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Silymarin-Laden PVP-Nanocontainers Prepared Via the Electrospraying Technique for Improved Aqueous Solubility and Dissolution Rate

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Abstract: The aim of the present research was to develop a silymarin-laden PVP-nanocontainer providing ameliorated aqueous solubility and dissolution of the drug. Several silymarin-laden formulations were formed with varying quantities of PVP and SDS via the solvent evaporation method using the electrospraying technique. The influence of the hydrophilic carriers on solubility and dissolution was explored. The solid-state characterization was carried out by particle-size analysis, PXRD, DSC, FTIR and SEM. All of the formulations demonstrated better solubility and dissolution than did silymarin plain powder. Both the SDS and PVP had positive effects on solubility and dissolution of silymarin in the aqueous media. An increased solubility was attained as the drug/PVP ratio was 1/4; however, further increase in PVP did not provide significant improvement. In particular, a nanocontainer formulation prepared with silymarin, PVP and SDS (1/4/0.5, w/w/w) exhibited the best solubility (26432.76 ± 1749.00 μg/mL) and an excellent dissolution (~92 % in 20 min) than did silymarin plain powder. Also, it demonstrated similar dissolution profiles compared to a commercial product; therefore, might be bioequivalent to the commercial product ($f_1 = 3$ and $f_2 = 69$). Moreover, cumulative undersize distribution values as represented by $X_{10}$, $X_{50}$ and $X_{90}$ were 201 ± 21.01 nm, 488 ± 36.05 nm and 392 ± 48.10 nm, respectively. The drug existed in the amorphous state in the PVP-nanocontainers with no strong chemical bonding with other excipients. Thus, this formulation might be used for more effective administration of silymarin via the oral route.

Keywords: silymarin; electrospraying; PVP-nanocontainers; aqueous solubility; dissolution.
INTRODUCTION

Silymarin, a mixture containing flavonolignans such as silybin, silychristin, isosilybin and silydianin, is extracted from milk thistle plant (Silybum marianum). Silybin is the main active pharmaceutical entity amongst these flavonolignans [1]. Silymarin exerts remarkable hepatoprotective effects [2]; therefore, it has been successfully playing its role in the supportive treatment of hepatic morbidities and adversities [3]. Solubility in the aqueous gastrointestinal fluid and permeation across cell membranes are essential for absorption of a drug through the GIT. The drugs whose absorption is impaired owing to low or no solubility are placed in class II of Biopharmaceutics Classification System (BCS). The oral bioavailability of silymarin is extremely poor owing to its insufficient aqueous solubility [4]. Several solubility enhancing approaches, such as self-emulsifying drug delivery systems [5], formulation with phosphatidylcholine [6], formulation with cyclodextrins [7], formulation with lecithin [8] and solid dispersion formation [9], have been effectively employed for improving the aqueous solubility of silymarin.

Nanotechnology has performed a significant role in the amelioration of the aqueous solubility and dissolution of numerous very slightly soluble or practically insoluble drugs [10]. Natural hydrophilic polymeric matrices are popular excipients in pharmaceutical nanotechnology owing to their excellent safety, biocompatibility and biodegradation [11]. A pharmaceutical nano-sized particle is a drug-loaded object possessing a diameter of about 1 \( \mu m \) or less [12,13]. Development of nano-sized solid dispersions can cope with the low aqueous solubility and dissolution related problems of a number of very slightly soluble or practically insoluble substances [14,15]. A solid dispersion is a drug delivery tool which contains a very slightly soluble or water-insoluble active pharmaceutical ingredient dispersed in a hydrophilic polymeric matrix [15]. It may be developed with or without a solubilizing agent. A ternary solid dispersion is a three component system which consists of a drug, a solubilizing agent and a hydrophilic polymeric excipient (Figure 1) while a binary solid dispersion is a two component system containing a drug and a hydrophilic polymeric matrix [16]. Ternary solid dispersions are more effective in resolving the aqueous solubility problems of a number of water-insoluble drugs than binary solid dispersions [16]. Amongst the solid dispersion preparation methods, the solvent evaporation method has augmented the aqueous solubility, dissolution and oral bioavailability of poorly water-soluble drugs the most [17].

