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Irbesartan-loaded electrospun nanofibers-based PVP K90 for the drug dissolution improvement: Fabrication, *in vitro* performance assessment, and *in vivo* evaluation

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ABSTRACT: Irbesartan with a low bioavailability is known as a poorly water-soluble drug. The purpose of this investigation is the improvement of physicochemical properties (such as solubility and dissolution rate) of Irbesartan using electrospun nanofibers-based solid dispersion preparation. Nanofibers were prepared using certain weight ratios of the drug and polyvinylpyrrolidone K90 (PVP K90). Then, dissolution studies were carried out. Moreover, selected samples were examined by many different tests such as Fourier transform infra red (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), high-performance liquid chromatography (HPLC), and scanning electron microscopy (SEM). Though solubility and dissolution rate of all Irbesartan-PVP nanofibers improved, but the best result was obtained through of ENSD5 (3% (*w/v*) : 7% (*w/v*)). In sink condition approximately 97% of this sample was released during 60 min. The drug content was among the different batches from 40.55 ± 1.01 to 245.32 ± 1.77 μg/mL. The maximum saturation solubility was belonged to this sample. According to the results of the thermal analysis and FTIR spectroscopy, there is no chemical reaction between drug and carrier, also samples has not changed during the process. Amorphous structure for nanofibers was confirmed by DSC thermograms and XRD diffractograms and morphological structure of samples were observed by SEM images. Ultimately, *in vivo* studies were performed in healthy grey rabbits and the results were satisfactory. The drug–polymer nanofibers showed an increase in relative bioavailability than the plain Irbesartan suspension. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42212.

KEYWORDS: drug delivery systems; electrospinning; hydrophilic polymers

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INTRODUCTION

Irbesartan is an angiotensin II type 1 (AT_1) receptor antagonist and/or an angiotensin receptor blocker. The drug competes with angiotensin II for binding at the AT_1 receptor subtype. Irbesartan is used mainly for the treatment of hypertension. It is given orally in hypertension.[1–6] Angiotensin II receptor antagonists are considered one of several preferred antihypertensive drugs for the initial management of hypertension in patients with chronic renal failure, diabetes mellitus, and heart failure.[5,7–12]

Irbesartan (a non-peptide tetrazole derivative) is a white to off-white crystals, odorless with melting point of 180 to 181°C and a molecular weight of 428.5 g/mol. It is practically insoluble in water [pKa = 4.5; Log *P* (octanol/water) = 10.1].[13,14] Irbesartan is soluble in organic solvents such as ethanol, acetone, chloroform, dioxane, and tetrahydrofuran.[13] The wide chemical structure of this drug ($C_{25}H_{28}N_6O$) was represented in Figure 1. Irbesartan belongs to group II of the BCS (Biopharmaceutics Classification System) categorizing system,[15] which means that it is an insoluble drug in the water, but it has a high permeability related to biologic membranes. Therefore, enhancement of solubility or dissolution rate is the best way to increase bioavailability of this drug.

Since the drug solubility makes desirable treating effects, it is very valuable. Poorly water-soluble drugs make problems for providing useful formulations and decrease drug effectiveness. The majority of drugs which are solved in less water have low bioavailability. Therefore, enhancing bioavailability of poorly water-soluble drug is one of the vital research fields of pharmacy. Low-soluble drugs have low rates of dissolution and their absorption is not done completely.[16] In some oral prescriptions, its amount of absorption decreases as a result of the limited time of its contact with the membrane of the considered zone. In the mentioned cases, the absorption rate is equal to dissolution rate. Therefore, the best way to increase bioavailability of low soluble drugs is to enhance solubility or dissolution rate of drugs.[17] At the present time, dissolution in the distribution

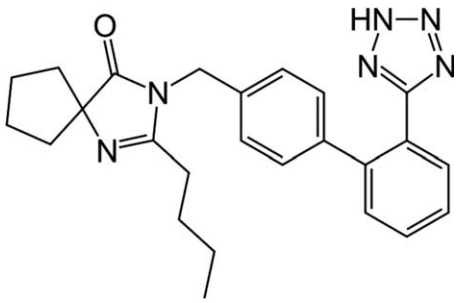

Figure 1. Chemical structure of Irbesartan.

layer and increase of contact level are the two methods to enhance the dissolution rate of digestive system and increase bioavailability. The issue of solubility associates with representing new drugs and this issue influences pharmaceutical systems.

There are many methods to enhance solubility of poorly soluble drugs and increase their bioavailability. The solid dispersion technique is one of the best methods to enhance solubility. Solid dispersion refers to dispersion of one or more effective substance in a neutral carrier.[18–21] The particle size reduction (abridged to submicron size or to the molecular size) and the change from crystalline to amorphous form are the mechanisms for the solubility and dissolution rate enhancement in the solid dispersion.[22] Several methods for preparing solid solutions exist such as solvent evaporation method, melting method, spray-drying method, melt extrusion method, melt agglomeration process, kneading technique, co-grinding method, lyophilization technique, and electrospinning method, and the study of preparing solid solution using the recent method is the object of this research.[23]

Electrospinning makes the process particularly suited to the production of nanofibers using large and complex molecules. A polymer solution is injected (it might be a solution[24] or melt as well) at a constant feed rate though a nozzle or needle which is charged to a high voltage, typically 10–30 kV. The applied voltage induces a charge on the surface of the liquid droplet and when this is sufficiently high, the hemispherical surface of the fluid elongates and a *Taylor cone* is established. On increasing the applied voltage further, a charged liquid jet is ejected from the *Taylor cone* and attracted to the earthed collector, which is positioned at a fixed distance from the needle. During this process, the solvent evaporates from the polymer solution, leaving dry polymer fibers on the collector.[24–30] The purpose of this study is the improvement of solubility and dissolution rate of Irbesartan using this method.

There are many medical applications of electrospinning technique. Some of these applications have been performed in various relevant areas such as tissue engineering,[31,32] wound healing,[33] and wound dressing.[34] Electrospinning is quite slightly known in pharmaceutical technology. This technique is relatively new in the field of pharmaceutical industry such as sustained drug release,[35] immediate release from the nonwoven system of electrospun nanofibers (called mat or web),[36,37] oral fast-dissolving drug delivery membranes for Acetaminophen,[38] fast dissolving oral dosage forms such as oral thin film technology (OTFs), and the orally disintegrating tablet formulation (ODTs).[39,40] The most recent researches in the orally dissolving solid dosage forms field have been dealing with OTF which is a relatively new area of interest regarding the oral administration.

