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PLGA Nanoparticles Improve the Oral Bioavailability of Curcumin in Rats: Characterizations and Mechanisms

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ABSTRACT: The overall goal of this paper was to develop poly(lactic-*co*-glycolic acid) nanoparticles (PLGA-NPs) of curcumin (CUR), named CUR-PLGA-NPs, and to study the effect and mechanisms enhancing the oral bioavailability of CUR. CUR-PLGA-NPs were prepared according to a solid-in-oil-in-water (s/o/w) solvent evaporation method and exhibited a smooth and spherical shape with diameters of about 200 nm. Characterization of CUR-PLGA-NPs showed CUR was successfully encapsulated on the PLGA polymer. The entrapment efficiency and loading rate of CUR were 91.96 and 5.75%, respectively. CUR-PLGA-NPs showed about 640-fold in water solubility relative to that of n-CUR. A sustained CUR release to a total of approximately 77% was discovered from CUR-PLGA-NPs in artificial intestinal juice, but only about 48% in artificial gastric juice. After oral administration of CUR-PLGA-NPs, the relative bioavailability was 5.6-fold and had a longer half-life compared with that of native curcumin. The results showed that the effect in improving oral bioavailability of CUR may be associated with improved water solubility, higher release rate in the intestinal juice, enhanced absorption by improved permeability, inhibition of P-glycoprotein (P-gp)-mediated efflux, and increased residence time in the intestinal cavity. Thus, encapsulating hydrophobic drugs on PLGA polymer is a promising method for sustained and controlled drug delivery with improved bioavailability of Biopharmaceutics Classification System (BCS) class IV, such as CUR.

KEYWORDS: curcumin, PLGA, nanoparticles, bioavailability, P-gp efflux, intestinal permeability

INTRODUCTION

Oral delivery of therapeutic agents and functional foods may improve compliance and comfort as well as the development of chronic treatment schedules. However, there are many drugs and foods with poor bioavailability by oral administration. Extensive efforts are being focused on resolving the issue of poor bioavailability of drugs by employing various pharmaceutical approaches.

Curcumin (CUR; (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Figure 1), a natural hydrophobic phenolic compound derived from the common food spice rhizome of *Curcuma longa* (turmeric), has a wide spectrum of healthy functions and pharmacological activities. Turmeric has been used to cure hepatic disorders, diabetic wounds, rheumatism, and sinusitis in Indian traditional medicine for centuries.[1] The pharmacological activities of CUR include antiamyloid, antibacterial activity, antidepressant effects, antiinflammatory properties, antioxidant, and antitumor with low intrinsic toxicity.[2,3] CUR had been shown to affect multiple targets and to interfere with cell signaling pathways, including inducing apoptosis (activation of caspases and down-regulation of anti-apoptotic gene products), inhibiting cell proliferation (HER-2, EGFR, and AP-1), inhibiting invasion (MMP-9 and adhesion molecules) and suppressing inflammation (NF-κB, TNF, IL-6, IL-1, COX-2, and 5-LOX).[4] Currently, sufficient data have been shown to advocate phase II and phase III clinical trials of CUR for a variety of cancer conditions including multiple myeloma, pancreatic, and colon cancers.[5]

Despite its efficacy and safety, the clinical application of CUR has been limited by its bioavailability.[6] Phase I clinical trials have shown that CUR is safe even at doses up to 12 g/day but exhibits poor bioavailability. Major reasons contributing to the low plasma and tissue levels of CUR appear to be due to poor absorption, rapid metabolism, and rapid systemic elimination.[7]

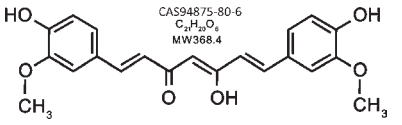

Figure 1. Chemical structure of CUR.