Moreover, the electrospaying technique of nanoparticle formation has successfully worked for numerous drug groups, such as antihyperlipidemics [10], antibiotics [18,19], anti-inflammatories [20,21] and hormones [22,23]. Accordingly, the solvent evaporation method in conjunction with the electrospaying technique is the best strategy to achieve nanoparticles of ternary solid dispersions [10,24]. In this technique, the drug, hydrophilic polymeric material and solubilizing agent are entirely dissolved in an appropriate solvent or solvent mixture. The resultant transparent solution is then electrosprayed (Figure 2) [10]. An electrosprayed nano-sized particle of a ternary solid dispersion is a spherical solid particle consisting of uniformly dispersed molecules of a drug and a solubilizing agent in a hydrophilic polymeric matrix (Figure 1) [11].
In the present study, a number of ternary solid dispersions were fabricated with silymarin, polyvinylpyrrolidone (PVP) and sodium dodecyl sulphate (SDS) via the solvent evaporation method using the electrospraying technique. The influence of PVP and SDS quantities on solubility and dissolution of silymarin in the solid dispersions was investigated. The optimized silymarin-laden PVP-nanocontainer, exhibiting the highest aqueous solubility and dissolution, was subjected to solid state characterization by particle-size analysis, powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Moreover, the dissolution profiles of the optimized formulation were compared with those of a commercial product.

MATERIAL AND METHODS

Materials

Silymarin (Purity ≥ 95%) was from Xi'an Xin Sheng Bio-Chem Co. (Xi'an, Shaanxi, China). Polyvinylpyrrolidone (PVP K30) and Sodium dodecyl sulphate (SDS) were from Sigma-Aldrich Co. (St. Louis, MO, USA). Legalon capsules (Bukwang, Seoul, South Korea) were used as the commercial product. All other chemicals were of reagent grade.

Preparation of silymarin-laden PVP-nanocontainers

All the formulations were electrosprayed using ESR 100 NanoNC electrospinning instrument (Seoul, South Korea). The compositions of formulations are shown in Table 1. For each formulation, silymarin, PVP and SDS were comprehensively dissolved in ethanol to obtain an absolutely transparent solution. Subsequently, the solution was filled in a glass syringe (Hamilton Co., Reno, NV, USA) and the syringe was mounted on the instrument. The solution was sprayed at a flow rate of 0.3 mL/hr and under the influence of an optimum voltage of 9.5-10.5 kV. The nanoparticles collecting plate was 20 cm apart from the spraying nozzle. The dried formulation was preserved in a clean, dry and air-tight conical tube placed at room temperature.
Table 1. Compositions of silymarin-laden PVP-nanocontainers.

| Constituents (g) | I  | II | III | IV | V  | VI | VII | VIII |
|------------------|----|----|-----|----|----|----|-----|------|
| Silymarin         | 1  | 1  | 1   | 1  | 1  | 1  | 1   | 1    |
| PVP              | 3  | 3  | 3   | 3  | 3  | 4  | 6   | 10   |
| SDS              | 0.1| 0.2| 0.3 | 0.4| 0.5| 0.5| 0.5 | 0.5  |

