Effect of particle size on solubility, dissolution rate, and oral bioavailability: evaluation using coenzyme Q_{10} as naked nanocrystals

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Abstract: In this paper work, four naked nanocrystals (size range 80–700 nm) were prepared without any surfactant or polymer using the solvent/nonsolvent method. The effects of particle size on their solubility, dissolution, and oral bioavailability were investigated. Solubility and dissolution testing were performed in three types of dissolution medium, and the studies demonstrated that the equilibrium solubilities of coenzyme Q_{10} nanocrystals and bulk drugs were not affected by the dissolution media but the kinetic solubilities were. Kinetic solubility curves and changes in particle size distribution were determined and well explained by the proposed solubilization model for the nanocrystals and bulk drugs. The particle size effect on dissolution was clearly influenced by the diffusion coefficients of the various dissolution media, and the dissolution velocity of coenzyme Q_{10} increased as particle size decreased. The bioavailability of coenzyme Q_{10} after oral administration in beagle dogs was improved by reducing the particle size. For 700 nm nanocrystals, the AUC\textsubscript{0–48} was 4.4-fold greater than that for the coarse suspensions, but a further decrease in particle size from 700 nm to 120 nm did not contribute to improvement in bioavailability until the particle size was reduced to 80 nm, when bioavailability was increased by 7.3-fold.

Keywords: particle size, solubility, dissolution, nanocrystal, bioavailability, coenzyme Q_{10}

Introduction

Since Sucker et al produced nanoparticles in the 1980s,\textsuperscript{1} nanonization has attracted much attention, especially for improving the bioavailability of poorly soluble drugs.\textsuperscript{2–7} For the oral administration of poorly soluble drugs, due to solubility limits and low dissolution velocity, there is a low concentration gradient between the gut and blood vessels, leading to limited transport, which consequently affects oral absorption. Increasing the solubility and dissolution rate is a promising approach. In this context, nanonization, ie, a reduction in particle size so that at least one dimension measures less than 1000 nm, has been developed based on theoretical considerations, such as the Ostwald–Freundlich equation,\textsuperscript{2,3,5,7,8} the Noyes–Whitney equation,\textsuperscript{2,4,6–8} the Prandtl equation,\textsuperscript{2,5,9} and Ostwald ripening.\textsuperscript{7}

Among these theories, the Ostwald–Freundlich equation is specific to nanonization as opposed to micronization, and has been used widely in the pharmaceutical industry, although it has attracted some controversy and has not as yet been thoroughly proven.\textsuperscript{10} It is derived from the Kelvin equation, and expresses the dependence of saturation solubility or equilibrium solubility on particle size,\textsuperscript{10} as follows:

\[ \rho v \frac{RT}{M} \ln \frac{S_r}{S_m} = \frac{2 \lambda}{r} \]  

(1)
where $S_r = \text{the solubility of particles of radius } r$, $S_s = \text{the solubility of the solid of a plane surface (or consisting of large particles)}$, $\gamma = \text{interfacial tension}$, $M = \text{the molar mass}$, $\rho = \text{density of the solid}$, $v = \text{represents the number of moles of ions formed from one mole of electrolyte}$, $\nu = 1 \text{ for non-electrolytes}$, $R = \text{the gas constant}$, $T = \text{absolute temperature}$. This equation is applicable to spherical particles smaller than 1000 nm in size, especially 200 nm.\(^\text{11}\) Equation 2 is the Noyes–Whitney equation:\(^\text{12,13}\)

\[
d\frac{dT}{dt} = \frac{DA}{Vh}(C_s - C_i)
\]  

where $dC/dt = \text{dissolution rate}$, $D = \text{diffusion coefficient}$, $A = \text{surface area}$, $V = \text{the volume of the dissolution medium}$, $C_s = \text{saturation solubility}$, $C_i = \text{the drug concentration of the bulk solution}$, and $h = \text{hydromic boundary layer thickness}$, which is also affected by particle size as expressed by the Prandtl equation.\(^\text{9}\) This indicates that, after reduction of particle size, increased saturation solubility, an enlarged surface area, and a thinner diffusion layer could dramatically increase the dissolution velocity, thereby simultaneously improving bioavailability.\(^\text{14}\) Ostwald ripening, like “a big fish eating a small fish,” is a process in which large crystals grow at the expense of smaller ones as a result of differences in solubility.

The particle size effect related to solubility, dissolution, and bioavailability has been documented by many researchers.\(^\text{4,15–17}\) However, the preparations used were stabilized by surfactants or polymers, so may not truly reflect the effects of particle size. Until now, investigations of the particle size effect using naked nanocrystals (ie, 100% drugs without any stabilizer) have rarely been reported.

