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Improved solubility, dissolution, and oral bioavailability for atorvastatin-Pluronic® solid dispersions

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ABSTRACT

Despite the status of atorvastatin (AT) as one of the top selling statins for prophylaxis against primary and secondary cardiovascular diseases, its limited oral absorption limits its full therapeutic benefits. Herein, formulations of AT with amphiphilic carriers (Pluronic F127® and Pluronic F68®) were developed in the form of hard capsules to improve *in vitro* solubility and dissolution, as well as *in vivo* oral bioavailability. Prepared formulas were characterized by assessing solubility improvements in the carrier solution and examining the FTIR, DSC, and X-RPD profiles for each formula. The dissolution rate and absorption were also examined after oral administration to New Zealand rabbits. The solubility of AT was improved by the incorporation of either Pluronic F127® or Pluronic F68®. No chemical changes or interactions were detected using X-RPD, DSC, and FTIR characterization. Dissolution profiles revealed an increase in the rate and maximum amount of dissolved AT and showed that up to 93% of the AT content was dissolved within 30 min. In vivo absorption of the tested formula (C_{max} = 1146 ng/ml and AUC0-12 to 9,993.4 ng.h/ml) was greater than Lipitor® (C_{max} = 642.3 ng/ml and AUC0-12 = 4427.4 ng.h/ml) and AT (C_{max} = 517.6 ng/ml and AUC0- 12 = 2,473.7 ng.h/ml). In conclusion, the formulation of AT with Pluronics® profoundly augments the dissolution behavior and absorption of AT and may serve as a useful approach for improving AT therapeutic and clinical efficacy.

1. Introduction

Hyperlipidemia is a serious health disorder characterized by abnormally elevated blood lipid levels, such as cholesterol, fatty acids, and triglycerides (Baila-Rueda et al., 2018). Although lipids are an imperative component of biochemical and anabolic processes, elevated plasma levels of cholesterol are associated with an increased risk for cardiovascular diseases (El Desoky et al., 2016). Hyperlipidemia can be inherited (primary/familial) or acquired (secondary/non-familial) through unhealthy diets and lifestyles (Humphries et al., 2017). In both types, hyperlipidemia can lead to serious health complications and may double the probability of developing cardiovascular disorders such as ischemic heart disease and stroke (Baila-Rueda et al., 2018; El Desoky et al., 2016). In the past decade, the number of individuals diagnosed with hyperlipidemia significantly increased, despite advancements in its recognition as a major risk factor for cardiovascular diseases. The development of various oral antihyperlipidemic drugs was the focus of a dynamic area of research achieving major advancements over the years (Humphries et al., 2017). These antihyperlipidemic drugs include, but are not restricted to, statins, bile acid binding resins, and cholesterol absorption inhibitors. Statins are a group of antihyperlipidemic drugs used to lower lipid blood levels through the inhibition of the cytoplasmic enzyme HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase. This enzyme is responsible for the *de novo* synthesis of plasma cholesterol through the mevalonate pathway in hepatocytes (Humphries et al., 2017; Laufs et al., 2017).

Among the marketed statins, Atorvastatin (Lipitor®) is considered the most popular choice in decreasing plasma levels of triglycerides and "bad" cholesterol (low-density lipoproteins), as well as elevating the plasma levels of "good" cholesterol (high-density lipoproteins) (Laufs et al., 2017). Currently, AT is also prescribed for hyperlipidemic patients (familial/non-familial) as prophylaxis against primary and secondary cardiovascular disorders (El Desoky et al., 2016; Laufs et al., 2017). Although the clinical importance of AT in lowering blood

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cholesterol has been adequately investigated, clinicians are addressing the issue of its low oral bioavailability by prescribing dramatically increased daily doses (Kim et al., 2008; Mahmoud et al., 2017). However, these dose incrementations have evoked clinicians' worries regarding the drastic risks of side effects, especially in light of frequent polypharmacy in elderly patients. Based on the Bio-pharmaceutics System for Drug Classification, AT is categorized under class II, which was assigned low bioavailability due to poor aqueous solubility in the gastric fluid (Mahmoud et al., 2017).

Several pharmaceutical formulations attempting to enhance AT oral bioavailability have been previously reported. However, research in this field is still challenging. The primary goal for these research attempts was decreasing or diminishing the unabsorbed fraction of the administered dose while maintaining effective therapeutic levels of AT in the blood. Previous research regarding enhanced solubility was performed via the manipulation of AT particle size (Zhang et al., 2009) or crystalline state (Kim et al., 2008), assembly as a self-emulsifying delivery system using water-soluble emulgents (Yeom et al., 2016), formulations as a gut sustained-release vehicle using a floating formula (Khan and Dehghan, 2012) or a mucoadhesive formula (Ramani et al., 2012), or encapsulation in biodegradable nanoparticles (Ahmed et al., 2016). Furthermore, the dispersion of AT within an inert pharmaceutical polymer is considered one of the best formulation attempts (Anwar et al., 2011; Jahangiri et al., 2015). Incorporation of AT with a water-soluble amphiphilic biopolymer is an attractive approach since biopolymers are capable of improving the physical characteristics of loaded AT and extensively solubilize resulting particles in a reasonable quantity and pattern (Liu et al., 2011).

Amphiphilic biopolymers are pharmaceutical co-polymers composed of alternative hydrophilic and lipophilic chains in their architecture (Svensson et al., 2004). Pluronics® are commonly used amphiphilic excipients manufactured by BASF Corporation (New Jersey, USA) and composed of a copolymer of polyoxypropylene (hydrophobic moiety) and polyoxyethylene (hydrophilic moiety) (Kabanov et al., 2002). According to BSAF nomenclature, Pluronics® are coded to indicate their respective physical state and shape [flake (F), paste (P) and liquid (L)], molecular weight of polypropylene oxide [the molecular mass divided by 300, represented as the first digit(s)] and the percentage of polyethylene oxide [the percentage divided by 10, represented as the last digit]. As such, Pluronic F127® and Pluronic F68® are flakes, having polyoxypropylene chains with a molecular mass of 3,600 and 1,800 g/mol, as well as polyoxyethylene contents of 70 and 80%, respectively (Kabanov and Alakhov, 2002; Kabanov et al., 2002). Pluronics® are profoundly employed in the solubility and bioactivity improvement of numerous pharmaceutical active ingredients, including genistein (Raval et al., 2017), paclitaxel (Raval et al., 2017; Wei et al., 2009), quercetin (Raval et al., 2017), lidocaine (Scherlund et al., 2000), oxcarbazepine (Singla et al., 2016), nevirapine (Jindal and Mehta, 2015), indomethacin (Chokshi et al., 2005), ibuprofen (Foster et al., 2009) and flurbiprofen (Alexander et al., 2011). However, use of the atorvastatin Pluronics® binary system for improving the solubility and oral bioavailability of AT has not been investigated comprehensively and, therefore, this was the focus of this study.

