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# Elevating Bioavailability of Curcumin via Encapsulation with a Novel Formulation of Artificial Oil Bodies

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**ABSTRACT:** Utilization of curcumin has been limited due to its poor oral bioavailability. Oral bioavailability of hydrophobic compounds might be elevated via encapsulation in artificial seed oil bodies. This study aimed to improve oral bioavailability of curcumin via this encapsulation. Unfortunately, curcumin was indissoluble in various seed oils. A mixed dissolvent formula was used to dissolve curcumin, and the admixture was successfully encapsulated in artificial oil bodies stabilized by recombinant sesame caleosin. The artificial oil bodies of relatively small sizes (150 nm) were stably solidified in the forms of powder and tablet. Oral bioavailability of curcumin with or without encapsulation in artificial oil bodies was assessed in Sprague−Dawley male rats. The results showed that encapsulation of curcumin significantly elevated its bioavailability and provided the highest maximum whole blood concentration ($C_{max}$), 37 ± 28 ng/mL, in the experimental animals 45 ± 17 min ($t_{max}$) after oral administration. Relative bioavailability calculated on the basis of the area under the plasma concentration−time curve (AUC) was increased by 47.7 times when curcumin was encapsulated in the artificial oil bodies. This novel formulation of artificial oil bodies seems to possess great potential to encapsulate hydrophobic drugs for oral administration.

**KEYWORDS:** *artificial oil bodies, bioavailability, caleosin, curcumin, encapsulation*

## INTRODUCTION

Curcumin, a hydrophobic polyphenol with yellow color, is a major constituent extracted from the dried root of turmeric, the rhizome of the herb *Curcuma longa*, a plant that grows on the Indian subcontinent and in tropical countries of Southeast Asia.[1] In addition to its antioxidative and anti-inflammatory activities, curcumin is proposed to possess pleiotropic pharmacological effects on neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune, and neoplastic diseases.[2−4] However, the applications of curcumin in pharmacological utilization have been impeded due to its extremely low water solubility and poor oral bioavailability.[5] Many techniques and protocols have been developed to increase the solubility and bioavailability of curcumin in the past decade, such as encapsulation in various types of emulsions to control its release and distribution.[6,7]

Plant seed oil bodies are intracellular lipid storage organelles of sizes ranging from 0.5 to 2 μm.[8] They are extremely stable both in vivo and in vitro as a consequence of their unique structural organization, a triacylglycerol matrix surrounded by a monolayer of phospholipids and proteins. The oil-body proteins comprise at least three classes: oleosin, caleosin, and steroleosin.[9−11] Stable artificial oil bodies have been technically reconstituted with the three essential components of oil bodies: triacylglycerols, phospholipids, and oil-body proteins.[12] Moreover, a single class of oleosin or caleosin, but not steroleosin, was found capable of stabilizing artificial oil bodies in the reconstitution.[13]

Several application platforms have been developed on the basis of artificial oil bodies, including a protein expression system, an oral delivery system for hydrophobic drugs, a new enzyme fixation technique, and a hapten presentation system for producing monospecific antibodies against small molecules.[14−22] In the oral delivery system, oil-soluble drugs encapsulated in artificial oil bodies are comparatively stable and seem to be gradually released in the digestive system of animals with an improved bioavailability.[16]

In this study, we aimed to improve the oral bioavailability of curcumin via encapsulation in artificial oil bodies. In the first attempt, curcumin could not be dissolved in various vegetable oils. Instead, a mixed dissolvent formula was used to dissolve curcumin, and the admixture was successfully encapsulated in artificial oil bodies. Particle sizes and storage stability of this new type of artificial oil body in the forms of solution, powder, and tablet were examined. The bioavailability of curcumin in whole blood of the rats fed curcumin with or without encapsulation in the artificial oil bodies was evaluated.

