Effects of Tween-80 on the Dissolution Properties of Daidzein Solid Dispersion *in Vitro*

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Abstract

Objective Solid dispersions of daidzein (DZ) were prepared using tween-80 as the surfactant to improve its dissolution and solubility. **Methods** Using tween-80 as the surfactant and polyethylene glycol (PEG)-10000 as the carrier, solid dispersions of DZ were prepared by solvent method. Infrared spectroscopy (IR) and X-ray diffraction spectroscopy were applied to determine the status of DZ in carriers. **Results** When appropriate amount of Tween-80 was added into the solid dispersion, the dissolution of DZ could be improved obviously. The data of IR showed the absence of well-defined drug-polymer interactions. The data of X-ray diffraction showed that the drug might exist in the form of amorphism or molecule in solid dispersions. **Conclusion** Appropriate amount of Tween-80 could increase the dissolution rate of DZ.

Keywords: Daidzein, PEG, Tween-80, Solid dispersion, Dissolution rate, IR, X-ray diffraction

Daidzein, extracted from Traditional Chinese Medicine (Guo, 1996, PP. 146-148), was synthesized in 1972 by China. DZ could expand coronary artery, reduce myocardial oxygen consumption, increase the coronary and cerebral blood flow, and ameliorate the symptoms of patients with hypertension such as headache, dizziness, nuchal rigid pain and so on obviously. It could significantly alleviate angina pectoris, possessed anti-arrhythmia and antioxidant properties, enhanced the body's immune system, and had pharmacological effects of lowering blood sugar. In clinical practice, it was principally applied as target medicine for angina pectoris, hypertension and cardiovascular disease (He, 2000, PP. 58-59). In addition, DZ could also improve the menopausal syndrome, strengthen calcium absorption, and had preventive and therapeutic effects on neurological sensory deafness and sudden deafness. However, due to its strong liposolubility, slow dissolution rate, poorly oral absorption and low bioavailability, the pharmacological effects were interfered. In recent years, , there has been a trend in the studies of solid dispersion using a single carrier or mixed carriers with surfactant, and meanwhile, the surfactant could promote the bioavailability of drug in gastrointestinal tract (Zhou, 2003, PP. 42-44). In the present paper, DZ solid dispersion was prepared by solvent method, using polyethylene glycol (PEG) as carrier, and surfactant Tween-80 was added during the preparation process. Effects of Tween-80 on the dissolution of DZ in PEG solid dispersion were investigated.

1. Apparatus and materials

1.1 Instrument

T6 UV spectrophotometer (Beijing Persee General Instrument Co., Ltd.); ZRS-8G Intelligent Dissolution Tester (Tianjin Tiandatianfa Technology Co., Ltd.); RE-2000A rotary evaporator (Gongyi Yuhua Instrument Co., Ltd.); DZF-6050 vacuum oven (Gongyi Yuhua Instrument Co., Ltd.); 100 mesh standard inspection sieve (pore size 0.15 mm, Shangyu Huafeng Hardware Instrument Co., Ltd. in Zhejiang); FT-IR 8400S Fourier Transform Infrared Spectrometer (Shimadzu, Japan); Y-2000 X-ray diffractometer (Dandong Ray Instruments Co., Ltd.).

1.2 Materials

DZ (Yangling Dongke Madison Pharmaceutical Co., Ltd.); PEG (relative molecular weight of 10,000, Tianjin Kermel Chemical Reagent Development Corporation); anhydrous ethanol (Chongqing Chemical Reagent Factory);

Tween-80 (Tianjin Donghua Reagents Factory); potassium dihydrogen phosphate (Beijing Chemical Factory); sodium hydroxide (Tianjin North Tianyi Chemical Reagent Factory.)

2. Experimental Methods

2.1 Determination of detection wavelength

Appropriate amount of DZ, PEG and Tween-80 were weighed accurately and added to pH 6.8 phosphate buffer and anhydrous ethanol 9:1 (v /v) mixed solutions. Solutions with proper concentration were prepared, and the same mixed solvent as blank. They were scanned at the wavelength from 200 to 400 nm. As seen from Figure 1, DZ had two absorption peaks at the wavelength of 248 and 305 nm while PEG and Tween-80 had none. Because PEG and Tween-80 both had absorption at the wavelength of 248 nm, and had none at 305 nm, 305 nm was selected as detection wavelength. Figure 1 shows the UV spectra of DZ, PEG and Tween-80.

