Research in Pharmaceutical Sciences, April 2020; 15(2): 123-136
Received: 10-07-2019
Revised: 14-11-2019
Accepted: 16-04-2020
Published: 07-05-2020

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Enhancement of dissolution of atorvastatin through preparation of polymeric solid dispersions using supercritical fluid technology

Bashar Altaani*, Rana Obaidat, and Walaa Malkawi

Department of Pharmaceutical Technology, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan.

Abstract

Background and purpose: This study aimed at preparation of solid dispersions in order to enhance dissolution of poorly water-soluble atorvastatin using supercritical CO_2 technology. Atorvastatin has poor bioavailability of 12%, mainly due to poor water solubility and dissolution. Dispersion of drugs in various hydrophilic carriers using supercritical fluid technology has been found to be an outstanding method to prepare solid dispersion.

Experimental approach: Four different polymers were employed. These were polyvinyl pyrrolidone K30 (PVP), polyethylene glycol 6000 (PEG), Soluplus®, and chitosan. Full physicochemical characterizations were performed in addition to *in vitro* dissolution study.

Findings / Results: The used polymers enhanced the dissolution rate of atorvastatin. However, supercritical parameters affected the dissolution profile and drug loading efficiency of the prepared dispersions. High performance liquid chromatography assay indicated the stability of the prepared PEG, Soluplus® and chitosan-based dispersions. On the other hand, PVP solid dispersions were not stable and formed sticky paste. Powder X-ray diffraction showed similar patterns for PEG-based dispersions after exposure to storage condition, while the intensity of atorvastatin peaks increased after three months of storage of Soluplus® and chitosan dispersions.

Conclusion and implications: Supercritical fluid technology proved to have great potential to prepare dispersions for biopharmaceutics classification system (BCS) class II drugs. Dissolution enhancement of atorvastatin was achieved through successful preparation of polymeric dispersions of the drug using the supercritical technology without further addition of solvents.

Keywords: Atorvastatin; Solublity enhancement; Polyethylene glycol; Solid dispersions; Soluplus; Supercritical fluid technology.

INTRODUCTION

Surface tension and solvent strength, which vary between gas-like and liquid-like values, depend on the temperature and pressure conditions (1). Many gasses have been used as supercritical fluid (SCF), but CO_2 has been considered the best gas as it is nontoxic, nonflammable, inexpensive, and readily available. Also, it has a mild critical temperature (Tc = 31 °C) and low critical pressure (Pc = 7.38 MPa). SCF offered many advantages in the pharmaceutical arena specially for heat sensitive materials, drying process, and preparation of carriers and solid dispersions (SDs) (2).

Dispersion of the active ingredient in an inert carrier matrix through SD is one of the most commonly used methods to enhance the dissolution and hence the bioavailability of poorly water-soluble drugs. Many traditional methods have been used to produce SDs including hot melt extrusion (3), fusion method (4), solvent-evaporation method (5), and spray drying (6). However, SCF technique proved its suitability for preparing SDs. This is attributed to the preparation of SD in a light and oxygen-free atmosphere (7).

*Corresponding author: B. Altaani
Tel: +96-2795562651, Fax:+96-227201075
Email: altaani@just.edu.jo

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.273812

SDs prepared by SCF technology showed good flow properties, small particle size, and absence of the residual organic solvent as opposed to conventional techniques (7). Obaidat *et al*. proved the significant enhancement of tacrolimus dissolution profile by preparing SDs using SCF method (8).

Many polymers proved to be utilized in preparing SDs such as polyvinyl pyrrolidone (PVP), polyethylene glycol (PEG), Soluplus®, and chitosan. These polymers have several advantageous properties including water solubility, biocompatibility, and safety. PVP is a common carrier which is used for preparing SDs of poorly water soluble drugs to improve their solubility and dissolution rate and hence their bioavailability. Different methods, including SCF technique has been used to improve aqueous solubility of lipophilic drugs (9). PEG is a hydrophilic and hygroscopic polymer. It has excellent ability to enhance the solubility of hydrophobic drugs; In addition, PEG can improve the physical and chemical stability of drugs and prevents drugs aggregation (7). It is one of the most widely and commonly used polymers for preparing SDs. Another unusual polymer is Soluplus®. It is a synthetic novel polymer. It has been applied in the recent years and considered as one of the third generation solid dispersion polymer. It can function as a matrix polymer for solid solutions as it behaves as a solubilizer through micelle formation. It is water soluble, nonionic and slightly surface active. Soluplus® has good flowability and low hygroscopicity (10). Chitosan is derived from chitin by deacetylation of chitin in alkaline conditions or by enzymatic hydrolysis (11). Chitosan is a hydrophilic, cationic, polysaccharide polymer. It is of great interest due to its biocompatibility, biodegradability, bioactivity, nontoxic, and non-allergic (12).

Atorvastatin is a synthetic lipid lowering-agent. It selectively and competitively inhibits the enzyme β-hydroxy β-methylbutyryl-CoA (HMG-CoA) reductase. It is commonly used as atorvastatin calcium salt. It exists as white to off-white crystalline powder. It is insoluble in aqueous solution below pH 4. It has a short half-life and needs 1-2 h to reach its maximum concentration (T_{max}) in the plasma (13). Atorvastatin has a good intestinal permeability but low bioavailability; the bioavailability of 40 mg oral dose is only 12%. Its low bioavailability is due to its first pass hepatic metabolism, poor water solubility, and crystalline nature (14). Atorvastatin is administered in high doses to overcome its insufficient bioavailability. High doses of atorvastatin can lead to undesirable side effects such as liver abnormalities, rhabdomyolysis, arthralgia, and kidney failure (13). Many approaches were utilized to enhance the dissolution and bioavailability of atorvastatin. This includes the formation of a dry emulsion containing atorvastatin using spray-drying method (15). Preparing of amorphous atorvastain is another approach. Amorphous atorvastatin was prepared using supercritical antisolvent by which the physichochemical properties and the bioavailability of atorvastatin were improved (16). In addition, prepartion of SDs by different techniques to enhance the dissolution of atorvastatin using several carriers like PVP (13), Soluplus®(15), gum karaya (17), and PEG 6000 (18) were reported.

The primary objective of this work was to employ SCF technology as a method to prepare SDs of atorvastatin without the use of any additional solvents in order to enhance the dissolution of the drug. The selected polymers were PVP, PEG, Soluplus®, and chitosan. The physicochemical properties of raw materials, physical mixtures, and prepared dispersions were studied. Also, *in vitro* drug release, drug-loading efficiency, and stability study were conducted.

MATERIALS AND METHODS

Atorvastatin calcium was supplied by Biocon, India. Kollidon 30 (PVP K30) as supplied by Aldrich Chemicals, USA. PEG 6000 and sodium triphosphate (STPP) were supplied by Sigma-Aldrich, USA. Soluplus® was kindly supplied by BASF Ludwigshafen, Germany. High molecular weight chitosan 600 kDa was provided by Shanghai Hanshare Industry CO., China. Potassium dihydrogen phosphate (KH_2PO_4), extra pure, was supplied by ScharlauChemie, Spain. Sodium hydroxide

(NaOH) and methanol were supplied by Fisher Scientific, UK. Potassium bromide (KBr) IR grade by Sigma-Aldrich, France. Ethanol was supplied by Solvochem, Holland. Hydrochloric acid (HCl, 37% w/w) was supplied by Biosolvo, France. Acetonitrile (purity 99.9%) was supplied by Anhuni Fulltime Specialized Solvents and Reagents Co., China. Acetic Acid was supplied by Xilong Chemical Industry Incorporated, China. Liquid CO_2 (purity 99.9%) was supplied by Jordanian Gas Company, Amman, Jordan. Distilled water was used for all experiments. All Chemicals were used without any modification.

Preparation of chitosan carrier

Chitosan oligomer (11 kDa) was prepared by allowing raw material chitosan (600 kDa) to react with 2 M HCl for 4 h, as described previously (19,20). The molecular weight of chitosan carrier oligomer was determined by viscosity analysis using a sine wave Vibro SV-10/SV-100 viscometer (KSV Instrument Helsinki, Finland) (20). The obtained chitosan oligomer powders were stored in glass vials at room temperature.

