Preparation and Evaluation of Andrographolide Solid Dispersion Vectored by Silicon Dioxide

Dingkun Zhang, Junzhi Lin, Fang Zhang, Xue Han, Li Han, Ming Yang, Wenquan Zou

INTRODUCTION

Many newly developed active pharmaceutical components exhibit low oral bioavailability due to their poor water solubility,[1] thereby posing widely acknowledged difficulties in drug development. For drugs in class II of the biopharmaceutics classification system (BCS), the dissolution rate after oral administration is the key factor that limits the absorption rate. Andrographolide (Andro, structure are shown in Figure 1), a diterpene lactone derived from the stems and leaves of Andrographis paniculata (Burm.f.), has been used in traditional Chinese medicine and is regarded as a “natural antibiotic” as well as a typical insoluble drug. The purpose of this study was to investigate the feasibility of commercially available silica (SiO2) as a carrier of solid dispersion to enhance the dissolution of Andro. Materials and Methods: The solvent evaporation method was adopted, and a series of process parameters were studied to prepare a solid dispersion. Andro, SiO2, physical mixture, and solid dispersion were characterized with respect to particle size distribution, special surface area, pore volume, and scanning electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction studies. Results: Single factor test suggested the best preparation of solid dispersion was the drug and carrier (SiO2:B) ratio of 1:8, with tetrahydrofuran as the solvent, and a recovery temperature of 50°C. Compared to crude drug and mixture, solid dispersion was found to form a unique structure to disperse the drug and displayed superior performance in rapid dissolution. Conclusion: The present study signifies the commercially available SiO2 is an excellent but cheap carrier to improve the dissolution of Andro. Our results provide a highly operability approach for improving the dissolution of insoluble natural products and are beneficial for the clinical effects improvement.

Key words: Andrographolide, dissolution, evaluation, silicon, solid dispersion

SUMMARY

• The potential of commercially available silica as a carrier for enhancing the insoluble drug dissolution was investigated
• Factors affecting the dissolution of solid dispersion were investigated
• Solid dispersion formed a unique structure to disperse the drug and release drug rapidly

• Commercially available silica is an excellent but cheap carrier to improve the dissolution of Andro.


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alumina,[18] calcium silicate,[19] and porous starch.[20] These materials have a pore structure throughout the whole bulk phase, as well as large specific surface area and porosity, which provides uniquely supports for the adsorption and release of water insoluble drugs.[21] Due to the confinement effect of the nanometer-sized pores, the dissolution rate of drugs can be improved by decreasing the particle size, increasing the surface area, or decreasing the crystallinity.

The release rate of the drug in the porous carrier is mainly affected by the pore size, pore shape, and surface functional groups. Pore size mainly affects the release kinetics, as dissolution rate depends on the degree of compatibility between the pore size and the drug molecule. To a certain extent, the release rate is accelerated as the aperture increases; however, if the pore size is too large, the crystalline state of the drug will increase.[22] Pore shape can generally be divided into two types: Ordered and disordered. The former is characterized by a uniform pore size, ordered dimension, periodic array, and constant release rate; on the other hand, the pore channels extend in all directions in the latter. Generally, disordered pores have improved dissolution rates. Surface functional groups affect the binding force between the drug and the carrier. For most candidate drugs, the release rate is decreased by an amino-modified carrier surface but increased by a methyl-modified one.[18,23]

Unlike mesoporous SiO₂, ordinary commercial SiO₂ has a disordered pore structure, with various pore sizes and connecting channels. This special structure has additional functional applications that include solidifying volatile oils to improve stability,[24,25] promoting the absorption of self-emulsifying drug delivery systems in the intestines,[26,27] decreasing the disintegration time of dispersion tablets,[28] and improving the yield and quality of spray-dried herbal extracts.[29] To investigate the feasibility and reliability of ordinary commercial SiO₂ as a carrier for improving the oral delivery of poorly water soluble drugs, we designed a series of screening tests to optimize the preparation. The capacity of the SiO₂ as a drug delivery system was demonstrated by in vitro tests. Next, the solid state properties and other pharmaceutical properties were characterized by particle size distribution, special surface area and pore volume, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). Finally, the stability of the solid dispersion was studied to ensure the application.

