

Spray-dried curcumin nanoemulsion: A new road to improvement of oral bioavailability of curcumin

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Abstract: In this study a new soluble solid curcumin nanoemulsion powder was prepared using spray-drying technology to improve the solubility and bioavailability of curcumin. The liquid nanoemulsion consisted of curcumin, Capryol 90, Transcutol P, and Cremophor RH40. The solid nanoemulsion was prepared by spray-drying the liquid nanoemulsion in laboratory spray dryer, using lactose as solid carrier. The *in vitro* release from powder formulation was 97.6% within 15 min while the release from the curcumin crystalline was about 10%. An oral pharmacokinetic study was conducted in rats and the relative bioavailability of spray-dried curcumin powder significantly increased compared with that of curcumin crystalline. The C_{max} value of solid curcumin nanoemulsion powder was 5.5-fold greater than the value of the curcumin crystalline in aqueous suspension. The absorption mechanism of the spray-dried curcumin powders was discussed. The results indicate that spray-drying in combination with nanoemulsion was a powerful methodology for improving the dissolution rate and oral bioavailability of curcumin.

Keywords: Curcumin, spray-drying, solubilization, oral bioavailability, nanoemulsion.

INTRODUCTION

Despite having good therapeutic effects, about 40% of new drug candidates fail in clinical situations due to their poor water solubility (Lipinski 2002). Poor water solubility leads to numerous issues such as poor bioavailability, high intrasubject/intersubject variability. Therefore, Attempts to overcome the solubility problem and improve oral absorption is very important for such drugs.

Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*, has a wide spectrum of biological activities and pharmacological actions, such as antioxidant, anti-inflammatory, antimicrobial and anti-tumor activities. The pharmacological studies proved that curcumin has a low toxicity even at very high doses, making it a promising compound for clinical treatment of a lot of human diseases (Araujo and Leon 2001, Hsu and Cheng 2007). Unfortunately, Formulating curcumin for clinical efficacy has presented many challenges due to its low solubility and poor bioavailability.

Several technologies have been attempted to improve the oral absorption of curcumin, the use of solid dispersions (Onoue *et al.*, 2010, Seo *et al.*, 2012, Chuah *et al.*, 2014), nanoparticles (Shaikh *et al.*, 2009), nanoemulsion or self-micro emulsifying drug delivery systems (Ahmed *et al.*, 2012, Yu and Huang 2012), phospholipid complexes (Maiti *et al.*, 2007) have been proposed to improve the oral bioavailability and the therapeutic efficacy of curcumin. Among these approaches, nanoemulsion is a

promising one owing to its considerable capacity to improve the solubility of hydrophobic drugs.

Nanoemulsion are transparent, thermodynamically stable systems of oil and water, stabilized by a surfactant or surfactant mixture, frequently in combination with a co surfactant (Lawrence and Rees, 2000). Nanoemulsion is already used as a drug carrier to increase hydrophobic drug solubility and adsorption (He *et al.*, 2010). However, the drawbacks such as inconvenient usage, short shelf life and instability limited its usage. In order to increase the drug stability and portability, solid nanoemulsion formulations have been investigated, as alternative approaches to improve dissolution rate and oral bioavailability of insoluble drugs. These systems require the solidification of liquid nanoemulsion into tablets (Ali *et al.*, 2013), pellets (Abdalla *et al.*, 2008) and powders (Yi *et al.*, 2008) to create various solid dosage forms.

Spray drying has long been a useful technique for the production of micro particulate powders suited for drug delivery applications. Spray-drying can transform a solution or suspension into a fine powder with rapid evaporation of the solvent. Recently, spray drying has been employed to improve the oral bioavailability of poorly water soluble drugs.

In this study, a new soluble spray-dried curcumin formulation was prepared using nanoemulsion solubilization technology in combination with spray-drying process. The spray-dried powder of curcumin nanoemulsion was prepared and optimized. The physicochemical characteristic was investigated. The dissolution of curcumin spray-dried solid powder was studied and compared with the curcumin crystalline. The

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oral bioavailability of curcumin in spray-dried solid powder was investigated. In addition, the dissolution and absorption mechanism of the spray-dried nanocurcumin formulation were also discussed.