Chitosan carrier was prepared by reacting 100 mL of 1% low molecular weight chitosan solution with 100 mL of 0.5% STPP. STPP solution was added at high mixing speed (4000 rpm), and the mixing was continued for 30 min. Then, the mixture was dehydrated by immersion in a series of successive ethanol baths of increasing ethanol concentration (20, 40, 60, 80, and 100%, V/V in water). After that ethanol was dried using SCF apparatus (Eurotechnica, GmbH, Germany) at 1450 psi and 40 °C for 2 h (2).

Preparation of atorvastatin solid dispersions

Atorvastatin was physically mixed with one of the polymers (PVP, PEG, Soluplus®, or chitosan carrier) to produce a mixture of the drug to polymer in ratio of 1:9. Then it was processed by SCF apparatus at different temperatures, pressures and loading times. Also, drug to polymer ratio for PEG-based SDs was changed (Table 1). The produced SDs were ground using a mortar and pestle and sieved through 300 μm sieve and then stored in an amber airtight container in the refrigerator.

Table 1. The prepared solid dispersions (SD) showing their preparation conditions with their loading efficiency.

SDs	Conditions				Drug: polymer	%Average drug content ± SD (w/w)
	Pressure (psi)	Temperature (ºC)	Time (h)	Polymers		
SD1	1450	40	2	PVP	1: 9	67.83 ± 1.88
SD2	1450	60	2	PVP	1: 9	79.73 ± 0.99
SD3	1450	80	2	PVP	1: 9	88.68 ± 0.36
SD4	1450	80	4	PVP	1: 9	81.98 ± 2.95
SD5	1450	80	6	PVP	1: 9	87.45 ± 6.69
SD6	1450	40	2	PEG	1: 9	79.23 ± 1.27
SD7	1450	50	2	PEG	1: 9	80.65 ± 1.63
SD8	1450	60	2	PEG	1: 9	104.8 ± 15.6
SD9	1450	50	2	PEG	2: 8	66.22 ± 7.16
SD10	1450	50	2	PEG	3: 7	90.17 ± 9.69
SD11	1450	40	2	Soluplus	1: 9	80.47 ± 0.41
SD12	1810	40	2	Soluplus	1: 9	83.80 ± 0.94
SD13	2170	40	2	Soluplus	1: 9	79.90 ± 0.31
SD14	1450	40	2	Chitosan	1: 9	94.67 ± 3.45
SD15	1450	60	2	Chitosan	1: 9	94.79 ± 6.53
SD16	1450	80	2	Chitosan	1: 9	97.33 ± 4.77
SD17	1810	80	2	Chitosan	1: 9	106.7 ± 11.2
SD18	2170	80	2	Chitosan	1: 9	102.5 ± 5.31

Preparation of physical mixture

Physical mixtures (PMs) were prepared by mixing atorvastatin physically with the polymers using a mortar and pestle to produce a PM in a ratio of 1:9. The PMs were sieved through 300 μm sieve then stored in an amber airtight container in the refrigerator.

Physicochemical characterization

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were carried out for atorvastatin, the used polymers, the prepared SDs, and their corresponding physical mixtures using DSC 204 (Netzch, Germany). Indium was used to calibrate temperature and energy scale. An accurately weighed sample was placed in a sealed aluminum pan (P/N 201-52943). Then, it was heated in the range 30-200 °C under constant nitrogen flow of 30 mL/min. An empty sealed aluminum pan was used as a reference. Sample crimper was used to seal the pans.

Powder X-Ray diffraction

Powder X-Ray diffraction (PXRD) pattern of atorvastatin, the used polymers, the prepared SDs and their corresponding physical mixtures were acquired using Ultima IV X-ray diffractometer (Rigaku, Japan) equipped with cobalt radiator at voltage of 40 KV and a current of 30 mA. The angle (2 Θ) scanning range of the samples was between 0º and 60º at step of 0.02º.

Scanning electron microscopy

Scanning electron microscopy (SEM) images were obtained using Quanta FEG 450, SEM (FEI, US), to study the surface morphology of atorvastatin, raw polymers, SDs, and their corresponding PMs. Before SEM analysis, the samples were placed on stubs and coated with platinum under vacuum atmosphere using Q150R rotary-pumped sputter coater/ carbon coater (Quorum Technologies, UK).

Determination of drug content

Drug content was determined by dissolving an amount equivalent to 1 mg atorvastatin from each formula in 50 mL methanol and then stirred for 30 min. The total amount of the drug was measured using UV-1800 spectrophotometer (Shimadzu, Japan) at λ_{max} 246 nm. Drug content was calculated using the following equation:

$$Drug\ content = \frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100$$

In vitro drug release study

Drug release was performed for the prepared SDs and their corresponding PMs, using dissolution test apparatus II (rotating paddle) at 37 ± 0.5 °C. The rotational speed were set at 75 rpm. The dissolution media was 900 mL of freshly prepared phosphate buffer solution (0.05 M) with pH 6.8. This media is recommended by Food and Drug Administration. A sample equivalent to 40 mg of atorvastatin from the prepared SDs and PMs were accurately weighed and filled manually into a gelatin capsule (size 0) and placed in the dissolution test. The samples were withdrawn at predetermined time intervals over 2 h. The withdrawn samples were filtered through a filter unit (pore size 0.45 μm, nylon filter membrane, Bonna-Agela Technologies). Each withdrawn volume was replaced by an equal volume of fresh dissolution media to maintain the volume and sink condition. The samples were diluted appropriately and assayed for atorvastatin by UV-VIS spectroscopy at λ_{max} of 241 nm.

Mathematical modeling of release kinetics

The *in vitro* drug release data were fitted to several release kinetic models including zero order, Higuchi and Korsmeyer-Peppas equations. The regression coefficient was calculated to determine the best-fitted model and the release mechanism

Stability study

Selected samples were subjected to specific stability studies conditions. Selection was based on samples showing the best dissolution profile. The samples were stored at two different conditions 30 ± 2 °C, 75 ± 5% relative humidity and 40 ± 2 °C and 75 ± 5% relative humidity for three months. The stability of the selected SDs was tested chemically and physically. Chemical stability was evaluated by determining the concentration of dug using high-performance liquid chromatography (HPLC) to detect any possible degradation. Other stability tests included drug loading efficiency and PXRD pattern.

HPLC method

Chemical stability and detection of any degradation were studied by performing a validated HPLC method for inter- and intraday variation (21). The retention time of atorvastatin and formulations were detected by HPLC apparatus, model LC-10AD VP with UV-VIS detector model SPD-10A VP and auto sampler model SIL-20A, Shimadzu, Japan. Methanol was used as the solvent to dissolve atorvastatin and the SDs. The chromatographic column was ACE C18 (250×4.6 mm, 5 µm). The mobile phase was acetic acid solution (0.05%):acetonitrile (35:65). A UV detector was set at 246 nm. The flow rate was 1 mL/min and the injection volume 20 µL. The experiment was done at ambient temperature.

Statistical analysis

All measurements were carried out repeatedly and the results were expressed as the mean ± standard deviation (SD). The data from the different groups were statistically analyzed using a paired t-test. *P* values less than 0.05 were considered statistically significant. Microsoft Office Excel application was used for the calculations.

RESULTS

Physicochemical characterization

Differential scanning calorimetry

DSC thermogram (Fig. 1) shows a sharp endothermic peak of atorvastatin at 157.7 °C. DSC thermograms of all polymers matched well with published literature with broad peak existing at 30-150 °C for PVP (13). On the other hand, a sharp peak indicating crystalline nature was shown for PEG at 65 °C (13). A broad endothermic hump related to glass transition (T_g) temperature for Soluplus® was detected at 72.2 ºC (22). A broad endothermic peak from 40-130 ºC has been detected for chitosan (11). The characteristic DSC thermograms of atorvastatin were not seen in all the prepared PMs and SDs. This indicates that thermal analysis could not provide sufficient data about physical state of the drug inside PMs and SDs. A clear shift of Tg of Soluplus® from 72.2 to 93 °C occurred in the prepared SD.