**Aqueous solubility test**

For each formulation, about 20 mg quantity was poured into a 300 μL of distilled water placed in a 2 mL microtube. After vortex-mixing, the tubes were placed in a water-bath at 25°C and shaken at 100 rpm for 5 days. Subsequently, the samples were vortex-mixed again and centrifuged at 5,000 x g for 5 min (Hanil Science Industrial Co., Smart 15, Gangneung, South Korea). One hundred fifty microliter of supernatant was poured in another clean microtube and diluted with 150 μL of the mobile phase. The diluent was passed through a syringe filter (0.45 μm) [10]. Fifty microliter filtrate was analyzed by the HPLC (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, CA, USA). A Capcell Pak ACR 5 μm C18 column having dimensions of 4.6 mm I.D. x 150 mm (Shiseido, Tokyo, Japan) was used for quantification of silymarin. The mobile phase was consisted of components A and B at the volume ratio of 95/5 respectively. The component A was comprised of methanol, acetic acid and phosphate buffer (pH 3.0) at the volume ratio of 34/6/60 respectively. Acetonitrile was used as component B. The mobile phase, eluting at a flow rate of 1 mL/min, was monitored at 285 nm for determination of silymarin titre [2].

**Estimation of the entrapment efficiency and silymarin content in PVP-nanocontainers**

In this method of PVP-nanocontainers formation, all the constituents were entirely dissolved in ethanol to get a clear solution before electrospraying; thus, it was assumed that the entrapment efficiency was equivalent to the drug content. For each formulation, an accurately weighed sample equivalent to 10 mg silymarin was completely dissolved in 100 mL of ethanol. The expected strength of the solution was 100 μg/mL. The clear solution was filtered (0.45 μm) and analyzed for silymarin content employing the HPLC method as explained above. For each formulation, the test was carried out in triplicate. The drug content was calculated by the following formula: \( S_c = \frac{S_a}{S_t} \times 100 \). Where, \( S_c \) is the silymarin content, \( S_a \) is actual concentration quantified by the HPLC, and \( S_t \) is the theoretical strength.

**Dissolution studies**

The dissolution study was accomplished using the USP dissolution tester II (Vision® Classic 6TM, Hanson Research Co., Los Angeles, CA, USA). Each formulation, equivalent to 30 mg of silymarin, was sealed in a dialysis bag (Spectra/Per® dialysis membrane, MWCO 3500, Spectrum Laboratories, Rancho Domínguez, CA, USA) and enclosed in the sinker. Then, the sinker was dropped in 500 mL of 2% (w/v) aqueous Tween 80 [25] placed in the dissolution vessel and maintained at 37 ± 0.1°C. The paddle rotated at an intentional speed of 75 rpm [26]. At fixed time points, 1 mL of the dissolution medium was taken with the help of a sampler and filtered (0.45 μm). One milliliter of fresh dissolution medium was poured into to the dissolution vessel after each sampling. Fifty microliter filtrate was assayed by the HPLC method as elucidated above. The same procedure was adopted to obtain the dissolution profiles of the commercial product.

**Particle-size analysis**

The selected PVP-nanocontainer formulation was perused using a laser diffraction particle-size analysis equipment (HELOS H1918, Sympatec GmbH, System-Partikel-Technik, Clausthal-Zellerfeld, Germany) for determining cumulative
undersize distribution. The values of $X_{10}$, $X_{50}$ and were recorded at the undersize of 10%, 50% and 90% of total volume, respectively.

**Powder X-ray diffractometry**

For examining the crystalline property of the samples, they were subjected to scanning using a Rigaku X-ray diffractometer (D/MAX-2500 PC, Rigaku Corporation, Tokyo, Japan). The perusal was accomplished using a Cu Kα1 monochromatic radiation ($\lambda = 1.54178 \ \text{Å}$) source with 30 mA current and 40 kV voltage. The diffraction spikes were transcribed in the range of 10–70°C with a 2θ scanning mode, an angular increment of 0.02°/second and a scan rate of 10°/minute.

**Differential scanning calorimetry**

The thermal physiognomies of the samples were determined using a differential scanning calorimeter (DSC Q20, TA Instruments, New Castle, Delaware, USA). About 2-5 mg of each sample was enclosed in an aluminium pan and heated at the rate of 10°C/min over a range of 30–120°C. The scanning was conducted in the presence of a nitrogen flow of 25 mL/min.