2.2 The calibration curve

DZ was recrystallized with anhydrous ethanol. Accurately weighted DZ (Dried to constant weight) 0.025 g was added into a 100 ml volumetric flask, and dissolved and diluted with anhydrous ethanol to the scale as stock solution. 0.1, 0.1, 0.2, 0.5, 0.7 and 1.0 ml of stock solution were added to 50, 25, 25, 25, 25 and 25 ml volumetric flask respectively, and anhydrous ethanol were supplemented to the flaks until 5, 2.5, 2.5, 2.5, 2.5 and 2.5 ml, respectively. Finally, pH 6.8 phosphate buffer solution was added to the final scale, respectively. Absorption at the wavelength of 305 nm was determined respectively. Linear regression was undertaken using drug concentration (C) and absorbance (A), and DZ calibration curve, Y = 23.58834X-0.01941 (r = 0.9999, linear range of 0.5~10 mg/L, n=6) was obtained.

2.3 Preparation of solid dispersions

Three groups of 1:2, 1:5 and 1:8 (w/w) of the DZ and PEG-10000 solid dispersion were prepared. Each group had three samples. One sample among each group was added to rotary evaporator with 50 mL anhydrous ethanol, heated by water bath to dissolve. The resultant solution was evaporated till constant weight, and frozen for 24 h. The obtained solid dispersion was placed into a vacuum oven for 12 h, grinded, and then undertaken 100 mesh sieve for further investigation. Solid dispersion preparation of the rest two samples among the three groups used the same proportion of DZ and PEG, with 0.4g and 0.8g Tween-80 respectively, and 50 mL anhydrous ethanol. The following steps were the same as the first group, and the solid dispersion of Tween-80 and DZ with surfactant was prepared.

2.4 Determination of dissolution

Dissolution medium was 1000 mL pH 6.8 phosphate buffer at the temperature of 37±0.5 ℃ with the paddling speed of 100 rpm. 5 mL solution was sampled at accurate time of 5, 10, 20, 40, 60, 90 and 120 min, and meanwhile the same volume of phosphate buffer was supplemented at the same temperature. The sample was rapidly filtered through a 0.45 μm microporous membrane. Proper amount of filtrate was selected to determine the absorbance at the wavelength of 305 nm. Drug concentration was obtained from the calibration curve, and then the drug dissolution rate was calculated. Pharmaceutical raw material and solid dispersions were all weighted according to the equivalence to 10 mg of DZ raw material. Sample of each group was replicated 6 times. Dissolution curves of drug were undertaken regression analysis using SPSS 13.0 software, and the equations with the largest R^2 were selected as the fitting equation of each dissolution curve.

2.5 FTIR spectra

FTIR spectra of DZ raw material, PEG and DZ PEG solid dispersion were investigated using potassium bromide tablet method. 1~2 mg sample was grinded into powders with an agate mortar, and 100~200 mg grinded and dried KBr was added. They were mixed evenly, prepared into transparent tablets with the thickness of 1 mm and the diameter of 10 mm by pressure machine and tested. Sample dosage and tablet thickness was considered to be good when FTIR spectrum with the baseline of over 80 % and the maximum absorption peak of about 20 % transmittance was obtained.

2.6 X-ray diffraction

DZ, PEG and solid dispersions were undertaken X-ray diffraction analysis respectively. Test condition: Cu target; pressure, 30 kV; pressure: 30 kV; tube current: 20 mA; scanning speed: 2 °/min; scan range: 5 °~90 °.

3. Results and discussion

3.1 Dissolution test in vitro

Figure 2 describes the dissolution profiles of pure DZ, and its solid dispersions. The dissolution rate of DZ solid dispersion with or without Tween-80 increased with the decreasing ratio of drug to PEG, and the drug dissolution rates were classified in this order: DZ<(drug: PEG, W/W) 1:2 <(drug: PEG, W/W) 1:5 <(drug: PEG, W/W) 1:8,

which might be attributed to the facts that the increment of carrier impeded the contact among drug molecule, and reduced the possibility of forming large particle size drug crystal. Consequently, the practical size of drug crystal decreased, or more drugs were dispersed in the form of molecule, and thus the drug dissolution rate increased.

