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# Preparation and characterization of honokiol nanoparticles

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**Abstract** In this paper, honokiol nanoparticles were prepared by emulsion solvent evaporation method. The prepared honokiol nanoparticles were characterized by particle size distribution, morphology, zeta potential and crystallography. Results showed that the obtained honokiol nanoparticles at size of 33 nm might be amorphous, and could be well dispersed in water. Due to the great dispersibility in water, the obtained honokiol nanoparticles might have great potential in medical field.

## 1 Introduction

Traditionally, almost half of new molecular entities identified by pharmaceutical industry screening programs have failed to be developed because of their poor water-solubility, which made their formulation difficult or even impossible and one essential factor in the drug development equation is drug solubility [1, 2]. Since Nobel Laureate Richard Feynman predicted the emergence of a new science called nanotechnology in 1959, nanotechnology as a novel technology has played a very important role in many fields of our society, and it is taking root in the drug and medical device industry especially drug delivery [3]. Nanotechnology provides a very interesting method to overcome the poor solubility of hydrophobic drugs [4–9]. After such hydrophobic drugs were manipulated to form nanoparticles at wanted particle size, they could be well dispersed in water to form stable suspension to meet the requirement of administration.

Honokiol, as a multi-functional drug, has great potential in disease therapy especially in cancer therapy [10–16]. Previously, a rapid separation approach had been developed using high-capacity high-speed counter-current chromatography (high-capacity HSCCC) to isolate and purify honokiol and magnolol by Chen et al. in our lab [17]. But due to poor water-solubility, administration of honokiol is greatly restrained. Now, the surfactant should be added to prepare aqueous honokiol solution (actually, the "solution" should be called "suspension"). So, in order to improve the hydrophilicity of honokiol, honokiol nanoparticles were prepared by emulsion solvent evaporation in this paper. The prepared honokiol nanoparticles might have great potential clinic application as a new dosage form.

## 2 Experimental

### 2.1 Materials

Pluronic® F127 (F127) was purchased from Sigma (USA). Ethyl acetate (Et Ac) was purchased from KeLong Chemicals (Chengdu, China). Honokiol was prepared in our library [17].

X.Y. Li and M. Dai are the co-first authors for this paper, who did the even work with M.L. Gou.

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### 2.2 Preparation of honokiol nanoparticles

Briefly, different amounts of honokiol (20, 60, 80,100 mg) were dissolved into Et Ac (2 ml) to form honokiol solution at different concentration. Then, the prepared honokiol-Et Ac solution was introduced into 4 ml of F127 aqueous solution at the concentration of 5% (w/w) under extreme stirring by T10 homogenizer (IKA, Germany). About 10 min later, oil in water (O/W) emulsion formed. Then, Et Ac was evaporated in rotator evaporator (BÜCHI, Switzerland) and the honokiol nanoparticles were obtained. The content of honokiol in the prepared honokiol nanoparticles suspension was determined by high-performance liquid chromatography (HPLC) and the concentration of honokiol and F127 was adjusted to 15 mg/ml and 5% (w/w) respectively. At last, the honokiol nanoparticles slurry was lyophilized and the powder was stored at 4°C before further use.

### 2.3 Detection of particle size and zeta potential of honokiol particles

Particle size distribution of nanoparticles was determined by laser diffraction particle sizer (Nano-ZS, Malvern Instrument, UK). The zeta potential of honokiol nanoparticles in water was measured by Malvern Zeta analyzer (Nano-ZS, Malvern Instrument, UK). The temperature was kept at 25°C during measuring process. And all results were the mean of 3 test runs.

### 2.4 Morphology study

The morphology of prepared honokiol nanoparticles was observed under a transmission electron microscope (TEM) (H-6009IV, Hitachi, Japan): nanoparticles were diluted with distilled water and placed on a copper grid covered with nitrocellulose. The sample was negatively stained with phosphotungstic acid and dried at room temperature.

### 2.5 Crystallographic assay

Crystallographic assay was performed on honokiol particles by X-ray Diffractometer (DX-2000, DanDong Fangyuan Instrument Company, China) using CuKα radiation.