**MATERIALS AND METHODS**

Andrographolide (99.32%) was purchased from Sichuan Houde Medical Technology Co. Ltd (Chengdu, China). SiO₂ A-E were of pharmaceutical grade and purchased or received as a gift from the Chengdu Kelong Chemical Reagent Company (Chengdu, China), Anhui Shanhe Pharmaceutical Excipients Co., (Anhui, China) Henan Chaoqun Chemical Plant (Henan, China), Wuhan Chu Fengyuan Pharmaceutical Co., Ltd. (Hubei, China) and Xi’an Yuelai Pharmaceutical Co., Ltd. (Shanxi, China), respectively; methanol, dehydrate alcohol, acetone, tetrahydrofuran, and sodium dodecyl sulfate (SDS) were of analytical grade and purchased from Chengdu Kelong Chemical Reagent Company (Sichuan, China); KBr was of spectral grade and purchased from Tianjin Guangfu Fine Chemical Institution (Tianjin, China); Methanol of high-performance liquid chromatography (HPLC) grade was purchased from Promtart Ltd. (Elk Grove, CA, United States); water was purchased from Watsons (China).

**Preparation of solid dispersion**

**Operating conditions**

Andro powder and all kinds of SiO₂ powder were dried in an oven at a temperature of 60°C for 5 h to remove moisture. An amount of 0.5 g of Andro powder and 120 mL of organic solvent were placed in a 250 mL round-bottomed flask and were subjected to ultrasonic treatment (250 W, 40 kHz) at room temperature for 10 min to completely dissolve the drug powder. An appropriate amount of SiO₂ was added, and the resulting mixture was stirred with a magnetic stirrer for 5 min. The solvent was then removed by a rotary evaporator (the bath temperature was 60°C, the vacuum pressure was ~0.08 MPa, and the speed was 100 rpm/min). The powder was collected, transferred to an evaporating basin, and dried at an oven temperature of 60°C for 1 h, until no residual solvent remained. After passing through a 100 mesh sieve, the solid dispersion was finally obtained.

**Drug loading and analysis**

Drug content was determined by dissolving 10 mg of solid dispersion in 50 mL of methanol and analyzing the sample using HPLC. HPLC was performed on a Agilent chromatographic system (1200, Agilent, United States), and an Welchrom C18 column (4.6 mm × 250 mm, 5 μm, Shanghai Yuexu Material Technology Co., Ltd., China) was used. Methanol–water (60:40, v/v) was used as the mobile phase at a flow rate of 1 mL/min. The ultraviolet detection was conducted at 225 nm. The calibration curve was y = 1.883x + 6.423 (r = 1.0000, n = 6), which exhibited an excellent linearity over a concentration range of 3.125–100 μg/mL of Andro. The drug content on solid dispersion was measured by HPLC as described above, and drug loading was calculated by the following equation:

\[
\text{Drug loading} \times 100\% = \left( \frac{\text{weight of drug in solid dispersion}}{\text{weight of solid dispersion}} \right) \times 100\%
\]

**Dissolution study**

The dissolution study was performed by placing the solid dispersion equivalent to 90 mg of Andro in 900 mL of 0.2% SDS, using the paddle method at 100 rpm and 37 ± 0.5°C. Dissolution medium (1 mL) was withdrawn at 5, 15, 30, 45, 60, and 90 min through a 0.22 μm microporous membrane, and analyzed using HPLC.

**Selection of carrier proportion**

While selecting the carrier proportion, ethanol was identified as solvent, due to its safety and environmental protection. SiO₂ was obtained from B (Anhui Shanhe Pharmaceutical Excipients Co.) and the solvent temperature was 60°C. Dissolution studies in 0.2% SDS were investigated at five ratios (2:1, 1:1, 1:2, 1:4, and 1:8), and the mean drug release rate was calculated.