After adding Tween-80, drug dissolution rate with relative more PEG content was classified in the order: (drug: PEG: Tween-80, W/W/W) 1:5:0.8 <(drug: PEG, W/W) 1:5 <(drug: PEG: Tween-80, W/W/W) 1:5:0.4; (drug: PEG: Tween-80, W/W/W) 1:8:0.8 <(drug: PEG: Tween-80, W/W/W) 1:8:0.4 <(drug: PEG, W/W) 1:8; X-ray diffraction data also proved this point (Figure 4), which indicated that Tween-80 could enhance the dissolution of DZ. But Tween-80 content shouldn't be too high. Too much Tween-80 could defer the dissolution of DZ, which might be ascribed to the facts that Tween-80 was sticky liquid, and when its content was relatively high, DZ was dried slowly, which afford enough time for the crystal to grow, made the drug failed to completely disperse in the carrier in the form of amorphism, and thus affected drug dissolution; Proper Tween-80 dosage not only couldn't affect the drying time of solid dispersion, but also could lower the surface tension during evaporating, in order to make the drug to distribute evenly and prevent further aggregation of drug particles. Drug dissolution rate with relative less PEG content was classified in the order: (drug: PEG, W/W) 1:2< (drug: PEG: Tween-80, W/W/W) 1:2:0.8 < (drug: PEG: Tween-80, W/W/W) 1:2:0.4, and the dissolution with Tween-80 increased. However, too much addition had less effects compared to the less amount of Tween-80, possibly because the dissolution at the ratio of 1:2 was low by itself. Effects of Tween-80 on the solubilization of DZ tended to be more conspicuous, and because Tween-80 was sticky solution, too much addition wouldn't be obvious compared to the less addition. Table 1 summarizes the results of Fitting equations of PEG solid dispersions and DZ dissolution curves. Drug dissolution curves of solid dispersion with or without Tween-80 at each ratio accorded with cubic curve, quadric curve and power function curve.

3.2 FTIR spectra

Figure 3 shows the FTIR spectra of different samples. Each solid disperse was basically similar in infrared spectra, and the spectra of solid disperses were very similar to that of PEG. PEG accounted for the larger proportion, and therefore each peak position of PEG covered that of drug. Hydrogen bond and other bonding interaction between DZ and PEG had not been found. All results indicated that there was no chemical change between DZ and PEG, but only physical interaction.

3.3 X-ray diffraction spectra

As seen from Figure 4, PEG had a distinct diffraction peak at about 20° or 23°, while drug had many peaks at about 10°, 18°, 19° and 25°. In the diffraction spectrum 3~11, peaks of DZ diminished significantly, and even disappeared, which suggested that carrier existed in the form of crystal, and drug might be dispersed in the carriers in the form of amorphism or molecule. All these also explained the reason why PEG solid disperse enhanced the dissolution of DZ significantly.

4. Conclusions

Using PEG 10000 as carrier, DZ solid dispersion prepared by solvent method could enhance the dissolution rate of DZ. The dispersion level of DZ in solid dispersions increased with the increasing weight ratio of PEG 10000. Appropriate amount of Tween-80 could enhance the dissolution of drug, but too much could impede inversely. In the present paper, the Tween-80 level was enhanced to 2.0 g, but could not be dried completely, and therefore its dosage shouldn't be too high. The optimum level of Tween-80 still needs further investigation.

During the preparation process of DZ solid dispersion, there had been no chemical bonding between DZ and PEG 10000, and preparation process didn't change the molecular structure of DZ. Because only the dissolution of DZ solid dispersion in vitro was observed, further investigation should be carried out on the comparison of dissolution in vitro among the current existing DZ dosage in the market in order to provide theoretical basis for the development of new DZ formulations.

References

Guo, J.P., & Sun, Q.R. (1996). Chemical composition and clinical application research status of Pueraria. *The Journal of Pharmaceutical Practice*, 14(3):146-148.

He, W.S. (2000). Clinical pharmacology research progress of effects of Pueraria and its extract on cardiovascular and cerebrobascular diseases. *Journal of Integrative Medicine on Cardio-/ Cerebrovascular Disease*, 17(3):58-59.

Zhou, Y.S., Jia, Y.Y., & Shen, X.Q. (2003). Studies on preparation and dissolution in vitro of puerarin solid dispersions. *Chinese Pharmaceutical Journal*, 38(1):42-44.

