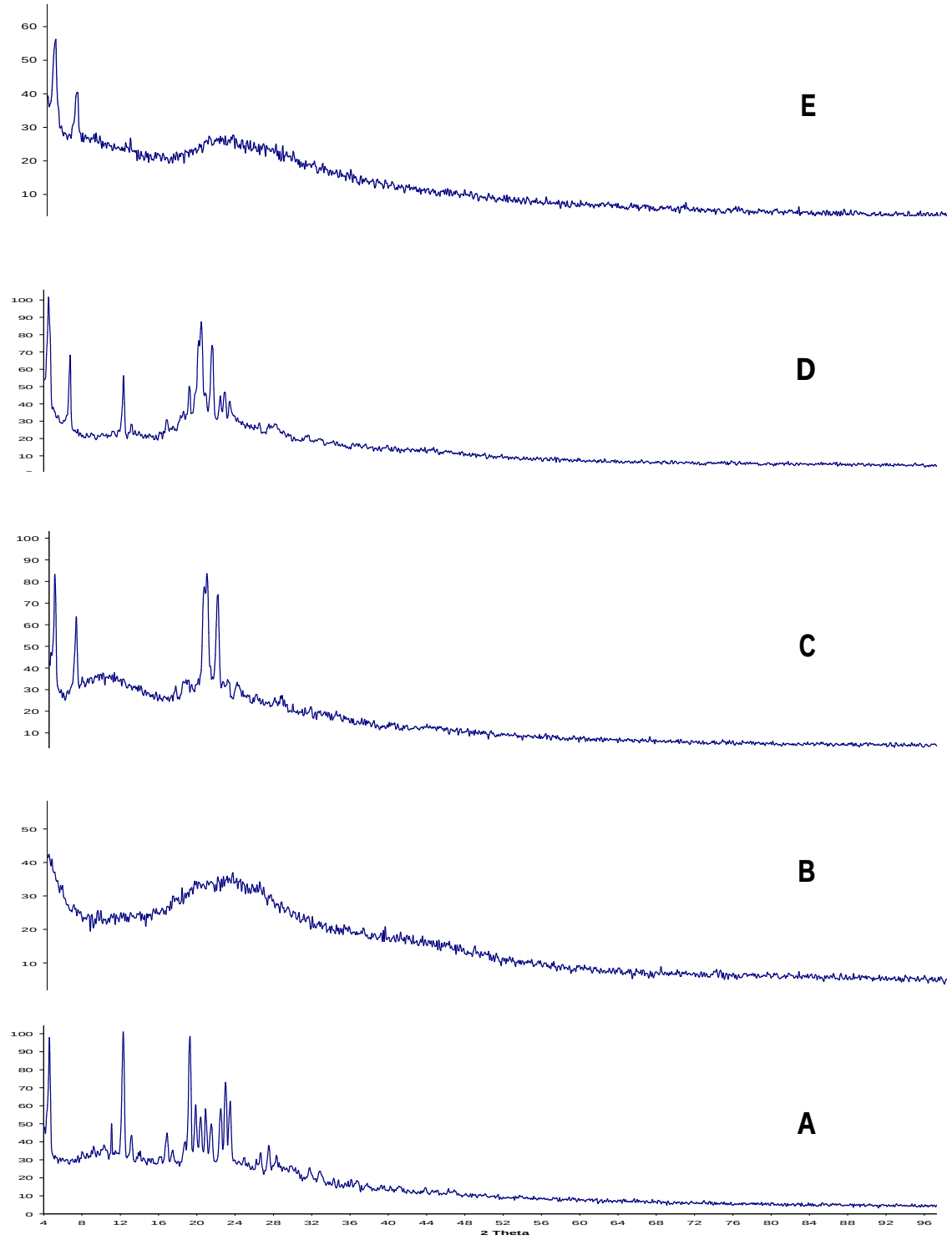

Figure 7: X-ray spectra of (A) Irbesartan, (B) HPMC, (C) SDS, (D) PM and (E) Nanoparticles

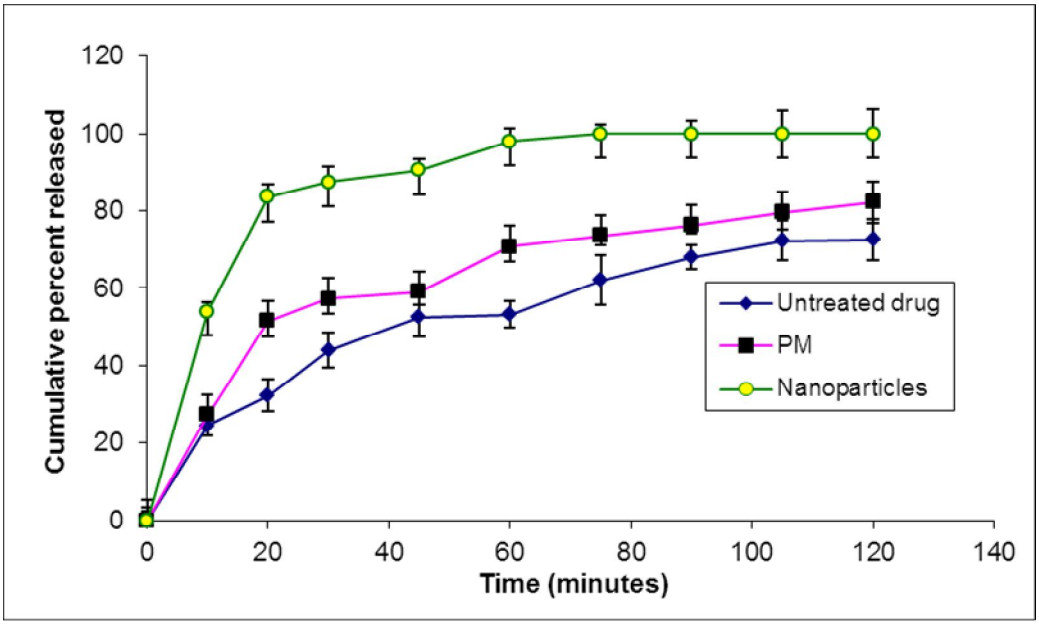

Figure 8: Dissolution profiles of Irbesartan, PM and nanoparticles

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