For nanocrystal systems, obtaining high drug loadings is a challenge. There is a tendency towards precipitation or agglomeration to reduce the enlarged surface area, which usually need to be resisted by adding surfactants or stabilizers,\(^\text{18}\) so that drug loadings of 86% (weight of itraconazole/total solid weight) for sub-300 nm itraconazole nanoparticles have been considered to be high.\(^\text{19}\) There are only a few studies about the use of naked nanocrystals in the pharmaceutical industry to date,\(^\text{20–24}\) and most of the relevant research has only involved the preparation method. Wang et al\(^\text{25}\) and Sun et al\(^\text{26}\) prepared naked nanocrystals of different sizes with coenzyme $Q_{10}$ using the solvent/nonsolvent method and studied the relationship between particle size and solubility in four ethanol–water solutions. However, there is still no systematic research on naked nanocrystals. Thus, the present study was designed to investigate further the effect of particle size on solubility and dissolution of coenzyme $Q_{10}$ from another angle. In addition, the relationship between particle size and bioavailability was investigated using beagle dogs, since this is the accepted model for obtaining more stable data about oral administration.

Materials and methods

Materials

Raw coenzyme $Q_{10}$ material was purchased from Yunnan Chuxiong Sun Pharmaceutical Co, Ltd (Yunnan, China). Coenzyme $Q_{10}$ capsules were obtained from Shanghai Pukang Pharmaceutical Co, Ltd (Shanghai, China). Absolute ethanol (99.7% v/v) and isopropanol (99.7% v/v) were provided by Tianjin Bodi Chemical Holding Co, Ltd (Tianjin, China). Tween 20 was purchased from Tianjin Kerem Chemical Reagent Co, Ltd (Tianjin, China). High-performance liquid chromatography (HPLC) grade methanol was obtained from Jiangsu Hanbon Science and Technology Co, Ltd (Jiangsu, China) and ethanol was obtained from Tianjin Kerem Chemical Reagent Co, Ltd. All other chemicals and reagents were of analytical grade.

Preparation of naked coenzyme $Q_{10}$ nanocrystal and coarse suspensions

A precipitation method was developed for producing suspensions of naked coenzyme $Q_{10}$ nanocrystals. Briefly, 30.0 mg and 10.0 mg of coenzyme $Q_{10}$ were separately dissolved into 3.0 mL of ethanol in a water bath at 60°C as the organic phase. This was then injected into 27.0 mL of double-distilled water at 14,000 rpm and stirred for 15 seconds (IKAT18 Ultra Turrax®, Germany) to obtain suspensions of 120 nm and 80 nm, respectively. The suspensions of 400 nm and 700 nm nanocrystals were prepared by dispersing 27.0 mL of double-distilled water into the organic phase in a 50°C water bath at 30 mL per minute and 15 mL per minute under stirring at 800 rpm and 400 rpm, respectively. The nanocrystal suspension of 80 nm was concentrated by ultrafiltration (Millipore, Bedford, MA). The coarse suspensions were produced by dispersing 30.0 mg of coenzyme $Q_{10}$ in 30.0 mL of distilled water containing 10% v/v ethanol. The coenzyme $Q_{10}$ content of the five suspensions was adjusted to about 1 mg/mL.

Characterization of nanocrystal suspensions

Particle size distribution and zeta potential

The particle size distribution of the naked nanocrystals was determined by dynamic light scattering using a PSS Nicomp

Materials and methods

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380 ZLS equipped with a He-Ne laser source at 632.8 nm (PSS Nicomp, Santa Barbara, CA) at 23°C under an angle of 90 degrees and characterized by intensity-weighted particle size. The suspension sample (about 1 mL) was placed inside the sample holder of the particle size analyzer and then determined directly without dilution. Once the required intensity was reached, an analysis was performed to obtain the mean particle size and polydispersity index. The zeta potential was also measured using the PSS Nicomp 380 ZLS.

Transmission electron microscopy
The samples of different-sized nanocrystal suspensions were diluted with distilled water, pipetted onto collodion film-coated copper grids with a mesh size of 300, dried using absorbent paper, stained with phosphorus tungsten acid for 4 minutes, and finally dried under ambient conditions. Morphological evaluation of the particles was performed using a transmission electron microscope (JM-1200EX; JEOL, Tokyo, Japan).

Differential scanning calorimetry
Thermographs of the nanocrystals and bulk drugs in suspension were obtained using a differential scanning calorimetry instrument (1 STARe; Mettler Toledo, Schwerzenbach, Switzerland). Samples equivalent to 20 μg of coenzyme Q₁₀ were placed in aluminum pans and heated from 20°C to 60°C at a scanning rate of 10°C per minute under a nitrogen purge of 40 mL per minute. The results were analyzed using STARe software. The liquid samples were then placed in separate aluminum pans and dried at room temperature for 12 hours to remove the solvent. Thermographs of dried samples were subsequently obtained under the same operating conditions.

Preparation of dissolution media
Three types of dissolution medium were utilized in this work to investigate the size effect in a series of in vitro dissolution and solubility experiments. Dissolution medium A contained 1.3% w/v Tween 20, dissolution medium B contained 1.3% w/v Tween 20 and 5.0% v/v isopropanol, and dissolution medium C contained 1.3% w/v Tween 20 and 10.0% v/v isopropanol.