**Selection of solvent type**

While selecting the type of solvent, the ratio of drug and carrier was identified according to previous data. The SiO₂ used was from B (Anhui
Sunhe Pharmaceutical Excipients Co., Ltd.) and the solvent recovery temperature was 60°C. Dissolution studies in 0.2% SDS were investigated using four kinds of solvents (methanol, dehydrate alcohol, acetone, and tetrahydrofuran), and the mean drug release rate was calculated.

**Selection of silica type**

While selecting the type of SiO2, the ratio of drug, carrier, and solvent was identified according to previous data. The solvent recovery temperature was 60°C. Dissolution studies in 0.2% SDS were investigated using five kinds of SiO2, provided by five manufacturers (A, B, C, D, and E), and the mean drug release rate was calculated.

**Selection of solvent recovery temperature**

While selecting the solvent recovery temperature, the ratio of drug and carrier, solvent and the source of SiO2 were identified according to previous data. Dissolution studies in 0.2% SDS were investigated at four temperatures (40, 50, 60, and 70°C), and the mean drug release rate was calculated.

**Verification of enlarged process**

Based on the optimal parameters determined by dissolution testing as described above, the feeding amounts of the drug, the carrier, and the solvent dosage were increased 10 times to prepare the solid dispersion.

**Preparation of the physical mixture**

Based on the ratio of drug and carrier as determined above, Andro and SiO2 B were accurately weighed and then ground and mixed in a mortar. After passing through a 100 mesh sieve, the physical mixture was finally obtained.

**Comparison of in vitro dissolution**

Based on the dissolution test conditions that have been determined, the dissolution behaviors of Andro, solid dispersion, and the mixture were investigated in 0.2% SDS and water, respectively, to verify the advantage of solid dispersion compared to the crude drug and mixture.

**Evaluation of solid dispersion**

**Particle size**

Particle size distribution was determined by the Laser diffraction particle size analyzer (Malven 2000, United States). The dispersion agent was water, the speed of impeller was 2400 r/min, the measurement time was 15 s, the ultrasonic power was 20 MHz, and the ultrasonic time was 2 min.

**Surface state**

The surface morphology and structure were measured under an environmental scanning electron microscope (JSM-7500E, Japan) operated with an acceleration voltage of 5.0 kV. Samples were coated with a thin layer of gold using the ion sputtering device (E-1010, Japan) and imaged under the microscope.

**Surface area and pore volume**

The special surface area was determined using nitrogen sorption isotherms with the Brunauer-Emmett-Teller (BET) protocol. Nitrogen sorption studies were performed using the TriStar3000 automatic specific surface area and porosity analyzer (Mack Company, United States). Before the initiation of the study, powder samples were stored in sample bulbs and then subjected to 60°C under a vacuum of 0.1 MPa overnight to facilitate moisture removal. The nitrogen sorption data were generated through a relative pressure (P/P0) with a range of 0.0-1.0.

**Infrared spectroscopy**

An FTIR spectrometer from PerkinElmer (United States) was used in an attenuated total reflectance manner to obtain FTIR spectra. The samples were ground thoroughly with potassium bromide at a 1:100 (sample/potassium bromide) weight ratio in an agate mortar and pestle until a uniform mixture was generated.

**X-ray diffraction**

To elucidate the physical status of Andro, SiO2 mixture, and solid dispersion, the XRD patterns were recorded on an XRD-6000X-ray diffractometer DX-2700 (Dandong Haoyuan Instrument Co., Ltd., China). The generator was operated in continuous mode with a voltage and current of 40 kV and 40 mA, respectively. Samples were analyzed in the scanning range of 10 and 70° at a step size of 0.03°.

**Density and flowability**

Both loose bulk density and tapped bulk density, as well as the angle of repose, were determined as previously described. Three determinations were performed.

**Stability study**

Solid dispersion samples were stored at 40°C and 75% (RH) relative humidity for 3 months for accelerated stability studies and then evaluated for drug content and dissolution relative to the original state.