The factors influencing the bioavailability of the drug include physical and chemical properties, such as hydrophobicity, pK_a, and solubility. In terms of chemical structure, CUR is a bis-R, α,β-unsaturated β-diketone, which exhibits keto−enol tautomerism having a predominant keto form in acidic and neutral solutions and a stable enol form in alkaline medium. CUR is an oil-soluble coloring compound, readily soluble in alkali, ketone, acetic acid, and chloroform, but insoluble in water at acidic or neutral pH.[5] To improve the bioavailability of CUR, some approaches have been used to develop its new drug delivery systems by oral administration, for instance, nanoparticles,[4,8] liposomes,[9,10] cyclodextrin inclusion complexes,[11] poly-(ε-caprolactone) nanofibers or nanodisks,[12] and biodegradable polymeric micelles. These research results showed that

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new delivery systems enhanced antioxidant, antihepatoma activities and bioavailability of CUR.[4,8,9]

On the other hand, oral bioavailability has some connection with permeability, efflux transporters (e.g., P-glycoprotein, P-gp), and enzyme induction or inhibition on intestinal epithelial cell. The intestinal P-gp efflux pump and enterocyte-based metabolism have been proposed to contribute a major barrier to the oral bioavailability for a number of compounds,[13] in particular, Biopharmaceutical Classification System (BCS) Class III or IV molecules and P-gp substrates. Recently, the poor permeability of CUR and its metabolism by CYP_{450} 3A4 on intestinal epithelial cells[14] and the Caco-2 cell line[15] have been reported.

The choice of carrier material in the oral delivery system is of high importance because it significantly affects the pharmacokinetics and pharmacodynamics of the drugs. A wide range of materials, such as chitosan, polymers, cyclodextrins, and dendrimers, have been employed as carriers to improve bioavailability. Poly(lactic-*co*-glycolic acid) (PLGA) is a copolymer that is used in a host of U.S. Food and Drug Administration (FDA) approved therapeutic devices because of its biodegradability and biocompatibility.[16] PLGA can be used as an efficient carrier of functional foods and for drug delivery.[17,18] Recently some authors reported that PLGA was used for CUR delivery by oral administration with increased bioavailability at different levels, but the mechanism has not been discussed.[19−21]

On the basis of these factors, to improve the oral bioavailability of CUR, we designed and prepared CUR-PLGA-NPs (PLGA nanoparticles loaded with CUR). In the present study, CUR-PLGA-NPs were characterized for surface morphology, CUR loading, and encapsulation efficiency and CUR release in vitro. The bioavailability of CUR-PLGA-NPs was compared to that of native curcumin (n-CUR) in rat. The mechanisms of improving bioavailability have been discussed.

MATERIALS AND METHODS

Materials. PLGA polymer (poly(lactic acid)/poly(glycolic acid) = 50:50; inherent viscosity 1.13 dL/g; MW 30000) was purchased from Jinan Daigang Co., Ltd. (Shangdong, China). Curcumin (CUR, ≥98%, synthetic) was purchased from TCI (Tokyo, Japan). Poly(vinyl alcohol) (PVA, MW 30000−70000) and verapamil (VRP) were acquired from Sigma-Aldrich (St. Louis, MO). All organic solvents were of HPLC grade, and other chemicals were of analytical grade.

Preparation of CUR-PLGA-NPs. CUR-PLGA-NPs were prepared according to a solid-in-oil-in-water (s/o/w) solvent evaporation technique with moderate modification.[16,20] Simply, 45 mg of PLGA was dissolved in dichloromethane for 12 h to obtain a uniform PLGA solution. Five milligrams of CUR was added to PLGA solution and sonicated at 55 W for 2 min to generate the s/o primary emulsion. The received solution was emulsified with 20 mL of PVA solution (1% w/v) by rotating at 300 rpm and again sonicated at 55 W for 3 min to generate the final s/o/w emulsion. The organic solvent was eliminated by rotary vacuum evaporation at 50 °C in a water bath. Larger aggregates and free PLGA/PVA polymers were removed by centrifugation at 3000 rpm on an Eppendorf centrifuge 5417R (Eppendorf AG, Hamburg, Germany) for 10 min. Finally, the solution was lyophilized using an FL-60 system. CUR-PLGA-NPs were stored at 4 °C for further use.