MATERIALS AND METHODS

Materials

Curcumin was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Transcutol P was obtained by Gattefosse (Shanghai, China). Lactose was purchased from shanhe Co. Ltd. (Anhui, China). Acetonitrile and methanol were HPLC grade and supplied by Kermel Chemical (Tianjin, China). Double-distilled water was used throughout the study. All other chemicals and solvents were analytical reagent grade.

Preparation of liquid curcumin nanoemulsion

Curcumin nanoemulsion was prepared as previously described with Capryol 90 as oil, Cremophor RH40 and Transcutol P, as surfactant and co-surfactant. In brief, excess curcumin was dispersed to the mixture of oil and surfactant. Then an appropriate amount of Transcutol P aqueous solution was added to the mixture drop by drop and the mixture was stirred for 48h at 25°C under light shielding. The undissolved drug was removed by centrifugation and the supernatant was filtered by 0.45µm membrane. After appropriate dilution with methanol, the concentration in the filtrate was measured by HPLC.

Spray drying of curcumin nanoemulsion

The spray dried powders were prepared using an YC-015 experimental spray dryer. Curcumin nanoemulsion solution containing 2% (w/v) lactose was spray dried. The impact of the outlet and inlet temperature on the yield of powder was investigated. The outlet temperatures varied from 70 to 110°C and the inlet temperatures tested ranged from 150 to 180°C. To determine the impact of the inlet temperature, the pump rate adjusted between 0.5 and 2mL/min and the aspiration rate was set at 100%. The spray dried powders were further dried in a vacuum oven at 40°C.

Scanning electron microscopy (SEM)

Morphological examination of the particles was determined by KYKY 2800b scanning electron microscope (KYKY Technology Development LTD. Beijing, China). Prior to examination, samples were gold sputter-coated to render them electrically conductive.

Differential scanning calorimetry (DSC)

DSC determinations were conducted on a Shimadzu DSC-60 thermal analyzer (Shimadzu Corporation, Japan). Indium was used to calibrate for the temperature scale and energy. Accurately weighted amounts of samples were placed in perforated aluminum pans and heated at a scanning rate of 10°C /min, under a nitrogen purge gas flow rate of 25ml/min.

Flow property study

The flow properties of untreated curcumin and spray dried samples were evaluated using the angle of repose and compression index. The angle of repose was assessed using the fixed funnel method. Flow time was established by recording the time. The compression was determined using a graduate and was calculated by the following formula below: $\text{Compression} = (\rho_a - \rho_t) / \rho_a \times 100$. Where ρ_a is the bulk density of material freely settled and ρ_t is the maximum packing density. Each experiment was performed in triplicate.

HPLC analysis

All samples were analyzed by HPLC that consisted of LC-20A liquid chromatogram and SPD-20A UV/VIS detector (Shimadzu, Kyoto, Japan) using a C18 column (5µm, 250 × 4.6mm) at room temperature. Curcumin was detected at 428nm. The mobile phase was a mixture of methanol: H₂O (containing 3.6% glacial acetic acid) (70:30, v/v) at a flow rate of 1.0ml/min.

In vitro dissolution test

The *in vitro* dissolution was used in order to identify the advantages of the spray-dried curcumin formulation compared to the curcumin crystalline. Powder dissolution tests was performed using a ZRS-8G dissolution equipment (Hai Yi Da Technology Limited Company, Tianjin). The dissolution test was performed at 37°C using the paddle method at 100 rpm with 900ml of 0.4% sodium dodecyl sulfate aqueous solution as dissolution media. At predetermined time intervals 5ml of the medium was sampled and filtered through a membrane filter (0.45µm). The concentration of curcumin in the resulting solution was analyzed using a spectrophotometer at 428nm. All samples were tested in triplicate.