**RESULTS AND DISCUSSION**

**Results of screening process**

The in vitro release profiles of different carrier proportions are shown in Figure 2a. The rate and extent of drug release increased as the carrier proportion increased. When the ratio reached 1:8, the dissolution rate was appreciably higher than the others. The cumulative dissolution rate was >50% within 10 min and was close to dissolution equilibrium in 30 min. These results determined the proportion of drug and carrier to be 1:8.

The in vitro release profiles of different evaporation solvents are shown in Figure 2b. It was shown that different solvents had different effects on the dissolution rate. The sample prepared from tetrahydrofuran demonstrated the fastest dissolution rate among all samples. The cumulative dissolution rate reached 75% within 10 min and approximately 90% at the end. This may be associated with the minimum surface tension of tetrahydrofuran and the highest solubility of the drug. The other samples were prepared from methanol and dehydrated alcohol, which produced nearly the same effects. The least soluble sample was prepared from acetone, which had a poor dissolution rate in the first half. Therefore, the best solvent was tetrahydrofuran.

The in vitro release profiles of different SiO2 sources are shown in Figure 2c. It was demonstrated that the source of SiO2 had a significant effect on the dissolution rate. Among all carriers, SiO2 B had the best effect on promoting dissolution rate, followed by SiO2 C, SiO2 A, SiO2 E, and SiO2 D. This may be related to the special surface area and porosity of carriers. Therefore, SiO2 B was chosen as the carrier.

The in vitro release profiles of different solvent recovery temperatures are shown in Figure 2d. It was demonstrated that the recovery temperature of the solvent had little effect on the dissolution rate. The sample, generated at 40°C, had a lower dissolution rate than the others, especially during the initial 30 min. However, samples prepared at 50, 60, and 70°C showed largely similar dissolution profiles. Taking into account the energy consumption, we chose 50°C as the ideal recovery temperature. After several single factor experiments, the solid dispersion process was determined to be “the drug and carrier (SiO2 B) ratio of 1:8, with tetrahydrofuran as the solvent, and a recovery temperature of 50°C.”

**Dissolution results of amplification process products**

Through the amplification process, it was beneficial to verify the reliability of single factor experiments and optimize the preparation process for future...
use. Figure 3 exhibits the dissolution profiles of amplification products in water and 0.2% SDS, respectively. The solid dispersion dissolved much faster than the mixture and crude drug, regardless of the medium. Specifically, >50% of the solid dispersion dissolved within 5 min in water while only 20% and 5% dissolved in mixture and crude drug, respectively. Moreover, the accumulative dissolution rate of solid dispersion was much greater than that of the mixture and crude drug. A similar observation was also made in 0.2% SDS. These results suggested that after preparing the solid dispersion, the majority of drug was released quickly within a short time, which may improve the bioavailability of Andro.

**Drug loading studies**

After calculations, the drug loading on solid dispersion was 12.78 ± 0.34% (n = 3).

**Particle size distribution studies**

Table 1 showed the results of the particle size distribution. It was shown that Andro is the biggest in size, followed by the mixture and solid dispersion, with SiO₂ being the smallest. The median diameter of the solid dispersion was 6.848 μm, which was much smaller than that of the crude drug. This decrease was considered to be due to the dispersion effect of the carrier. In addition, it was also observed that the diameter of mixture was not due to linear additivity of the crude drug and SiO₂, but that it instead became smaller, a phenomenon that may be related to the grinding action of SiO₂.