Characterization of CUR-PLGA-NPs. *Fourier Transform Infrared (FTIR) Spectra.* FTIR spectra were investigated to detect the functional groups of compounds through a NEXUS 670 FTIR spectrometer (Nicolet, USA). PLGA polymer, n-CUR, and CUR-PLGA-NPs were mixed with the spectroscopic grade KBr to result in a translucent KBr pellet, and then the pellets were prepared for examination.

Differential Scanning Calorimetry (DSC). DSC curves of PLGA polymer, n-CUR, and CUR-PLGA-NPs were measured with a thermal analysis data system (Diamond, Perkin-Elmer, USA). Each sample (3−5 mg) was heated in an aluminum pan from 25 to 450 °C at a flow rate of 10 °C/min under dry nitrogen. Comparison of the DSC curves provided certain useful information about the physical state of CUR in the carriers and possible interaction between CUR and polymer.

X-ray Powder Diffractometry. The patterns of PLGA polymer, n-CUR, and CUR-PLGA-NPs were received using a Ricoh Dmax 2500 diffractometer (Ricoh, Japan) with a tube anode copper over the interval 5−45°/2θ. Measurements were operated at a voltage of 40 kV, 200 mA, and the scanning rate was 2°/min.

Scanning Electron Microscopy (SEM). The morphology of CUR-PLGA-NPs was observed using a SEM (JSM-5900, Japan) at an accelerating voltage of 5 kV. One drop of CUR-PLGA-NP suspension was placed on a graphite surface. After the sample had reached dryness, it was coated with gold using an ion sputter.

High-Performance Liquid Chromatography (HPLC) Method of CUR. CUR levels were determined by HPLC using a Diamonsil C18 column (250 × 4.6 mm, 5 μm particle size, Dikma Technologies, Beijing, China) with the mobile phase consisting of methanol/5% glacial acetic acid (65:35, v/v). The mobile phase was filtered through a 0.22 μm nylon membrane filter and ultrasonically degassed before use. The system was run isocratically at a flow rate of 1 mL/min, and CUR was detected at 425 nm. The injection volume was 20 μL, and the analysis time was 40 min per sample. The retention times for emodin (the internal standard) and CUR were about 2 and 14 min, respectively. The linear equation was $A = 0.6702C + 0.1995$ ($R^2 = 0.9991$), where A was the HPLC area and C was the concentration of CUR (μg/mL). The limit of detection of CUR was determined to be 25 ng/mL in rat plasma, and both intraday and interday precisions were <3%.

CUR Loading and Encapsulation Efficiency. The amount of CUR loading and encapsulation in CUR-PLGA-NPs was measured by UV−vis spectrophotometer. A calibration curve was established using standard solutions of CUR. The calibration curve was linear between 500 ng and 3.0 μg with good linearity ($r^2 = 0.9987$). Lyophilized CUR-PLGA-NPs (1−2 mg) were dissolved in 1 mL of methanol completely to extract CUR to methanol for the loading and encapsulation detection. The samples in methanol were gently shaken on a shaker for 24 h at 37 °C to leach out CUR entirely. Then the solutions were centrifuged at 13500 rpm for 10 min, and supernatant was gathered. The supernatant (100 μL) was diluted to 2 mL for loading and encapsulation detection using a UV−vis spectrophotometer at 425 nm, as previously described. The amount of CUR loaded and encapsulated in nanoparticles was expressed as loading efficiency or encapsulation efficiency calculated as follows:

$$\text{loadingefficiency}\ (\%) = \frac{\text{wtofCURinnanoparticles}}{\text{wtofnanoparticles}} \times 100 \quad (1)$$

$$\text{encapsulationefficiency}\ (\%) = \frac{\text{wtofCURinnanoparticles}}{\text{wtoftotalCUR}} \times 100 \quad (2)$$