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## MATERIALS AND METHODS

**Chemicals and Materials.** D-(−)-Mannitol was obtained from Merck KGaA (Darmstadt, Germany). Curcumin, dioleoylphosphatidylcholine, IGEPAL CA-210, Cremophor EL, polyethylene glycol 400 (PEG 400), ethyl oleate, acetonitrile, acetic acid, and methanol were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Sesame oil and olive oil were purchased in a local market. Castor oil and cottonseed oil were purchased from Acros (Fair Lawn, NJ, USA). Sesame (*Sesamum indicum* L.) seeds were provided and authenticated by Dr. Yiu of the Tainan District Agricultural Research and Extension Station, Taiwan. Water was purified by a Millipore clear water purification system (Millipore Direct-Q, USA).

**Dissolution of Curcumin.** To evaluate its dissolubility in various seed oils, 10 mg of curcumin was mixed with 1 mL of sesame, castor, cotton, or olive oil, and the mixture was vortexed for 30 min. Moreover, a mixed dissolvent formula composed of 30 mL of IGEPAL CA-210, 30 mL of Cremophor EL, 30 mL of PEG 400, and 10 mL of ethyl oleate was prepared. The resulting dissolvent of 400 μL was used to dissolve 10 mg of curcumin by vortex for 30 min.

**Purification of Sesame Oil Bodies.** Sesame seeds were soaked in water for 10 min prior to the purification of oil bodies according to the method developed by Tzen et al.[23] The method included two-layer flotation by centrifugation, detergent washing, ionic elution, treatment with a chaotropic agent, and integrity testing with hexane.

**Production of Recombinant Sesame Caleosin.** A full-length cDNA clone of sesame caleosin (accession no. AF109921) was constructed in the nonfusion expression vector pET29a(+) (Novagen), transformed to *Escherichia coli* strain BL21 (DE3), and overexpressed in a bacteriophage T7 RNA polymerase/promoter system as described previously.[24] Proteins extracted from *E. coli* cells were mixed with the sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 0.02% bromophenol blue, 10% glycerol, and 5% β-mercaptoethanol and resolved by SDS-PAGE using 15% acrylamide. Following electrophoresis, the gel was stained with Coomassie Blue R-250. The recombinant caleosin eluted from the SDS-PAGE gel was quantitated by using the ImageJ 1.41 program (http://rsb.info.nih.gov/ij/) and used to generate artificial oil bodies for encapsulation of curcumin.

**Encapsulation of Curcumin in Artificial Oil Bodies.** Artificial oil bodies were generated with 60 mg of the dissolvent containing curcumin, 150 μg of dioleoylphosphatidylcholine, and 250 μg of the recombinant caleosin in 1 mL of 10 mM sodium phosphate buffer, pH 7.5.[24] Dioleoylphosphatidylcholine dissolved in chloroform was placed at the bottom of an Eppendorf tube, and the chloroform was allowed to evaporate in a chemical hood overnight. After evaporation, the curcumin-loaded dissolvent and the recombinant caleosin suspended in 1 mL of the sodium phosphate buffer were incorporated, followed by sonication with a 3 mm diameter probe in a Sonics & Materials VCX750 ultrasonic processor (Newtown, CT, USA) with 30% amplification for 20 s, and samples were cooled in an ice bucket for 5 min. The sonication was repeated two more times to generate artificial oil bodies encapsulating curcumin. Relative sizes between artificial oil bodies with or without encapsulation of curcumin and isolated sesame oil bodies suspended in 50 mM sodium phosphate buffer, pH 7.5, were observed under a Nikon type-104 light microscope.

**Solidification of Artificial Oil Bodies.** Five hundred milligrams of mannitol was used as an excipient to facilitate the solidification of the artificial oil bodies of 1 mL in a freeze-drying process commonly used for food products; the sample was precooled at −80 °C and then solidified in an FD-8530 dryer (Pan Chum Scientific Corp, Taiwan) by heating at 30 °C under vacuum of 200 mTorr for 24 h.[25] Tablets of 200 mg were made of powdered artificial oil bodies under the pressure of 500 lb for 10 s using a flat-face round tooling on a tablet machine (Carver press, model C, Carver Inc., Wabash, IN, USA). In this preparation, the tablet formulation was composed of 50% mannitol. Particle charge (zeta potential) and size distributions of curcumin-loaded artificial oil bodies in the forms of solution, powder, and tablet, before and after storage at 4 °C for a week, were determined using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK) at 25 °C with the He−Ne laser beam scattering angle of 173°.