Pharmacokinetics studies in rats

Sprague-Dawley rats were fasted overnight prior to the experiment, and water was available ad lib. They were randomized to be administered orally with curcumin spray-dried solid powders (curcumin 400mg/kg) and curcumin crystalline (curcumin 1000mg/kg). Curcumin crystalline was dispersed in 0.4% sodium carboxymethylcellulose (CMC-Na) solution to form a suspension for administration. Blood samples were collected in tubes containing heparin at various intervals after administration. Samples were centrifuged after collection and stored at -20°C.

The plasma concentration of curcumin was determined by HPLC mentioned above. Briefly, 0.6ml ethanol was added to 0.2ml plasma and vortexed for 5min. Then the mixture was centrifuged at 10000rpm for 10min. The concentration of curcumin in the supernatant was determined by HPLC reported above. The pharmacokinetics parameter was calculated by Winnonlin software.

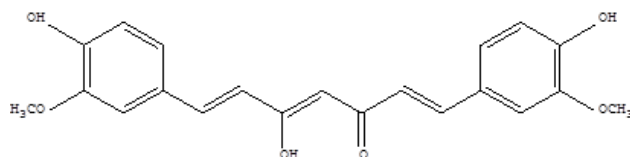


Fig. 1: The chemical structure of curcumin

Data analysis

The pharmacokinetic parameters were calculated based on a non-compartmental model. Winnonlin software was used in the calculation of pharmacokinetics parameter. The area under the concentration-time curve from time zero to time t (AUC₀ (t)) was calculated using the trapezoidal method. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma concentration-time profiles. The data obtained from the release rate and pharmacokinetic parameters were analyzed statistically by one-way ANOVA and Student's t -test using SPSS version 11.0 software. Statistically significant differences were assumed when $p < 0.05$. All values are expressed as their mean $\pm$ SD.

RESULTS

Physical properties and in vitro dissolution of the spray-dried powders

The shape and the surface morphology of spray-dried powders and curcumin crystalline are presented in (fig. 2A). The powders were observed as spherical agglomerated particles. The particles appear a smooth surface with no drug crystal on it. Curcumin crystalline appeared as irregular-shaped crystals (fig. 2B).

Powders had very low moisture content. The moisture content is $1.34 \pm 0.25\%$ with good stability and storage of powders. The powders also had a high bulk density 0.43 g/cm^3 , compressibility of 19.1%, repose angle values of 35.8.

Fig. 3 shows the dissolution profiles of spray-dried curcumin nanoemulsion, in comparison to drug crystalline. There was an obvious difference in dissolution rate between these samples. The spray-dried formulation showed a significant increase in the dissolution of curcumin (97.6% within 15 min) compared to the curcumin crystalline (10% within 2 h) ($p < 0.05$). By comparing the result, it was concluded that the dissolution was very rapid from the spray-dried nanoemulsion.

The improved dissolution of spray-dried powders could be attributed to these reasons: (a) the solubilization by the nanoemulsion (b) After spray-drying, the nanoemulsion composition adsorption on the carrier, leading to an increase in surface area to the dissolution medium which further enhances the dissolution rate. (c) The carriers used in this study for spray-drying is lactose, its hydrophilic

affinity to facilitate the dissolution medium rapid penetration into the particles, hence improved wettability and dissolution rate.