MATERIALS AND METHODS

Materials

Irbesartan (USP Reference Standard grade) was purchased from Fluka, USA. PVP K90 (average MW = 360,000) and Phosphoric acid (HPLC grade) were purchased from Fluka of USA (Analytical grade). Hydrochloric acid, 37%, (Pharmacopeia grade) was purchased Sigma-Aldrich, USA. Ethanol (EMSURE®, Pharmacopeia grade) was purchased from Merck Millipore, USA. Ethanol 99.5% was purchased from Sigma-Aldrich of USA. Water has been twice distilled just before use and all other chemicals used were analytical grade. Diethyl ether, dichloromethane (both CHROMASOLV®, HPLC grade, ≥99.9%), acetonitrile (CHROMASOLV® Plus, for HPLC, ≥99.9%) and triethylamine (HPLC grade, ≥99.5%) were purchased from Sigma-Aldrich, USA. Methyl Paraben (analytical standard) was purchased from Supelco. Potassium phosphate monobasic (for molecular biology, ≥98.0%) was purchased from Sigma.

Methods

Preparation of PVP-Drug Fibers. First, prespinning solutions of the polymer polyvinylpyrrolidone K90 with concentrations of 1, 2, 3, 5, and 7% (*w/v*) were prepared by dissolving the appropriate amount of PVP K90 in 80% ethanol aqueous solution [ethanol : water, 8 : 2 (*v/v*)] and stirring at ambient temperature (24 ± 1°C) and ambient humidity (55 ± 5%). In addition, a 3% (*w/v*) presolution of the drug was prepared by dissolving the appropriate amount of pure Irbesartan in ethanol aqueous solutions before the electrospinning process at the same conditions. The final spinning solution was obtained by mixing PVP solution and drug solution. In this study, electrospinning process was performed under ambient conditions (relative humidity [55 ± 5%] and room temperature [24 ± 1°C]).

In order to provide the final spinning solution for the various formulations, a certain concentration of the PVP solution was added to drug solution (Table I). To obtain a homogeneous spinning solution, the final spinning solution was mixed and stirred by a magnetic stirrer with fixed speed of 50 RPM for 30 min at room temperature and ambient humidity. The solutions were degassed using a SK5200H Ultra-sonator (350 W, Shanghai Jinghong Instrument Co., Ltd. Shanghai, China) for 10 min. In order to avoid any air bubbles, the spinning solutions were carefully loaded into a 10 mL syringe. The single-syringe infusion pump (115 VAC, **Cole-Parmer®**, Vernon Hills, IL, USA) was used for injecting the spinning solution. The feed rate was fixed at 1.5 mL/h. A high-voltage power DC supply (CZE1000R 30kV Auto-Reversing Pwr Supply Rack Mount; **Spellman®**, USA) was used as the positive electrode at a voltage of 14 kV. A metal collector was covered with the aluminum foil and was applied as the grounded electrode. The electrospun nanofibers were collected with this set (15×20 cm^2). The metal needle tip has 0.25 mm inner diameter.

All samples were placed in silica gel beads in a desiccator to facilitate the removal of moisture and residual organic solvents.

Table I. Solubility Studies of Pure Irbesartan, Electrospun Nanofiber-Based Solid Dispersions, and the Physical Mixture Containing PVP K90 ($n = 3$, mean ± SD)

Batch	Process	Drug (w/v) : Polymer (w/v) (% : %)	Theoretical drug content		Assayed drug content		Saturation solubility (μg/mL)
			Amount (mg)	Expressed (%)	Amount (mg)	Expressed (%)	
Irbesartan	–	3 : 0	100	100	99.96 ± 1.27	99.96	40.55 ± 1.01
ENSD1	Electrospinning	3 : 1	75	100	74.80 ± 1.35	99.74	195.29 ± 1.28
ENSD2	Electrospinning	3 : 2	60	100	59.79 ± 1.84	99.66	205.35 ± 2.19
ENSD3	Electrospinning	3 : 3	50	100	49.79 ± 1.16	99.57	214.20 ± 1.50
ENSD4	Electrospinning	3 : 5	37.5	100	37.28 ± 1.68	99.41	222.89 ± 1.64
ENSD5	Electrospinning	3 : 7	30	100	29.75 ± 1.11	99.18	245.32 ± 1.77
PM5	Physical mixture (w/w)	3 : 7	30	100	29.95 ± 0.84	99.83	52.45 ± 1.78

In the next step, samples were collected for doing different tests. All samples were kept in plates with closed lids, out of sun radiation, and at the room temperature up to the next tests.

Preparation of Physical Mixtures as Controls. For the sake of comparison, physical mixtures having the same composition of the solid dispersions were prepared (drug and carrier were blended in a glass mortar by a spatula for 5 min) by simply triturating the drug and the polymer (%, w/w) in a porcelain mortar. The mixtures were then sieved and stored in amber glass-capped containers.

Assay of the Drug. For assessment of the drug and other samples, an optimum wavelength is required. To this end, an Irbesartan solution with known concentration was prepared (by pure drug and 0.1 *N* hydrochloric acid as solvent); then its ultraviolet (UV) absorption spectrum was depicted at a wavelength range between 200 and 400 nm (**SHIMADZU** UVmini 1240 Spectrophotometer, Kyoto, Japan). The maximum wavelength absorption of the drug was determined at 244 nm.[41] The mentioned wavelength accorded with previously studied. In this wavelength (244 nm), we have the maximum amount of Irbesartan absorption and the best R^2 ($R^2 = 0.999$) is depicting for calibration curve. This curve determines amount of Irbesartan.

Drug Content. First, the certain concentrations of Irbesartan including 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 μg/mL (by dissolution of appropriate amount of Irbesartan in 0.1*N* hydrochloric acid) were prepared. Second, the absorbance was measured at 244 nm wavelength by an ultraviolet spectroscopy device (SHIMADZU UVmini 1240 Spectrophotometer, Kyoto, Japan) and the amount of drug in each formulation was calculated.[41] This stage was repeated three times on one day, and six other times on two other days. Finally its final standard curve was depicted. According to the obtained curve, linear equation, linear regression, drug concentration of the samples was determined.