Herein, we prepared AT as a solid dispersion formulation with Pluronic F127® or Pluronic F68® to increase the extent of solubility, improve the dissolution rate, and enhance the oral bioavailability of AT. We examined the prepared AT-Pluronic solid dispersion formulation for possible interactions between AT and Pluronics® using X-RPD, FTIR, and DSC. The enhancement of the dissolution rate and solubility was evaluated. Finally, *in vivo* oral bioavailability studies were performed for mini-capsules containing the AT-Pluronic binary system, in comparison with capsules filled with free AT, and AT tablets (Lipitor®).

2. Material and methods

2.1. Materials

Atorvastatin was kindly supplied as a gift from Jamjoom Pharmaceuticals Company in Jeddah, Saudi Arabia. Pluronic F127® and Pluronic F68® were provided by BASF Corporation (New Jersey, USA). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO USA) and were used as received without any modifications. Millipore® deionized water (18.2 MΩ cm) was used for all experiments.

2.2. Solubility improvement

The solubility of AT in a Pluronic aqueous solution was examined as previously described (Higuchi and Connors, 1965). In 40 ml glass scintillation vials, pre-weighed excess amounts of AT were added to 20 ml of deionized water containing Pluronic F127® or Pluronic F68® at different concentrations. Capped vials were then sonicated under ambient temperature (25 ± 2 °C) for 1 h followed by shaking in a 37 °C water bath for two days. The contents of each vial were filtered using 0.22 Millipore membrane filters, and the AT absorbances were measured at λ = 267 nm using UV spectrophotometry (Evolution 201, Thermo Scientific). These determinations were performed in triplicate.

2.3. Preparation of atorvastatin-Pluronic® solid dispersions

A fusion method was employed for the preparation of solid atorvastatin-Pluronic® solid dispersions (AT-P) with various constitutions and weight proportions, as presented in Table 2. In an appropriate porcelain dish, accurately weighed amounts of Pluronic® were first allowed to melt properly at (60 ± 2 °C). The pre-weighed AT was then added and stirred carefully using magnetic stirring until the melted AT and Pluronic® were thoroughly blended. Using a pre-warmed stainless-steel syringe, the melted atorvastatin–Pluronic® mixture was added dropwise onto a stainless-steel plate mounted over an ice bath to rapidly yield a congealed solid mass. The collected solid masses were ground in a glass mortar and passed through a vibrating 315-μm sieve shaker. The resulting powder from the sieve was collected and stored in a tightly closed container at room temperature until further characterization or usage.

The adapted weight ratios for the obtained atorvastatin-Pluronic® solid dispersions were 10/90, 25/75, and 50/50 percent, respectively. Equivalent amounts of 40 mg of AT were accurately weighed and filled in size two capsules. The filled hard gelatin capsules were weighed and stored in a closed container at room temperature (25 ± 2 °C). To ensure that dose uniformity for each formula was achieved, the AT content was measured. From each formula, three capsules were each dissolved in fifty milliliters of methyl alcohol. These alcoholic solutions were diluted to one liter using water. Fifteen-milliliter samples were withdrawn, filtered, and their absorbances measured at λ = 267 nm using UV spectrophotometry (Evolution 201, Thermo Scientific). These measurements were performed in triplicate.

2.4. In vitro characterization of the obtained AT-P solid dispersions

2.4.1. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopic scans for all prepared formulas were performed using a Shimadzu Prestige-21 FTIR spectrometer. The spectrophotometer contains a MIRacle ATR (attenuated total reflection) sampling technique. Each sample was scanned 32 times with a scan range from 4000 cm^{-1} to 500 cm^{-1}. The absorbance was measured using a mercury cadmium telluride detector, and the obtained spectra showed a resolution of 4 cm^{-1} for all step-scans.

2.4.2. Differential scanning calorimetry (DSC)

The thermal behavior of all obtained atorvastatin-Pluronic® solid dispersions and corresponding physical mixtures (PMs) was examined using Q200 DSC. The differential scanning calorimeter has a heat flow sensor and is connected to interface TA controller (TC15) computer software. The DSC cell was flushed at a rate of 50 ml/min with nitrogen prior to sample scanning. First, the machine was calibrated by running an indium standard sample with a characteristic melting point at 156.6 ± 0.3 °C. Samples were sealed inside aluminum mini-pans (standard 40 µl size pans), and the lid was manually punctured to avoid boiling and bucking of samples, or pan explosion. Each sample was heated from 0 to 250 °C at a rate of 10 °C/min, and an empty pan of the same type (blank) was utilized as a reference.

2.4.3. Powder X-ray diffraction (X-RPD) analysis

The X-ray diffraction for obtained atorvastatin-Pluronic® solid dispersions and corresponding physical mixtures was examined using X-ray diffractometry (Bruker D8-Advance, Germany). The X-ray diffractometer emits Cu Kα radiation at a voltage of 40 kV and 30 mA. Samples were scanned at an adjustable angle (5–60°) with a rate of 4°/min.

2.5. In-vitro dissolution studies

The AT release from the prepared atorvastatin-Pluronic® solid dispersions and corresponding physical mixtures (PMs) was examined using the DT 720 ERWEKA (GmbH, Germany) paddle dissolution apparatus (apparatus II), as described in the USP XXVIII. The dissolution medium was 900 ml of a 0.1 N HCl aqueous solution, at a temperature of 37 ± 0.5 °C. The stirring rate was 75 rpm. The dissolution study was performed with three filled capsules and either 40 mg of AT or the equivalent amount of PM or AT-P (Table 2). Samples of 5 ml were manually collected at pre-determined time intervals (5, 10, 15, 20, 30, 45, 60, 90, and 120 min) and directly substituted with the same volume of fresh dissolution medium. Those collected samples were filtered through a 0.2 µm membrane filter, and AT content was directly determined using UV absorption measurements at 267 nm. The results are reported as the mean ± standard deviation of four independent trials for each experimental condition.

2.6. In-vivo oral bioavailability and pharmacokinetics

White male rabbits (New Zealand origin weighing 1.8–2.0 kg and 8–9 weeks old) were selected to test the oral bioavailability of the chosen AT-P formula. The study protocol was approved by the ethical committee (approval number: HU-REC-FPPS-52–9/18). Experimental procedures were performed following the ethical standards as provided through the guidelines of the European regulations for animal experimentation. All rabbits were kept in a light-controlled room (equal periods of light and darkness every day) at a temperature of 25 ± 3 °C, with free access to food and water. Before initiating the experiment, the ear veins of each rabbit were cannulated with 23-gauge cannulas. The cannulated rabbits were distributed randomly into three groups (A, B, and C), each containing five animals. Using an oral gavage tube, each rabbit was administered one mini capsule (size 9el, Harvard apparatus, Holliston, MA, USA) followed by 2 ml of drinking water. Each mini capsule was filled with a premeasured weight of AT (equivalent to 10 mg/kg of the rabbit weight). Group A received mini capsules filled with free AT, while groups B and C were administered the AT trade product (Lipitor®), and a selected representative AT-P formula (formula AP11), respectively. At predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h), 1 ml blood samples were drawn from the ear vein cannulas into heparinized microcentrifuge tubes. A total of 0.2 ml of heparin solution (50 IU/ml) was then injected to maintain the patency of the cannula between samplings. Immediately after blood collection, plasma was separated by centrifuging at 6,000 × g for 5 min, after which plasma specimens were collected in glass tubes and kept at −20 °C until further analysis. The AT content was quantified using a previously reported LC-MS method with slight modification (Kim et al., 2008).