### 2.6 High performance liquid chromatography

The concentration of honokiol in the obtained honokiol nanoparticles suspension was determined by HPLC Instrument (Waters Alliance 2695). Solvent delivery system equipped with a column heater and a plus autosampler. Detection was taken on a Waters 2996 detector. Chromatographic separations were performed on a reversed phase $C_{18}$ colume (4.6 × 150 mm-5 μm, Sunfire Analysis column). And the column temperature was kept at 28°C. Acetonitrile/water (60/40, v/v) was used as eluent at a flow rate of 1 ml/min.

## 3 Results and discussion

Previously, the method to extract honokiol had been reported by our lab, and the molecular structure of honokiol was shown in Fig. 1 [17]. Unfortunately, its application was restrained due to its great hydrophobicity. To overcome such shortcoming of honokiol crystal, we prepared a kind of honokiol nanoparticles here.

Emulsion solvent evaporation method has been widely used to prepare nanoparticles [18]. During preparation of honokiol nanoparticles in this article, the concentration of honokiol-Et Ac solution has great effect on the particle size distribution of honokiol particles, which could be seen in Fig. 2. With increase in honokiol concentration, the particle size increased and the size distribution became wider. At the same time, it could be observed that the size distribution was splitted into two peaks above a certain concentration which might be due to agglomeration of honokiol nanoparticles to increase the particle size and decrease the total particle surface while the constant F127 was not enough to coat and stabilize more amounts of honokiol nanoparticles. When it was considered to gain honokiol nanoparticles with small particle size and narrow particle size distribution at high concentration, 30 mg/ml was chosen as the honokiol concentration and the obtained honokiol nanoparticles were characterized in detail, which was shown in Fig. 3. The average particle size of honokiol