Saturation Solubility. To evaluate saturation solubility of the drug, solid dispersions, and physical mixtures, this test was carried out. Approximately 100 mg of the samples was added in glass flasks including distilled water and then they were mixed for 48 h at the temperature equal to 25°C with fixed speed of 400 RPM (**VARIOMAG®** Poly 15, Multipoint Stirrer, Germany). Samples were filtered then being centrifuged with fixed speed of 10,000 RPM for 10 min in two sessions and crystallization separating stage. Moreover, the amounts of saturated soluble, after doing needed diluting, were determined by a UV spectrophotometer at 244 nm. This test was repeated three times for each of the samples and their means were recorded. Data were analyzed by ANOVA and *T*-test.

***In Vitro* Dissolution Rate.** *In vitro* drug release was performed in order to study of dissolution rate of Irbesartan and other samples. Dissolution medium was chosen based on Irbesartan tablet by 0.1 *N* hydrochloric acid. Volume and temperature of dissolution medium were, respectively, considered 500 mL and 37 ± 0.5°C.[41] Moreover, the paddle device (USP Apparatus II) with a speed of 100 RPM was considered (**Erweka** DT6R, Heusenstamm, Germany). Ten milligrams of pure Irbesartan or its nanofibers equivalent was put on the device, after being passed through sieve of 80 and 100 meshes. After the start of the test in certain periods of time (0, 5, 10, 15, 20, 30, 45, and 60 min), aliquot of 2 mL of the samples of dissolution medium was withdrawn and replaced with the same amount of dissolution medium (sink conditions). Samples were decanted for 15 min and then filtered. After doing the needed dilution, drug concentration of samples was spectrophotometrically measured using a UV device at 244 nm then by a standard curve; the amount of the drug was calculated. The test was repeated thrice for each of the samples.

Statistical Analysis. All data were analyzed by analysis of variance (ANOVA) with Tuckey's multiple comparison tests using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA) also; $P < 0.05$ was used as the criterion to assess statistical significance.[42]

Dissolution Efficiency (DE). In order to the evaluation of *in vitro* dissolution rate efficiency, the calculations of DE parameter were performed. The DE percentage of a pharmaceutical dosage form is defined as the area under the dissolution curve up to a certain time "*t*" and expressed as a percentage of the

area of the rectangle described by 100% dissolution at the same time. In other words, the DE is equal to the total area under the dissolution curve at the certain time.[43] This parameter calculated by the following equation:

$$\mathrm{DE}(\%) = \left(\frac{\int_0^t y \cdot dt}{y_t^{100}}\right) \cdot \mathbf{100}$$

where "y" is the percent drug dissolved at the time "t".

Fourier Transform Infra-Red (FTIR) Spectroscopy. FTIR test was done to diagnose the occurrence of probable changes in the chemical structure of drugs and determine internal intervention between carrier and drug. The FTIR spectra were despised using an FTIR spectrophotometer (**Perkin-Elmer** 843 System, Shelton, USA). Therefore, 2–3 mg of each of the samples was blended with 50–100 mg potassium bromide (KBr) in a glass mortar. Then using a tablet making machine, a disk with a diameter of 12 mm under 10 tons of pressure was prepared. Finally, absorbing spectrum of FTIR disk was provided. The range of scans was 200–4000 cm^{-1} with 1 cm^{-1} resolution. The scanning average was taken 20 cm^{-1}.

Differential Scanning Calorimetry (DSC). Investigating thermal properties of samples were carried out by differential scanning calorimetric technique. The reason is that any intervention between drug and carrier leads to some differences in their thermal behavior. Before doing the test, the device was calibrated by a standard Indium sample (99.99%) at temperature 156.6 ± 1°C. Five milligrams of sample was precisely weighed and was placed on aluminum DSC pan. Another pan was also considered as control pan. An empty pan was considered as a reference (**SHIMADZU** DSC-60, Kyoto, Japan). Tests began from 25°C, and then their temperature rose up with 10°C/min speed, this process continued up to 200°C or better to say melting temperature. Then, the onset temperature of testing samples was determined. Moreover, the nitrogen gas atmosphere of the test was equal to a flow of 30 mL/min.

X-ray Diffraction (XRD). X-ray diffraction was used to study the probability of changes occurring in the crystalline structure of drug particles during the processes of solid dispersion and physical mixture. Also XRD was performed to examine the physical state of the nanofiber samples. Samples were put in the device whose diameter was 25 mm and height equaled 2 mm; they were subjected to X-ray from different angles varying from 4 to 40° 2θ. The wavelength of radiated light was 1.564056 Å, its type was Cu-Kα, recording distances were equal to 0.02° 2θ and scanning speed was equal to 1° min^{-1} (**PHILIPS** PW 1800 X-ray Diffraction Machine, The Netherlands). It should be noted that Irbesartan has no major peak less than about 2° (2θ) and more than about 40° (2θ).

Scanning Electron Microscopy (SEM). The surface morphology of pure drug, physical mixtures, and the electrospun nanofiber was observed utilizing an analytical scanning electron microscope (**VEGA\\TESCAN-LMU**, Brno, Czech Republic), equipped with thermoemission cathode (**Oerlikon Balzers Union Ltd**, Balzers, Lichtenstein). The samples were mounted onto the carbon stubs (10 mm diameter, 3 mm height) using double-sided adhesive tape and then coated with gold–palladium alloy under vacuum atmosphere (0.25 Torr) using fine coat ion sputter (**Joel**, fine coat ion sputter, JPC-1100). Then the coated samples were placed in the scanning electron microscope chamber. The samples were subsequently generated using a 30 kV electron beam and analyzed.

High-Performance Liquid Chromatography Analysis. The drug concentration in plasma was analyzed by a high-performance liquid chromatography (HPLC) method. The liquid chromatographic system consisted of an **Agilent** 1200 Series HPLC System (Santa Clara, CA, USA) equipped with auto sampler (G1329A), UV detector (G1314B), degasser (G1379B), and binary pump (G1312A) (**GenTech Scientific**, NY,USA). **ChemStation** Software Rev.B.03.01 was considered as HPLC parameter controller. Analysis was carried out with a **Lichrospher®** C_{18}, 250 × 4.60 mm, 5 μm column. The detection wavelength was 244 nm. The mobile phases were degassed by vacuum for 15 min. The mobile phase consisted of acetonitrile and 0.01 M NaH_2PO_4 buffer (34 : 66, *v/v*) at a flow rate of 1 mL/min.[44] Potassium dihydrogen phosphate buffer was prepared by a 0.07% triehylamine. The pH was adjusted with phosphoric acid to pH 3.0. The injection volume was 20 μL.