In brief, 400 µl of acetonitrile was used to extract 200 µl of plasma containing 100 µl of internal standard (methaqualone 100 ng/ml) with vortex mixing. After extraction, 200 µl of the obtained acetonitrile was evaporated in small glass tubes under argon gas flow. The residual content was then dissolved in 200 µl of methyl alcohol and transferred to the autosampler glass vials. Employing 10 µl as an injection volume, AT was determined using LC-MS (Accela, Thermo scientific, USA) using hypersil gold C18 (Phenomenex 2.1 µm, 50 × 20 mm) as the separating column, and a Gemini C18 column (Phenomenex 5 µm, 4 × 3 mm) as a guard cartridge. The LC-MS system consisted of an LC- pump, auto-injector, UV detector (the detection λ = 270 nm), and LC-MS 2010A mass spectrometer (Shimadzu, Kyoto, Japan). The mobile phase contained an equal volume of acetonitrile and ammonium acetate buffer (pH = 4.0) with a flow rate of 1 ml/min. The mass spectrometer operated under these specifications: a drying gas flow of 1.5 l/min, a CDL temperature of 250 °C, a block temperature of 200 °C, and a probe voltage of + 4.5 kV. The MS was connected to the chromatographic system, using an API electrospray interface and was operated in positive ion mode ($[M + H]^+$, *m/z* 559.00 for the internal standard and *m/z* 559.00 for AT). The pharmacokinetic parameters were determined using non-compartmental analysis via the WinNonlin® software program (Version 1.5, Apex, NC, USA).

2.7. Statistical analysis

Statistical analysis for the collected data was performed using GraphPad software (GraphPad prism version 5.02, Inc. La Jolla, CA USA). Two-way ANOVA tests were used, and the significance confirmed by Bonferroni post-tests. Results are reported as means ± SD (standard deviation). The intergroup statistical differences were considered significant if the calculated *p*-value was < 0.05.

3. Results and discussion

3.1. Solubility improvement

The determined AT solubilities in aqueous Pluronic F127® and Pluronic F68® solutions at different concentrations are illustrated in Table 1. As shown, the aqueous Pluronics® solution strongly improved the solubility of AT. The increased solubility was linearly correlated with the concentration of Pluronics® in water. At a 5% concentration of Pluronic F68® and Pluronic F127®, the solubility increases were 280- and 400-fold relative to the AT solubility, respectively. At 10% concentration, the increases in solubility were 576- and 705-fold of the AT solubility for Pluronic F68® and Pluronic F127®, respectively. Such solubility improvement is mainly associated with the lowering of surface tension and the increased wettability of AT, which is attributed to the surfactant properties of Pluronics®, in addition to the direct

Table 1
Measured solubility and calculated Gibbs free energy of transfer (ΔG_t) for Atorvastatin in water- Pluronics® system at 37 °C.

% Pluronic F 127®	% Pluronic F 68®	Measured solubility (mg/mL)	Calculated ΔG_t (kJ/mol)
–	–	0.0206 ± 0.006	–
–	15	22.11 ± 1.16	−17.99
–	10	11.85 ± 0.63	−16.38
–	5	5.77 ± 0.34	−14.53
15	–	25.6 ± 1.27	−18.37
10	–	14.52 ± 0.84	−16.91
5	–	8.26 ± 0.42	−15.45

Table 2
Constitution, filled capsule size, capsule weight and determined AT content for hard gelatin capsules filled with different Atorvastatin-Pluronics® formulas and corresponding physical mixtures.

Formula		AT (mg)	Pluronic F 127® (mg)	Pluronic F 68® (mg)	Filled capsule code	Selected Capsule size	Filled weight (mg)	AT Content (%)
Solid dispersions	**AP 61**	40	–	360	CAP61	0	399.6 ± 5.8	98.9 ± 1.9
	AP 62	40	–	120	CAP62	2	160.5 ± 1.7	99.1 ± 1.8
	AP 63	40	–	40	CAP63	3	79.8 ± 0.6	98.7 ± 1.6
	AP 11	40	360	–	CAP11	0	398.9 ± 9.5	99.3 ± 2.9
	AP 12	40	120	–	CAP12	2	159.9 ± 1.0.9	99.8 ± 2.1
	AP 13	40	40	–	CAP13	3	80.2 ± 0.5	98.9 ± 1.7
Physical mixtures	**PM 61**	40	–	360	CPM61	0	398.9 ± 6.4	99.8 ± 1.8
	PM 62	40	–	120	CPM62	2	158.8 ± 2.1	98.1 ± 2.9
	PM 63	40	–	40	CPM63	3	78.9 ± 0.9	97.7 ± 1.7
	PM 11	40	360	–	CPM11	0	398.9 ± 9.5	98.8 ± 2.4
	PM 12	40	120	–	CPM12	2	159.9 ± 1.0.9	99.1 ± 2.3
	PM 13	40	40	–	CPM13	3	79.7 ± 0.8	99.7 ± 1.9

solubilization effect of the assembled micelles of Pluronics®. The determined Gibbs free-energy of transfer (ΔG_t) is a precarious thermodynamic parameter related to the AT solubility (Table 1). The decrease in ΔG_t value is a considerable reflection of the increase in AT solubility. This ΔG_t was determined by the following equation:

$$\Delta G_t = -\;2.303\;RT\;\log\;S_o/S_s$$

Where R is the gas constant, T is the absolute temperature of the solubility media, S_o is the molar solubility of AT in Pluronics solution and S_s is the molar solubility of AT in water. The results in Table 1 demonstrate that all of the calculated ΔG_t values were negative, revealing the spontaneity of AT solubilization (Damian et al., 2000). Simultaneously, the ΔG_t values decreased by increasing the percentage of Pluronic®, demonstrating that the AT solubilization process become more attainable as Pluronic® concentrations increased (Agafonov et al., 2019; Pillai et al., 2018).