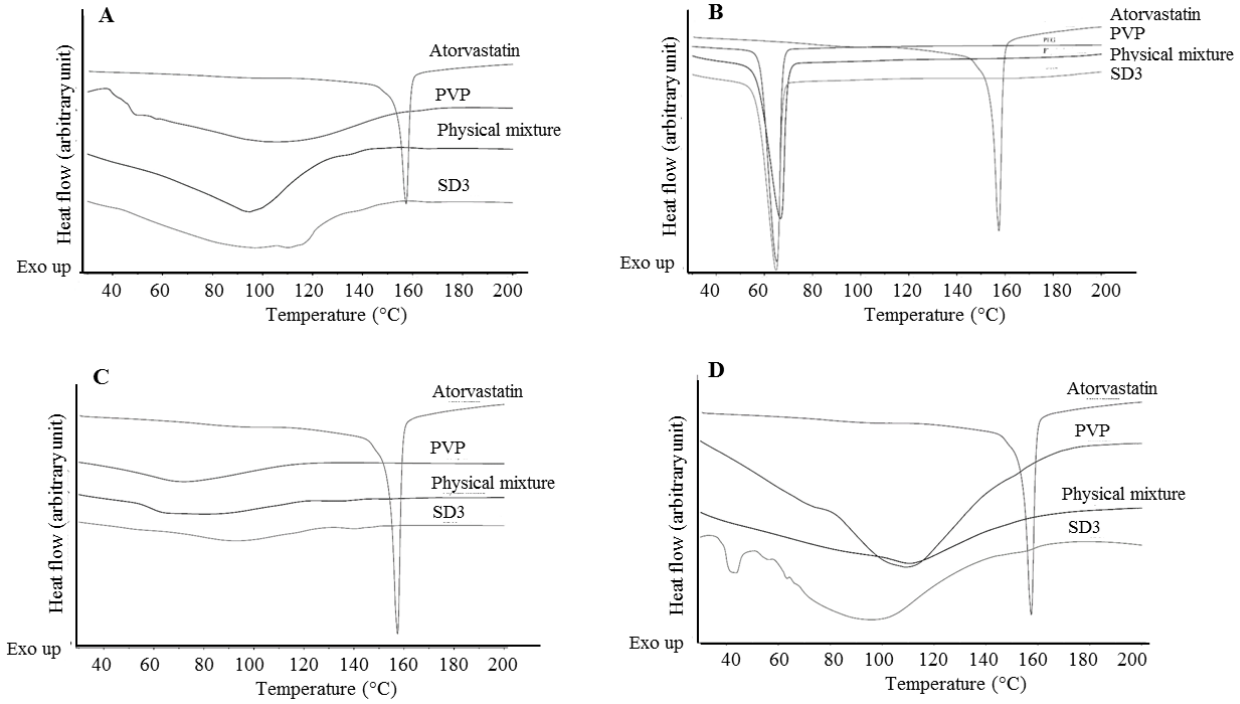

Fig. 1. Differential scanning calorimetry thermograms of (A) atorvastatin, PVP, atorvastatin-PVP physical mixture and atorvastatin-PVP solid dispersion (SD3); (B) atorvastatin, PEG, atorvastatin-PEG physical mixture and atorvastatin-PEG solid dispersion (SD7); (C) atorvastatin, Soluplus®, atorvastatin-Soluplus® physical mixture and atorvastatin-Soluplus® solid dispersion (SD11); and (D) atorvastatin, chitosan, atorvastatin-chitosan physical mixture and atorvastatin-chitosan solid dispersion (SD14). PVP, polyvinyl pyrrolidone K30; PEG, polyethylene glycol; SD, solid dispersion.

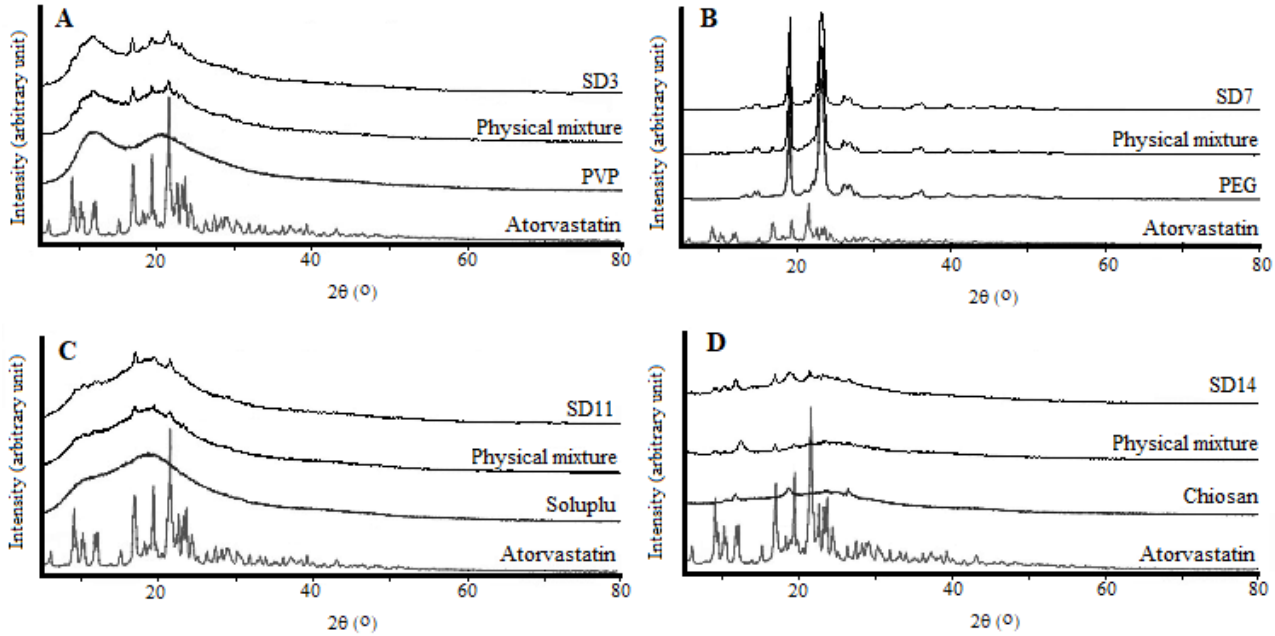

Fig. 2. Powder X-ray diffraction pattern of (A) atorvastatin, PVP, atorvastatin-PVP physical mixture and atorvastatin-PVP solid dispersion (SD3); (B) atorvastatin, PEG, atorvastatin-PEG physical mixture and atorvastatin-PEG solid dispersion (SD7); (C) atorvastatin, Soluplus®, atorvastatin-Soluplus® physical mixture and atorvastatin-Soluplus® solid dispersion (SD11); and (D) atorvastatin, chitosan, atorvastatin-chitosan physical mixture and atorvastatin-chitosan solid dispersion (SD14). PVP, polyvinyl pyrrolidone K30; PEG, polyethylene glycol; SD, solid dispersion.

Powder X-ray diffraction

PXRD of atorvastatin (Fig. 2) showed presence of sharp peaks at 2Θ equals to 6.10, 9.11, 9.42, 10.22, 10.50, 11.80, 12.13, 16.93, 18.79, 19.40, 21.54, 22.65, 23.25, 23.65, and 24.33 degrees. PXRDs of pure polymers matched well with published literature, without the appearance of any sharp peak for PVP with PXRD showing only two broad peaks in the range of 5-15, and 15-25 degrees (13). PXRD of PEG shows two characteristic peaks at 2Θ equal to 19.12, and 23.3 degrees. Also, PXRD diffractogram of Soluplus® indicates the absence of any characteristic peaks. Moreover, chitosan carrier shows a sharp characteristic peak at 2Θ equals to 15 degrees with two peaks at 2Θ equals to 10 and 20 degrees (23). These peaks were maintained in the prepared PM and SD.