***In Vivo* Evaluation.** All experimental procedures were conducted according to the ethical standards and protocols were approved by the Animal Experimentation Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran; (**AECSBMU**). All efforts were made to minimize the number of animals and their suffering. The animals were fed with a normal standard chow diet and tap water *ad libitum*.

The bioavailability of ENSD5 (as selected electrospun nanofiber sample) was determined in comparison with pure Irbesartan in healthy rabbits (New Zealand grey) of average weight 2.5 ± 0.5 kg. The rabbits were divided into two groups of 6 animals each ($n = 6$). One group as a control received pure Irbesartan and the second group received optimized formulation containing Irbesartan/PVP nanofibers of the same dose. Preparation of Irbesartan solution in water is very difficult because the solubility of Irbesartan is very less in water and it floats on water. Therefore, suspension of the drug was prepared (hence, the dose equivalent to 200 mg (1/20 of LD_{50}) of pure drug and ENSD5 suspension in the 0.01 HCl). The samples administered orally with the help of a syringe. The solubility of Irbesartan is very less in water and it floats on water, so it is difficult to prepare the solution in water.[45] During the study, the rabbits had free access to water only.[46] Blood samples were collected from marginal ear vein at intervals of 0 (before drug administration), 15, 30, 45, 60, 90, 120, 180, 240, and 300 min after administration of the drug. Blood was collected into heparinized tubes containing dilute heparin and centrifuged at 5000 RPM for 25 min.[44] The plasma was separated and stored at 20°C until analysis. During the whole study, rabbits remained conscious.

Biological Samples Preparation. Aliquot of 0.5 mL of the plasma was transferred into test tube and 10 mL of methylparaben with a 20 ng/mL concentration as the internal standard working solution was transfixed. Solution was vortexed (**IKA** Vibrax Vibrating Vortex mixer, VXR-S1, Germany) and acidified

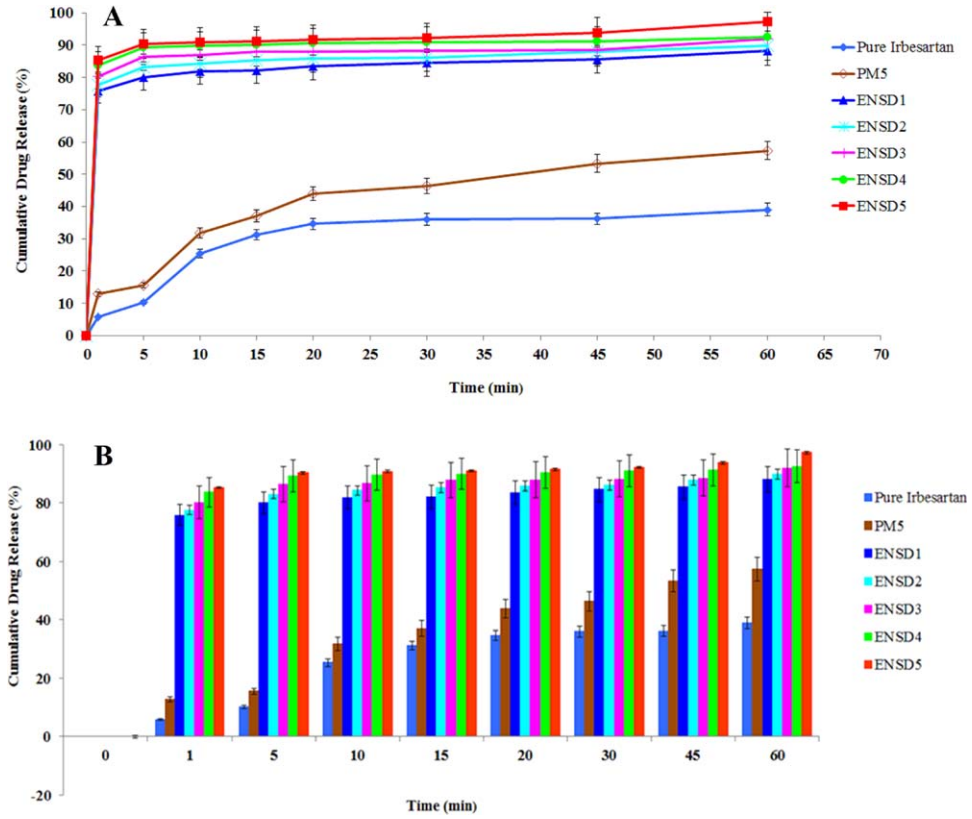

Figure 2. (A) *In vitro* dissolution drug release profiles of pure Irbesartan, electrospun nanofiber samples, and the physical mixture ($n = 3$, mean ± SD). (B) Column charts of *in vitro* dissolution drug release of pure Irbesartan, electrospun nanofiber samples, and the physical mixture for compare values across categories ($n = 3$, mean ± SD). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with 150 mL of 1 M phosphoric acid. Then 3 mL of extraction solvent (7 (*v/v*) : 3 (*v/v*)) diethyl ether : dichloromethane was added. The sample was stirred for 5 min and centrifuged for 10 min at 2000 RPM.[44] The organic layer was transferred to vials and evaporated at 70°C to remain residue. At the time of analysis, the sample was reconstituted using mobile phase.[47]

Pharmacokinetic Analysis. Data were generated assuming first-order absorption. The different pharmacokinetic parameters of samples were calculated using unpaired *t*-test. The maximum plasma concentration (C_{max}) and time of its occurrence (T_{max}) were directly calculated from the plasma concentration vs time plot. The AUC_{0-5}, T_{max}, and C_{max} were calculated. Also, relative bioavailability was calculated with reference to oral suspension of pure Irbesartan.

RESULTS AND DISCUSSION

Solubility Studies

Data of saturation solubility of samples was shown in Table I. Saturation solubility of all ENSD samples has been noticeably increased in comparison to pure drug (from 195.29 to 245.32 μg/mL, the saturation solubility of pure drug is 40.55 μg/mL). Table I shows that saturation solubility in all samples was increased by electrospun nanofibers preparation. (For ENSD1–ENSD5 samples, 4.82, 5.06, 5.28, 5.50, and 6.05 times more than the pure drug respectively.) Statistical studies show that saturation solubility all samples of nanofiber-based solid dispersions has a significant difference with the pure drug ($p < 0.001$). Since ENSD5 has more saturation solubility with 29.75% of assayed drug content than the other samples, it has a significant difference with the other samples ($p < 0.001$). Also saturation solubility of PM5 (as a physical mixture of selected samples) is more than a pure drug; this is because of the solubilizing effect of PVP K90. Saturation solubility of ENSD5 has increased up to 6 times more than pure Irbesartan and its solubility equals 245.32 ± 1.77 μg/mL. Therefore, this sample is known as the best sample. ENSD1, ENSD2, and ENSD3 samples do not have a significant difference with each other ($P > 0.05$). Moreover, ENSD5 with ratios of 3% (*w/v*) drug : 7% (*w/v*) polymer has statistically significantly different from other samples ($p < 0.001$).