3.2. FTIR spectroscopy

Fig. 1 represents the obtained FTIR spectrum for AT, Pluronic F127®, or Pluronic F68® and the prepared AT-P formulas. As shown in Fig. 1, AT characteristic peaks are: 3,360 cm^{-1} (allocated to intermolecular hydrogen bonds and the stretching of OH bonds); 3,375 cm^{-1} (allocated to the stretching of N-H bonds); 3,250 cm^{-1} and 3,050 cm^{-1} (allocated to the stretching of OH asymmetry and symmetry, respectively); 3,000–2,800 cm^{-1} (allocated to the stretching of C-H bonds); 1,657 cm^{-1} and 1,590 cm^{-1} (allocated to the stretching of C = 0 asymmetry and symmetry, respectively); 1,521 cm^{-1} and 1,443 cm^{-1} (allocated to the stretching of aromatic C-C bonds); 1,326 cm^{-1} (allocated to the deformation of $-CH_3$ and $-CH_2$ bonds); 1,228 cm^{-1} (allocated to the stretching of C—N bonds); 1,170 cm^{-1} (allocated to the stretching of C—F bonds); 1,121 cm^{-1} and 1,063 cm^{-1} (allocated to the bending of aromatic C—H bonds in-plane); 848 cm^{-1}, 819 cm^{-1} and 750 cm^{-1} (bending of aromatic C—H out of plane); and 692 cm^{-1}, 633 cm^{-1} and 594 cm^{-1} (deformation bending of C—H bonds). The typical peaks for Pluronics® detected in both; Pluronic F127® and Pluronic F68® are: 3,665–3,320 cm^{-1} (allocated to the stretching of OH bonds); 2,985 cm^{-1} and 2,898 cm^{-1} (allocated to the stretching of C—H bonds); 1,470 cm^{-1}, 1,350 cm^{-1} and 971 cm^{-1} (allocated to the bending of C—H bonds); 1275 cm^{-1} and 1249 cm^{-1} (allocated to the stretching of C—O—C bonds); 1119 cm^{-1} (allocated to the stretching of C—O bonds); and 846 cm^{-1} (allocated to the stretching C—C bonds). The obtained spectra of all the formulas presented all of the characteristic peaks of AT and Pluronic® without any additional peaks. The recognition of AT peaks conclusively indicates the incorporation of AT within all formulas without any change in chemical structure or integrity (Branca et al., 2018). However, the observed broadening in the stretching peak of the hydroxyl group at 3,250 cm^{-1} and the carbonyl group at 1,657 cm^{-1} of AT in all of the prepared AT-P formulas may reflect the possible AT amorphization and the hydrogen bonding between AT and Pluronic® (Jahangiri et al., 2015). Those hydrogen bonds could also inhibit AT re-crystallization and subsequently prolong the *in vitro* stability and *in vivo* bio-performance of AT-P formulas (Cherian et al., 2019; Jahangiri et al., 2015).

3.3. DSC analysis

Differential scanning calorimetric examination was performed mainly to find out changes in the crystalline state of the incorporated AT within the prepared AT-P solid dispersion formulas and the corresponding physical mixtures. In addition to investigating the probability of interaction between the incorporated AT and Pluronic® within the prepared AT-P formulas. Fig. 2 shows the thermal profiles of the prepared PMs and AT-P formulas in comparison with AT and the Pluronics® formulations used in this study. The figure showed that Pluronic F127®, Pluronic PF68®, and AT are semi-crystalline and exhibit melting/endothermic peaks at 62.1, 61.6, and 161 °C, respectively. Thermal profiles of the physical mixtures showed a slight decrease in the melting temperature and enthalpy of the utilized Pluronic® with the total disappearance of the AT endothermic peak. Nonetheless, thermal profiles of AT-P solid dispersion formulas exhibited a considerable decrease in the melting enthalpy of the utilized Pluronic®, as well as a total disappearance of the AT endothermic peak. This lowering of melting enthalpy and melting point is directly proportional to the AT ratio in the AT-P formula. Increasing the AT ratio in AT-P decreases the melting point and enthalpy of incorporated Pluronic® (Shaker et al., 2010).

The results revealed that pure AT shows a low degree of crystallinity as indicated by its low melting enthalpy ($\Delta H = -80.54$ J/g) (Jahangiri et al., 2015). The complete disappearance of the AT melting peak in PMs and AT-P formulas is most appropriately attributed to a decrease in AT crystallinity and solubilization of AT in the melted Pluronics® during the heating associated with thermal scanning. However, the amorphization of AT during the preparation of solid dispersion is the most probable cause and illustrated with FTIR data and previously reported studies (Jahangiri et al., 2015).

Additionally, the incorporation of AT disrupts the crystalline configuration and the lattice energy of Pluronic®, as estimated from the measured ΔH (heat of infusion). Presuming the proportional relationship between crystallinity and measured enthalpy, the 100% crystallinity was estimated from the fusion enthalpy, for Pluronic F127® (ΔH = -229.1 J/g) and Pluronic F68® ($\Delta H = -268.5$ J/g) (Shaker et al., 2010). The melting ΔH for AP11, AP12, and Ap13 declined to −107.4, −44.7 and −21.5 J/g, that matches 46.8%, 19.5%, and 9.4% crystallinity, respectively. Similarly, the melting ΔH for AP61, AP62, and AP63 declined to −161.1, −89.5, and −26.8 J/g, matching 60%, 33.3%, and 10% crystallinity, respectively. Such a decrease in crystallinity is because the incorporation of AT molecules disrupts the movability of Pluronic's poly (ethylene oxide) moieties and hinders their easy arrangement and consequently diminishes the crystallization