Fourier transform infra-red spectroscopy

Fourier transform infra-red spectroscopy (FTIR) spectroscopy (Fig. 3) of atorvastatin shows, the absorbance peak at 3665 cm^{-1} indicating free O-H stretching. The peaks at 3364 and 1649 cm^{-1} indicate the stretching vibrations of N-H and C=C bond of the aromatic ring, respectively. The absorbance peak at 1105 cm^{-1} indicated O-H bending, and at 746 and 690 cm^{-1} indicated C-F stretching (24). FTIR spectrum for pure polymers also matched with previous reported data. For PVP, FTIR spectrum shows a broad peak from 3200 to 3700 cm^{-1} which were related to the presence of water in the polymer, this confirmed the result of DSC thermogram. Another two important peaks at 2953 and 1676 cm^{-1} were related to C-H stretching and C=O, respectively (13). Also for PEG, its spectrum shows important peaks at 3441, 2893, and 2099 cm^{-1} which were related to O-H, C-H, and C-O-C stretches, respectively (25). As well FTIR spectrum of Soluplus® shows aliphatic C-H stretching at 2924 cm^{-1} and C=O stretching at 1732 and 1634 cm^{-1}, the spectrum has been confirmed by comparing it to the previously studied spectrum (15). For chitosan carrier, FTIR spectroscopy illustrated a peak in the area between 1593 and 1643 cm^{-1} represented NH_2 scissoring vibration, the region between 1600 and 1400 cm^{-1} represented N-H bending vibration and the region between 1000 and 1150 cm^{-1} represented vibration of C-O-C, C-OH, and C-C in the ring. The presence of theses peaks indicated depolymerization of chitosan (20).

The peaks of atorvastatin were observed in the prepared PMs and SDs of PVP and PEG, while disappeared for chitosan and Soluplus® based SDs as well as for chitosan PM.

Scanning electron microscopy

SEM morphology study for atorvastatin prepared using different carriers by SCF process shown in Fig. 4 in comparison with their corresponding PMs. Atorvastatin appeared as rod-shaped crystals with smooth surfaces and partially agglomerated in bundles. In PMs, PVP K30 appeared as spherical balls, PEG 6000 appeared as bulky crystalline particles with a smooth surface, while, Soluplus® appeared as regular shapes with smooth surfaces, and chitosan carrier showed that the particles are less uniform in size. In all the prepared PMs and SDs, the particles of the drug were observed. It was noticed the change of Soluplus® and chitosan surfaces after exposing to SCF CO_2.

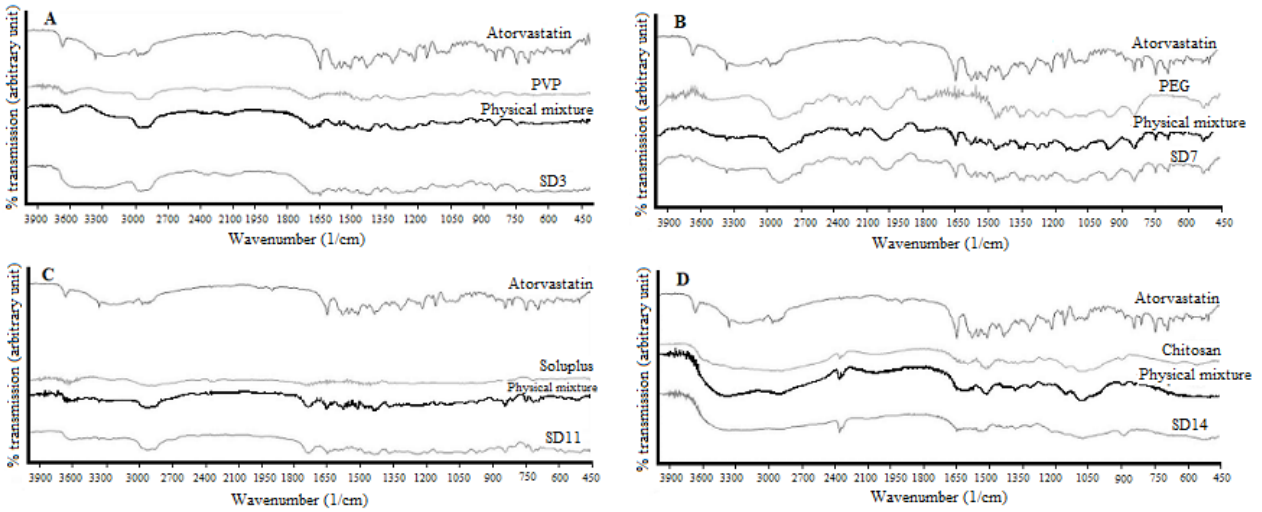

Fig. 3. Fourier transform infra-red spectroscopy of (A) atorvastatin, PVP, atorvastatin-PVP physical mixture and atorvastatin-PVP solid dispersion (SD3); (B) atorvastatin, PEG, atorvastatin-PEG physical mixture and atorvastatin-PEG solid dispersion (SD7); (C) atorvastatin, Soluplus®, atorvastatin-Soluplus® physical mixture and atorvastatin-Soluplus® solid dispersion (SD11); and (D) atorvastatin, chitosan, atorvastatin-chitosan physical mixture and atorvastatin-chitosan solid dispersion (SD14). PVP, polyvinyl pyrrolidone K30; PEG, polyethylene glycol; SD, solid dispersion.

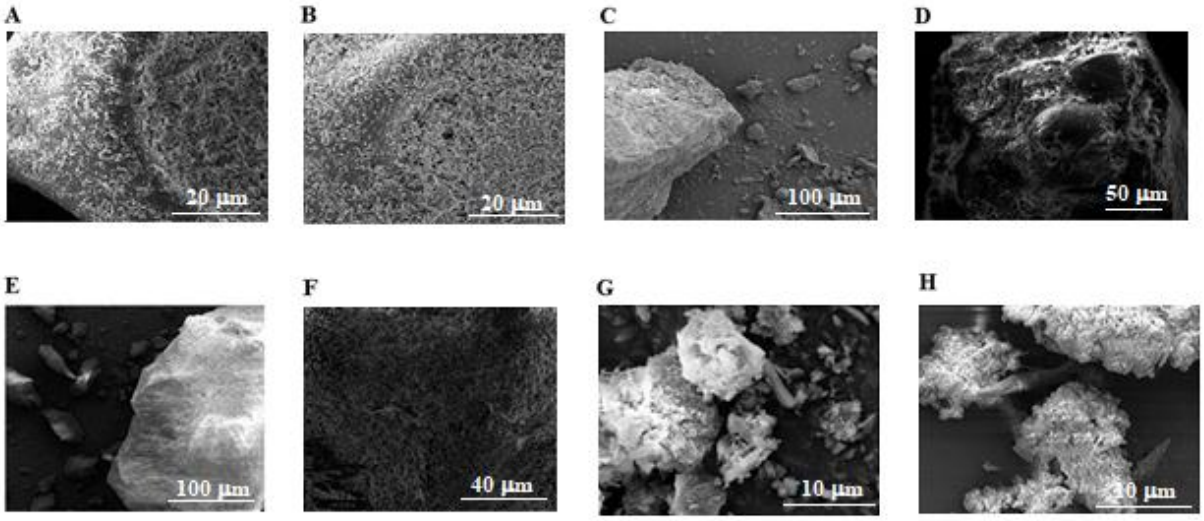

Fig. 4. Scanning electron microscopy photos of (A) atorvastatin-PVP solid dispersion (SD3), (B) physical mixture of atorvastatin and PVP, (C) atorvastatin-PEG solid dispersion (SD7), (D) physical mixture of atorvastatin and PEG, (E) atorvastatin-Soluplus® solid dispersion (SD11), (F) physical mixture of atorvastatin and Soluplus®, (G) atorvastatin-chitosan solid dispersion (SD14), and (H) physical mixture of atorvastatin and chitosan. PVP, polyvinyl pyrrolidone K30; PEG, polyethylene glycol; SD, solid dispersion.