**Animal Experiment.** Sprague−Dawley male rats weighing 250−350 g were obtained from BioLASCO Taiwan Co. (Taipei, Taiwan). The animals were kept under standardized conditions, in a light- and temperature-controlled room (12:12 h light−dark cycle with humidity at 60 ± 5% and a temperature of 22 ± 2 °C), with free access to rat chow and water at all times. They were acclimatized to laboratory conditions over 1 week before the experiments. The rats were fasted overnight, and only water was supplied before treatments. The experimental protocol was approved by the Animal Committee of the National Chung-Hsing University (IACUC Approval 99-52). The rats were divided into two groups of five rats each. Group I animals received a single oral administration equivalent to 1000 mg/kg curcumin, whereas group II animals received 50 mg/kg curcumin encapsulated in artificial oil bodies of tablets. Blood samples (0.4 mL each) were collected through the tail vein at 0, 10, 20, 30, 60, 90, 120, and 180 min postadministration. The blood samples were centrifuged at 15000*g* for 15 min, and the supernatants were collected as plasma samples in tightly sealed plastic tubes (heparin lithium anticoagulation) and kept frozen at −20 °C until analysis.

**Pharmacokinetic Analysis.** Maximum whole blood concentration ($C_{max}$) of curcumin and the time to reach $C_{max}$ ($t_{max}$) were recorded directly from the experimental results. The area under the plasma concentration−time curve (AUC) was calculated by the linear trapezoid rule. Body clearance (CL) was calculated by dose (mg)/AUC (mg × h/L), elimination rate constant (Ke) was obtained from the ratio of $0.693/t_{1/2}$, and volume of distribution (Vd) was calculated by CL/Ke. Elimination half-life ($t_{1/2}$) was calculated by dividing ln 2 by elimination rate constant.[26] All data are reported as means ± SEM. A $p$ value <0.05 was considered statistically significant.

**Quantitation of Curcumin.** To quantitate curcumin, a linear standard regression equation was established by loading standard solutions of a serial dilution of known curcumin concentrations, 0.02, 0.05, 0.1, 0.5, 1, 2, 4, and 8 μg/mL (in blood or water), onto HPLC, and the cover areas of the peaks were recorded. One hundred milligrams of curcumin was first dissolved in 1 mL of acetonitrile and further diluted serially with blood or water to the desired concentrations. Similar values were detected for curcumin dissolved in either blood or water. The contents of curcumin in the artificial oil bodies as well as in the blood samples were analyzed by HPLC and quantitated by using the linear standard regression equation ($y = 53411X + 1373.1$, $R^2 = 0.9978$).

Encapsulation efficiency of curcumin in artificial oil bodies was determined by quantitating curcumin inside and outside artificial oil bodies after preparation. A preparation of artificial oil bodies composed of the dissolvent (120 μL) containing 3 mg of curcumin was subjected to centrifugation at 10000*g* for 60 min. After centrifugation, artificial oil bodies encapsulating curcumin were precipitated, and the supernatant was collected and subjected to quantitation of curcumin. Approximately 5% of curcumin (154 μg) was detected in the supernatant. For the blood samples, each plasma sample of 0.2 mL was transferred to a clean tube and mixed with 2 times the volume of acetonitrile (1:2, v/v). The resulting solutions were vortexed for 30 s and centrifuged at 15000*g* for 10 min. The upper organic layer was transferred to a 250 μL tube and injected into the HPLC system comprising an UV−vis detector (L-2420), an autosampling device (L-2200), and a pump (L-2130) (Hitachi, Japan). An analytical C18 column (RP-18e, LichroCART Purosphere STAR, 250 mm × 4.6 mm, 5 μm) was used. The mobile phase was a mixture of acetonitrile/5% acetic acid (52:48, v/v). The injection volume was 20 μL, run time was 15 min, flow rate was 1 mL/min, and wavelength was 425 nm. Between two consecutive pipettings, the multiprobe needles were washed with water and methanol.