Solubility measurement
Shaking behavior
The kinetic solubility of bulk coenzyme Q₁₀ with different amounts of drug content was examined in dissolution medium A, and two types of shaking behavior were investigated. Samples of coenzyme Q₁₀ 2 mg, 6 mg, 10 mg, and 20 mg were each dispersed in 60 mL of dissolution medium, after which 3 mL of samples were transferred into vials that were capped and sealed with Parafilm®. Samples with the same drug content were taped in two directions, ie, horizontally and vertically, to the shaker platform, and then continuously agitated at 100 rpm in a water bath maintained at 25°C. The amount of drug dissolved was determined at different time points by passing each sample through a 0.1 μm Millipore filter and analyzing the drug concentration using reverse-phase high-performance liquid chromatography (HPLC). The measurements were repeated three times.

Solubility of CoQ₁₀ nanocrystals and bulk drugs
The equilibrium solubility values for the four coenzyme Q₁₀ nanocrystals and bulk drugs in the three types of dissolution medium were determined using the dilution method. Taking dissolution medium B as an example, 1 mL of the coenzyme Q₁₀ nanocrystal suspensions of different particle size (drug content equals approximately 1 mg) was added to 2 mL of medium (containing 1.95% w/v Tween 20 and 7.5% v/v isopropanol) in a capped vial to give dissolution medium B (containing 1.3% w/v Tween 20 and 5.0% v/v isopropanol). The vial was sealed with Parafilm, taped horizontally to the shaker platform, and then continuously agitated at 100 rpm in a water bath maintained at 25°C. At different time points, the equilibrated samples were passed through a three-layer membrane filter (pore size from the top to bottom layer being 0.1 μm, 0.05 μm, and 0.1 μm), while a one-layer 0.05 μm Millipore filter was also investigated. The filtrate was analyzed by reverse-phase HPLC.

In vitro dissolution profile measurement
Dissolution studies of five coenzyme Q₁₀ suspensions were conducted in a drug dissolution test apparatus (ZRS-8G, Tianda Tianfa Technology Co, Ltd, Tianjin, China) using the paddle method. Based on the solubility values obtained in the previous section, 0.6 mL of coenzyme Q₁₀ nanocrystal suspension (containing 0.6 mg of coenzyme Q₁₀) was dispersed in 900 mL of dissolution medium to create sink conditions. Dissolution was fixed at 100 rpm and temperature was maintained at 25°C. At predetermined time intervals, 3 mL of dissolution medium was withdrawn and replaced with the same volume of fresh medium. A 0.05 μm membrane filter was used to remove undissolved drug particles. Drug concentrations were quantified by HPLC and dissolution studies were performed in triplicate.
Bioavailability studies in beagle dogs

Twelve healthy beagle dogs (body weight 10.02 ± 0.57 kg) were supplied by the Experimental Animal Center at Shenyang Pharmaceutical University, Shenyang, China. Animal studies were performed in accordance with the guidelines for animal experimentation of Shenyang Pharmaceutical University and approved by the animal ethics committee of the institution. Four different sizes of coenzyme Q₁₀ₐ nanocrystal suspensions, coarse suspensions, and commercial capsules were orally administered to the 12 beagle dogs at a dose of 60 mg/body in a crossover manner. There was a washout period of one week between each consecutive dose. The dogs were fasted for 12 hours before dosing but had access to water. Blood samples (1.5 mL) were collected from an antecubital vein into heparinized syringes at 0 (pre-dose), and at 0.17, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours after dosing. Serum samples were obtained by centrifugation of the blood samples and stored at −20°C until further analysis.

Analytical method

CoQ₁₀₁₀ in dissolution media

Samples for solubility and dissolution investigations were subjected to HPLC using a system consisting of an HPLC pump (Model P230; Dalian Elite Analytical Instruments Co. Ltd, Dalian, China) and an ultraviolet detector (Model UV228; Dalian Elite Analytical Instruments Co. Ltd) set at 212 nm. A C₁₈ column (Haito pack ODS C₁₈, 4.6 mm × 100 mm, 5 μm; Zhongshan Haito Biomaterials Technology Ltd, Guangdong Province, China) operated at 30°C was used as an analytical column. The mobile phase, a mixture of methanol and ethanol (40:60, v/v), was delivered at a flow rate of 1.0 mL per minute and the injection volume was 100 μL.

Coenzyme Q₁₀₁₀ in serum

Coenzyme Q₁₀₁₀ in serum was determined by a validated HPLC method. First, 10 μL of internal standard solution (vitamin K₁, 15 μg/mL in ethanol) was added to 100 μL of serum kept for 20 minutes at 37°C. After mixing thoroughly on a vortex mixer, 200 μL of methanol and 600 μL of hexane were added to the mixture and mixed well on the vortex mixer for 3 minutes. The mixture was then centrifuged at 10,000 rpm for 10 minutes, and 500 μL of supernatant was removed. The extraction was repeated twice in a similar fashion and 600 μL of supernatant was removed. The total volume of supernatant (1100 μL) was collected and dried under nitrogen. The residue was reconstituted in 100 μL of mobile phase which was a mixture of methanol and ethanol (30:70, v/v), followed by centrifugation at 10,000 rpm for 10 minutes and then determined by HPLC consisting of an HPLC pump and an ultraviolet detector (Model UV200II; Elite) set at 275 nm. A 20 μL sample was injected into a C₁₈ column (Hypersil BDS C₁₈, 4.6 mm × 150 mm, 5 μm; Elite) kept at 30°C and using the mobile phase for analysis at a flow rate of 1.0 mL per minute for 10 minutes. All experiments were performed under low lighting conditions.