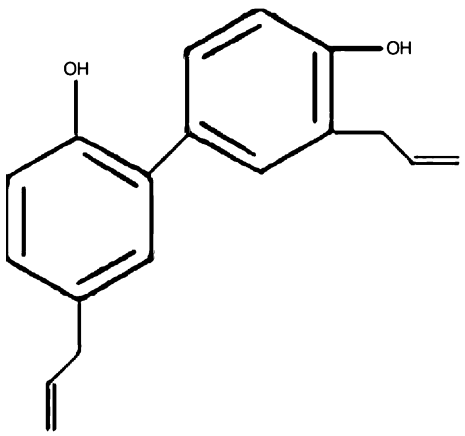


**Fig. 1** The molecular structure of honokiol

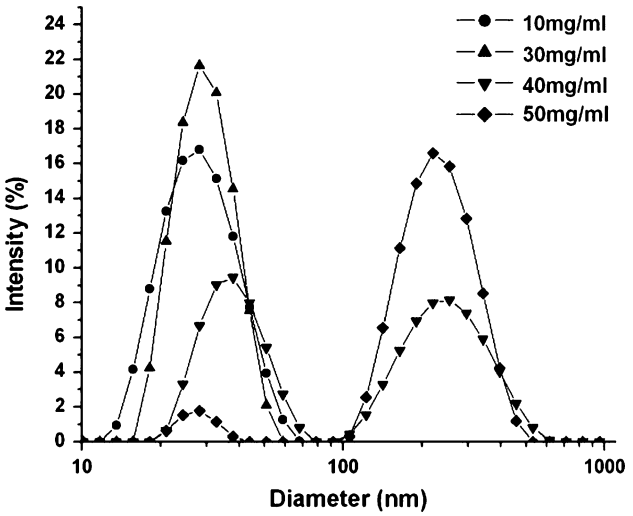


**Fig. 2** The effect of honokiol concentration on particle size distribution of obtained honokiol nanoparticles

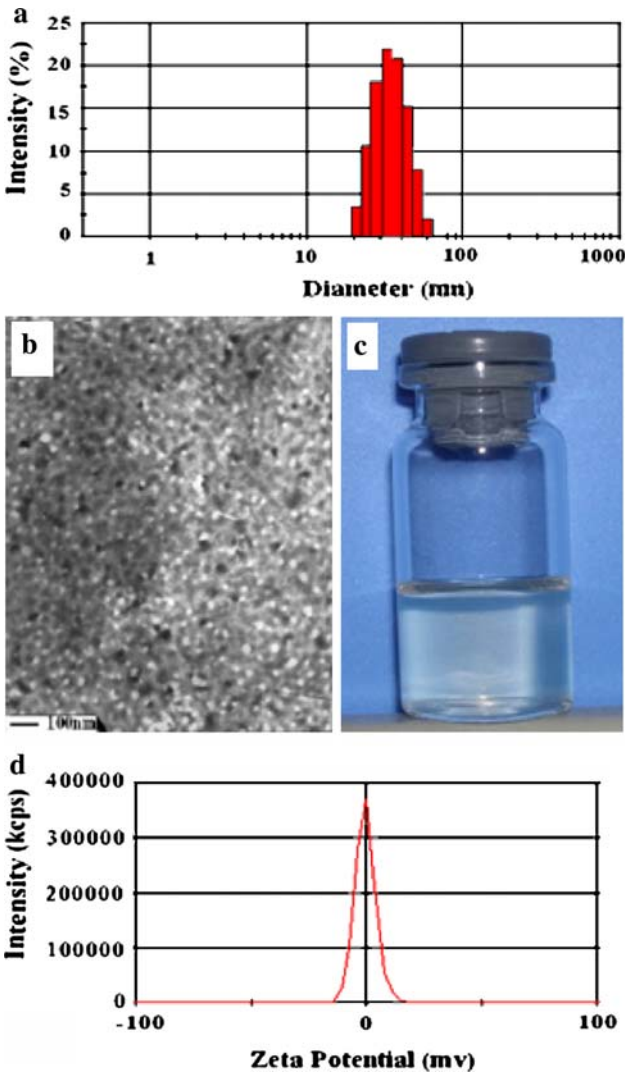


**Fig. 3** Characterization of honokiol nanoparticles. (**a**) Particles size distribution spectrum; (**b**) TEM image; (**c**): optic image; (**d**): zeta potential distribution spectrum

nanoparticles was about 33 nm and polydisperse index (PDI) was 0.036. Then, TEM image of honokiol nanoparticles was presented in Fig. 3b. From which, the spherical morphology of honokiol particles could be observed. The appearance of prepared honokiol nanoparticles suspension was shown in Fig. 3c and the clear solution could be seen. Meanwhile, the zeta potential of prepared honokiol nanoparticles was −0.385 mv and zeta potential distribution spectrum could be seen in Fig. 3d. From Fig. 3, it could be found that the obtained nanoparticles could be well-dispersed in water, and it was stable. Because of its high surface/volume ratio, nanoparticles always tend to form aggregates. However, due to stereospecific blockade or electrostatic interaction, stable nanoparticles suspension also could be obtained. The zeta potential of honokiol nanoparticles is only −0.385 mv which implied that the surface charge could be regarded as neutral surface charge. Meanwhile, Pluronic F127 is an amphiphilic triblock copolymer consisted of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) monomers in the arrangement of $PEO_{100}PPO_{70}PEO_{100}$. In aqueous solvent, the relative difference in hydrophobicity between PPO and PEO allows the formation of self-assembled micelle, whereby cores of PPO and water are surrounded by coronas consisting of PEO [19]. So, the stability of honokiol nanoparticles suspension obtained here might be mainly contributed to stereospecific blockade formed by PEO hydrophilic chain at the surface of honokiol nanoparticles and the might be micro-structure of honokiol nanoparticles was shown in Fig. 4 which implied that the prepared honokiol nanoparticles might have core-shell structure: honokiol as core and PEO chains as shell. At last, crystallographic assay was performed by XRD and the result was presented in Fig. 5. It is obvious from the XRD diagrams that there is no specific diffraction peak of honokiol in the honokiol nanoparticles. Taking into consideration the drug-crystal-free particle, it is apparent that honokiol is amorphously dispersed inside the F127 shell and good affinity formed between the honokiol (core) and F127 (shell).