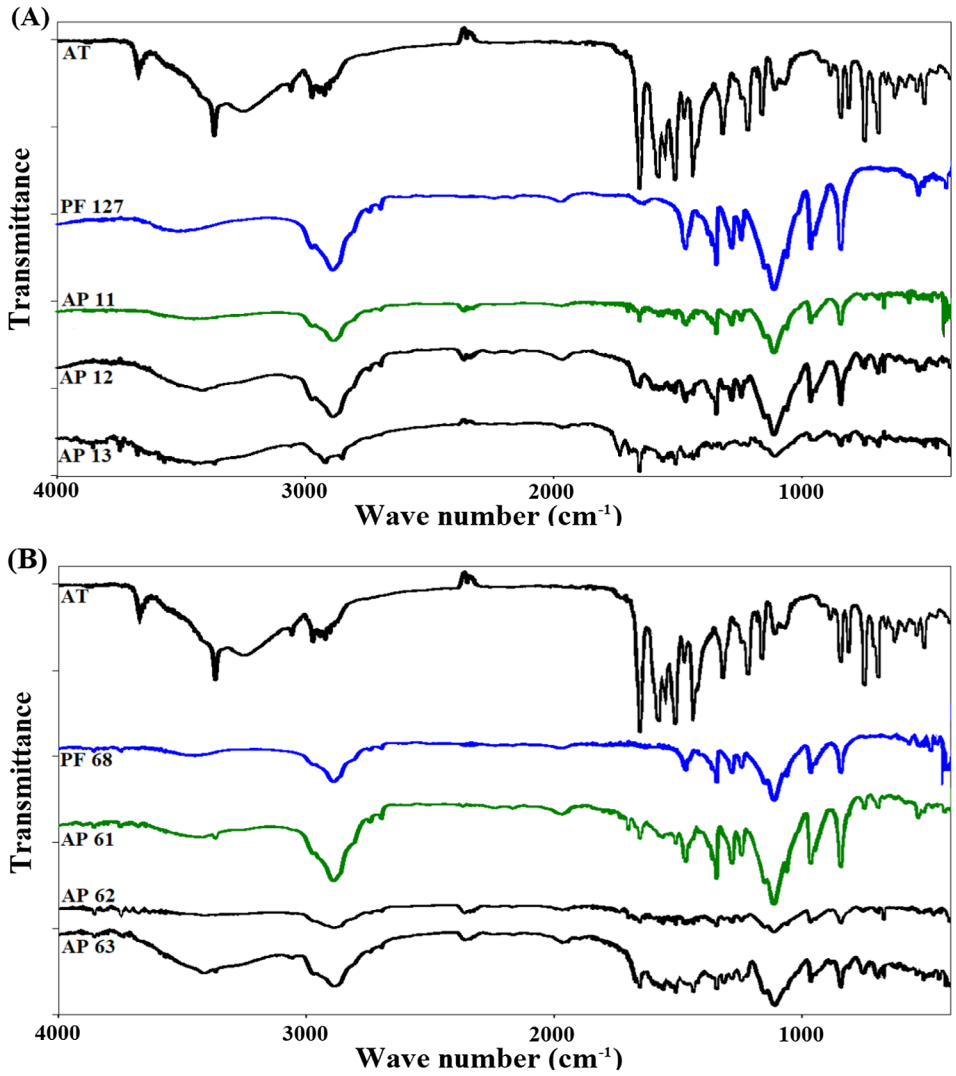

Fig. 1. FTIR graphs for the prepared Atorvastatin formulas using (A) Pluronic PF127 and (B) Pluronic PF68.

during the AT-P preparation (Branca et al., 2018; Jannin et al., 2006).

3.4. X-RPD analysis

Fig. 3 represents the obtained X-ray diffraction spectra for atorvastatin, Pluronic F127®, or Pluronic F68®, and the prepared AT-P formulas. As shown in Fig. 3, AT has a characteristic crystalline diffractogram with sharp peaks at 2θ (diffraction angles) of 9°, 10.1°, 11.5°, 16.9°, 19.4°, 21.5°, 22.6°, 23.2°, and 23.6°, together with blunt peaks at different 2θ, confirming the semi-crystalline nature of AT as illustrated by DSC. Moreover, Pluronic F127® and Pluronic F68® exhibited a distinctive crystalline pattern with tapered crystalline peaks at 2θ (diffraction angles) of 19.0° and 23.19°, and small and broad pulse peaks at 14.9°, 26°, 26.9°, 36.3°, 39°, and 44.6°. The diffractogram for the physical mixtures showed the crystalline peaks for both AT and Pluronic but at different intensities, which clarified that the crystallinity of AT is partially disrupted but still exists.

Also confirming that, the solid dispersion formulas showed the distinctive peaks for the employed Pluronic® without AT diffraction peaks. This finding confirms the conversion of AT from a semi-crystalline to an amorphous state, which can be attributed to the fact that the structural arrangement order of AT in the AT-P formula is entirely disrupted (Branca et al., 2018; Jannin et al., 2006; Karolewicz et al., 2016). In addition to FTIR and DSC results, the above data also confirmed the amorphization of AT during the formulation as AT-P.

3.5. In vitro dissolution

The dissolution profiles for the obtained AT-P formulas in hard gelatin capsules in 0.1 N HCL, compared with that of free AT and PMs are shown in Fig. 4. The results clearly illustrated the increase in the rate of AT dissolution with either mixing with Pluronics® or dispersion in Pluronics®.

AT shows slow dissolution behavior; only 14% of the initial amount dissolved in 20 min. Such a slow dissolution has been previously attributed to the poor aqueous solubility of AT, in addition to the low wettability, aggregation, and surface floating of AT powder in the dissolution medium (Agafonov et al., 2019). The physical mixtures with Pluronic F127® (Fig. 4A) and Pluronic F68® (Fig. 4B) increased the dissolution to 27–57% and 24–66%, respectively, in 20 min. However, both Pluronic F127® (Fig. 4A) and Pluronic F68® (Fig. 4B) solid dispersion formulas showed rapid dissolution, which is in the range of 37–87% and 34–86%, respectively, in 20 min. The rate of AT dissolution in the Pluronic F127® formulas (solid dispersions and PMs) were in the following order AP11 > AP12 > AP13 and PM11 > PM12 > PM13. Furthermore, the Pluronic F68® formulas (solid dispersions and PMs) were in the following order AP61 > AP62 > AP63 and PM61 > PM62 > PM63.

