Preparation of Zaleplon Microparticles Using Emulsion Solvent Diffusion Technique

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Abstract

Zaleplon is a BCS class II drug suffering from poor solubility. In a trial to improve its dissolution, emulsion solvent diffusion method (ESD) was used to prepare zaleplon microparticles using sodium lauryl sulfate (SLS) as surfactant. Optimization of drug system was achieved. Particle size values of the prepared microparticles were ranged from 6.57 μm to 20.30 μm with narrow particle size distribution range. Differential scanning calorimetry (DSC) and x-ray diffraction (XRD) were used for the characterization of the prepared systems. DSC and XRD patterns showed that the zaleplon microparticles possessed decreased crystallinity. Dissolution studies demonstrated that the systemized zaleplon microparticles exhibited significantly enhanced dissolution rate when compared to pure zaleplon. A correlation could be observed between the microparticle morphology and the dissolution rate, where zaleplon microparticles that exhibited the fastest dissolution profiles had a distinctive rod shape compared to the other systems. In contrast, pure zaleplon powder appeared as irregularly-shaped particles. In conclusion, the dissolution rate of zaleplon can be enhanced to a great extent by ESD technique.

Keywords: Zaleplon; Poor water solubility; BCS class II drug; Emulsion solvent diffusion; Microparticles

Introduction

Zaleplon is a nonbenzodiazepene hypnotic drug that is used for the treatment of insomnia [1]. It has a pyrazolopyrimidine structure that selectively binds to the benzodiazepine (BZ1) - receptor subtype (located on the gamma-aminobutyric acid (GABA) receptor complex in the brain) resulting in demonstrated sedative, anxiolytic, muscle relaxant and anticonvulsant activity [2]. Zaleplon also is a potential anticonvulsant against phenylenetetrazole- and electroshock-induced convulsions [1].

Zaleplon suffers from poor aqueous solubility, which has a negative effect on its bioavailability. It has been determined that zaleplon has an absolute bioavailability of -30%, because of extensive presystemic metabolism during the first pass [3,4].

To overcome the problems caused by poor aqueous solubility of zaleplon, some researchers have developed new drug delivery systems of zaleplon. Zaleplon loaded proliposomes prepared using hydrogenated soyphosphatidylcholine and cholesterol and solid dispersions of zaleplon using different hydrophilic carriers were prepared and have shown enhancement in the dissolution rate of zaleplon [5,6].

Recent advances in particle engineering technology significantly enhance delivery of poorly water-soluble drugs [7]. According to the Noyes–Whitney equation; the dissolution rate of poorly water-soluble drugs could be increased by reducing the particle size to the micro scale thus increasing the interfacial surface area [8,9]. Micro-sized particle systems could be applied to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility and so its bioavailability [10]. Therefore, micro-particle technology could play a major role in the successful development and marketability of a poorly water soluble drug.

Emulsion solvent diffusion (ESD) method was developed from sphaerical crystallization technique [11] and proposed by Kawashima et al. [12,13]. ESD is an effective way to prepare drug-loaded polymeric micro/nanoparticles for masking taste, drug targeting, controlling release, etc. [14,15]. In ESD method, drug and polymer are dissolved in a suitable partially water soluble solvent with or without a bridging liquid. Subsequently, the drug- solvent phase is emulsified in an aqueous solution (poor solution) containing stabilizer, leading to solvent diffusion to the external phase under stirring and immediately the emulsion droplets are formed in the external poor solution. The solvent diffuses out of the droplets as the ESD proceeds and water diffuses into the droplets. Therefore, the drug and polymer are co-precipitated, leading to emulsion droplets solidification. Finally, the solvent is eliminated by evaporation or filtration [16,17].

ESD technique was chosen to prepare micro-sized drug particles due to its simplicity, low cost, success with poor aqueous solubility drugs and the production of micro-sized particles of relatively high drug loading [18,19]. This technique was used to prepare aspirin, riboflavin and roxithromycin microspheres [14,20]. As with some of the other techniques, ESD is efficient in enhancing water solubility of lipophilic drugs [21]. Disadvantages of ESD technique are the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency and the high water volumes to be eliminated from the suspension.

The aim of this study was to prepare zaleplon microparticles utilizing ESD method using sodium lauryl sulfate (SLS) as surfactant. In this study, no bridging liquid was introduced. Different process and formulation variables were optimization to obtain zaleplon microparticles. Also, the possible particle formation mechanism was explained and the experimental parameters were investigated, and the resulting zaleplon microparticles were characterized in detail.

Materials and Methods

Materials

Zaleplon (Batch No. 1401032009) was kindly supplied by The Egyptian Co. for Pharmaceutical and Chemical Industries, S.A.E.
(EPCI), Industrial Zone, Bayad El-Arab, Beni Suef, Egypt. Its purity was reported to be 98.5% according to the company analysis certificate. Acetone, Sodium lauryl sulphate (SLS) and Methanol were obtained from Adwic, El-Nasr pharmaceutical company (Egypt).