Determination of drug content

The overall drug content (Table 1) exceeded 66.22% reaching values more than 100% in some preparations. Chitosan carrier based SDs showed the highest content of drug. It can be seen that PVP K30 and PEG 6000 based SDs showed an increase in drug content with increasing SCF processing temperature. Also, results showed that when processing time was 4 h for PVP K30-based SDs, drug content was lower than when processing time was 2 and 6 h. Also, drug content did not depend on SCF processing pressure for Soluplus® and chitosan based SDs.

In vitro release study

The dissolution profiles of atorvastatin, the prepared PMs, and SDs are shown in Fig. 5. The prepared PMs enhanced the dissolution profile of atorvastatin, except for the PM prepared using Soluplus®. PVP K30 revealed a slower dissolution rate. For all the dissolution profiles, the maximum reported error bar was ±14. The results of the mechanism of atorvastatin release are summarized in Table 2. Dissolution profiles of the prepared SDs were best fitted to Korsmeyer and Peppas except for SD5 which was best fitted to Higuchi equation.

Effect of pressure, temperature, loading time, and polymer to drug ratio on drug release

The effect of SCF processing parameters and the effect of polymer to drug ratio over drug release were evaluated (Fig. 5). Soluplus® and chitosan dispersions were used to study the effect of operational pressure while PEG 6000 and chitosan dispersions were used to study the effect of operational temperature. Also, the effect of polymer to drug ratio was evaluated for PEG 6000 SDs.

The drug release was affected by changing the pressure operator for Soluplus®, and chitosan dispersions; it was found that the drug release enhanced by decreasing the operational pressure. Also, the drug release was affected by changing the operational temperature for PEG 6000 chitosan-based SDs. The drug release enhanced by decreasing the operational temperature.

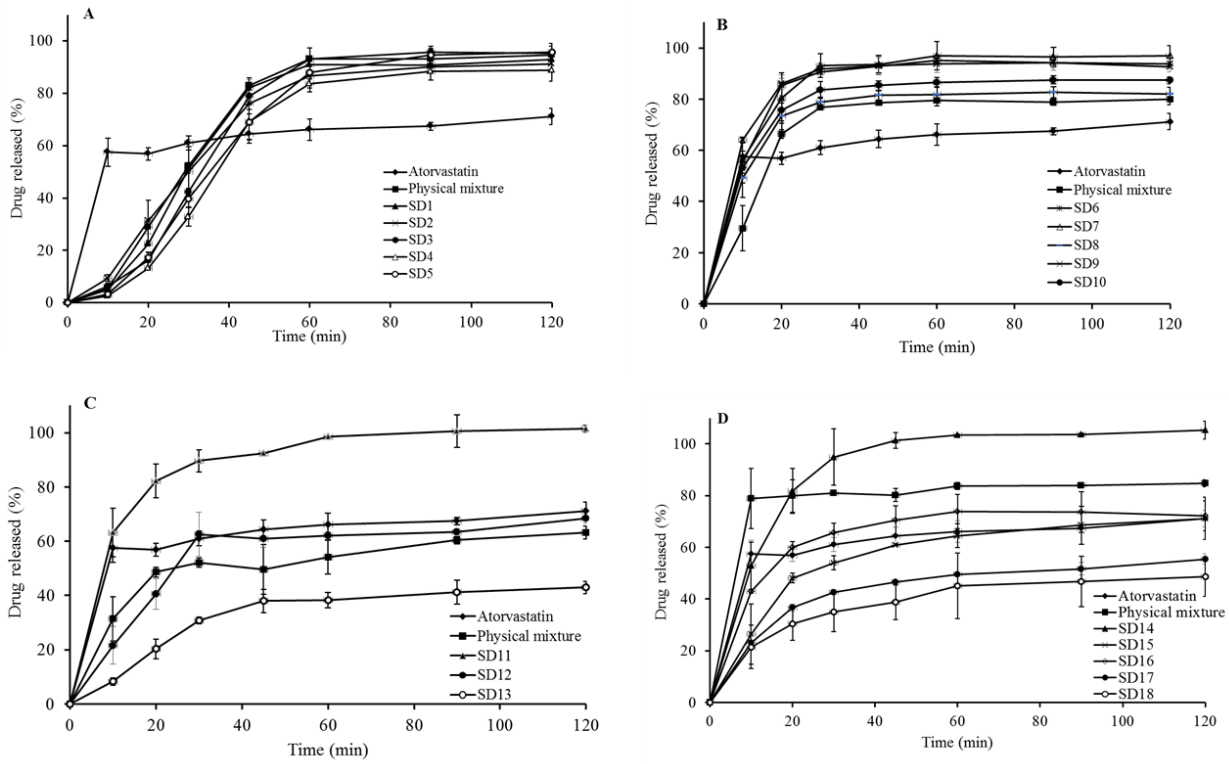

Fig. 5. Release profile of (A) atorvastatin, atorvastatin-PVP physical mixture and different atorvastatin-PVP solid dispersions; (B) atorvastatin, atorvastatin-PEG physical mixture and different atorvastatin-PEG solid dispersions; (C) atorvastatin, atorvastatin-Soluplus® physical mixture and different atorvastatin-Soluplus® solid dispersions; and (D) atorvastatin, atorvastatin-chitosan physical mixture and different atorvastatin-chitosan solid dispersions PVP, polyvinyl pyrrolidone K30; PEG, polyethylene glycol; SD, solid dispersion.