The results indicate the effectiveness of electrospun nanofibers of drug-PVP K90 in improving drug saturation solubility. It might be because of amorphous natures and very fine and uniform with higher hydrophilicity of prepared samples.[21] In this study, using PVP K90 and utilizing an electrospinning process, solubility of Irbesartan was enhanced (about 5 times more).

Drug release profiles of pure Irbesartan, nanofiber solid dispersion, and physical mixtures were depicted in Figure 2. Also Table II shows the dissolution parameter of the pure drug, PVP K90, and samples. According to the results, dissolution rate of ENSD1–ENSD5 samples has increased significantly. The percentage of drug released from samples ENSD4 and ENSD5

Table II. *In Vitro* Dissolution Parameters and Calculated Kinetic Parameters for Pure Irbesartan, Electrospun Nanofiber-Based Solid Dispersions, and Physical Mixtures ($n = 3$, mean ± SD)

Batch	Drug : Polymer (% : %)	Concentrations	Morphology	Diameter (nm)	DE_{10} (%)	DE_{30} (%)	*n*
Irbesartan	3 : 0	(*w/v*) : (*w/v*)	Mixed	-	25.14 ± 1.77	35.85 ± 2.03	0.57
ENSD1	3 : 1	(*w/v*) : (*w/v*)	Linear	903 ± 103	81.04 ± 2.17	85.35 ± 1.17	0.25
ENSD2	3 : 2	(*w/v*) : (*w/v*)	Linear	905 ± 180	84.33 ± 1.33	87.45 ± 1.29	0.27
ENSD3	3 : 3	(*w/v*) : (*w/v*)	Linear	905 ± 178	85.82 ± 1.99	90.03 ± 1.00	0.30
ENSD4	3 : 5	(*w/v*) : (*w/v*)	Linear	905 ± 223	87.20 ± 1.01	90.53 ± 2.08	0.28
ENSD5	3 : 7	(*w/v*) : (*w/v*)	Linear	907 ± 119	88.95 ± 1.22	91.77 ± 1.29	0.22
PM5	3 : 7	(*w/w*) : (*w/w*)	Mixed	-	30.98 ± 1.30	47.69 ± 1.55	0.85

equals 92.59 and 97.25, respectively (at 60th min). In comparison with the pure drug, this increase for ENSD5 is 2.5 times more than the pure Irbesartan (39.01%); among all electrospun nanofiber samples (ENSD1–ENSD5) and physical mixtures, ENSD5 formulation with ratios of 3% (*w/v*) drug : 7% (*w/v*) polymer has the maximum cumulative drug released percentage (97.25%). According to the results (Figure 2 and Table II), the selected nanofiber samples have significant differences with pure drug and other samples. For the sake of comparison, physical mixture of ENSD5 with the same weight ratio of 3% drug : 7% polymer (*w/w*) was prepared (PM5) and dissolution test has been done on it. The enhancement of dissolution drug released of poorly soluble drugs by solid dispersion preparation is influenced by many factors such as carrier type (PVP K90 has good filament-forming and high hydrophilicity) particle size reduction, and the area surface increasing of drug; in addition, viscosity of carrier around drug particles in dissolution medium, concentration gradient of the drug and balance between them, and the chemical nature of the drug are important factors.[48]

PVP (Povidone) has been used in pharmaceutical formulations for many years, being first used in the 1940s as a plasma expander, it has now been superseded for this purpose by dextran. PVP is widely used as an excipient, particularly in oral tablets and solutions. Povidone formulations are widely used in the pharmaceutical industry due to their ability to dissolve in both water and oil solvents. It has multiple uses, including as a binder for tablets and capsules, a film former for ophthalmic solutions, to aid in flavoring liquids and chewable tablets, and as an adhesive for transdermal systems. When consumed orally, PVP may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes. PVP additionally has no irritant effect on the skin and causes no sensitization.[49]

According to the obtained results of drug dissolution tests, there is a considerable increase (2.5 times more) in drug release for some of the formulations. In comparison with the pure drug, all samples have shown a significant increase in dissolution rate of solid dispersions ($p < 0.001$). The provided samples with concentration ratios of 3% : 1% and 3% : 2% (drug : polymer, *w/v*) show less dissolution rate than other samples. However, samples with the relative drug : polymer (*w/v*) of 3% : 5% and 3% : 7% show the maximum dissolution rate statistically. The addition of PVP to the drug solution would reduce the surface tension and increase the permittivity of the main fluid, and thus in turn decrease the applied voltage value needed to initiate electrospinning.

The release of Irbesartan was analyzed using a variety of kinetic models. The Higuchi, Weibull, Baker–Lonsdale, Elovich, Parabolic, Michaelis–Menten, Freundlich, Hixson–Crowell, Korsmeyer–Peppas models, zero-order, and first-order were all investigated. Only the Korsmeyer–Peppas model and zero-order were found to provide a good fit to the experimental data. The results of this analysis are presented in Figure 3. The Korsmeyer–Peppas model states that $\boldsymbol{Q = k \cdot t^n}$ where: *k* is the release

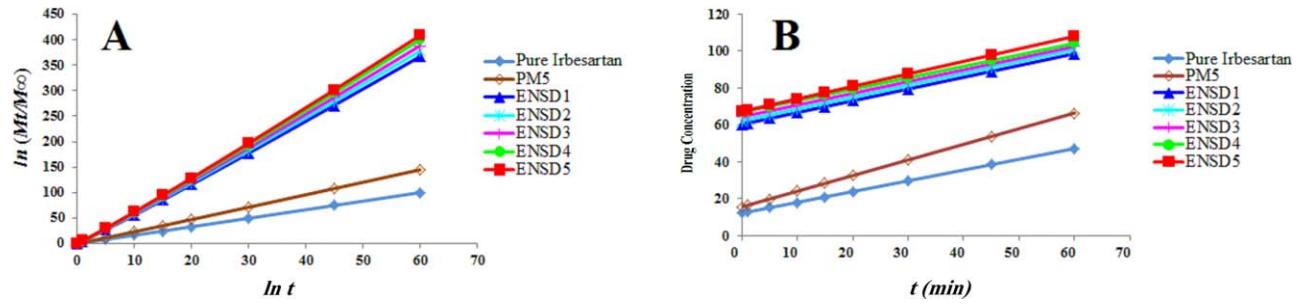

Figure 3. Plots of the drug release from Irbesartan nanofibers formulations according to the (A) Korsmeyer–Peppas model and (B) zero-order model ($n = 3$, mean ± SD). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