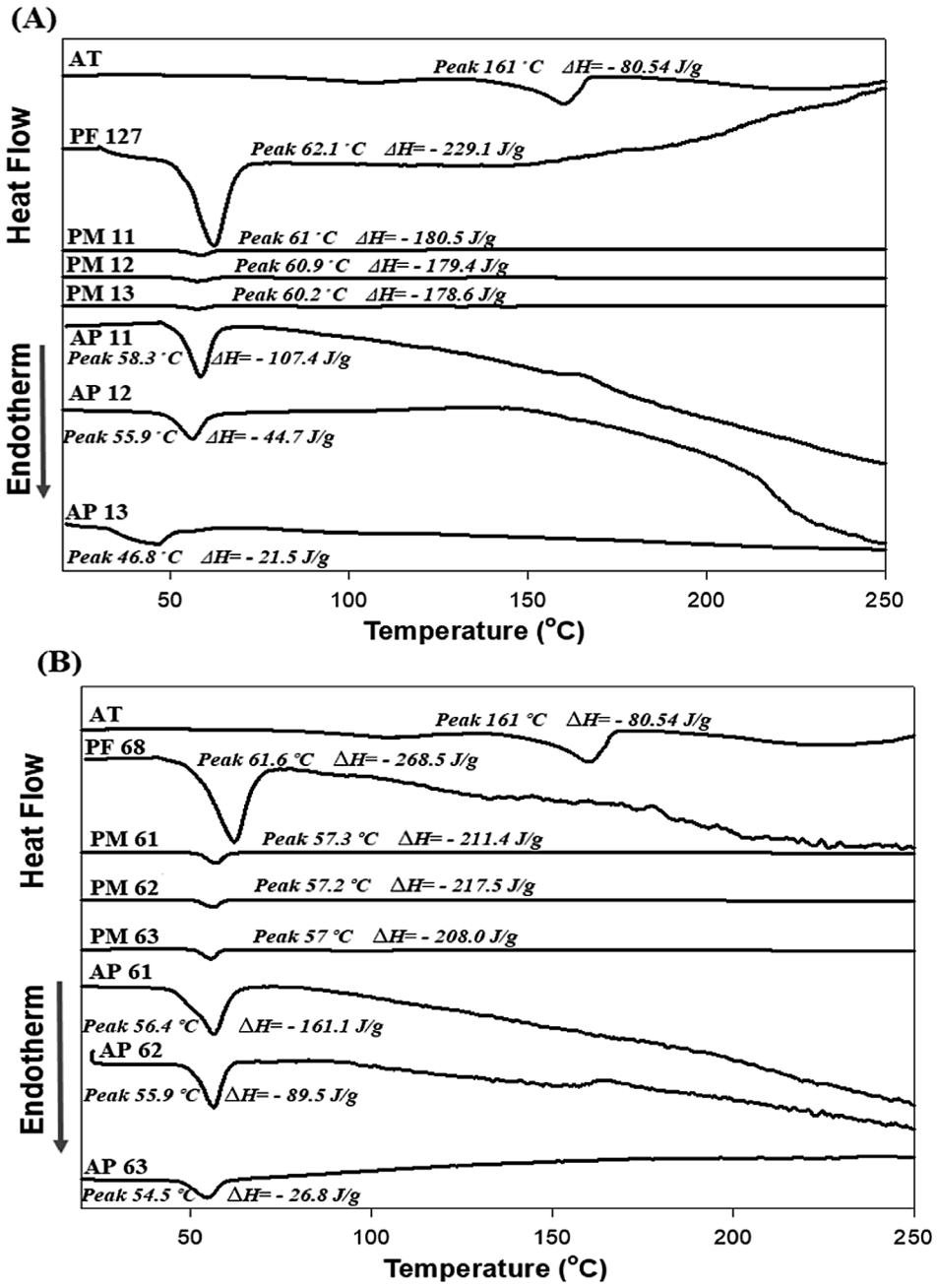

Fig. 2. DSC curves for the prepared Atorvastatin solid dispersion formulas and corresponding physical mixtures using (A) Pluronic PF127 and (B) Pluronic PF68.

The significant increase in the dissolution rate and the maximum amount of dissolved AT in the PMs can be ascribed to the direct solubilization effect of the amphiphilic carrier (Al Khateb et al., 2016; Patil et al., 2019). The surface and interfacial tension lowering effects of Pluronic® participates in the wetting of AT powder within the dissolution media and hindering AT crystals from re-aggregation (Karolewicz et al., 2016; Pillai et al., 2018). In addition to the surface tension effects of Pluronic®, the solubilization effect of the assembled micelles from the dispersed Pluronic molecules in the dissolution media (Karolewicz et al., 2016; Pillai et al., 2018) also contributed to the dissolution rate and the amount of dissolved AT. Additionally, further increases in AT dissolution are shown by the solid dispersion formulas. This finding is mainly attributed to decreased particle size as well as complete amorphization of AT during formulation, as clarified by FTIR data and confirmed through the DSC and X-RPD results. The high internal energy content of amorphous AT promotes the rapid dissolution when compared to the semi-crystalline form of AT (Jahangiri et al., 2015).

It is also important to highlight that the AT formulation with an equal mass of Pluronic F68® (AP63) demonstrated a slight improvement in the maximum amount of dissolved AT, while the formulation with an equal mass of Pluronic F127® (AP13) demonstrated significant improvement in the maximum amount of dissolved AT. This anticipated difference in solubility is mainly due to the affinity of AT (hydrophobic drug) to the polyoxypropylene oxide chain (hydrophobic moiety) in Pluronics® (Batrakova and Kabanov, 2008). The higher the polyoxypropylene oxide chain length, the greater the affinity of AT to the assembled hydrophobic core of the Pluronic® micelle. Furthermore, Pluronic F127® has a polyoxypropylene chain with a molecular mass of 3,600 g/mol, while Pluronic F68® has a polyoxypropylene chain with a molecular mass of 1,800 g/mol (Kabanov and Alakhov, 2002). Hence, the micellular solubilization capacity of Pluronic F127® to AT is higher than that of Pluronic F68®, leading to higher initial saturated solubility

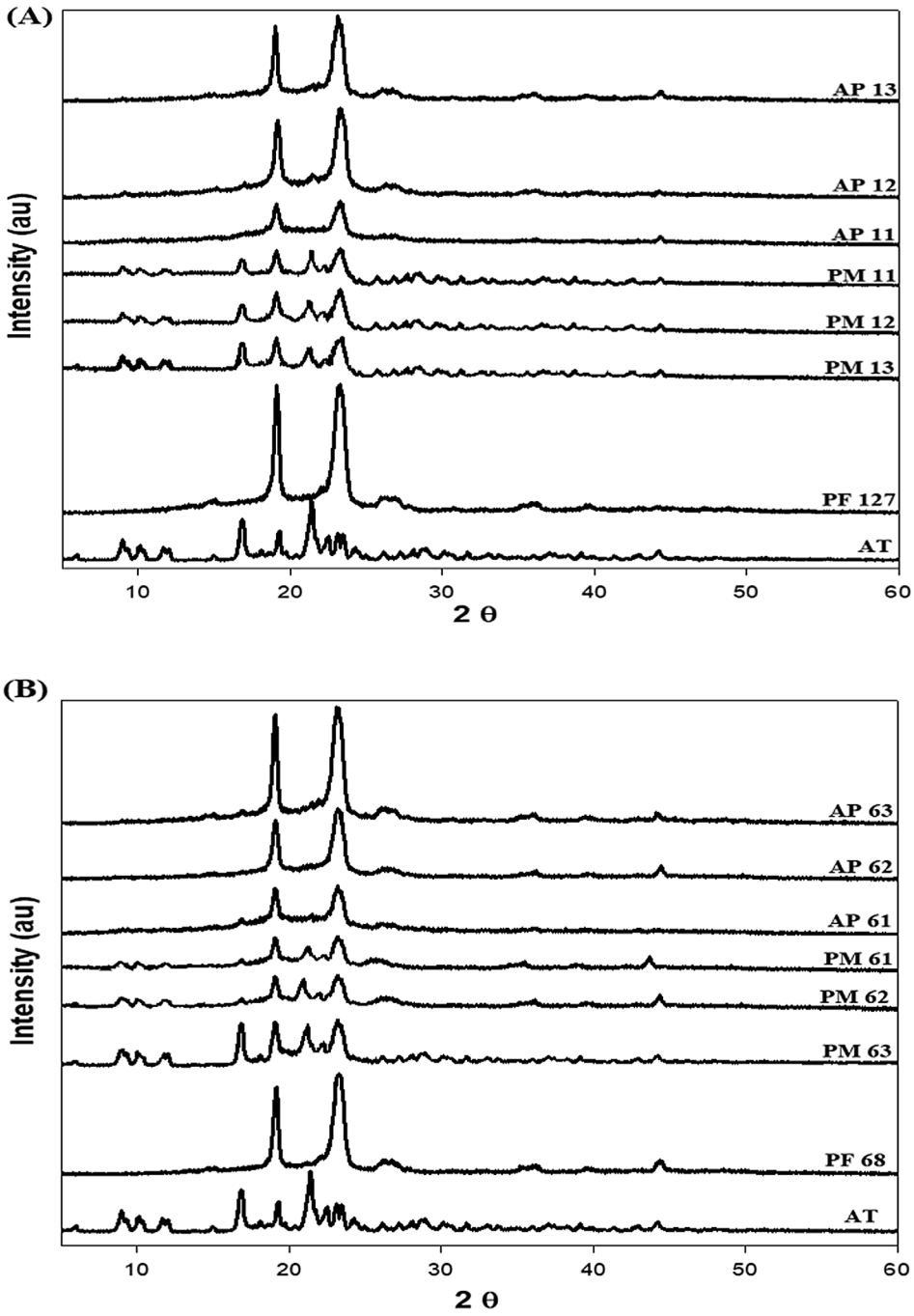

Fig. 3. X-ray diffractograms for the prepared Atorvastatin solid dispersion formulas and corresponding physical mixtures using (A) Pluronic PF127 and (B) Pluronic PF68.