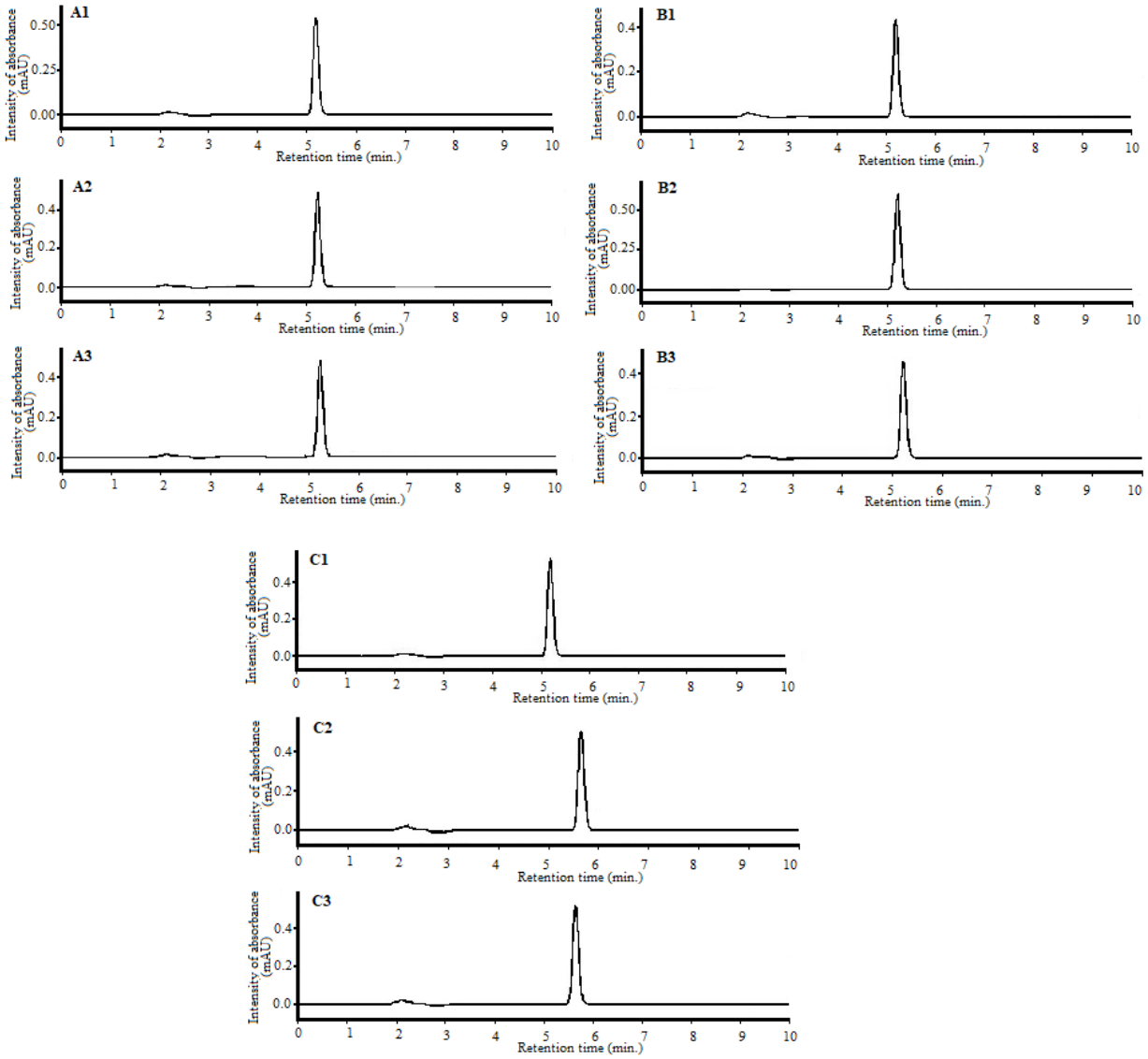

Fig. 6. High-performance liquid chromatography chromatograms for the stability studies of (A_1-A_3) atorvastatin-PEG solid dispersion (SD7), (B_1-B_3) atorvastatin-Soluplus® solid dispersion (SD11), and (C_1-C_3) atorvastatin-chitosan solid dispersion (SD14). The designated numbers, 1-3, in each part indicate (1) day 0, (2) day 90, at 30 ± 2 °C and 75 ± 5% relative humidity, and (3) day 90 at 40 ± 2 °C and 75 ± 5% relative humidity. SD, solid dispersion.

Stability

Chemical and physical stability was studied for selected SDs (SD3, SD7, SD11, and SD14). HPLC assay (Fig. 6), the drug content (Table 3), and PXRD (Fig. 7) were evaluated for PEG, Soluplus®, and chitosan based SDs. For HPLC assay, the results showed the presence of a single peak which was related to atorvastatin.

Moreover, the results of drug content indicated that there was no significant difference after three months ($P \leq 0.05$), except for PEG-based SD *i.e.* SD7 when it was stored at 40 ± 2 °C and 75 ± 5% relative humidity. Also, the PXRD pattern of PEG-based SD did not change after three months of storing. However, the PXRD of Soluplus® and chitosan based SDs, SD11 and SD14, respectively, exhibited an increase in the intensity of some peaks related to atorvastatin. On the other hand, a sticky paste has been formed by SD3 which indicates the instability of SD prepared using PVPK30.

Due to the formed sticky paste, samples could not be obtained for HPLC and PXRD analysis.

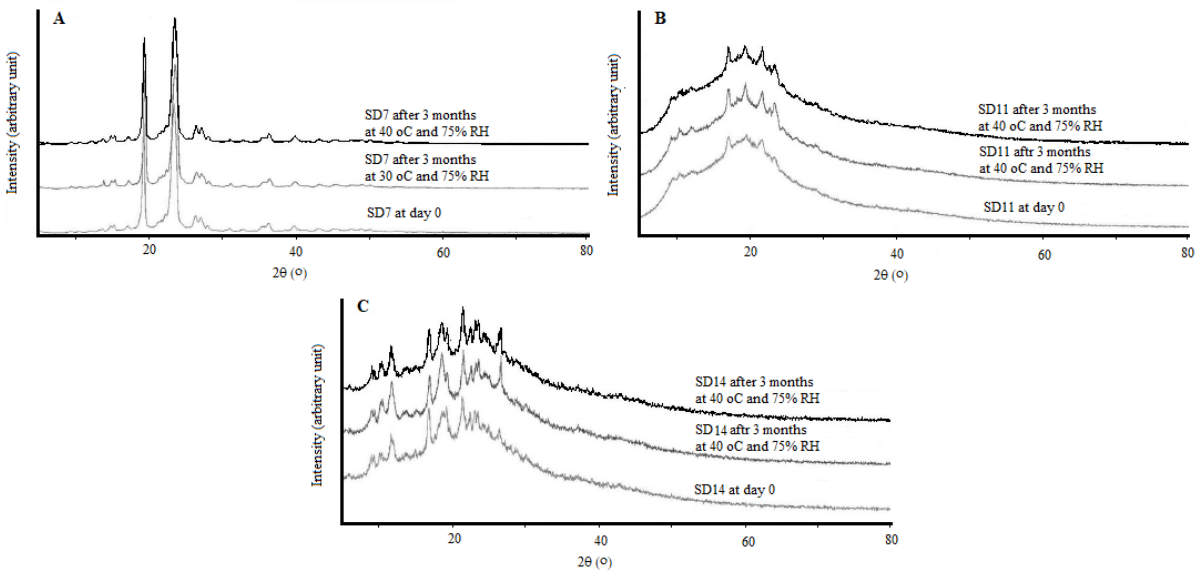

Fig. 7. Powder X-ray diffractions patterns during stability studies of atorvastatin, (A) atorvastatin-polyethylene glycol solid dispersion (SD7), (B) atorvastatin-Soluplus® solid dispersion (SD11), and (C) atorvastatin-chitosan solid dispersion (SD14).

Table 3. Loading efficiency (LE) of SD7, SD11, and SD14 at day 0 and 90. Data are presented as mean ± SD; n =

SDs	LE ± SD, Day 0	LE ± SD, Day 90 30 ± 2 °C and 75 ± 5% RH	*P* values	LE ± SD, Day 90 40 ± 2 °C and 75 ± 5% RH	*P* values
SD7	82.88 ± 3.34	90.33 ± 7.82	0.32	55.22 ± 4.65	**< 0.05**
SD11	77.79 ± 3.38	85.65 ± 5.43	0.22	83.26 ± 12.95	**0.62**
SD14	97.33 ± 4.77	109.52 ± 10.73	0.23	112.24 ± 6.59	**0.12**

DISCUSSION

The sharp endothermic peak of atorvastatin, which was obtained by DSC thermogram, was attributed to the melting point indicating the crystalline nature of the drug (26). Also, PXRD confirmed the presence of polymorph I. The results of DSC and PXRD indicated amorphous nature of PVP K30 (27). On the other hand, the crystalline nature of PEG 6000 was confirmed by the presence of a melting point in DSC thermogram and two characteristic peaks in PXRD. The absence of any characteristic peaks in the diffractogram pattern of Soluplus® indicated the amorphous nature of the polymer demonstrated by DSC thermogram too. Moreover, the PXRD of chitosan carrier indicated the formation of annealed polymorph (2). The disappearance of atorvastatin peak in PEG 6000-based SDs and PMs was due to the complete miscibility between the drug and the melted polymer which occurred during the heating cycle at 10 °C/min. Patil *et al*. has reported the same behavior for Gliclazide- PEG 6000 SD (25). Although DSC analysis of PEG 6000-based SDs and PMs showed complete miscibility. PXRD indicated the presence of crystalline material of atorvastatin (25). This emphasizes that thermal analysis alone is not enough to characterize polymers especially those with low melting point. While the shift in Tg of Soluplus® was related to dissolving of the drug in the polymer; Tg does not exhibit a shift in its value unless the two components are in one phase. It has been reported that Tg of a polymer is affected by the dissolved polymer and can be explained by the intermolecular interactions where a low molecular weight compound is dissolved in the polymer, modified its intermolecular interactions and replaced them with new secondary bonds, therefore the mobility of the system is altered and leads to increase or decrease in the

polymer`s Tg (28). FTIR spectrum results for dispersions prepared using PVP K30, PEG 6000, and chitosan indicated the absence of any chemical interaction between the drug and the polymers (13). While FTIR spectrum for Soluplus®-based SDs showed the interaction between the drug and the polymer; demonstrated by decrease peak intensity of N-H stretching bond at 3364 cm^{-1} such result has been reported previously (25). The weakening or disappearing of N-H stretching bond was due to the interaction of atorvastatin with Soluplus®. N-H of atorvastatin can form H-bond with C=O group of amide group of Soluplus®. SEM morphology indicated the presence of the drug in its crystalline nature, as it has been previously proved by PXRD. After exposing of Soluplus® to supercritical CO_2, its surface has been foamed and bubbles have been formed. Similar behavior reported for poly (methyl methacrylate) after it was exposed to supercritical CO_2 (29). Foaming can be related to the solubility of CO_2 in Soluplus® that is illustrated from the presence of characteristic FTIR peak at 1600 cm^{-1} indicating the presence of CO_2 inside the polymer. However, the foaming behavior of Soluplus®decreased by the presence of the drug, hence further future studies for Soluplus® should be performed.