**Fourier transform infrared spectroscopy**

The FTIR spectra of silymarin powder, PVP, SDS, physical mixture and PVP-nanocontainers were recorded. Each sample was perused over a range of 600–4000 cm$^{-1}$ with 2 cm$^{-1}$ resolution using a Nicolet-6700 FTIR spectrophotometer (Pittsburgh, PA, USA). The physical mixture was a combination of triturated silymarin, PVP and SDS at the optimized ratio of 1/4/0.5 (w/w/w).

**Scanning electron microscopy**

The morphological features of particles of silymarin plain powder and selected PVP-nanocontainer formulation were observed using a scanning electron microscope (S-4800, Hitachi, Tokyo, Japan). A carbon tape was clung on a metallic disc using its one adhesive side while the other exposed adhesive side of the tape was used as a platform for adhering the samples. Subsequently, the adhered samples were subjected to platinum coating (EMI Teck Ion Sputter - K575K) for 5 min under 8 × 10$^{-3}$ mbar pressure using 15 mA current and 100% turbo speed. The purpose of the coating was to make the samples electrically conductive for subsequent scanning by the electron microscope for imaging.

**Statistical methods**

For solubility testing, analysis of three samples was performed for each formulation or plain drug powder (n = 3). The mean value and standard deviation were calculated using MS Excel. Release test was carried out in sextuplicate (n = 6). The mean and standard deviation of six values were determined using MS Excel. Furthermore, six values of percent drug released achieved at a particular time point for the optimized formulation were compared with the corresponding values of each formulation separately using t-test, and p-value was calculated. Likewise, comparison of release rates of the optimized formulation with those of the commercial product was done.

**RESULTS AND DISCUSSION**

Nanoparticles can resolve the aqueous solubility, dissolution rate and oral bioavailability related problems of various BCS class II drugs [27]. Electrospraying technique is a promising strategy to fabricate pharmaceutical polymeric nanoparticles [28]. Ternary solid dispersions remarkably optimize the aqueous solubility, dissolution rate and bioavailability of several low water-soluble or water-insoluble chemical entities [16]. The solvent evaporation method is considered as the best method for the preparation of solid dispersions as far as enhancement in the aqueous solubility, dissolution rate and oral bioavailability are
concerned [17]. The concomitant use of PVP and SDS as hydrophilic carriers in the formation of ternary solid dispersions has enormously ameliorated the aqueous solubility and dissolution of numerous drugs [29-32]. Accordingly, in the present research, silymarin-laden PVP-nanocontainers of ternary solid dispersions were prepared with PVP and SDS via the solvent evaporation method using the electrospraying technique.