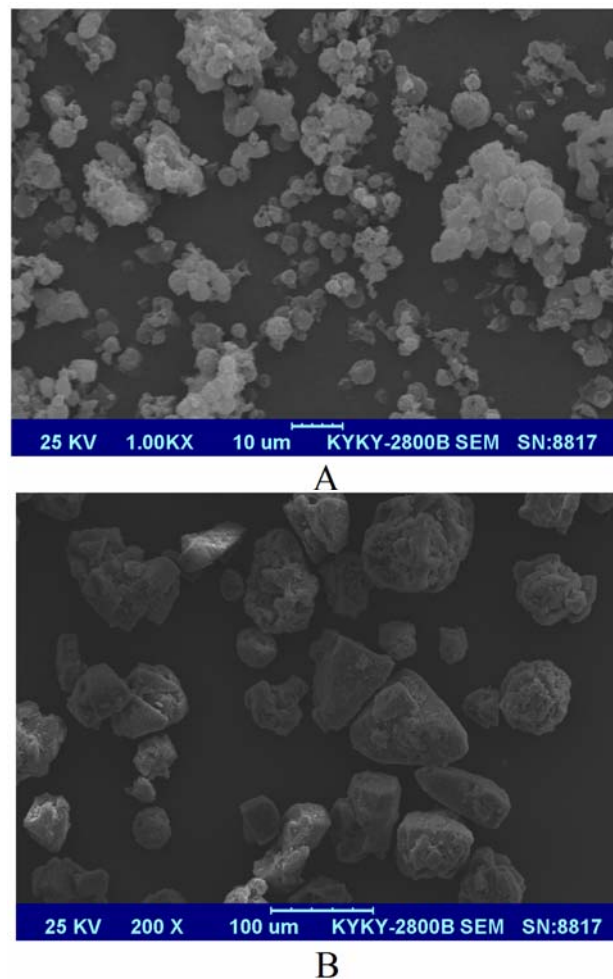


Fig. 2: SEM images of (A) spray-dried powders (B) bulk curcumin powder.

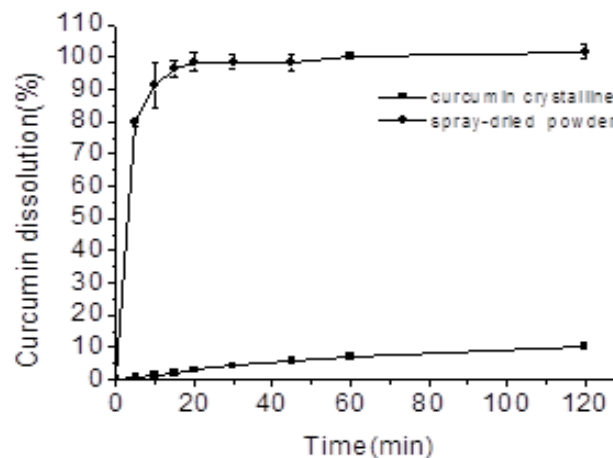


Fig. 3: The dissolution profile of curcumin from curcumin crystalline and spray-dried powders

Pharmacokinetic study

The oral concentration-time curve (AUC) of spray-dried powder and curcumin crystalline in rats is shown in fig. 5. The pharmacokinetic parameters are listed in table 4. The spray-dried powder exhibited significantly increased in AUC_{0-6h} and C_{max} compared with curcumin crystalline. Pharmacokinetic parameters were calculated by noncompartmental analysis. At the same dose equivalent to 400mg/kg of curcumin, the AUC_{0-6h} of the curcumin from spray-dried powder were about 5.5-fold higher than that of the curcumin crystalline ($p < 0.05$). Incorporation nanoemulsion into spray-dried powder resulted in increased absorption of curcumin. From these results, we can conclude that curcumin absorption was enhanced significantly by employing spray-dried powder compared with a curcumin crystalline.

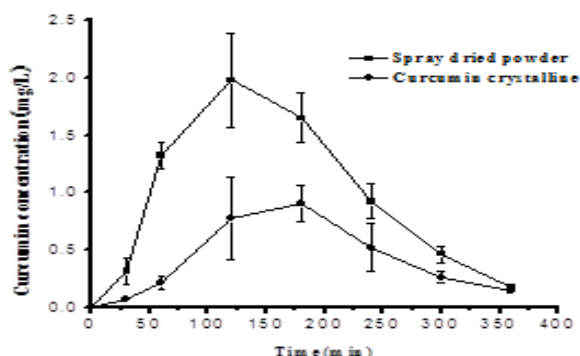


Fig. 4: The mean concentration-time curve after a single oral administration of spray-dried powders and curcumin crystalline in rats.