Solubility and Stability Study. To compare the solubility of CUR before and following the encapsulation process, saturation solubility was determined. Excessive samples (n-CUR and CUR-PLGA-NPs) were dispersed into 20 mL of water and incubated in a shaker at 200 rpm, 37 ± 0.5 °C. After 24 h, samples were taken out and filtered through a 0.22 μm Millipore membrane. Filtrate was diluted appropriately, and the absorbance was measured by a UV−vis spectrophotometer at 425 nm. The solubility was calculated according to a standard equation and dilution times.

n-CUR and CUR-PLGA-NPs with an equal quantity CUR were dissolved in PBS (0.01 M, pH 7.4) to research the aqueous solubility of our formulation. CUR at 20 μg/mL and equal quantity of CUR of CUR-PLGA-NPs were prepared in phosphate buffer solution (PBS, 0.01 M, pH 7.4) and shaken in a shaker at 200 rpm, 37 ± 0.5 °C, for 12 h. n-CUR was dissolved in PBS with the aid of methanol (<0.5%). At designated time points, the tube was removed and detected using a UV−vis spectrophotometer at 425 nm. The stability of CUR was calculating according to the formula

$$\text{stability of CUR } (\%) = C_t/C_0 \times 100 \quad (3)$$

C_0 and C_t represent the concentrations of CUR in PBS at 0 h and t h, respectively (t = 0.5, 1, 2, 4, 6, 8, and 12 h).

Release Kinetics in Vitro. The release of CUR from CUR-PLGA-NPs was carried out by dissolving 100 mg of NPs in artificial gastric juice (PBS adjusted to pH 2.0 with HCl) and intestinal juice (PBS at pH 7.4) without enzymes, 15 mL of PBS (0.01 M, pH 7.4), and the solution was divided into 30 Eppendorf (0.5 mL each) tubes. The samples were put in a shaker at 37 ± 0.5 °C at 200 rpm. At designated time intervals, the tube was taken out and centrifuged at 3000 rpm for 10 min. The pellet was resuspended in 100 μL of methanol to determine the amount of CUR released by HPLC. All of the operations were carried out in triplicate.

Pharmacokinetics and Bioavailability in Vivo. Male Sprague−Dawley rats (weighing 200−240 g, Southeast University Experimental Animal Center, Nanjing, China) were maintained in an environmentally controlled room (23 ± 3 °C, 12 h dark−light cycle) with free access to standard laboratory food and water for 7 days before experiments. They were fasted overnight before administration. Animal experiments were performed according to the *Principles of Laboratory Animal Care* (NIH Publication 86-23, revised 1986) and local regulations. Furthermore, all experiments were approved and supervised by the Animal Care and Use Committee and Animal Ethics Committee at Nanjing Normal University.

For the pharmacokinetic study, the 15 rats were divided randomly into three groups (n = 5). One group of rats received n-CUR with 0.5% Tween 80 at a dose of 10 mg/kg by intravenous injection into the tail vein. Other groups received n-CUR suspension with 0.5% CMC-Na and CUR-PLGA-NPs with aqueous solubility at a dose of 100 mg/kg by oral administration, respectively. Blood samples (about 0.3 mL) were collected into heparinized centrifuge tubes at 15, 30, 60, 90, 120, 180, 240, 300, 360, and 720 min following oral administration and at 3, 5, 15, 30, 45, 60, 90, 120, 240, and 360 min following intravenous injection. Rats were given normal saline to compensate for the blood loss during experimentation. The plasma was separated by centrifugation and stored at −70 °C before HPLC analysis.