In the solvent evaporation method of solid dispersion formation, relatively higher quantities of excipients are required [33]; therefore, three times higher quantity of PVP was used against that of silymarin initially. The compositions of all the formulations are shown in Table 1. In order to determine the influence of SDS concentration on the aqueous solubility and dissolution rate of silymarin, formulations I-V were prepared with different quantities of SDS while keeping silymarin/PVP quantity constant at 1/3 (w/w). A significant enhancement in solubility and dissolution was observed with increasing quantities of SDS owing to its solubilizing property. In particular, amongst the formulations I-V, formulation V consisting of silymarin, PVP and SDS (1/3/0.5, w/w/w) exhibited the most optimized solubility (17555.78 ± 1424.25 μg/mL) and dissolution rate (84.98 ± 2.59 % in 20 min) of the drug (Figure 3A and Figure 3B). Thus, this formulation was chosen for further testing the effect of PVP concentration on the aqueous solubility and dissolution rate of the drug. Formulations VI-VIII were formed with different concentrations of PVP while keeping the drug/SDS quantity constant at 1/0.5 (w/w). The increase in the quantity of PVP in formulations VI-VIII significantly improved the aqueous solubility and dissolution rate of the drug as compared to formulation V (P < 0.05). On the other hand, an apparent decrease in the aqueous solubility was witnessed with increasing quantity of PVP in formulations VI-VIII; however, their solubility and dissolution results were not significantly different (P > 0.05) from one another (Figure 3A and Figure 3B). This apparent decrease in solubility with increasing concentration of PVP in formulations VI-VIII was owing to higher quantity of PVP against the quantity of loaded drug which resulted in supersaturation [10,34]. As a result, further dissolution of PVP-nanocontainers, and thereby solubility of the loaded silymarin, was ceased. In short, amongst all the formulations, the nanocontainer formulation VI containing silymarin, PVP, and SDS (1/4/0.5, w/w/w) showed apparently the most optimized solubility (26432.76 ± 1749.00 μg/mL) and dissolution rate (92.69 ± 2.69 % in 20 min) of the drug. The enhancement in the aqueous solubility of silymarin was ~1100-fold (26432.76 ± 1749.00 vs. 22.76 ± 7.89 μg/mL) and dissolution rate (92.69 ± 2.69 vs. 25.18 ± 2.59 % in 20 min) compared to plain drug powder; therefore, this formulation was selected for solid state characterization by particle-size analysis, PXRD, DSC, FTIR and SEM.
Figure 3. Effect of SDS (I-V) and PVP (VI-VIII) on the aqueous solubility (A) and the dissolution rate (B) of silymarin in the electrospayed nanocontainers. Each value of solubility and dissolution rate denotes the mean ± S.D. (n = 3 and 6, respectively). *p < 0.05 compared with the silymarin plain powder and formulations I-V.

Prior to the dissolution rate determination, the drug content in all the formulations was investigated. All the formulations showed drug content in the range of 99-101 %. This suggested that during the preparation of PVP-nanocontainers via the solvent evaporation method using the electrospraying technique neither of the components was lost and the product contained uniformly intermingled constituents. In the solvent evaporation method of PVP-nanocontainers formation, all the constituents were entirely dissolved in ethanol to get a clear solution before electrospaying. Also, higher quantity of PVP was used against the quantity of drug in each PVP-nanocontainer formulation. Accordingly, it was assumed that all the drug remained entrapped in the polymeric matrix in the dried PVP-nanocontainer after...
electrospraying. Thus, entrapment efficiency was considered equivalent to drug content in the present study.

The dissolution profiles of PVP-nanocontainer formulation VI and the commercial product were compared using similarity factor ($f_2$) and difference factor ($f_1$) as recommended by the Food and Drug Administration (FDA) [35]. As a general rule, $f_1$ value should be $\leq 15$ while $f_2$ value should be $\geq 50$ for a dissolution profile of a product similar to that of a reference standard [35]. The silymarin-laden PVP-nanocontainer formulation VI furnished $f_1$ and $f_2$ value of 3 and 69, respectively. This suggested that the dissolution profiles exhibited by PVP-nanocontainer formulation VI were similar to those of the commercial product (Figure 3B). Thus, our optimized formulation might be bioequivalent to the commercial product.

The particle-size cumulative distribution data of PVP-nanocontainer formulation VI is shown in Figure 4. The values of $X_{10}$, $X_{50}$ and $X_{90}$ were 201 ± 21.01 nm, 488 ± 36.05 nm and 392 ± 48.10 nm, respectively. Moreover, polydispersity index (PDI) calculated for this sample was 0.405. This confirmed that the particles were the ‘nanoparticles’ as the particle-size was $< 1000$ nm [12,13].