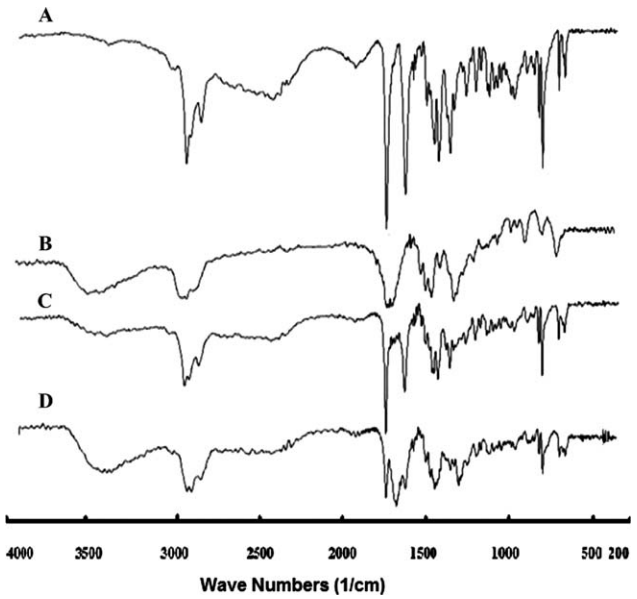

Figure 4. FTIR spectra of (A) pure Irbesartan, (B) PVP K90, (C) PM5, and (D) ENSD5.

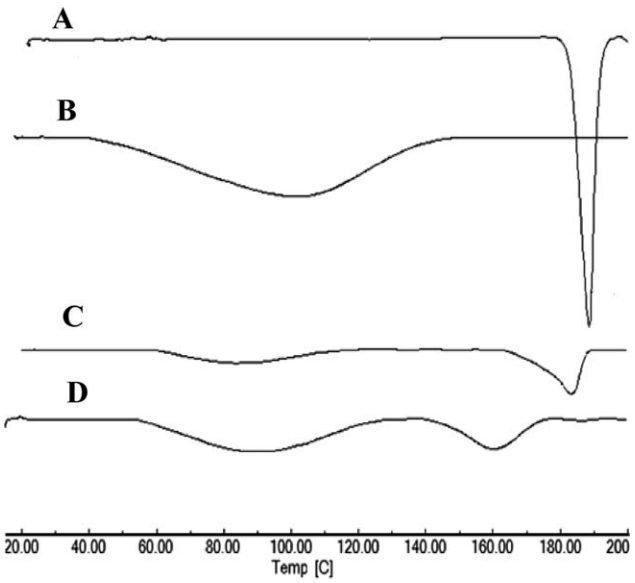

Figure 5. DSC multi thermograms of (A) pure Irbesartan, (B) PVP K90, (C) PM5, and (D) ENSD5.

rate and n is the diffusion exponent (n = release exponent); $\boldsymbol{Q = M_t/M_\infty}$ where M_t is the amount of drug released at time t and M_∞ is the total amount of drug in the nanofibers. A plot of $\ln(M_t/M_\infty)$ versus ln t should thus yield a straight line of gradient n and intercept ln k if the model is applicable. The Korsmeyer–Peppas model is applicable here and it is clear from Figure 3A. The values of n calculated are listed in Table II. The values of n are generally 0.5 or less, indicating that Fickian diffusion. Where n lies between 0.5 and 1, suggesting the drug is released via non-Fickian transport may control the drug release here. Irbesartan release is found to occur at comparable rates for the PM5 in both releases whereas it is around five times slower for all the electrospun nanofibers. The data were also analyzed by zero-order, and this model states that $\boldsymbol{Q = kt + Q_0}$. A plot of Q (drug concentration) versus t (min) was depicted. The zero-order kinetic model also is applicable here, and it is clear from Figure 3(B).

Finally, with regard to saturation solubility and dissolution tests, ENSD5 have been selected as the best sample and more tests have been done on it.

FTIR Spectroscopy Studies

Figure 4 shows the FTIR spectrum of pure Irbesartan, PVP K90, and other samples. The main peaks of Irbesartan have appeared in 1610, 1728, 2934, and 2960 cm^{-1} zones and accorded with standard spectra of drug mentioned in the drug.[44] The major peaks of the drug in 1610 cm^{-1} related to C—N stretch, 1728 cm^{-1} C=O stretch, 2934 cm^{-1} and 2960 cm^{-1} N—H stretch. The main peaks of PVP K90 have appeared in 1668 cm^{-1} (C=O stretch) and 1293 cm^{-1} (C—N stretch) zones and accorded with standard spectra of drug mentioned in the drug.[44] FTIR spectrum related to a selected sample of electrospinning nanofiber including ENSD5 has been represented in Figure 4D. Moreover, pure drug and PVP K90 have also been illustrated in Figures 4(A,B). The main peaks of the drug and the polymer for all samples have been appeared; the intensity of the peaks is the only difference of them, which is related to changes in concentration. Since peaking related to Irbesartan and PVP K90 in solid dispersions as well as physical mixtures [Figure 4(C)] are the same in Figure 4(D), the possibility of chemical intervention among Irbesartan and carrier is rejected. On the other hand, the peak related to C=O of the drug in 1728 cm^{-1} of ENSD5 sample has shifted to higher wavelengths. It could be the result of *Van der Waals* force between Irbesartan and carrier. Integration of drug–polymer molecules in the flat network of solid dispersion systems may lead to omission of the drug peaks.