Pharmacokinetic analysis

Standard pharmacokinetic parameters for coenzyme Q₁₀₁₀ were obtained from the plasma concentration-time curves. These pharmacokinetic parameters included peak concentration of the drug in plasma (Cₘₚₘₚ), the time taken to reach peak concentration, and the area under the curve (AUC₀→₄₈), which was calculated from 0 to 48 hours using a linear trapezoidal rule.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance and the Student’s t-test with SPSS version 16.0 software (IBM Corporation, Armonk, NY). P-values less than 0.05 were considered to be statistically significant.

Results and discussion

Size distribution and zeta potential of coenzyme Q₁₀₁₀ nanocrystals in suspension

As shown in Figure 1, suspensions of the bulk drug and the naked coenzyme Q₁₀₁₀ nanocrystals of different sizes had different appearances. The drug contents of the five formulations were the same, and the difference in appearance was caused only by variations in size. It was found that all the nanocrystals were homodispersed, with a shift from a
darker to lighter color on reduction in size. In contrast, the
bulk drug dispersed in suspension was quite unstable and
had sedimented completely by 2 days.

The average size, size distribution, and zeta potential for
each of the four different-sized coenzyme Q_{10} nanocrystals
are shown in Table 1, and the physical stability results are
shown in Figure 2. The average size of the coenzyme Q_{10}
nanocrystals in the different suspensions was found to be
80.6 nm, 122.2 nm, 391.6 nm, and 680.1 nm (expressed as
80 nm, 120 nm, 400 nm, and 700 nm, respectively). The
400 nm- and 700 nm-sized nanocrystals were stable for at
least 2 weeks, but the 80 nm- and 120 nm-sized nanocrystals
were physically stable for up to 1 month at room temperature.
Zeta potential measurement demonstrated that the nanocrystals
in suspension were negatively charged, which might account for the adsorption caused by the large surface area,
and contributed to their physical stability. Strong Brownian
motion and small density differences between the coenzyme
Q_{10} nanocrystals and the solvent could also have contributed
to their stability.

**Evaluation of morphology and crystal form**

Transmission electron micrographs clearly show that the
nanocrystals were rounded (Figure 3), which is crucial for
investigation of the relationship between size and solubility
proposed by the classical Ostwald–Freundlich equation.

Differential scanning calorimetry and X-ray powder
diffraction analysis are usually used to detect if the crystal
form has changed. Because the coenzyme Q_{10} nanocrystals
were prepared without any stabilizer and dispersed in
an ethanol–water mixture, additional processes, such as
lyophilization or other dry methods, could lead to a change
in particle size. Therefore, nanocrystals in suspension
were used in subsequent experiments. Small-angle X-ray
scattering showed disappointingly low sensitivity for
80 nm nanocrystals in suspension, so liquid differential
scanning calorimetry was chosen to investigate the original
suspension sample. The results of liquid differential scanning
calorimetry (see Figure 4) show that the nanocrystals failed
to demonstrate the melting point of coenzyme Q_{10} even
when concentrated 50-fold. Interestingly, the raw materials
dispersed in water had a typical melting point of about
50°C, which disappeared after 4 hours of storage at room
temperature, showing that the crystal transformation of
coenzyme Q_{10} might be induced by the solvent. In order
to validate this hypothesis, dried samples were used as a
reference preparation. All the samples showed an obvious
endothermic peak (see Figure 5), especially for the 50-fold
concentrated 120 nm nanocrystal suspension, in which the
amount of drug was high enough to produce sensitivity, and
further showed solvent-induced crystal transformation.

**Solubility**

For poorly soluble coenzyme Q_{10}, the aqueous solutions
provided solubility and dissolution results which were too
low for measurement, so the nonionic surfactant Tween 20
was used, in accordance with an already reported method. Furthermore, different concentrations of isopropanol, which
has lower volatility than ethanol, were added to prepare
three different media for investigating the effects of size.
Although the surfactant was used, it was added after the
nanocrystals were prepared so as to develop an appropriate
dissolution condition. This is different from the situation
in which the surfactant was used as a stabilizer when
nanocrystals were prepared. Even though all formulations
were prepared using the same concentrations of surfactant,
the density of the surfactants dispersed in different-sized
nanocrystals was different due to the curvature and surface
area.