Loading efficiency was good (≥ 66.22%) in prepared SDs. Exceeding 100% in loading efficiency can be attributed to polymer loss during the preparation process. Also, the increase in the loading efficiency with increasing of processing temperature for PVP K30 and PEG 6000 dispersions is in agreement with what was previously reported (8).

The results of HPLC indicated the chemical stability of SDs after exposing to the storage conditions for three months except for PVP-based SDs that showed sticky paste. PVP usually suffers from such long-term instabilities especially in high relative humidity conditions (30). Reported changes in both Soluplus® and chitosan is related to solubilization of CO_2 inside in a supercritical state (29). This can lead to changes upon storage.

Although the results of this study proved the ability of solvent-free SCF in dispersing the drug inside the polymer, yet precipitation of atorvastatin in these polymers was as a crystalline form unlike with tacrolimus SDs that were reported by Obaidat *et al*. (31). This illustrates the importance of studying physicochemical characteristics of the drug as well as the used polymer in SCF. Solubilization of the drug is very critical in precipitation of the drug in the amorphous form. Solubilization of the drug is supposed to occur in supercritical CO_2, or inside the polymeric material in the supercritical state with precipitation during depressurization. Utilizing solvent-free CO_2 is not enough to disperse all types of drugs in the amorphous form, and utilizing additional co-solvent would be necessary.

The enhancement of dissolution profile by PMs prepared using PVP K30, PEG 6000, and chitosan can be attributed to bringing the drug in close contact with hydrophilic polymers, which increased the wettability of the drug. However, the reduction of the dissolution profile for PMs prepared using Soluplus® may be related to gel formation and sticky nature of Soluplus® when it contacted with water. All the carriers showed an increase in the dissolution profile of atorvastatin; because of the hydrophilic properties and the solubilization power of the polymers (13). PVP K30 revealed a slower dissolution rate compared to other polymers which can be related to the formation of a gel layer by the hydrated polymer which led to slow diffusion of the drug from the polymer (32).

Higuchi equation describes the release of drugs from the insoluble matrix as a square root of time (33). Korsmeyer-Peppas equation is a semi-empirical model that correlates drug release with time by a simple exponential equation for a fraction of drug released. When $n \leq 0.43$ indicates Fickian release which means that release is diffusionally controlled. Intermediate values $0.43 < n < 0.85$ indicate a non-Fickian kinetics, anomalous or non-typical behavior, which means that the release is controlled by both diffusion and polymer relaxation. Values of $n > 0.85$ indicate a super case kinetic II transport which is transport or relaxation controlled release. This model is useful when there is more than one mechanism of the release or when the drug release mechanism is unknown (34).

All atorvastatin-PVP dispersions have n values above 0.85, which indicates super case kinetic II transport. Therefore the mechanism

of drug release could be a polymer relaxation, the initial slow release of the drug from PVP-based SDs is attributed to incomplete hydration of the polymer which led to incomplete relaxation. SD5 best fitted to Higuchi model, this mechanism was reported for PVP before (35). Atorvastatin-PEG 6000 dispersions had $n < 0.43$ that indicate Fikian diffusional characteristics. Atorvastatin-Soluplus® dispersions showed Fickian release for all the formulations, except for SD13 where the mechanism has been shifted to non-Fickian release. Soluplus®-based SDs have reported both mechanisms (36). Atorvastatin-chitosan dispersions release data fitting in Korsmeyer-Peppas model showed that n value was less than 0.43 (range: 0.199 - 0.365) which indicate Fickian diffusionally controlled release. This result is in agreement with what previously reported by Zou *et al* (37). They studied the release of bovin serum albumin from chitosan microspheres and they have found that the release behavior is Fickian diffusion suggesting drug diffusion through the swelled polymer spheres. The drug release was influenced by changing the operational pressure for Soluplus® and chitosan solid dispersions. It was generally found that decreasing the operational pressure facilitate drug release. This finding is a result of enhancing the partitioning between the drug and the polymer. Operational pressure also affects the transport properties of SCF CO_2. The viscosity of SCF CO_2 decreases along with diffusivity improvement with decreasing operational pressure. Therefore, the transport properties and plasticization are enhanced by decreasing operational pressure and the partitioning between the drug and the polymers enhances too (8). Also, the drug release was affected by changing the operator temperature for PEG 6000 and chitosan dispersions. The release of drug enhanced by decreasing the operational temperature. Finally, ratio between the used polymer to the drug is not the only factor that affects drug release. Operational condition has a profound effect on drug performance.

CONCLUSION

SCF technology proved to have great potential in preparing dispersions for BCS class II drugs. A good loading efficiency was obtained for all the polymers. Dissolution enhancement of atorvastatin was achieved through successful preparation of polymeric dispersions of the drug using SCF technology without further addition of solvents. Great enhancement of dissolution profile was obtained using Soluplus® and PEG solid dispersions showing high rate and percentage of release for the drug. The possibility of hydrogen bonding between the drug and Soluplus® was proved using FTIR.

ACKNOWLEDGEMENTS

This work was financially support by the Deanship of Research at Jordan University of Science and Technology (JUST) under the Grant No. 206/2015. The authors would like to acknowledge Scientific Research Funds (SRF) at Ministry of Higher Education (Amman, Jordan) for providing our lab with SCF unit (MPH/2/15/2013).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest for this study.

AUTHORS' CONTRIBUTION

B. Altaani, R. Obaidat, and W. Malkawi proposed the experiments and research design. W. Malkawi performed the experiments with supervision of R. Obaidat and B. Altaani . R. Obaidat analyzed the results of solid-state characterizations with contribution of other authors. B. Altaani analyzed the results of in vitro drug release and drug assay with contribution of others authors. B. Altaani wrote the manuscript for publication with help of other authors.

REFERENCES

1. Badens E, Majerik V, Horvath G, Szokonya L, Bosc N, Teillaud E, *et al*. Comparison of solid dispersions produced by supercritical antisolvent and spray-freezing technologies. Int J Pharm. 2009;377(1-2):25-34.
   DOI: 10.1016/j.ijpharm.2009.04.047.
2. Obaidat RM, Tashtoush BM, Bayan MF, Al Bustami RT, Alnaief M. Drying using supercritical