**Special surface area and pore volume studies**

The nitrogen sorption curve of Andro [Figure 4] illustrated that the adsorption curve and desorption curve were closed at low pressure and...
high pressure, there was essentially no hysteresis loop, and the pore shape and size of the crude drug were uniform. These results suggested that the isotherm of Andro belonged to Type II adsorption isotherms, which usually occurred in macroporous materials (the diameter was < 50 nm with no upper limit). However, the nitrogen sorption curves of other samples were different from that of Andro. The typical characteristics were that the adsorption and desorption curves suddenly increased in the high-pressure zone, where the P/P₀ value was approximately 0.8–1, and the hysteresis loop appeared. These phenomena were related to the capillary condensation phenomenon of mesoporous materials (diameter was between 2 and 50 nm). Therefore, the isotherms of SiO₂, mixture, and solid dispersion belonged to Type IV adsorption isotherms. Combining the data in Table 2, it was determined that the pore of SiO₂ was opened at two sides and was uniformly cylindrical. By calculations, the BET value and pore volume value of SiO₂ were 94.99 m²/g and 0.5257 cm³/g, respectively, indicating that the carrier had a large surface area and pore volume and provided conditions for drug loading. Further, these values of solid dispersion were lower than those of the mixture, suggesting that some drug had entered the carrier. In addition, the three-dimensional structure of Andro was measured according to the Chemical Database Software (Institute of Process Engineering, Chinese Academy of Science, China). The results suggested that the molecular size was approximately 1.024 nm × 0.494 nm × 0.7 nm, which demonstrated that Andro molecules could theoretically enter the mesoporous internal of SiO₂. Through the analysis of the above results, we concluded that in the solid dispersion preparation, the dense drug was dispersed by the porous carrier, and the specific surface area and porosity of the drug increased sharply; this may be the key mechanism underlying the increased dissolution rate.

Scanning electron microscopic studies

Andro displayed a regular appearance, smooth surface, and good crystallization, but its size was heterogeneous and much larger than that of the other 3 samples [Figure 5]. SiO₂ displayed a tiny size and similar globose shape, and the diameter of the single particles was in the nanometer range; however, most particles aggregated in clusters due to high surface free energy. The mixture displayed the characteristics of both android and SiO₂, but we found that some large android particles were coated by masses of tiny SiO₂ particles. Solid dispersion displayed an aggregated appearance, but the inequality of the sizes made it difficult to identify the regular Andro particles, and the physical state was different from that of the crude drug and the mixture.

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<th>Table 1: Results of particle size distribution (±, n=3)</th>
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Versus andro group, *P<0.01; versus SiO₂ group, †P<0.01. SiO₂: Silicon dioxide

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<th>Table 2: Results of surface area and pore volume (±, n=3)</th>
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⁺BJH adsorption cumulative surface area of pores between 1.700 nm and 300.000 nm diameter, †BJH desorption cumulative surface area of pores between 1.700 nm and 300.000 nm diameter. Versus andro group, *P<0.01; versus SiO₂ group, †P<0.01. BET: Brumauer-Emmett-Teller; BJH: Barret-Joyner-Halenda; SiO₂: Silicon dioxide

Figure 4: N₂ adsorption-desorption isotherm of Andrographolide (a), silica (b), mixture (c) and solid dispersion (d)
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Fourier transform infrared spectroscopy studies

The FTIR spectra were obtained for the crude Andro, SiO₂, mixture and solid dispersion [Figure 6]. The carbonyl peak of Andro was observed at 3397.6, 2928.4, 2868.6, 2849.5, 1727.8, 1674.6, 1455.3, and 1366.7/cm, whereas SiO₂ had no apparent absorption peaks. In addition, in the 1400–1000/cm period, Andro exhibited a series of sharp peaks while SiO₂ only produced a large, smooth peak. These features could be used to compare the samples. Typical features of solid dispersion were different from that of the crude drug and mixture: A series of characteristic peaks of intensity of the drug were significantly reduced while the general features were similar to those of the carrier. These results suggested that solid dispersion was not a simple linear additivity of carrier and drug but that it formed a special structure with drug loading on the internal or surface of the carrier.