Table 1. Fitting equations of dissolution profiles of DZ and PEG solid dispersions at each ratio

Ratio	Equation	R^2	P
1:2	$Y=-7.412+1.404X-0.02X^2+0.003X^3$	1.000	0.000
1:5	$Y=-31.79+6.083X-0.371X^2+0.011X^3$	0.999	0.000
1:8	$Y=54.092-0.241X^2+0.007X^3$	0.998	0.000
1:2:0.4	$Y=11.478-0.077X^2+0.004X^3$	0.998	0.000
1:5:0.4	$Y=-0.583-0.03X^2+0.003X^3$	0.997	0.000
1:8:0.4	$Y=109.993-12.249X+0.362X^2$	0.995	0.000
1:2:0.4	$Y=-4.623+0.722X+0.005X^3$	0.998	0.000
1:5:0.4	$Y=2.573X^{0.013}$	0.996	0.000
1:8:0.4	$Y=-8.722+2.587X-0.208X^2+0.007X^3$	0.998	0.000
DZ	$Y=-0.144+0.393X-0.02X^2$	0.998	0.000

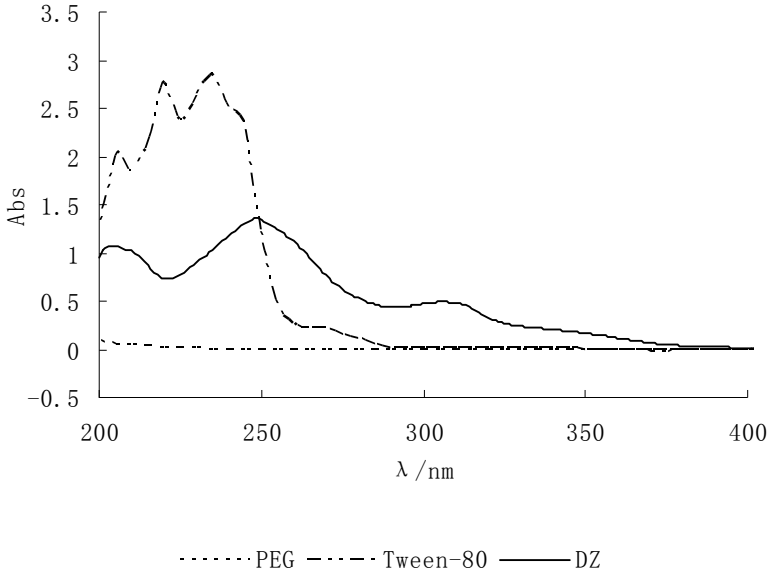

Figure 1. UV spectra of DZ, PEG and Tween-80

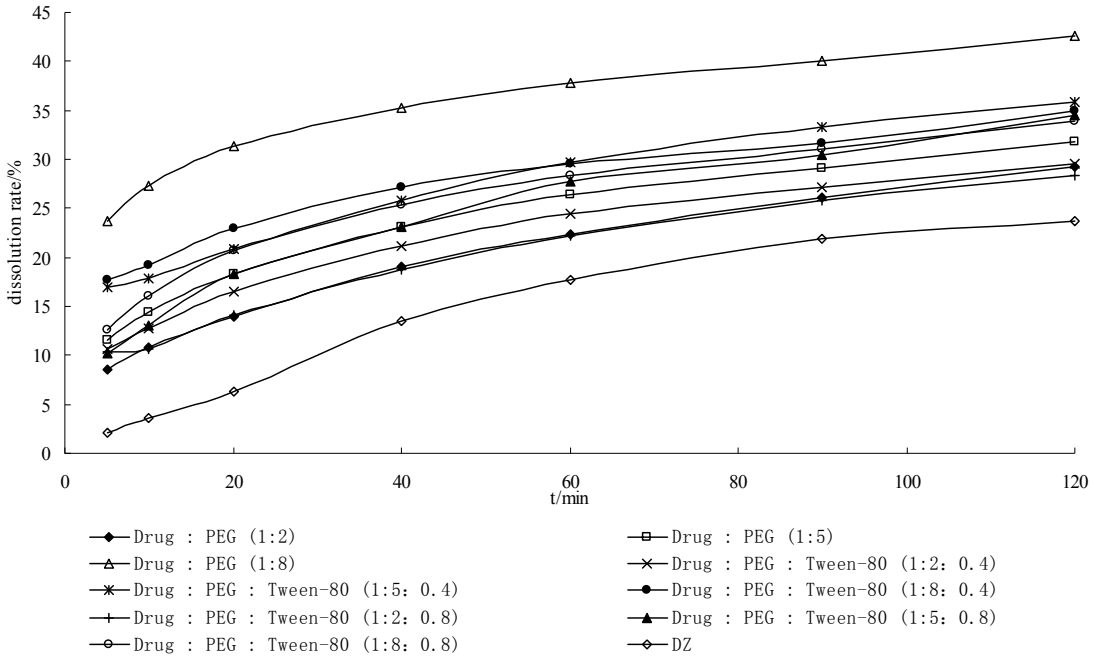

Figure 2. Dissolution profiles of DZ and PEG solid dispersions at each ratio