## RESULTS

**Dissolution of Curcumin with a Mixed Dissolvent Formula.** To encapsulate curcumin in artificial seed oil bodies, various vegetable oils were first tested to dissolve curcumin.


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Unfortunately, curcumin was apparently indissoluble in all four oils examined (sesame, castor, cotton, and olive seed oils), although slight dissolubility of curcumin was observed in sesame oil (Figure 1A). As an alternative, curcumin was

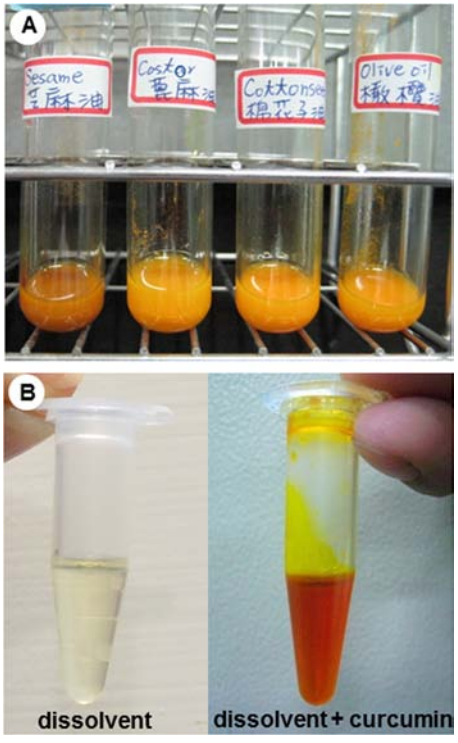


**Figure 1.** Photos of curcumin mixed with various vegetable oils and a dissolvent. (A) Curcumin was mixed with sesame oil, castor oil, cottonseed oil, or olive oil. (B) A dissolvent containing IGEPAL CA-210, Cremophor EL, PEG 400, and ethyl oleate was used to dissolve curcumin.

effectively dissolved in a mixed dissolvent formula comprising IGEPAL CA-210, Cremophor EL, PEG 400, and ethyl oleate (Figure 1B). The transparent dissolvent was originally colorless but changed to orange-red after the dissolution of curcumin. The curcumin-loaded dissolvent was used to replace the conventional seed oils (triacylglycerols) as the lipid matrix for the generation of artificial oil bodies.

**Generation of Artificial Oil Bodies Encapsulating Curcumin.** A cDNA clone of sesame caleosin was transformed and overexpressed in *E. coli* cells to produce recombinant caleosin (Figure 2). As expected, the molecular mass (27 kDa) of the recombinant caleosin was similar to that of the native caleosin extracted from sesame oil bodies. Artificial oil bodies were successfully generated as a yellow emulsified solution by encapsulating curcumin-loaded dissolvent with the recombinant caleosin, and they were further solidified in the forms of powder and tablet (Figure 3). In contrast with the flotation of native oil bodies and artificial oil bodies composed of seed oils, the novel artificial oil bodies composed of curcumin-loaded dissolvent were precipitated after centrifugation. Encapsulation efficiency of curcumin in artificial oil bodies was approximately 95% under the current preparatory conditions. As observed in a microscope, sizes of artificial oil bodies with or without encapsulation of curcumin were similar and, apparently, much smaller than those of native sesame oil bodies (Figure 4). Average particle sizes of curcumin-loaded artificial oil bodies in