DSC Studies

DSC thermogram and thermal behavior of Irbesartan, electrospun nanofiber-based solid dispersions, and their relevant physical mixtures are depicted in Figure 5. Figure 5(A–D) demonstrates the DSC thermogram of Irbesartan, PVP K90, PM5, and ENSD5, respectively. The DSC curve of Irbesartan showed a sharp endothermic peak ($T_{peak} = 185.14°C$) corresponding to its crystalline nature, indicating its melting point. This temperature associates with melting the substance and also accords with thermogram of previous studies.[50] The endothermic peak of PVP K90 was observed around 155.12°C. The thermal behavior of the PVP K90 is that expected for hygroscopic, amorphous substances, with a large endothermic effect in the 50–150°C range due to polymer dehydration. (PVP K90 is an amorphous polymer and so does not show any phase transitions or endothermic peak; it exhibits a broad endotherm because of dehydration; that broad endothermic peak is the water loss of the sample.) The DSC thermogram of the PM5 shows a broad shallow endothermic peak because of the dehydration of PVP K90 followed by the melting of Irbesartan at 161.52°C and drug degradation above 200°C. Clearly, both DSC and XRD data show that the PM5 contains crystalline Irbesartan. For ENSD5, two endothermic peaks are observed. The DSC curve of ENSD5

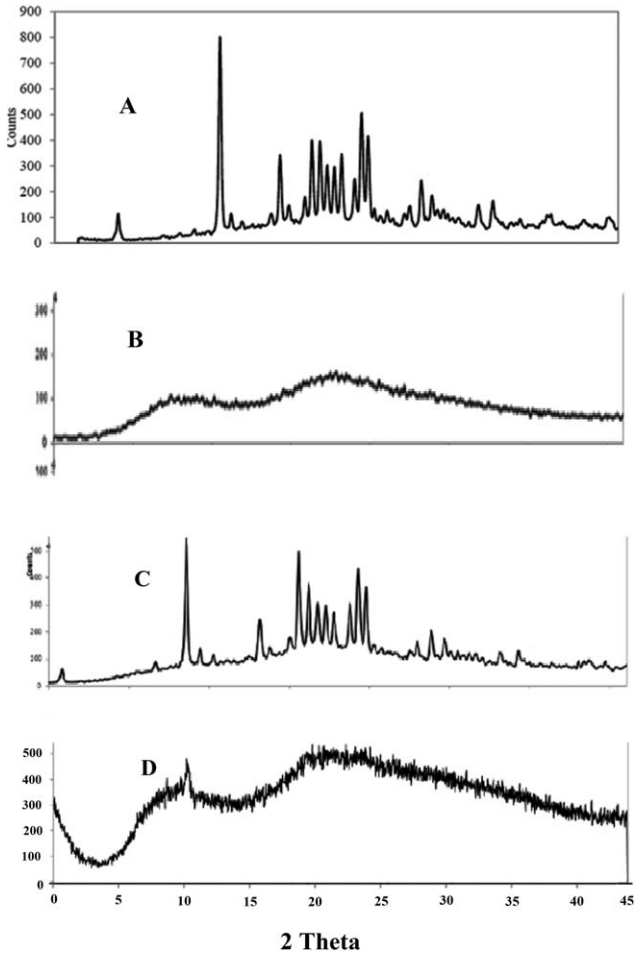

Figure 6. XRD diffractograms of (A) pure Irbesartan, (B) PVP K90, (C) PM5, and (D) ENSD5.

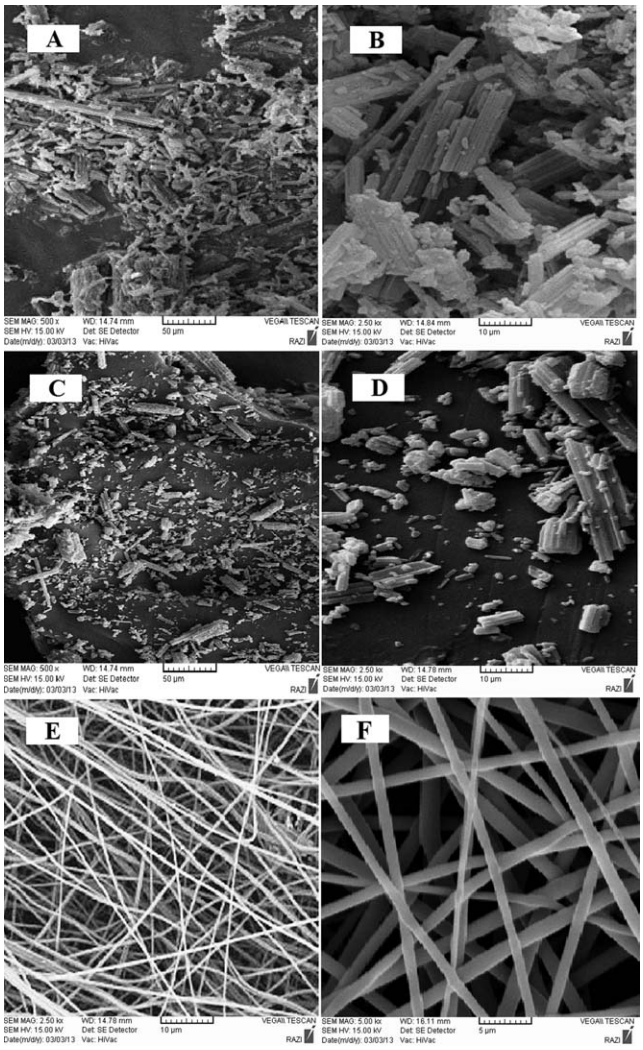

Figure 7. SEM of (A) pure Irbesartan (magnification: 500×), (B) pure Irbesartan (magnification: 2500×), (C) PM5 (magnification: 500×), (D) PM5 (magnification: 2500×), (E) ENSD5 (magnification: 2500×), and (F) ENSD5 (magnification: 5000×).

exhibited two broad endotherm peaks, first peak between 60 and 100°C attributed to dehydration, and the second ranging from 140 to 170°C (169.44°C), which is believed to be because of the drug melting. The small size of the T_g does indeed indicate that the amount of the sample that is amorphous is quite small. The T_g is also shifting to higher values as a function of heating rate. The DSC traces show drug degradation (~220°C), polymer degradation (~180°C), and only broad endotherms below 100°C (dehydration). These data suggest that Irbesartan in the PVP-fibers are present either as an amorphous dispersion or a solid solution. The Irbesartan melting endotherm peak is not visible from any of the electrospun nanofibers, confirming the amorphous nature of the drug in these samples.