(Table 1).

Nonetheless, an additional increase in the mass percentage of the employed Pluronic® demonstrated remarkable improvement in the rate of dissolution, as well as the maximum amount of dissolved AT. This finding is mainly attributed to the aforementioned DSC and XRD results. The presence of Pluronic® in the AT-P is associated with a diminished AT crystallinity. Simultaneously, such a profound improvement in the maximum amount of dissolved AT and its dissolution rate is correlated with the improved wetting behavior of AT and the disappearance of AT floating agglomerates (Agafonov et al., 2019; Jannin et al., 2006).

3.6. *In vivo* pharmacokinetics

Atorvastatin concentrations in the plasma of New Zealand rabbits after the oral administration of either AT (1 mg/100 g of body weight), or an equal amount of formula AP11 and Lipitor®, are shown in Fig. 5. The calculated pharmacokinetic parameters are listed in Table 3. As demonstrated, the greatest plasma C_{max} value of AT has been achieved after the administration of the AP11 formula. Additionally, the oral absorption of AT from the AP11 formula is substantially better than either Lipitor® or free AT. The estimated AT bioavailability is in the sequence of the AP11 formula > Lipitor® > free AT. Rabbits administrated Lipitor® and free AT exhibited maximum plasma concentrations (C_{max}) of 642.3 and 517.6 ng/ml, respectively, almost 2 h after

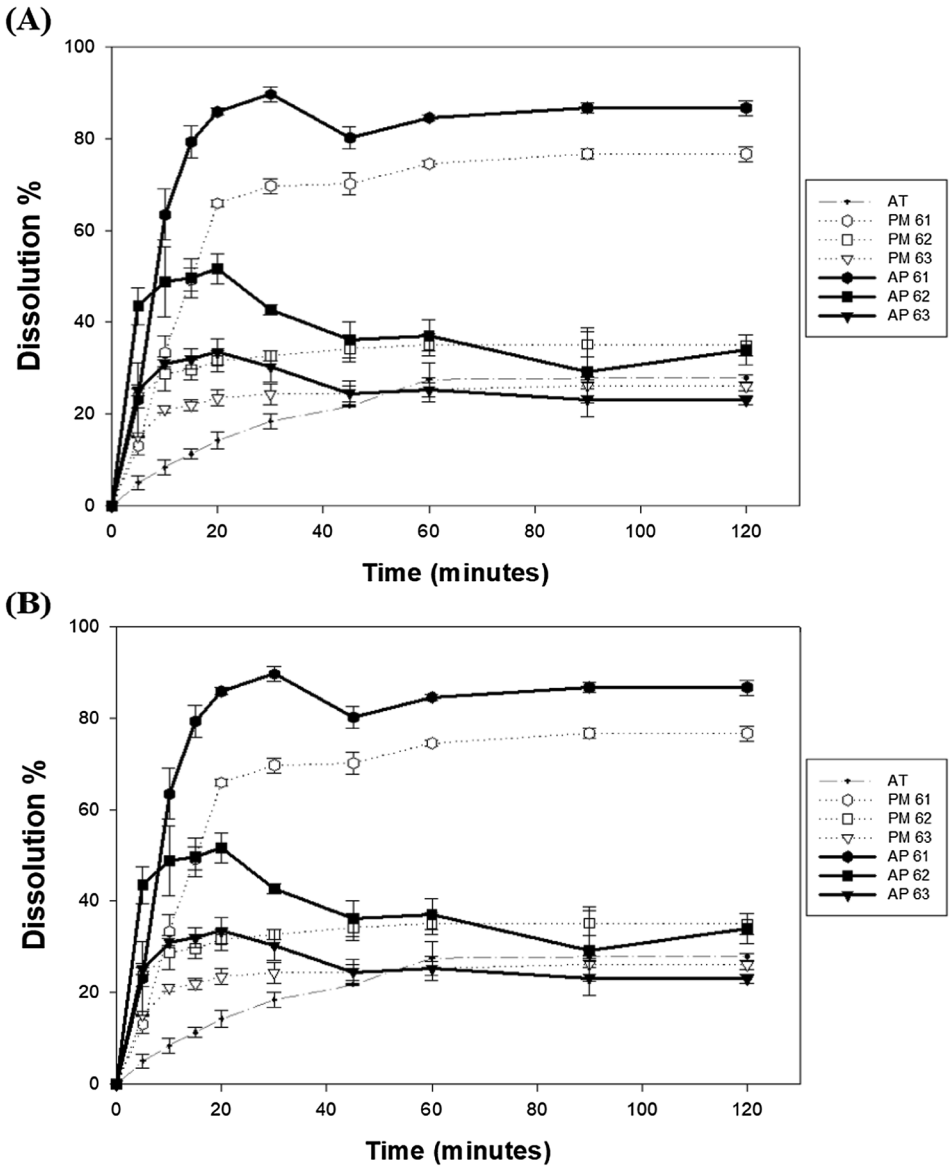

Fig. 4. Dissolution profiles of Atorvastatin, prepared solid dispersion formulas and the corresponding physical mixtures (PM) using (A) Pluronic PF127®, (B) Pluronic PF 68®. All the prepared formulas filled in hard gelatin capsules and dissolution is measured in 0.1 N HCL as a dissolution medium. Results are represented as mean ± S.D. (n = 4).

administration. The absorption rate constant (K_a) was equal to 0.876 and 0.561 h^{-1} as well as an AUC_{0-t} equivalent to 4,427.4 and 2,473.7 ng.h/ml for Lipitor® and free AT, respectively. Nonetheless, rabbits given an equivalent amount of AT as the AP11 formula presented a notable increase in the maximum plasma concentration peak (C_{max}) up to 1,146 ng/ml, which was reached almost 3.2 h after dose administration, and was associated with an increase in AUC_{0-t} value to 9,993.4 ng.h/ml and a decline in the K_a value to 0.313 h^{-1}.