In the PXRD analysis, silymarin plain drug powder showed some minute peaks suggesting the presence of some crystalline entity in it (Figure 5A, a). PVP did not show any peak suggesting its amorphous nature (Figure 5A, b). SDS generated some sharp peaks reflecting its crystalline nature (Figure 5A, c). The physical mixture also exhibited some minute peaks corresponding to silymarin and SDS (Figure 5A, d). The physical mixture was prepared by mixing silymarin, PVP and SDS (1/4/0.5, w/w/w) using a pestle and mortar. The PVP-nanocontainer formulation VI did not reveal any peak suggesting that all the crystalline components were converted to the amorphous state (Figure 5A, e). In essence, PVP blocked recrystallization during electrospraying process [10]. In the solvent evaporation method of solid dispersion preparation, the crystalline constituents are converted to the amorphous state which also enhances the aqueous solubility and dissolution rate of the drugs [17].
In the DSC analysis, silymarin (Figure 5B, a) and PVP (Figure 5B, b) showed broad endotherms suggesting that they retain moisture as these broad endotherms are characteristics of escape of moisture from the samples [2]. SDS was crystalline in nature (Figure 5B, c). The physical mixture also showed the broad endotherm which was owing to the presence of moisture in silymarin and PVP (Figure 5B, d). On the other hand, in case of PVP-nanocontainer formulation VI, no endotherm was witnessed (Figure 5B, e). Thus, in the preparation of PVP-nanocontainers via the solvent evaporation method using the electrospraying technique, all the crystalline components were converted to the amorphous
form [10]. Moreover, considerable moisture was evaporated during the preparation process which might improve stability by preventing any possible hydrolysis of the product [36].

The FTIR spectra are shown in Figure 5C. The physical mixture was a blend of triturated silymarin, PVP and SDS at the optimized ratio of 1/4/0.5 (w/w/w). Silymarin plain powder produced sharp peaks at 643, 737, 782, 823, 996, 1024, 1083, 1127, 1164, 1269, 1364, 1463, 1513, 1632 cm\(^{-1}\) (Figure 5C, a). Both the PVP (Figure 5C, b) and SDS (Figure 5C, c) furnished high-pitched peaks at the same points; accordingly, the peaks of silymarin were overlaid by them. Therefore, there was no differentiating peak of silymarin in the spectrum of physical mixture (Figure 5C, d). However, the spectrum generated by the PVP-nanocontainer formulation VI (Figure 5C, e) was very similar to that produced by the physical mixture (Figure 5C, d) and there was no major shift of peaks; this suggested non-existence of covalent interactions amongst components of the formulation.

The scanning electron micrographs of silymarin plain drug powder and silymarin-laden PVP-nanocontainer formulation VI are shown in Figure 6A and Figure 6B, respectively. Silymarin powder was consisted of irregular-shaped particles with uneven surfaces (Figure 6A). On the other hand, PVP-nanocontainers were smooth-surfaced round-shaped particles with a dimple on one side (Figure 6B). Furthermore, a pharmaceutical nanocontainer is a round-shaped drug-loaded particle possessing a diameter of about 1 \(\mu\)m or less [12,13]. In addition to the abovementioned results of particle-size analysis, Figure 6B also revealed that all the particles were nano-sized having diameter less than 1 \(\mu\)m.

**CONCLUSION**

All the formulations demonstrated ameliorated aqueous solubility and dissolution rate of the drug in the order of VI=VII=VIII>V>IV>III>II>I>Silymarin powder. In particular, the PVP-nanocontainer formulation VI composed of silymarin, PVP and SDS (1/4/0.5, w/w/w) had a particle-size of <1000 nm. All the components were present in the amorphous state in this formulation. It considerably ameliorated silymarin solubility (26432.76 ± 1749.00 \(\mu\)g/mL) and dissolution (~92% in 20 min) compared to plain drug powder. The enhancement in solubility and dissolution rate might be attributed to the hydrophilic matrices in the formulation which improved wettability and solubilization, complete conversion of all the components to the amorphous state and decreased particle-size of the formulation which resulted in greater surface area available for rapid dissolution. Moreover, the optimized formulation might be bioequivalent to the commercial product; accordingly, it can be a useful candidate to administer silymarin with improved bioavailability. Nevertheless, in vivo experimentation in animal models will be conducted in our next studies to further establish the influence of this PVP-nanocontainer formulation on the oral bioavailability and hepatoprotective efficacy of silymarin.

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