X-ray diffraction studies

The XRD patterns of samples were recorded to identify whether a crystalline phase could be detected in solid dispersion. As shown in Figure 7, the diffraction pattern of Andro was highly crystalline in nature as indicated by the numerous peaks, while that of SiO₂ was a smooth curve with a wide and flat peak at 15–30°. For solid dispersion, most characteristic peaks of drug disappeared and only a significant peak at 15.9° was observed. Moreover, it was surprising that most characteristic peaks of the drug also disappeared in the mixture except the peak at 14.9° and 15.9°. It is interesting that there was a little difference between the physical mixture and solid dispersion. We think it was associated with the microstructure of physical mixture. According to the Table 1, the diameter of SiO₂ was even less than one-tenth of Andro. Based on the purification principle in the micromeric subject, when the diameter of the large particles is 10 times larger than that of the small particles, the latter is likely to adsorb on the surface of the former in the grinding process. In the grinding process, SiO₂ particles were easily attached to the surface of Andro, and finally formed many coated particles. At this time, the surface of the mixture mainly dominated by SiO₂ particles, and it was supported by the results of SEM. In the solid dispersion, Andro was highly dispersed by amount of SiO₂, and the diffraction peak signal of drug was sharply weakened. Therefore, the XRD of mixture was similar with that of solid dispersion. This may be the reason for the disappearance of most drug peaks. From the above discussion, we conclude that the crude drug was absorbed by the porous carrier, making an amorphous form, which contained more free energy and served as a driving potential for solubility. Therefore, solid dispersion was beneficial for the drug’s rapid release from the carrier.

Density and friability studies

Bulk density and tap density are often used to predict the filling properties of powder while the angle of repose is used as a fast and simple means to characterize the flow property. Table 3 illustrates a significant decrease in filling properties of solid dispersion compared to crude drug, but there was no difference in friability. This change was closely related with the low density of the carrier.

Stability studies

Table 4 shows the results of the accelerated stability study. It was shown that there was no striking difference between 0 month and 3 months in drug content and dissolution. It suggested that solid dispersion can remain stable for 3 months under the conditions of 40°C and 75% RH.

DISCUSSION

Technological conditions are the important factors affecting the dissolution of solid dispersion. Besides the dosage of SiO₂, SiO₂ type, solvent type, and solvent recovery temperature all have certain influence on the rate of dissolution. We investigated the particle size and specific surface area of different sources SiO₂ and found that there was a little difference. The value of d₅₀ of five kinds of SiO₂ was 14.77, 6.27, 9.44, 20.8, and 16.19 μm, respectively. Moreover, the BET specific surface area was 85.22, 94.99, 92.17, 62.38, and 77.53 m²/g, respectively. SiO₂ B owned the minimum particle size and largest specific surface area. The bigger the specific surface area, the faster the drug release rate. It was thus the specific surface area was the main factor for the difference. Solvent type and temperature are the important factors affecting drug recrystallization results. During four kinds of solvents, the polarity and surface tension of tetrahydrofuran was the minimum, and the solvent is easier to penetrate into the tiny holes in SiO₂. There was no doubt that it could increase the dispersion degree of drug, and decrease the crystallinity. It is worth mentioning that the polarity of acetone was smaller than methanol and
considered. On one hand, it is meaningful to properly reduce the carrier proportion to improve bulk density, in the condition of not significantly reduce the drug release rate; on the other hand, the solid dispersion can be used as intermediates in the preparation of dispersible tablets, and add some greater density fillers such as calcium sulfate or calcium hydrogen phosphate. In addition, we have carried out exploratory research on the bioavailability of Andro by HPLC method, but the results found it was hard to detect the chromatographic peak of Andro in the serum after administration, even given a high dose (100 mg/kg). Due to the limitation of detection instruments, the bioavailability of solid dispersion was not carried out.

**CONCLUSION**

In the present study, a solid dispersion was successfully prepared by the solvent evaporation method, and the optimal conditions were determined. The physical structure of the solid dispersion was characterized by particle size distribution, special surface area and pore volume, SEM, XRD, and FTIR. The solid dispersion showed a significant improvement in in vitro performance compared to the crude drug. Therefore, SiO₂ may be used as a solubility enhancer and carrier for various poorly soluble drugs in BCS class II.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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