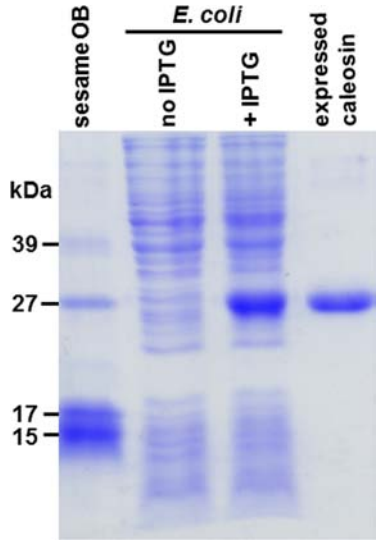


**Figure 2.** SDS-PAGE of the recombinant sesame caleosin in *E. coli*. Along with sesame oil body proteins (10 $\mu$g) and the purified recombinant caleosin (5 $\mu$g), the total proteins (20 $\mu$g) of *E. coli* with the recombinant sesame caleosin overexpressed via a nonfusion vector before or after isopropyl $\beta$-D-1-thiogalactopyranoside (IPTG) induction were resolved in SDS-PAGE. Labels on the left indicate the molecular masses of two oleosin isoforms (15 and 17 kDa), caleosin (27 kDa), and steroleosin (39 kDa).

the forms of solution, powder, and tablet were approximately 150, 180, and 220 nm, respectively (Table 1). Similar particle sizes of these artificial oil bodies as well as their size distribution in the three forms were observed after they were stored at 4 °C for a week. Comparable negative charge on the surface of these particles in the three forms before and after storage was consistently observed with an average zeta potential of approximately −50 mV.

**Bioavailability of Curcumin with or without Encapsulation.** Curcumin with or without encapsulation in artificial oil bodies was used for the measurement of oral bioavailability in rats. The mean blood concentration−time patterns of curcumin after single-dose oral administration are shown in Figure 5. The result showed that bioavailability of curcumin was significantly elevated when it was encapsulated in the artificial oil bodies. Pharmacokinetic parameters calculated from the noncompartmental analysis of a single dose of curcumin with or without encapsulation are listed in Table 2. The rats fed curcumin (50 mg/kg) encapsulated in artificial oil bodies possessed an evidently high $C_{max}$ (37 ± 28 ng/mL) of blood curcumin at $t_{max}$ of 45 ± 17 min, whereas those fed curcumin (1000 mg/kg) without encapsulation displayed a relatively low $C_{max}$ (15 ± 12 ng/mL) at $t_{max}$ of 50 ± 32 min. Relative bioavailability calculated on the basis of the area under the plasma concentration−time curve (AUC) was increased by 47.7 times when curcumin was encapsulated in artificial oil bodies for the animal feed.

## DISCUSSION

Oral delivery is still the most important and frequently used application route for drug administration.[27] However, oral bioavailability of many hydrophobic drugs, for example, curcumin, is extremely low, thus reducing their efficacy in medicinal utilization.[28] Formulation of artificial oil bodies comprising seed oils (mainly triacylglycerols) sheltered with


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**Figure 3.** Photos of curcumin-loaded artificial oil bodies in the forms of solution, powder, and tablet. Photos were taken for the three preparation forms, solution (before and after centrifugation), powder and tablet, of curcumin-loaded artificial oil bodies.

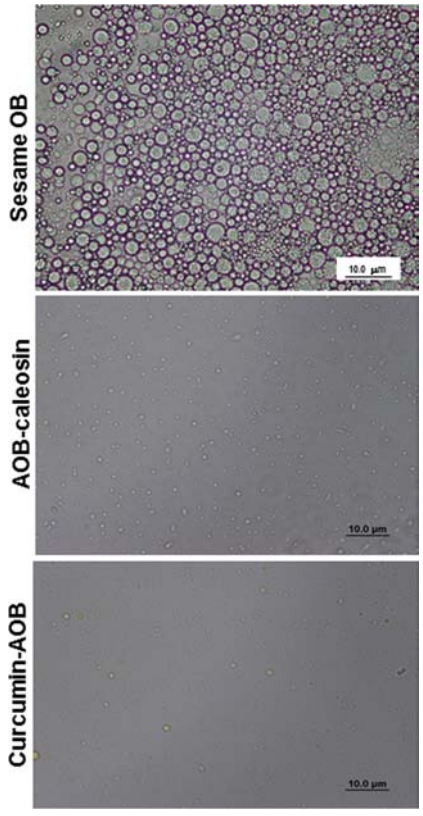


**Figure 4.** Light microscopy of native sesame oil bodies and artificial oil bodies with or without encapsulation of curcumin. Purified oil bodies from sesame seeds and caleosin-based artificial oil bodies with or without encapsulation of curcumin were suspended in the sodium phosphate buffer, pH 7.5, and observed under a light microscope. Bars represent 10 $\mu$m.