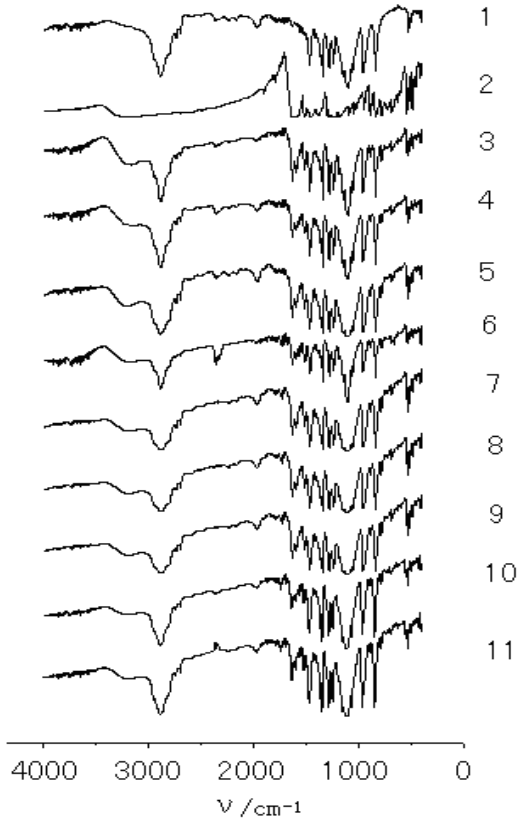

Figure 3. FTIR spectra of different samples: (1) PEG, (2) DZ, (3) Drug: PEG (1:2), (4) Drug: PEG (1:5), (5) Drug: PEG (1:8), (6) Drug:PEG:Tween-80 (1:2:0.4), (7) Drug:PEG:Tween-80 (1:5:0.4), (8) Drug:PEG:Tween-80 (1:8:0.4), (9) Drug:PEG:Tween-80 (1:2:0.8), (10) Drug:PEG:Tween-80 (1:5:0.8), (11) Drug:PEG:Tween-80 (1:8:0.8)

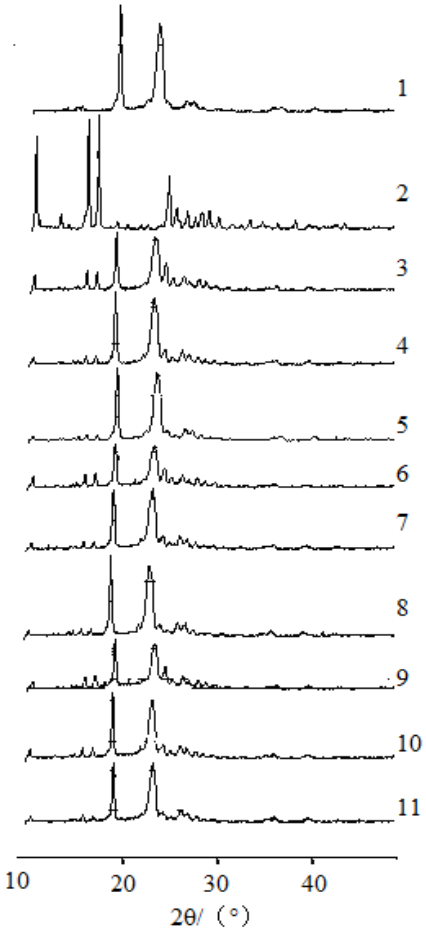

Figure 4. X-ray diffraction spectra of different samples: 1) PEG, (2) DZ, (3) Drug: PEG (1:2), (4) Drug: PEG (1:5), (5) Drug: PEG (1:8), (6) Drug:PEG:Tween-80 (1:2:0.4), (7) Drug:PEG:Tween-80 (1:5:0.4), (8) Drug:PEG:Tween-80 (1:8:0.4), (9) Drug:PEG:Tween-80 (1:2:0.8), (10) Drug:PEG:Tween-80 (1:5:0.8), (11) Drug:PEG:Tween-80 (1:8:0.8)