The chemical structure of zaleplon is shown as follows (Structure 1):

![Chemical structure of zaleplon](image)

The chemical structure of zaleplon.

Methods

Preparation of Zaleplon microparticles: Zaleplon powder was dissolved in acetone, and the solution was filtered through a 0.45 μm filter membrane to remove any solid impurities. Ten ml of zaleplon solution (20 mg/ml) was poured at once into 100 ml distilled water containing SLS under magnetic stirring (1000 rpm). The system turned opaque instantly after pouring the solution into water and was stirred for different time intervals. The mixture was then kept for about 6–10 minutes till the translucent emulsion droplets turned into opaque particles. The solidified particles were obtained by filtration and washed with distilled water then the resultant filter paste was dried in an oven at 60°C for 12 hours [16]. The composition of the prepared systems is shown in table 1.

In vitro evaluation of the prepared Zaleplon microparticles:

Percentage drug yield and drug content study: The dried Zaleplon microparticles were weighed and the percentage yield of the prepared particles was calculated by using the following formula:

\[
\text{Percentage drug yield} = \frac{\text{Actual weight obtained of dried particles}}{\text{Calculated weight}} \times 100
\]

Drug content was determined by dissolving dried particles equivalent to 10 mg of zaleplon in methanol and kept in sonicator for 20 minutes. The volume was adjusted to 100 ml with distilled water. The solution was suitably diluted and absorbance was measured at 232 nm.

Studies of Zaleplon microparticles dissolution: The dissolution rate studies of pure zaleplon powder and dried particles were performed in a dissolution apparatus using the paddle method, according to USP Type II. Dissolution study was carried out in a 900 ml of distilled water at 37 ± 0.5°C at a rotation speed 75 rpm according to US FDA guidelines [22]. 10 mg of pure drug or its equivalent amount of dried particles was added to 900 ml of distilled water. At time intervals of 2, 4, 6, 8, 10, 15, 20, 25, 30, 45 and 60 min, 5 ml of samples were withdrawn and replaced with an equivalent amount of the fresh distilled water in order to maintain the volume in the vessel constant. The solution was immediately filtered through a 0.45 μm filter membrane and analyzed spectrophotometrically for zaleplon content by measuring the absorbance at λmax 232 nm. The results given are mean of three runs ± SD.

Characterization of Zaleplon microparticles:

Particle size analysis: The size of the pure drug and of dried particles of different system was determined by light scattering using a laser diffraction particle size analyzer (masterizer). In addition to mean particle size, span was calculated to indicate the width of particle size distribution curve [23]. Span could be calculated by the following equation:

\[
\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}}
\]

where DN% (N=10%, 50% and 90%) means that the volume percentage of microparticles with diameters DN% equal to N%. The smaller the span value, the narrower the size distribution [24].

Fourier Transformation Infrared Spectroscopy (FTIR): Samples of 2-3 mg were mixed with about 400 mg of dry potassium bromide powder then pressed to obtain self-supporting disks. The IR spectra between 4000 and 400 cm⁻¹ of the pure drug and different systems were recorded.

Differential scanning calorimetry (DSC) studies: Differential scanning calorimetry was performed using Shimadzu differential scanning calorimeter, DSC-50. The apparatus was calibrated with purified indium (99.9%). Samples (3-4 mg) were placed in flat-bottomed aluminum pan and heated at a constant rate of 10°C/minute, in an atmosphere of nitrogen in a temperature range of 20-350°C. The DSC studies were performed for the pure drug and the selected systems.

X-ray diffraction (XRD): The X-ray diffraction patterns were recorded at room temperature using Scintag XGEN-4000 diffractometer. The samples were irradiated with Ni filtered Cu Ka radiation, at 45 K voltage and 40 mA current. The scanning rate employed was 2°/minute over a diffraction angle (2θ) range of 3–70°. X-ray diffraction was performed for the pure drug and the selected systems.

Scanning electron microscope imaging: Pure zaleplon and the selected systems were undergone scanning electron microscope imaging. The samples were coated with gold and imaged with scanning electron microscope (Jeol JSM-6400, Tokyo, Japan). Imaging was done at magnification 500 for zaleplon and 3500 for the other systems.

Results and Discussion

In vitro evaluation of the prepared Zaleplon microparticles:

Percentage drug yield and drug content study: Results show
that all the systems showed percentage drug yield above 75%. Some of zaleplon (after being water soluble) were lost into the dispersing medium during processing [25]. Since the particles were produced from SLS aqueous solution, some SLS might remain in the Zaleplon microparticles. Therefore, purity of the drug was examined using UV-spectrophotometer. The results showed that zaleplon contents for the particles reached more than 86% (86.83 ± 0.23 to 99.69 ± 0.36%), which proved that most of the SLS had been removed by washing the filter paste several times with distilled water.