Pharmacokinetic parameters were determined by the 3p97 software provided by the Chinese Pharmacological Society. The data were represented by the following parameters: area under the curve (AUC); plasma half-life ($t_{1/2}$); peak concentration (C_{max}); and time of peak concentration (t_{max}).

Absolute bioavailability compares the bioavailability of n-CUR in the systemic circulation following intragastrical administration (ig) with intravenous injection (iv). Absolute bioavailability (F_{abs}) was calculated according to the following formula:

$$F_{abs}\ (\%) = 100\frac{\text{AUC}_{ig}\text{dose}_{iv}}{\text{AUC}_{iv}\text{dose}_{ig}} \quad (4)$$

Relative bioavailability measures the bioavailability (estimated as the AUC) of n-CUR when compared with CUR-PLGA-NPs following intragastrical administration. Relative bioavailability (F_{rel}) was calculated by using the following formula:

$$F_{rel}\ (\%) = 100\frac{\text{AUC}_A\text{dose}_B}{\text{AUC}_B\text{dose}_A} \quad (5)$$

AUC_A and AUC_B represent the area under the blood concentration−time curve of CUR-PLGA-NPs and n-CUR, and $dose_A$ and $dose_B$ mean the dose of CUR-PLGA-NPs and n-CUR following intragastrical administration.

In Situ Single-Pass Intestinal Permeability and P-gp Inhibition Studies. The procedure of in situ single-pass perfusion experiments was performed according to the methods described by Varma et al.[22] with moderate modification. Fifteen SD rats were divided into three groups of five each. After overnight fasting, rats were anesthetized with 40 mg/kg sodium pentobarbital and fixed in a supine position. During the surgical process, the body temperature of the rats was kept at 37 °C by a heating lamp. The abdomen was opened with a midline incision approximately 3 cm and a 10−12 cm jejunum segment was exposed and cannulated at both sides with catheters connected to a peristaltic pump (BT50b-DG2, PreFluid Co., China). Then the segment was mildly washed with prewarmed normal saline (37 °C) and purged by air. CUR intestinal circulating perfusion solution (50 μg/mL) was prepared by dissolving n-CUR or CUR-PLGA-NPs in 50 mL of Krebs balanced salt solution (D-glucose 7.78 mM, NaCl 133 mM, KCl 4.56 mM, NaH_2PO_4 1.50 mM, $MgCl_2$ 0.20 mM, $NaHCO_3$ 16 mM, $CaCl_2$ 3.33 mM; pH was adjusted to 7.4).

At first, 50 mL of the solution was perfused through the segment at a flow rate of 5.0 mL/min. After 20 min, the flow rate was adjusted to 1.0 mL/min for another 2 h. Samples were collected at predetermined intervals of time (0, 30, 60, 90, and 120 min), and equivalent perfusion solutions were supplemented each time. Each experiment was triplicated. The remaining CUR ratio (RCR, %) was calculated according to the following formula:

$$\text{remaining drug ratio } (\%) = C_t/C_0 \times 100\% \quad (6)$$

In the above equation, C_0 and C_t represent the concentrations of CUR intestinal circulating perfusion solution at 0 and t min, respectively (t = 30, 60, 90, and 120 min).

A two-step perfusion procedure was followed to determine the permeability of CUR (n-CUR or CUR-PLGA-NPs, 50 μg/mL) with and without P-gp inhibitor (VRP, 50 μM). The experimental section was the same as depicted above. Then the permeability coefficient of CUR in the jejunum with and without P-gp inhibitor was calculated using the formula[23]

$$P_{eff} = \frac{-Q\ \ln(C_{out}/C_{in})}{2\pi rL} \quad (7)$$

where Q is the flow rate, C_{in} and C_{out} are the respective inlet and outlet concentrations of CUR measured by HPLC, r is the radius of intestine, and L is the length of intestine measured after completion of perfusion.