The poor solubility of honokiol was overcome by nanotechnology here. The obtained nanoparticles are injectable and can be administrated easily. Meanwhile, only F127, which is approved by FDA, is remained in the honokiol nanoparticles except honokiol. This might ensure the safe of the novel dosage form. The prepared honokiol nanoparticles might have great potential in clinic application. In addition, as a new dosage form, its pharmacokinetics and body distribution should be well studied later.

Otherwise, F127 exhibits reversible thermal gelation in aqueous solution at concentration above 20% (w/v). F127

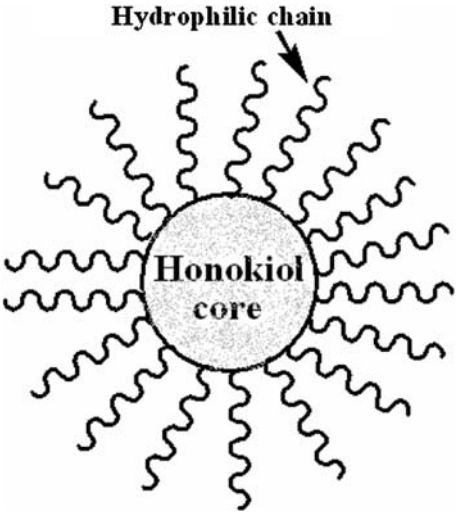


**Fig. 4** The schematic picture of prepared honokiol nanoparticles

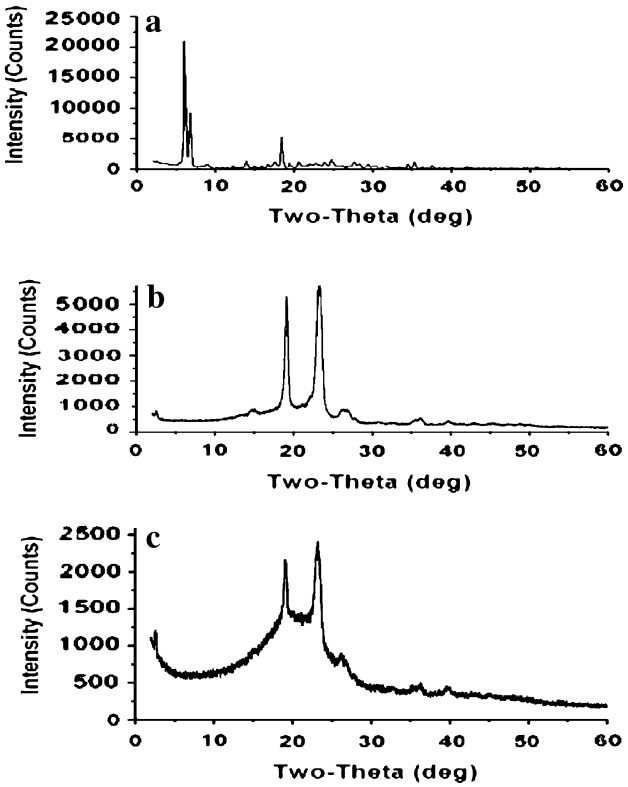


**Fig. 5** XRD diagrams. (**a**) Honokiol crystal; (**b**) F127 powder; (**c**) prepared honokiol nanoparticles powder

solution is liquid, as sol phase, at low temperatures, but it rapidly gels when temperature achieved ca. 25°C. The sol phase containing drugs at low temperature forms gel after subcutaneous injection and it act as a depot for the controlled release of drugs in situ. When the prepared honokiol nanoparticles power was re-dispersed in water, the concentration of F127 could be adjusted to 20% and injectable thermo-sensitive F127 matrix (20%) containing honokiol nanoparticles (60 mg/ml) could be employed to delivery honokiol in situ. At last, it is widely reported that Pluronic copolymers have great potential applications in overcoming multidrug resistance (MDR) in cancer therapy [20], so the prepared honokiol nanoparticles also might be a novel anti-MDR formulation.

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