**Studies of Zaleplon microparticles dissolution**: The dissolution profiles of zaleplon and different prepared systems in distilled water at 37°C ± 0.5°C are shown in figure 1. Figure 2 show the results of in-vitro dissolution data and the percentage drug dissolved in four minutes for zaleplon and its systems respectively.

All prepared systems have shown improvement in dissolution rate compared to pure drug alone. Within the prepared systems; S4 was found to be superior to the corresponding systems. S4 system (prepared by stirring for 15 minutes at 30°C using 0.01% SLS) showed significantly higher percentage of drug dissolved in four minutes compared with other systems (p<0.05). This is attributed to that stirring for 15 minutes allowed enough time to the ESD technique to proceed resulting in good emulsion droplet formation unlike stirring for 5 and 10 minutes (S1 and S2) which was not sufficient for the ESD process to complete. S4 showed 88% of drug dissolved after four minutes compared to 48% and 44% from S1 and S2 respectively.

Also, different SLS concentrations were investigated to understand the effects of SLS on dissolution of the prepared Zaleplon microparticles. It was found that 0.01% SLS (lowest SLS concentration used) resulted in the highest zaleplon dissolved during the first four minutes. This is in accordance with results obtained by Lee et al. [26] where low surfactant concentration resulted in decreasing size of the produced particles and so fast dissolution was obtained.

**The possible particle formation mechanism of zaleplon**: Zaleplon molecule contains hydrophilic parts (methylacetamide group) and hydrophobic parts (phenyl group), and can be generally regarded as an amphiphilic drug. After dissolving zaleplon in a good solvent (acetone) and pouring it into the aqueous phase (poor solvent), the zaleplon hydrophilic parts tend to align with the aqueous phase, whereas the hydrophobic parts are repelled from the aqueous phase. Then, the zaleplon molecules, which exist in the contact surface of water-miscible solvent and water, spontaneously assemble into bilayers [27,28]. To minimize the surface energy, these bilayers close up into small vesicles in which the good solvent and zaleplon molecules are enriched and then small emulsion droplets are formed. As a result of the miscibility of the solvent system, the good solvent and water counter-diffuse out of and into the droplets, respectively [29]. At the same time, these small droplets can coalesce into big droplets due to the collision and interfacial tension variation [30,31]. Zaleplon in the droplets becomes supersaturated with the progression of solvent diffusion process. The droplets are gradually solidified to form uniform drug particles. SLS was used as surfactant and stabilizer.

**Characterization of Zaleplon microparticles**

**Particle size analysis**: Figure 3 shows mean particle size values (µm) and span of the particles prepared by ESD method. It was observed that particle size values were ranged between 6.57 µm and 20.30 µm.

It was noticed also that span values were ranged between 1.61 and 2.34 which indicated narrow particle size distribution curve.

S4 system (15 minutes stirring at 30°C using 0.01% SLS) showed a particle size of 6.57 µm and the span as low as 1.61, which indicated smaller and narrower particle size distribution curve than the other systems, showing that ESD method at this conditions resulted in micro-particles uniform in size. However, micro-particles of other systems were of larger size and have wider particle size distribution compared to S4 system. Without SLS addition (S6), relatively larger particles (a mean size of 20.30 µm) with irregular size distribution were obtained. SLS decrease the interfacial tension and the diffusion property was modified. As a result, smaller particles with lower span values were formed [16]. However, the emulsion droplets formed at
the initial stage could no longer adsorb more SLS molecules when its concentration increased over a certain level (0.01%); meanwhile, the interfacial tension kept increasing with the decrease of solvent concentration in the droplets. So the coalescence of droplets occurred in almost the same way under different SLS concentrations, causing nearly the same mean size of the other resultant particles.

The diffusion and evaporation rate of solvents at lower temperature became slow and the transient emulsion droplets could not maintain their shape until the solidification [26]. With the increase of temperature above 30°C, the interfacial tension of the droplets increased due to the decreasing density of SLS adsorbed on the droplet surface which caused the droplets to coalesce into bigger ones. Also, the kinetic energy of the droplets was increased with the increase of temperature, resulting in higher opportunity and intensity of the mutual collision, which promoted the droplets coalescence [32]. Figure 4 shows the percentage of zaleplon dissolved at four minutes in comparison with mean particle size (μm) of different prepared systems. System S4 which has smallest particle size gives the highest percentage of zaleplon dissolved at four minutes. This is in accordance to the Noyes–Whitney equation which states that the dissolution rate of poorly water-soluble drugs increased by reducing the particle size to the micro-scale [8,9].