**Investigation of shaking behavior**

In the literature, solubility is always measured by adding
an excess amount of drug to the dissolution medium, while
shaking continuously in a water bath and sampling at a fixed
time (such as 24 hours). Because determination of solubility
at equilibrium takes a long time, and many problems,
such as chemical instability, biodegradation, and solvent
volatilization, can adversely affect the results, “kinetic”
solubility is preferable and is frequently determined.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Particle size, polydispersity index, and zeta potential of coenzyme Q_{10} nanocrystals (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>Particle size (nm)</td>
</tr>
<tr>
<td>A</td>
<td>80.6 ± 4.3</td>
</tr>
<tr>
<td>B</td>
<td>122.2 ± 10.5</td>
</tr>
<tr>
<td>C</td>
<td>391.6 ± 14.3</td>
</tr>
<tr>
<td>D</td>
<td>680.1 ± 63.1</td>
</tr>
</tbody>
</table>

**Abbreviation:** PI, polydispersity index.
As shown in Figure 6, drug content had obvious effects on kinetic solubility. The higher the drug content, the faster the dissolution rate and the shorter the time taken to reach equilibrium. The shaking behavior did not have a negligible effect. Solubility experiments are usually conducted at 100 rpm, but in our study, this rate of agitation was found to be weak and the vibration was distributed nonuniformly when the samples were placed vertically so that drug particles sedimented at the bottom of the tubes. The reduced surface area exposed to the dissolution medium resulted in a longer equilibrium time, as shown in Figure 6. When the samples were placed horizontally, a full vibration effect was obtained and samples with a drug content of 0.10 mg/mL, 0.17 mg/mL, and 0.33 mg/mL all reached equilibrium by day 25.
If equilibrium is not reached, a false impression that solubility is higher as drug content increases is created, leading to inaccuracy when comparing different drugs or formulations. Thus, when kinetic solubility is used as an index for comparison, it is essential to identify the amount of drug being used compared to the vague phrase “excess amount.” In addition, the method mentioned above could also be used for assessing equilibrium, and when the solubilities of more than two samples in a series of samples with varying drug content exhibit the same value after full agitation, equilibrium is reached, and the same value is the equilibrium solubility. It is worth noting that this method is not used for ionized drug, the solubility of which is dependent on drug content.

Solubility of coenzyme Q_{10} nanocrystals and bulk drug

Solubility experiments are usually conducted using drugs in the solid state. Considering that drying naked nanocrystals without any surfactant may change the particle size, and the adsorbed gas as a barrier would influence the dissolution rate, the suspensions were used directly, ie, by adding a certain volume of nanosuspension or coarse suspension to a solution to obtain the final dissolution medium. In each final dissolution system, about 3.3% of ethanol residue was introduced by the nanosuspensions. Dialysis in water was initially used to remove ethanol from the nanosuspensions, but this was abandoned because the particle size changed and the particles became unstable. Therefore, each formulation was treated in the same way to ensure consistency. In addition, previous experiments indicated that the influence of 3.3% ethanol on solubility could be ignored. 

As shown in Figure 7, the solubility values obtained using different Millipore filters differed greatly, especially for the 80 nm and 120 nm particle sizes. For 80 nm nanocrystals, after one day in dissolution medium A, the value obtained with the one-layer filter was 25.4-fold higher than that using...
the three-layer filter. Although both minimum pore sizes were 0.05 μm, small particles passed through the one-layer filter more easily. Further reduction of pore size, such as using a 0.03 μm filter, was not feasible, because water or ethanol had difficulty passing through. For this reason, a three-layer filter was used for the first time to obtain a better solid–liquid separation. The upper layer trapped large particles and prevented blockages, the middle layer, which is chosen based on the Nicomp distribution analysis intercepted small particles, while the bottom layer acted as a supporting surface, and the crisscrossing of layer to layer provided better solid-liquid separation and accurate solubility data as far as possible. For different sized nanocrystals, the kinetic solubility obtained using three-layer filters increased as the particle size decreased in this experiment.

In a previous study, we proposed a new concept of “interfacial solubility” (C*)_x, which is the average concentration of the boundary layer and describes the dissolution process of nanocrystals and bulk drugs. As shown in Figure 8, “solubilization” is a kinetic process and involves both dissolution and precipitation, which occur at the same time but in different ratios, and usually proceed by diffusion. The driving force is the difference between the interfacial solubility (C*)_x and the concentration of the bulk solution (C_x). The disjoining pressure of small particles is greater than that of large particles, so small particles have a higher interfacial solubility. Due to their higher differential concentration, thinner diffusion layer, and increased surface area, small particles dissolve faster (Figure 8A). For small particles, extremely fast dissolution quickly increases the concentration of the bulk solution to achieve a supersaturated state. On account of the presence of residual particles which induce precipitation and an increasing degree of supersaturation, the rate of precipitation gradually increases until it becomes equal to the rate of dissolution when the bulk solution concentration reaches its maximum, C_x(max). The rate of precipitation then exceeds that of dissolution, the bulk solution concentration decreases, and desaturation increases. As the driving force of precipitation is gradually reduced, the concentration of the bulk solution finally reaches a plateau, which is the constant saturation solubility, C_s (ie, the equilibrium solubility). The concentration of the bulk solution C_s, usually measured in practice, is called the kinetic solubility, which is a metastable state, and eventually returns to the equilibrium solubility (Figure 8B). This process happens when the concentration gradient is high enough. It happens faster after dilution, so that at last small particles would dissolve and precipitate into large particles, a process known as “Ostwald ripening.”