Statistical Analysis. Results are expressed as the mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance, and differences among the means of groups were analyzed by an unpaired, two-sided Student's t test. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Preparation and Characterization of CUR-PLGA-NPs. In our study, CUR-PLGA-NPs were formulated by a s/o/w solvent evaporation technique with PLGA as the carrier. As microparticles are most often prepared by emulsion techniques that include aqueous phases, the solubility of the drug in these media is an important value that needs to be determined in the initial phase of every microencapsulation study. Such external phases are commonly aqueous solutions containing PVA, the predominant emulsifier in emulsion-based encapsulation techniques.[24] Thence, PVA, which is a widely used stabilizer for PLGA polymer

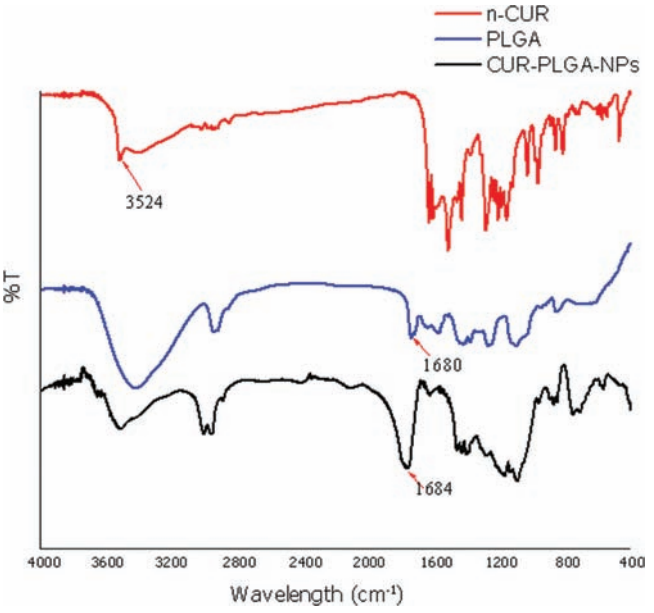

Figure 2. FTIR spectra of n-CUR, PLGA, and CUR-PLGA-NPs.

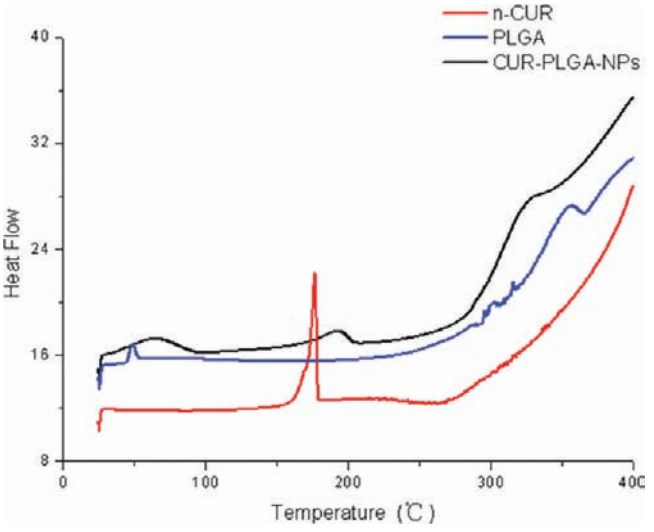

Figure 3. DSC curves of n-CUR, PLGA, and CUR-PLGA-NPs.

and other pharmaceutical formulations, acts as the linker in our study. With the selected carrier and stabilizer, we can get uniform nanoscale products in larger quantities.

The biodegradable and biocompatible PLGA is an important advanced delivery system for the week-to-month controlled release of hydrophobic drugs (e.g., from BCS class IV), which often display poor oral bioavailability.[24] The incorporation of antitumor drugs into PLGA polymers would be a possible approach to improve oral bioavailability. So far, many hydrophobic drugs have been encapsulated easily on PLGA polymers. PLGA, approved by the FDA, has good qualities of biocompatibility, biodegradability, and high stability in biological fluids and during storage. Moreover, PLGA is degraded into nontoxic lactic acid and glycolic acid in the body.