XRD Studies

XRD patterns of samples were shown in Figure 6. Diffractogram of Irbesartan [Figure 6(A)] demonstrates the nine peaks at 2θ value diffraction angles of 4.7, 12.5, 13.3, 17.1, 19.4, 21.2, 22.6, 23.2, and 27.3°. The appearance of these peaks indicates that used Irbesartan in this study is "A" crystal form (polymorph A).[45] Diffractogram of PVP [Figure 6(B)] has appeared at 2θ of 12° and 23°. PVP pattern has two the characteristic broad humps peaks; this is due to the amorphous nature of the polymer. The XRD patterns of the selected sample (ENSD5) do not show any diffraction peaks (consistent with the amorphous nature of the polymers). These reflections are also visible in the PM with PVP K90. The characteristic peaks of Irbesartan are almost absent from the diffractogram patterns of electrospun nanofibers [Figure 6(D)]. Hence, Irbesartan is amorphous in its nanofibers, suggesting the formation of a solid solution. Considering that amorphous forms and crystallinity reduction of the drug are the significant reasons for the dissolution enhancement is focused in the present study. These findings agree fully with those from other studies involving electrospun nanofibers.

Morphological Studies by SEM

The surface morphologies of pure Irbesartan, selected samples using scanning electron microscopy are shown in Figure 7. Figure 7(A) shows the needle-shaped crystals of Irbesartan with different sizes that are placed side by side. Electrospun nanofibers of the drug and carrier have uniform structures without any "beads on a string" morphology and smooth surface. There were no particles visible on the surface of drug–nanofibers

Table III. Drug Content of Selected Samples after Stability Studies According to ICH Guidelines; Storage Conditions: 40 ± 2°C; 75 ± 5% RH, ($n = 3$, mean ± SD)

Time (days)	Irbesartan (%)	ENSD4 (%)	ENSD5 (%)
0	99.96 ± 1.27	99.36 ± 1.10	99.06 ± 1.45
30	99.96 ± 0.24	99.35 ± 1.02	99.06 ± 1.23
60	99.95 ± 0.88	99.34 ± 2.25	99.05 ± 1.16
120	99.95 ± 1.80	99.33 ± 1.77	99.04 ± 1.43
180	99.92 ± 0.87	99.29 ± 1.03	99.01 ± 1.99

[Figure 7(E,F)], whereas there are many drug particles on the surface of prepared physical mixture [Figure 7(C,D)]. The ENSD5 drug–PVP nanofibers had an estimated average diameter of 907 ± 119 nm. Also samples ENSD4, ENSD3, ENSD2, and ENSD1 had an estimated average diameter of 905 ± 223, 905 ± 178, 905 ± 180, and 903 ± 103 nm, respectively. These results verified that the particle size of PVP–Irbesartan nanofibres had good ability for solubility improvement. Transformed crystallinity (amorphous) of the drug substance is one of the main reasons for the solubility and dissolution rate increasing of Irbesartan in this research.

Physical Stability

In order to the optimized formulations, accelerated stability studies were performed according to ICH* guidelines. Samples (each 100 mg, $n = 3$) were kept for a period of 180 days studied at 40 ± 2°C and 75 ± 5% RH by Environmental Test Chamber (**Cooper Group** 450/ME/-40, Albert Court, United Kingdom). The samples were kept in glass vials sealed with rubber plugs. Ten milligrams of stored samples (ENSD5) was taken out on 0, 30, 60, 90, 120, and 180th day, and then were analyzed for drug content and physical change. The results did not show a significant change (Table III).

In Vivo Studies

In vivo study was performed to evaluate the pharmacokinetic parameters of the drug from Irbesartan suspension and Irbesartan/PVP nanofibers, which were administered orally to rabbits. The pharmacokinetic parameters of Irbesartan as AUC_{0-5}, T_{max}, and C_{max} are illustrated in Figure 8. Figure 8 shows the plasma drug concentration as a function of time after oral administration. Peak plasma concentration value (C_{max}) of ENSD5 was found to be 340.25 ± 1.89 ng/mL and C_{max} for Irbesartan suspension was found to be 175.68 ± 2.10 ng/mL after oral administration. The T_{max} (The time occurrence for peak plasma concentration) for peak plasma concentration of Irbesartan suspension was obtained at 120 min and that of electrospun nanofiber sample is ∼ 85 min. The relative bioavailability of Irbesartan from ENSD5 (as selected nanofiber sample) was found to be 206.45 ± 3.36% (∼3 times more) at a dose of 200 mg to rabbits was shown significant enhancement in AUC and C_{max} ($p < 0.001$). In other words, the drug/polymer nanofibers showed an increase in relative bioavailability than the plain Irbesartan suspension.[44,46] These results are in congruence with the previously reported results.[16,51]

*International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

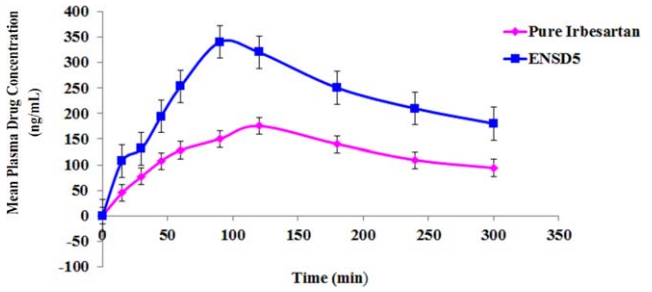

Figure 8. Mean plasma drug concentration–time profile (0–300 min) of Irbesartan after oral administration of Irbesartan suspension and ENSD5 ($n = 3$, mean ± SD). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSIONS

Using an effective method such as electrospinning and a suitable carrier as PVP K90, dissolution (based on *in vitro* drug released) and solubility of Irbesartan significantly were enhanced. ENSD5 as the best sample with the relative concentrations of (3% : 7%, *w/v*) has a maximum saturation solubility with 6.05 times more than the pure Irbesartan. Also the dissolution rate (in the 60th moment) of the drug using an electrospun nanofiber preparation was improved up to 2.5 times more than the pure Irbesartan. After 1 h of doing the dissolution test for ENSD5 sample, 97% of the drug was released, while at the same duration of time, only 39% of pure Irbesartan was solved in the same dissolution medium. In general, the amorphization may explain the solubility enhancement of the drug in this study.

This method for dissolution enhancement of poorly water-soluble drugs is scalable and valuable in a manufacturing process in future. In most of the cases, studied samples showed better dissolution properties than the intact Irbesartan. Therefore, the main purpose of the research, which was improving Irbesartan solubility and dissolution rate using electrospinning process and solid dispersion technique, was achieved. The optimized formulation of this investigation can be used to OTF or ODT systems.

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