As shown in Figure 7, the content of isopropanol had no obvious effect on the final equilibrium solubility of coenzyme Q_{10}, while the kinetic solubility was different for the three types of dissolution medium, perhaps due to their different diffusion coefficients. As the content of isopropanol in the dissolution medium increased from 0% to 10%, ripening became faster, and the concentration of the bulk solution reached its maximum at days 10, 3, and 1, respectively. For the different-sized nanocrystals, the smaller the size, the faster the ripening process (see Table 2). The trend of nanocrystal solubilization was that concentrations first increased to reach a supersaturated state and then decreased. In contrast, the concentrations increased gradually for bulk drugs.

Ripening is a problem when investigating the relationship between particle size and equilibrium solubility in addition to solid-liquid separation, because it is an inevitable process and occurs at the same moment as dilution (as shown in Table 2). When ripening happens, the values obtained represent kinetic solubility rather than equilibrium solubility, and small particles change to large ones when absolute equilibrium is reached. The best method is to determine the equilibrium solubility of coenzyme Q_{10} nanocrystals in suspension directly without dilution, after nanocrystal suspensions of uniform particle size have been prepared. Unfortunately, this is limited by absorption of coenzyme Q_{10} onto the Millipore filter.

**In vitro dissolution study**

In vitro dissolution of coenzyme Q_{10} from the suspensions containing 80 nm, 120 nm, 400 nm, or 700 nm naked nanocrystals and bulk drugs was also investigated in the three types of dissolution medium. Dissolution of nanocrystals smaller than 200 nm is seldom documented. In this paper, dissolution conditions were selected based on the following considerations: the barrier effect for diffusion caused by dialysis bags, which is a common method for dissolution
testing, should be avoided;\textsuperscript{25,26} the centrifugation method is used extensively for solid-liquid separation, but is not able to separate out small coenzyme Q\textsubscript{10} nanocrystals (a suspension of 139 nm nanocrystals was centrifuged at 30,000 rpm for one hour, and the size distributions of the top, middle, and bottom layers were all the same). There are also reports of using ultracentrifugation or centrifuging twice,\textsuperscript{29,30} but this takes a considerably longer time and is conducted at a low temperature, which changes the drug dissolution conditions; submicron particles, eg, 80 nm, can easily pass through common filters (\(\geqslant 0.1\) μm),\textsuperscript{4,31} and the three-layer filters described in the previous section are not appropriate for timely sampling. Although in vitro dissolution is often used to predict the in vivo behavior of drugs and experiments are usually carried out at 37°C, the temperature was set at 25°C considering the change in temperature during filtration with a 0.05 μm filter and the conditions being consistent with solubility measurement.

As shown in Figure 9, the dissolution rate of coenzyme Q\textsubscript{10} in the suspensions was clearly affected by particle size. In dissolution medium A, only the 80 nm and 120 nm nanocrystals showed marked dissolution. The peaks observed at 2 hours (51.4% and 45.2% for 80 nm and 120 nm, respectively) may be due to undisolved small particles passing through the 0.05 μm filter. In the case of the 80 nm nanocrystals, 65.4% of coenzyme Q\textsubscript{10} was dissolved in 24 hours, while for the 120 nm nanocrystals this value was only 13.2%. In dissolution medium B, the difference in dissolution profiles for the 80 nm and 120 nm nanocrystals was further demonstrated. Both showed rapid dissolution in 30 minutes, with the former producing dissolution of 62.8% and the latter producing dissolution of 37.0%. The 120 nm nanocrystals then dissolved slowly, and the fraction only reached 43.3% in 24 hours. In contrast, the 80 nm produced a steady increase to 85.4%. Although the 400 nm and 700 nm nanocrystals and bulk drugs showed very little dissolution, their dissolution profiles were similar until the ratio of isopropanol increased to 10% v/v (ie, dissolution medium C). Under this condition, the 80 nm and 120 nm nanocrystals rapidly dissolved in 30 minutes and both reached 100% dissolution at 2 hours and 4 hours. The three different dissolution profiles were clearly not influenced by solubility, because the solubilities of coenzyme Q\textsubscript{10} in the three types of medium were close, as shown in Figure 7. The perfect sink conditions avoided supersaturation and consequent ripening, so that our results only reflect different diffusion behavior. As the content of isopropanol increased, diffusion became easier and dissolution proceeded faster. These findings indicate that the diffusion coefficient of the dissolution medium is an important factor, in addition to the solubility that influenced the particle size effect on dissolution.