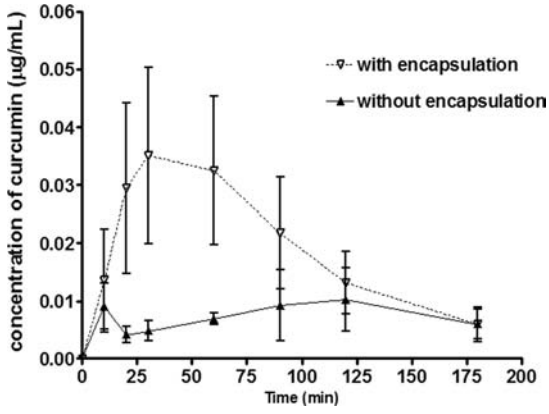


**Figure 5.** Mean blood concentration−time profile of curcumin in experimental rats following oral administration. Sprague−Dawley rats were fed curcumin with or without encapsulation in artificial oil bodies, and curcumin concentrations in the whole blood samples were detected at various time intervals after oral administration. Results are the mean ± SEM of five rats.

caleosin has been demonstrated to be a suitable technique to encapsulate hydrophobic drugs for oral administration.[16] Unfortunately, curcumin as well as many other hydrophobic compounds is indissoluble in seed oils. In this study, we replaced seed oils with a mixed dissolvent formula to constitute a novel type of artificial oil body and demonstrated that this new formulation seemed to be a superior protocol to elevate oral bioavailability of hydrophobic compounds. It will be interesting to see in the follow-up investigation if caleosin may serve as an adequate stabilizer to encapsulate many other lipids or oily mixtures to form artificial emulsions for diverse applications.

Native oil bodies isolated from diverse seeds are structurally stable with particle sizes ranging from 0.5 to 2 $\mu$m.[11] Artificial oil bodies encapsulating triacylglycerols with caleosin are also structurally stable, and their particle sizes (50−200 nm) are 10 times smaller than the native ones.[13] In this study, the sizes (approximately 150 nm) of the newly developed artificial oil bodies encapsulating the curcumin-loaded dissolvent with caleosin were also 10 times smaller than native oil bodies. In

**Table 1. Particle Size Distribution and Zeta Potential (ZP) of Curcumin-Loaded Artificial Oil Bodies in the Three Preparation Forms before and after Storage for 1 Week (Mean ± SD, $n$ = 3)**

| | solution | | powder | | tablet | |
|---|---|---|---|---|---|---|
| | fresh | stored | fresh | stored | fresh | stored |
| size (nm) | 151.4 ± 1.5 | 149.1 ± 1.2 | 184.7 ± 1.5 | 181.4 ± 2.6 | 226.9 ± 3.2 | 219.1 ± 6.2 |
| PDI[a] | 0.285 ± 0.026 | 0.252 ± 0.003 | 0.468 ± 0.003 | 0.449 ± 0.0030 | 0.587 ± 0.082 | 0.547 ± 0.030 |
| ZP (mV) | −42.1 ± 0.8 | −40.7 ± 1.1 | −50.8 ± 0.4 | −55.3 ± 0.80 | −49.6 ± 0.4 | −48.9 ± 2.0 |

[a]PDI, polydispersity index.