fluid technology as a potential method for preparation of chitosan aerogel microparticles. AAPS PharmSciTech. 2015;16(6):1235-1244. DOI: 10.1208/s12249-015-0312-2.
3. Maniruzzaman M, Boateng JS, Snowden MJ, Douroumis D. A Review of hot-melt extrusion: process technology to pharmaceutical products. ISRN Pharm. 2012;2012:436763-436771. DOI: 10.5402/2012/436763.
4. *Kamalakkannan V, Puratchikody A, Ramanathan L.* Development and characterization of controlled release polar lipid microparticles of candesartan cilexetil by solid dispersion. Res Pharm Sci. 2013;8(2):125-136.
5. Sharma A, Jain CP. Preparation and characterization of solid dispersions of carvedilol with PVP K30. Res Pharm Sci. 2010;5(1):49-56.
6. Dixit M, Kini AG, Kulkarni PK. Preparation and characterization of microparticles of piroxicam by spray drying and spray chilling methods. Res Pharm Sci. 2010;5(2):89-97.
7. Tabbakhian M, Hasanzadeh F, Tavakoli N, Jamshidian Z. Dissolution enhancement of
glibenclamide by solid dispersion: solvent evaporation versus a supercritical fluid-based solvent - antisolvent technique. Res Pharm Sci. 2014;9(5):337-350.
8. Obaidat RM, Tashtoush BM, Awad AA, Al Bustami RT. Using supercritical fluid technology (SFT) in preparation of tacrolimus solid dispersions. AAPS PharmSciTech. 2017;18(2):481-493. DOI: 10.1208/s12249-016-0492-4.
9. Vo CL, Park C, Lee BJ. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. Eur J Pharm Biopharm. 2013;85(3 Pt B):799-813. DOI: 10.1016/j.ejpb.2013.09.007.
10. Shamma RN, Basha M. Soluplus®: a novel polymeric solubilizer for optimization of carvedilol solid dispersions: formulation design and effect of method of preparation. Powder Technol. 2013;237:406-414. DOI: 10.1016/j.powtec.2012.12.038.
11. Maestrelli F, Zerrouk N, Chemtob C, Mura P. Influence of chitosan and its glutamate and hydrochloride salts on naproxen dissolution rate and permeation across Caco-2 cells. Int J Pharm. 2004;271(1-2):257-267. DOI: 10.1016/j.ijpharm.2003.11.024.
12. Zhong L, Zhu X, Luo X, Su W. Dissolution properties and physical characterization of telmisartan-chitosan solid dispersions prepared by mechanochemical activation. AAPS PharmSciTech. 2013;14(2):541-550. DOI: 10.1208/s12249-013-9937-1.
13. Hu L, Gu D, Hu Q, Shi Y, Gao N. Investigation of solid dispersion of atorvastatin calcium in polyethylene glycol 6000 and polyvinylpyrrolidone. Trop J Pharm Res. 2014;13(6):835-842. DOI: 10.4314/tjpr.v13i6.2.
14. Yin YM, Cui FD, Kim JS, Choi MK, Choi BC, Chung SJ, *et al*. Preparation, characterization and *in vitro* intestinal absorption of a dry emulsion formulation containing atorvastatin calcium. Drug Deliv. 2009;16(1):30-36. DOI: 10.1080/10717540802481380.
15. Ha ES, Baek IH, Cho W, Hwang SJ, Kim MS. Preparation and evaluation of solid dispersion of atorvastatin calcium with soluplus(R) by spray drying technique. Chem Pharm Bull (Tokyo). 2014;62(6):545-551. DOI: 10.1248/cpb.c14-00030.
16. Kim JS, Kim MS, Park HJ, Jin SJ, Lee S, Hwang SJ. Physicochemical properties and oral bioavailability of amorphous atorvastatin hemi-calcium using spray-drying and SAS process. Int J Pharm. 2008;359(1-2):211-219. DOI: 10.1016/j.ijpharm. 2008.04.006.
17. Aggarwal S, Gupta GD, Chaudhary S. Solubility and dissolution enhancement of poorly aqueous soluble drug atorvastatin calcium using modified gum karaya as carrier: *in vitro-in vivo* evaluation. Int J Drug Deliv. 2012;4(3):341-365. DOI: 10.5138/ijdd.v4i3.75.
18. Lakshmi Narasaiah L, Kalyan Reddy K, Kishore K, Raj Kumar R, Srinivasa Rao S, Venkateswara Reddy V. Enhanced dissolution rate of atorvastatin calcium using solid dispersion with PEG 6000 by dropping method. J Pharm Sci & Res. 2010;2(8):484-491.
19. Qandil AM, Obaidat AA, Mohammed Ali MA, Al-Taani BM, Tashtoush BM, Al-Jbour ND, *et al*. Investigation of the interactions in complexes of low molecular weight chitosan with ibuprofen. J Solution Chem. 2009;38(6):695-712. DOI: 10.1007/s10953-009-9405-4.
20. Obaidat R, Al-Jbour N, Al-Sou'd K, Sweidan K, Al-Remawi M, Badwan A. Some physico-chemical properties of low molecular weight chitosans and their relationship to conformation in aqueous solution. J Solution Chem. 2010;39(4):575-588. DOI: 10.1007/s10953-010-9517-x.
21. Shen H, Zhong M. Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin. J Pharm Pharmacol. 2006;58(9):1183-1191. DOI: 10.1211/ jpp.58.9.0004.
22. Lee JY, Kang WS, Piao J, Yoon IS, Kim DD, Cho HJ. Soluplus(R)/TPGS-based solid dispersions prepared by hot-melt extrusion equipped with twin-screw systems for enhancing oral bioavailability of valsartan. Drug Des Devel Ther. 2015;9:2745-2756. DOI: 10.2147/DDDT.S84070.
23. Ioelovich M. Crystallinity and hydrophility of chitin and chitosan. Res Rev: J Chem. 2014;3(3):7-14.
24. Lv HX, Zhang ZH, Jiang H, Waddad AY, Zhou JP. Preparation, physicochemical characteristics and bioavailability studies of an atorvastatin hydroxypropyl-beta-cyclodextrin complex. Pharmazie. 2012;67(1):46-53. DOI: 10.1691/ph.2012.1082.
25. Patil MP, Gaikwad NJ. Characterization of gliclazide-polyethylene glycol solid dispersion and its effect on dissolution. Braz J Pharm Sci. 2011;47(1):161-166.

DOI: 10.1590/S1984-82502011000100020.
26. Bathool A, Vishakante GD, Khan MS, Shivakumar HG. Development and characterization of atorvastatin calcium loaded chitosan nanoparticles for sustain drug delivery. Adv Mat Lett. 2012;3(6):466-470.
DOI: 10.5185/amlett.2012.icnano.153.
27. Abd El-kader M, Abu-Abdeen M. Thermal and mechanical studies of PVP/2-HEC blend films. Aust J Basic & Appl Sci. 2012;6(13):454-462.
28. Terife G, Wang P, Faridi N, Gogos CG. Hot melt mixing and foaming of soluplus® and indomethacin. Polym Eng Sci. 2012;52(8):1629-1639.
DOI: 10.1002/pen.23106.
29. Rende D, Schadler LS, Ozisik R. Controlling foam morphology of poly(methyl methacrylate) via surface chemistry and concentration of silica nanoparticles and supercritical carbon dioxide process parameters. J Chem. 2013;2013:864926,1-13.
DOI: 10.1155/2013/864926.
30. Caron V, Hu Y, Tajber L, Erxleben A, Corrigan O, McArdle P, *et al*. Amorphous solid dispersions of sulfonamide/soluplus® and sulfonamide/PVP srepared by ball milling. AAPS PharmSciTech. 2013;14(1):464-474.
DOI: 10.1208/s12249-013-9931-7.
31. Obaidat RM, Tashtoush BM, Awad AA, Al Bustami RT. Using supercritical fluid technology (SFT) in preparation of tacrolimus solid dispersions. AAPS PharmSciTech. 2017;18(2):481-493.
DOI: 10.1208/s12249-016-0492-4.
32. Roohullah, Iqbal Z, Nasir F, Akhlaq M, Sadozai SK, Zada A, *et al*. Sustained release carbamezapine matrix tablets prepared by solvent-evaporation technique using different polymers. Middle East J Sci Res. 2013;15(10):1368-1374.
DOI: 10.5829/idosi.mejsr.2013.15.10.7553.
33. Shoaib MH, Tazeen J, Merchant HA, Yousuf RI. Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pak J Pharm Sci. 2006;19(2):119-124.
34. Parize A, Stulzer H, Laranjeira M, Brighente I, Souza T. Evaluation of chitosan microparticles containing curcumin and crosslinked with sodium tripolyphosphate produced by spray drying. Quim Nova. 2012;35(6):1127-1132.
DOI: 10.1590/S0100-40422012000600011.
35. Shah I, Bhatt S, Yadav A. Enhancement of solubility and dissolution of nebivolol by solid dispersion technique. Int J Pharm Pharm Sci. 2014;6(7):566-571.
36. Sambath L, Muthu AK, Kumar MA. Soluplus complexation influence the release of lovastatin from porous osmotic pump tablet. World J Pharm Pharm Sci. 2013;2(5):3506-3521.
37. Zou X, Zhao X, Ye L, Wang Q, Li H. Preparation and drug release behavior of pH-responsive bovine serum albumin-loaded chitosan microspheres. J Ind Eng Chem. 2015;21:1389-1397.
DOI: 10.1016/j.jiec. 2014.06.012.