### Bioavailability study in dogs

All serum samples were analyzed following the HPLC method described earlier. Calibration curves (10–1000 ng/mL, eight concentrations) and quality control samples (50, 200, and 1600 ng/mL) were freshly prepared for each analysis. The lower limit of quantification using this method was 10 ng/mL. The linear regression coefficients for the calibration curves ranged from 0.99 to 0.9999. The accuracy and precision of the quality control samples ranged from 97.3% to 103.5% of nominal values, and 1.3% to 10.6% for the coefficient of variation, respectively. The results show that

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**Table 2: Size and size distribution of different coenzyme Q\textsubscript{10} nanocrystals after dissolution in three types of dissolution medium for different predetermined times\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Day</th>
<th>80 nm Size (nm)</th>
<th>80 nm PI</th>
<th>120 nm Size (nm)</th>
<th>120 nm PI</th>
<th>400 nm Size (nm)</th>
<th>400 nm PI</th>
<th>700 nm Size (nm)</th>
<th>700 nm PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>78.8 ± 0.8</td>
<td>0.05 ± 0.02</td>
<td>132.9 ± 0.9</td>
<td>0.08 ± 0.00</td>
<td>376.9 ± 2.0</td>
<td>0.15 ± 0.00</td>
<td>698.7 ± 2.4</td>
<td>0.23 ± 0.01</td>
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<td></td>
<td>1</td>
<td>81.1 ± 3.7</td>
<td>0.07 ± 0.00</td>
<td>149.4 ± 4.8</td>
<td>0.06 ± 0.00</td>
<td>322.9 ± 4.4</td>
<td>0.18 ± 0.00</td>
<td>667.2 ± 5.1</td>
<td>0.23 ± 0.00</td>
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<td></td>
<td>3</td>
<td>146.7 ± 22.8</td>
<td>0.08 ± 0.01</td>
<td>139.7 ± 9.3</td>
<td>0.05 ± 0.01</td>
<td>330.4 ± 17.0</td>
<td>0.16 ± 0.00</td>
<td>657.8 ± 22.9</td>
<td>0.24 ± 0.01</td>
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<td></td>
<td>30</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>506.8 ± 87.0</td>
<td>0.43 ± 0.06</td>
<td>648.8 ± 66.3</td>
<td>0.26 ± 0.03</td>
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<tr>
<td>B</td>
<td>1</td>
<td>104.4 ± 18.5</td>
<td>0.27 ± 0.09</td>
<td>189.6 ± 13.0</td>
<td>0.21 ± 0.04</td>
<td>385.4 ± 1.2</td>
<td>0.07 ± 0.01</td>
<td>688.4 ± 17.3</td>
<td>0.10 ± 0.01</td>
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<td>312.8 ± 74.4</td>
<td>0.39 ± 0.05</td>
<td>198.0 ± 43.0</td>
<td>0.30 ± 0.04</td>
<td>384.4 ± 39.2</td>
<td>0.18 ± 0.01</td>
<td>706.1 ± 79.8</td>
<td>0.24 ± 0.02</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>763.7 ± 131.1</td>
<td>0.40 ± 0.10</td>
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<tr>
<td>C</td>
<td>1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>459.7 ± 38.2</td>
<td>0.08 ± 0.01</td>
<td>745.3 ± 83.2</td>
<td>0.22 ± 0.04</td>
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<td></td>
<td>3</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>620.1 ± 127.3</td>
<td>0.46 ± 0.08</td>
<td>924.4 ± 139.7</td>
<td>0.49 ± 0.19</td>
</tr>
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</table>

**Notes:** \(\ast\) All data were obtained from three experiments and expressed as the mean ± standard deviation. x represents large visible particles which aggregated into very large bundles and subsequently sedimented.

**Abbreviation:** PI, polydispersity index.
Pharmacokinetic parameters obtained after oral administration of different-sized nanocrystal suspensions, coarse suspensions, and capsules in beagle dogs are presented in Figure 10 and Table 3, respectively. These results show that the absorption of coenzyme Q₁₀ markedly improved as the particle size was reduced to the nanoscale range. Compared with coarse suspensions of the bulk drug, the $C_{\text{max}}$ and AUCₘₐₜ of the naked coenzyme Q₁₀ nanocrystal suspensions with particle sizes of 80 nm, 120 nm, 400 nm, and 700 nm were increased by 10.8-fold, 5.8-fold, 5.0-fold, and 4.5-fold, and by 7.3-fold, 5.1-fold, 4.7-fold, and 4.4-fold, respectively. Compared with commercial capsules, the values were increased by 9.2-fold, 4.9-fold, 4.2-fold, and 3.8-fold, and by 6.8-fold, 4.8-fold, 4.4-fold, and 4.1-fold, respectively. Although the differences in particle size were significant for coenzyme Q₁₀ nanocrystals that were 120 nm to 700 nm in size, the increases in $C_{\text{max}}$ and AUCₘₐₜ observed for these nanocrystal suspensions were almost the same (not statistically significant). This indicates that the particle size effect on oral absorption has a fixed range, and controlling

Figure 9 Dissolution profiles of coenzyme Q₁₀ nanocrystals and bulk drugs from the suspensions in three types of dissolution medium. (A) Distilled water containing 1.3% w/v Tween 20, (B) distilled water containing 1.3% w/v Tween 20 and 5.0% v/v isopropanol, and (C) distilled water containing 1.3% w/v Tween 20 and 10.0% v/v isopropanol.

Notes: The dissolution study was performed at 100 rpm and 25°C. About 0.6 mg of coenzyme Q₁₀ was used in 900 mL of dissolution medium. The results are expressed as the mean ± standard deviation of three replicates. ● = 80 nm; ○ = 120 nm; ▲ = 400 nm; △ = 700 nm; ■ = bulk drugs.