Fourier transformation infrared spectroscopy (FTIR): Figure 5 shows IR spectra of zaleplon and different systems of zaleplon. IR spectrum of zaleplon is characterized by principal absorption peaks at 3,081 cm\(^{-1}\) (C–H aromatic), 2,933 cm\(^{-1}\) (C–H aliphatic), 2,228 cm\(^{-1}\) (C≡N), 1,650 cm\(^{-1}\) (C=O), 1,545 cm\(^{-1}\) (C=N), 1,224 cm\(^{-1}\) (C–N), 1,478 cm\(^{-1}\) (C=C aromatic) and 695 cm\(^{-1}\) and 796 cm\(^{-1}\) (m substituted benzene). Zaleplon shows strong absorption peaks at 2,228 cm\(^{-1}\) and 1,650 cm\(^{-1}\) indicating presence of cyanide and amide carbonyl group respectively while, peaks at 695 and 796 cm\(^{-1}\) may be assigned to aromatic stretching of the phenyl group in the molecule which is m-substituted.

The IR spectra of all systems show the same characteristic peaks of zaleplon in the same regions and at the same ranges and there was no new bands observed. This might indicate that the addition of SLS and the employment of ESD process did not change the chemical composition of zaleplon.

S4 which showed the highest percentage of drug dissolved after four minutes and smallest particle size was selected for further investigation. Plain zaleplon powder and zaleplon systems; S3 and S6 were also evaluated. Systems S3 and S6 were chosen based on that they contained the highest and lowest percentages of SLS (0.1% and 0% for S3 and S6 respectively) to examine the effect of SLS concentration on zaleplon microparticles formation.

Differential scanning calorimetry (DSC) studies: The DSC thermograms of zaleplon and systems (S3, S4 and S6) are represented in Figure 6. The DSC thermograms of zaleplon alone showed endothermic Tmax of 186.67°C, corresponding to the melting point of crystalline form of the drug zaleplon.

The sharp melting point peak of pure zaleplon appeared at 186.67°C, corresponding to the melting point of crystalline form of the drug, whereas this peak appeared but with decreased intensity.
especially in S4 system indicating that zaleplon was molecularly dispersed [33].

Zaleplon has a high melting point (186.67°C), which is indicative of strong crystal lattice energy. This high melting point is one of the factors responsible for lower aqueous solubility [34]. Therefore, any method which can disrupt the crystalline nature and/or result in lower crystal lattice energy would improve the aqueous solubility of zaleplon from the solid state. The crystalline nature of zaleplon can be disrupted by dissolving the drug in a suitable solvent then the solution is added into an aqueous medium where the emulsion droplets are immediately formed. As the ESD proceeds, the drug is co-precipitated, leading to the solidification of emulsion droplets which replace the drug molecule in the crystal lattice. This leads to a total or partial loss of crystallinity, resulting in a significant increase in solubility and dissolution rate of zaleplon.

X-ray diffraction (XRD): XRD study was performed to determine the physical state of the zaleplon powder, S3, S4 and S6 systems. The corresponding patterns are displayed in figure 7. The characteristic peaks of the drug were still existing in S3 and S6 systems but with lower intensity. Whereas, the intensity of characteristic peaks of the drug was greatly minimized in the case of system 4, which could be attributed to the destruction of the drug crystal lattice because of progressive amorphization [35]. It is obvious that poorly water soluble pharmaceuticals with lower crystallinity usually have higher dissolution rate and bioavailability [36,37]. Accordingly, the decrease in crystallinity of as-prepared Zaleplon microparticles is expected to improve its dissolution rate and bioavailability.

Scanning electron microscope imaging: Scanning electron micrographs of pure zaleplon and S3, S4 and S6 systems were shown in figure 8. Zaleplon powder showed irregular-shaped particles with a wide particle size distribution. Comparatively, S4 particles prepared by ESD method at 30°C using 0.01% SLS and stirred for 15 minutes appeared to be uniform short rods with smooth surface. However, irregular rods with coarse surface could be observed when using 0.1%
SLS (S3 system). Without the addition of SLS (S6 system), relatively big particles were obtained. This suggests that the morphology were strongly dependent on the SLS concentration.

**Conclusion**

ESD method was employed to prepare uniform micronized Zaleplon microparticles with enhanced dissolution. In this process, the particle size and morphology could be well controlled by temperature, stirring time and SLS concentration. Particle size values ranging from 6.57 μm to 20.30 μm with span values ranged between 1.61 and 2.34 were obtained. The solid-state properties of the micronized particles characterized by powder XRD and DSC showed gradual decrease in the crystallinity of drug. The dissolution rate of zaleplon was significantly enhanced by ESD technique. Based on the results, Optimized system containing 0.01% SLS and stirred for 15 minutes at 30°C could be a promising system for the preparation of solid dosage forms containing zaleplon.

**References**


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