DISCUSSION

The effect of the spray drying outlet temperature

The effect of outlet temperature was investigated in this study, at first the inlet temperature was fixed at $150 \pm 1^\circ\text{C}$, the varied range of outlet temperature was between 70 and 100°C , the results showed that changing the outlet temperature can significantly affect the yield of curcumin powder. As the increase of outlet temperature, the obtained curcumin powder is also increased. As the temperature was increased, the moisture content of the powder was decreased and less of the powder stickiness to the inside wall of the drying chamber and cyclone, which resulted in higher yield of powder. In subsequent experiments, the outlet temperature is set at 100°C .

The effect of the spray drying inlet temperature

Fixed the outlet temperature at $100 \pm 1^\circ\text{C}$, inlet temperature range of $150 - 180^\circ\text{C}$, the results showed that changing the inlet temperature insignificantly affect the yield of curcumin powder. Therefore, the optimal inlet temperature was set at 150°C .

Optimization of spray-drying process conditions

The operating parameters play a significant role in obtaining high quality product during spray-drying. Some parameters of the spray-drying process such as outlet temperature, flow rate, pump rate, aspiration rate and heat have greater influence on the characteristics of resultant product. In our experiment, Tout is a dependent variable, influenced by Tin and the solution flow. The inlet temperature was set at 150°C . At higher temperatures, the evaporation of the solvent takes place in the atomisation cone with the consequent powder deposition in this region.

Mechanisms of improving oral absorption

The bioavailability differences between spray-dried powders and curcumin crystalline should be attributed the following mechanisms.

Firstly, in the nanoemulsion formulations, oil phases, surfactants and co-surfactants were used for producing nanoemulsion of a small size and good physical stability (Reiss 1975). The presence of oil phases, surfactants and co-surfactants not only improved drug dissolution but also increased the permeability of the intestinal membrane or improved the affinity between lipid particles and the intestinal membrane (Gupta, Kesarla *et al.* 2013). Furthermore, incorporation of curcumin in nanoemulsion can protect the drug from chemical and enzymatic degradation, thereby delaying the *in vivo* metabolism.

Secondly, *In vitro* dissolution tests have confirmed that the dissolution rate of nanoemulsion is significantly faster compared with the rate of curcumin crystalline. An increase in dissolution rate allow drug to reach high concentrations in the GI tract (Neslihan and Benita 2004). Drug absorption involves a drug dissolution step that transfer drug into the luminal fluid followed by transport across the gastrointestinal tract (Craig, Lievens *et al.* 1993). Due to their small particle size, nanoemulsion may exhibit bioadhesion to the GI tract wall and enter the intervillar spaces thus increasing their residence time in

Table 1: The pharmacokinetics parameters after oral administration of spray-dried powders (400mg curcumin/kg body weight) and curcumin crystalline (1000mg/kg body weight) to rats (mean $\pm$ SD)

Parameters	Spray-dried powders	Curcumin crystalline
$T_{1/2}(\text{min})$	48.4 ± 4.13	64.1 ± 7.96
$C_{\text{max}}(\text{mg/L})$	1.98 ± 0.21	1.01 ± 0.13
$T_{\text{max}}(\text{min})$	120 ± 0	156.00 ± 32.86
$\text{AUC}(\text{min} \cdot \text{mg/L})$	374.0 ± 61.7	162.78 ± 23.11
$\text{MRT}(\text{min})$	166.9 ± 4.91	202.64 ± 7.37

the GI tract. This increase in adhesion will result in enhanced bioavailability (Balata *et al.*, 2016). Thus, spray-dried powders could exhibit enhanced bioavailability compared with curcumin crystalline.

CONCLUSIONS

The optimized curcumin nanoemulsion in spray-dried powders was successfully prepared. The *in vitro* drug release results showed that the spray-dried nanoemulsion formulation produced an improved dissolution rate of curcumin. Pharmacokinetic studies in rats revealed that spray-dried powders given at a dose of 400 mg/kg showed 5.5-fold greater absorption of curcumin crystalline. Therefore, our study illustrated the potential use of the new spray-dried curcumin nanoemulsion for enhanced oral bioavailability.

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