Table 3 Pharmacokinetic parameters obtained after oral administration of different coenzyme Q₁₀ nanocrystal suspensions, coarse suspensions, and capsules (n ≥ 4)*

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$T_{\text{max}}$ (hours)</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>AUCₘₐₜ (µg h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 nm</td>
<td>3.25 ± 0.50*</td>
<td>1.19 ± 0.18***</td>
<td>17.52 ± 1.55***</td>
</tr>
<tr>
<td>120 nm</td>
<td>4.86 ± 3.34*</td>
<td>0.64 ± 0.24***</td>
<td>12.18 ± 5.47***</td>
</tr>
<tr>
<td>400 nm</td>
<td>5.60 ± 1.67*</td>
<td>0.55 ± 0.09***</td>
<td>11.25 ± 1.16***</td>
</tr>
<tr>
<td>700 nm</td>
<td>6.00 ± 3.58</td>
<td>0.50 ± 0.06***</td>
<td>10.53 ± 1.79***</td>
</tr>
<tr>
<td>Coarse</td>
<td>19.50 ± 9.00</td>
<td>0.11 ± 0.01</td>
<td>2.40 ± 0.74</td>
</tr>
<tr>
<td>Capsules</td>
<td>19.60 ± 12.52</td>
<td>0.13 ± 0.03</td>
<td>2.56 ± 0.63</td>
</tr>
</tbody>
</table>

Notes: Results are expressed as the mean ± standard deviation. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with the corresponding parameters of coarse suspensions.

Abbreviations: $T_{\text{max}}$, time taken to reach peak plasma concentration; $C_{\text{max}}$, peak plasma concentration; AUC, area under the concentration-time curve.
the sizes in this range could achieve the same bioavailability, which makes application on an industrial scale more feasible. The significant discrepancy between the 80 nm and 120 nm nanocrystals in this regard may be attributed mainly to different degrees of uniformity. The 120 nm nanocrystals displayed a wide distribution (polydispersity index 0.156, see Table 1) and had two major particle size groups (82 nm and 191 nm, respectively), which led to unstable behavior in vivo which was evident in the pharmacokinetic parameters, with large standard deviations (see Table 3). This unusual result indicates that particle size uniformity is crucial for nanocrystals, because inhomogeneous nanocrystals were not only invalid but also showed increased individual differences.

Because coenzyme Q\textsubscript{10} is classified as a Class II drug according to the Biopharmaceutics Classification System,\textsuperscript{32} its absorption could be rate-limited by the dissolution process. Although there was no correlation between in vitro dissolution and in vivo bioavailability, the profiles shown in Figure 9 may still reflect the in vivo dissolution behavior. In the paper published by Jinno et al.,\textsuperscript{4} the absorption of cilostazol nanocrystals was lower in the fed state than in the fasted state, with results that were different from those for coarse suspensions. The authors considered that dissolution of cilostazol nanocrystals was rapid enough for absorption to be limited by permeability. The results of this dissolution experiment indicate that lesser absorption of nanocrystals in the fed state than in the fasted state might be partly due to a reduced diffusion coefficient after eating.

The particle size effect was not related to solubility at equilibrium, because the solubility at equilibrium was almost the same for all the four sizes of nanocrystals and the bulk drugs. The improvement in kinetic solubility, dissolution rate, and adhesion\textsuperscript{18} should be responsible for the improved bioavailability of coenzyme Q\textsubscript{10}. Taken together, reduction in particle size from the microscale to the nanoscale could enhance bioavailability, but a further reduction in particle size did not contribute to the improvement in bioavailability until it was reduced to less than 100 nm.

**Conclusion**

Four naked stable coenzyme Q\textsubscript{10} nanocrystals of different size were prepared and characterized to generate a drug model for investigating the effects of particle size. The kinetic solubility of four sizes of nanocrystals and bulk drugs was determined in three types of medium. The relationship between size and various solubilities (ie, kinetic solubility, equilibrium solubility) was studied. The results clearly show that particle size was related to kinetic solubility rather than equilibrium solubility. The biggest challenge in investigating the relationship between particle size and equilibrium solubility was found to be solid-liquid separation and the ripening process. A new concept of “interfacial solubility” and a solubilization model of nanocrystals and bulk drugs is proposed, based on the results of this study and is verified by trends in particle size and kinetic solubility. Dissolution testing of four nanocrystals and bulk drugs in three types of dissolution medium also clearly showed the effect of particle size, which was influenced by the diffusion coefficient. The effect of particle size on oral absorption has a fixed range, with coenzyme Q\textsubscript{10} nanocrystals from 700 nm to 120 nm in size having similar bioavailability. Further, for small nanocrystals 120 nm in size, a wide size distribution (polydispersity index 0.156) increased the individual differences, which indicates that small is not always optimal. Our findings for naked nanocrystals may have significant implications for understanding problems of nanonization, ie, effects of particle size on solubility, dissolution, and in vivo bioavailability, and assist in the design of new nanodrug delivery systems.

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**