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**Table 2. Pharmacokinetic Parameters of Curcumin after Its Oral Administration with or without Encapsulation in Artificial Oil Bodies (AOB) (Mean ± SEM, $n = 5$)[a]**

| group | $t_{max}$ (min) | $C_{max}$ (ng/mL) | $t_{1/2}$ (min) | Ke ($h^{-1}$) | CL (L/h) | Vd (L) | AUC (min ng/mL) | AUC/dose | Fr |
|---|---|---|---|---|---|---|---|---|---|
| curcumin (1000 mg/kg) | 50 ± 32 | 15 ± 12 | 95 ± 35 | 0.438 | 3.378 | 7.712 | 1480 ± 1290 | 14.8 | 1 |
| curcumin in AOB (50 mg/kg) | 45 ± 17 | 37 ± 28 | 54 ± 17 | 0.770 | 0.071 | 0.092 | 3530 ± 3010* | 706* | 47.7* |

[a] $t_{max}$, time to $C_{max}$; $C_{max}$, maximum plasma concentration; $t_{1/2}$, half-life; Ke, terminal elimination rate constant; CL, body clearance; Vd, volume of distribution; AUC, area under the plasma concentration−time curve; fr, relative bioavailability; *, $p < 0.05$, vs curcumin group.

other words, sizes of artificial oil bodies sheltered by caleosin were virtually unchanged when the encapsulated lipid matrix was changed from triacylglycerols to the mixed dissolvent. Possibly, the sizes of artificial oil bodies are mainly determined by caleosin no matter what kind of encapsulatable material is used as the lipid matrix. Of course, this speculation needs to be further verified after more artificial oil bodies sheltered by caleosin are successfully generated with diverse formulations of encapsulatable material.

In comparison to liquid formulas, the low water content in solid forms (powder or tablet) prevents the growth of microorganisms and generally increases the stability of drug compounds during long-term storage.[29] Although solidification of this novel type of artificial oil body might lead to a slight increase of particle size (approximately from 150 to 180 and 220 nm in powder and tablet, respectively) possibly due to inevitable damage during the preparation processes, they remained very stable in any of the three forms (solution, powder, and tablet) in the 1 week storage. According to the measurement of zeta potential (Table 1), electrorepulsion of the negatively charged surface seems to contribute to the structural stability of this novel type of artificial oil body besides steric hindrance sheltered by caleosin as observed in native oil bodies.

In the animal study, the relative bioavailability of curcumin was elevated by nearly 50 times when it was dissolved in the mixed dissolvent (IGEPAL CA-210, Cremophor EL, PEG 400, and ethyl oleate) and encapsulated with caleosin (Table 2). Igepal CA-210 is a nonionic dispersant and emulsifier for nonpolar solvents and also used in cosmetic formulations. Cremophor EL is a nonionic surfactant suitably added in formulation vehicles for insoluble pharmacological compounds. PEG 400 is widely used in a variety of pharmaceutical formulations as a consequence of its low toxicity and high compatibility with many solvents. Ethyl oleate, a fatty acid ester produced by the body during ethanol intoxication, is regarded as a food additive by the U.S. Food and Drug Administration and commonly used as a solvent for pharmaceutical preparations of hydrophobic drugs. A synergistic effect of these four compounds used in the dissolvent on the dissolubility of curcumin was observed in this study. It is possible that the dissolubility and bioavailability of curcumin as well as other hydrophobic drugs may be further elevated when the ratio of these four compounds is optimized. Surely, inclusion of other surfactant and solvent compounds in the encapsulated dissolvent of artificial oil bodies is feasible for the improvement of this drug delivery system.

In summary, we developed a new formulation of artificial oil bodies for the encapsulation of hydrophobic compounds as exemplified by curcumin and demonstrated that oral bioavailability of curcumin could be significantly elevated via encapsulation in this novel type of artificial oil body in an animal test. Although the elevated bioavailability is supposed to enhance the therapeutic effects of curcumin, the exact biological effects in vivo need to be further examined in follow-up studies. Moreover, it is very likely that this novel type of artificial oil bodies can be applied to improve the oral bioavailability of many hydrophobic drugs.

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### Notes

The authors declare no competing financial interest.

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