Also noteworthy is the finding that the oral absorption of AT is prolonged with the Pluronics® formulation (T_{max} of formula AP11 = 3.2 h). This increase in absorption time is related to the micellar incorporation of AT. The absorption of free AT from the formula AP11 contributes to the overall rapid absorption within the first 2 h of dose administration. Nonetheless, the slow diffusion of incorporated AT from the core of the self-assembled Pluronic® micelles sustained the oral absorption of AT longer (Raval et al., 2017; Shaarani et al., 2017). Accordingly, this slow diffusion led to sustained plasma levels of AT and extended the elimination half-life, as well as decreasing the calculated elimination rate constant of the tested formula (Shaarani et al., 2017).

According to this data, enhancement of the dissolution and oral absorption of AT can be considered the adjustable variable for any oral AT strategy to succeed. Numerous approaches have been endorsed to achieve this bioavailability improvement goal. These approaches successfully increased the oral bioavailability of oral doses, including, but not restricted to, the use of either supercritical anti-solvent or spray drying techniques by Kim et al., (2008) to improve the absorbed fraction by 2.9- and 1.9-fold, respectively (Kim et al., 2008). Furthermore, the formulation of AT as a nano-suspension was shown to improve the absorption by 2.1-fold (Zhang et al., 2009). Using another approach, Kim et al. (2013) were able to incorporate AT into different biopolymers to enhance the absorption. Using hydroxypropylmethylcellulose and polyvinylpyrrolidone vinyl acetate improved the absorbed fraction by 1.2- and 1.4-fold, respectively (Kim et al., 2013). The Palanisamy research group used the same strategy but incorporated cyclodextrin for 2.4-fold improved bioavailability (Palanisamy et al., 2016). Our previous work has also used the same strategy, incorporating Gelucire 44/14 for a 4.5-fold improved the bioavailability (Shaker, 2018). In 2016, another published work by Yeom and his group were able to improve

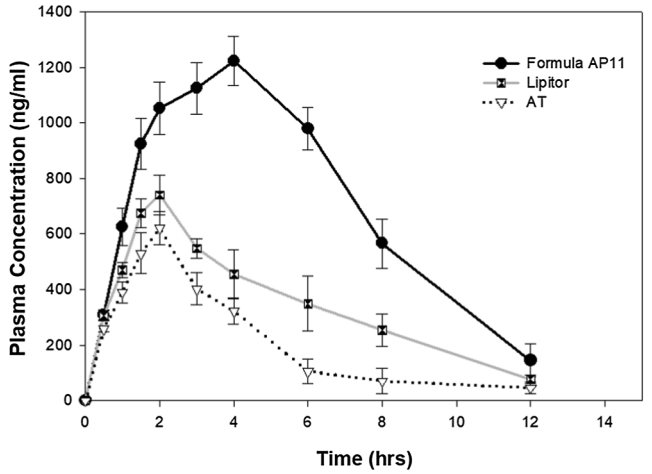

Fig. 5. Atorvastatin concentrations in the plasma of male New Zealand rabbits, at different time after given a 10 mg/kg dose of Atorvastatin, Lipitor® or formula AP11. Results are presented as mean ± S.D.

Table 3
Calculated pharmacokinetic parameters for AT after given as a 10 mg/kg oral dose to male New Zeeland rabbits of AT, Lipitor® or formula AP11. Results are presented as mean ± SD (n = 5).

Calculated Parameters	Atorvastatin	Lipitor®	Formula AP11
C_{max} (ng/mL)	517.6 ± 59	642.3 ± 73*	1146 ± 131*@
T_{max} (hr)	1.76 ± 0.3	2.05 ± 0.3	3.21 ± 0.5*@
$t_{1/2\ ka}$ (hr)	1.24 ± 0.2	0.79 ± 0.1*	2.22 ± 0.3*@
K_a (hr^{-1})	0.561 ± 0.09	0.876 ± 0.14*	0.313 ± 0.07*@
$t_{1/2}$ (hr)	1.20 ± 0.2	2.74 ± 0.4*	2.23 ± 0.4*@
K_e (hr^{-1})	0.576 ± 0.09	0.235 ± 0.03*	0.311 ± 0.04*@
$AUC_{0\text{-}12}$ (ng.hr/mL)	2473.7 ± 147	4427.4 ± 255*	9993.4 ± 575*@
CL/F (mL/hr)	4.04 ± 0.6	2.26 ± 0.3 *	1.01 ± 0.17*@
V_d/F (mL)	7.02 ± 0.9	9.61 ± 1.1*	3.22 ± 0.4*@

C_{max}: maximum AT concentration T_{max}: time to reach C_{max}; $t_{1/2\ Ka}$: absorption half-life; K_a: absorption rate constant; $t_{1/2}$: elimination half-life; K_e: elimination rate constant; $AUC_{0\text{-}12}$: area under the AT concentration time curve from time 0 to 12 hr; Cl/F: AT clearance divided by its bioavailability fraction; V_d/F ratio of the apparent volume of AT distribution over its bioavailability fraction:.
@Significantly different at $p < 0.05$ versus Lipitor®.
* *Significantly different at* $p < 0.05$ versus *Atorvastatin.*

the bioavailability of AT by 3.4-fold through the preparation of an AT self-micro emulsifying formula (Yeom et al., 2016). Whereas the formulation of AT as a gastro-resident tablet by *Khan and Dehghan* was shown to enhance the oral bioavailability by 1.7-fold (Khan and Dehghan, 2011).

In this study, our AT formulation with the amphiphilic carrier (Pluronic F127®) improved the bioavailability by 4.04-fold relative to untreated AT, as well as 2.26-fold relative to Lipitor®. The diminished AT crystallinity through the incorporation with Pluronics® significantly increased the water solubility and dissolution rate of AT. Increasing the incorporated ratio of Pluronics® was associated with a direct increase in AT solubility and dissolution. This boost in the *in vivo* absorption of AT corresponded with the considerable increase in the *in vitro* dissolution characters (Palanisamy et al., 2016; Yeom et al., 2016). Consequently, this remarkable increase in the absorbed fraction and relative bioavailability of AT can reflect an anticipated strengthening in the therapeutic effect of AT via the incorporation with Pluronics ® as an amphiphilic carrier.

4. Conclusions

In this study, the formulation of AT as a solid dispersion with Pluronics® has been demonstrated to optimize the bioavailability of AT, which can help in reducing the required dose and improve therapeutic outcomes. With the use of either Pluronic F127® or Pluronic F68®, the improvement in the extent of solubility, as well as the rate of dissolution over that of AT, have been achieved. Hard gelatin capsules filled with AT incorporated with Pluronic F127® improved the oral bioavailability by 4.04-fold relative to untreated AT. The AT-P binary system provides a different approach over the aforementioned strategies and can be considered as a convenient choice for optimizing the oral delivery of AT. This preference is due to the demonstrated uniformity for both fill weight and AT content of the prepared capsules as well as the well-known stability of prepared solid dispersion formulas. Moreover, the recognized biocompatibility, biodegradability, and manipulability of the Pluronics® matrix can facilitate their use in pharmaceutical production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2019.118891.

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