FTIR Spectroscopy. The infrared spectra of n-CUR, PLGA, and CUR-PLGA-NPs are shown in Figure 2. The characteristic absorption peak of n-CUR was found at 3524 cm^{-1} (O—H stretch). The

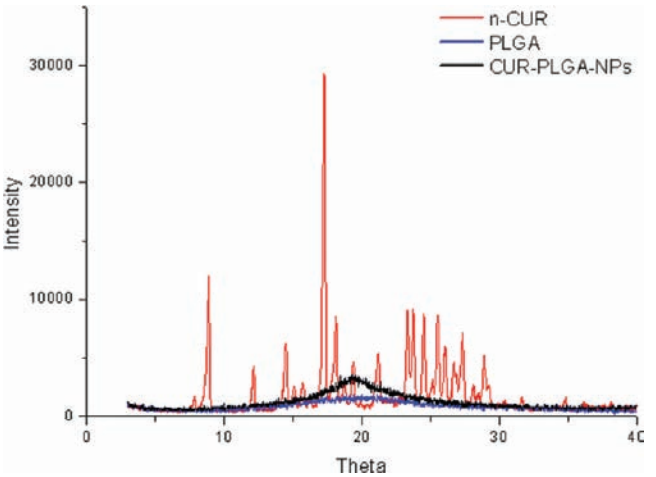

Figure 4. XRD of CUR, PLGA, and CUR-PLGA-NPs.

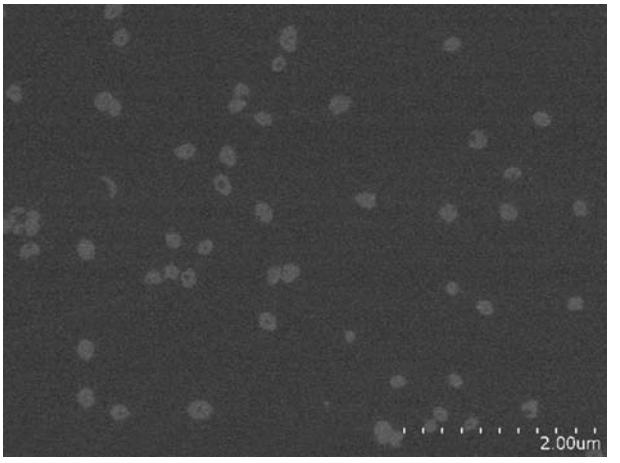

Figure 5. SEM photograph of CUR-PLGA-NPs.

spectrum of PLGA showed the C═O absorption band at 1680 cm^{-1}. Although in the encapsulated nanoparticles, the C═O peak was slightly moved to a lower wavelength and the O—H absorption band of n-CUR was not observed. This could be attributed to the formation of intermolecular hydrogen bonds between the O—H band of CUR and the C═O band of PLGA.

DSC Study. DSC studies were employed to research the crystal transformation of the nanoparticles system. The DSC curves of n-CUR, PLGA, and CUR-PLGA-NPs are shown in Figure 3. n-CUR gives rise to a sharp peak at 176.8 °C corresponding to the melting point of crystalline regions. The PLGA polymer shows a small peak at around 50 °C, referring to the relaxation peak that follows the glass transition, which is consistent with the previous study.[20] No notable melting point was observed because PLGA is amorphous in nature. Moreover, it can be found from Figure 3 that the PLGA polymer had the same melting peak as CUR-PLGA-NPs, indicating that the encapsulation process did not affect the polymer structure. However, the characteristic peak of n-CUR was not observed in CUR-PLGA-NPs, which could be due to the conversion of the crystalline form of CUR to the amorphous form.

XRD Study. To learn more about the interactions between CUR and PLGA polymer, X-ray diffraction (XRD) was used.

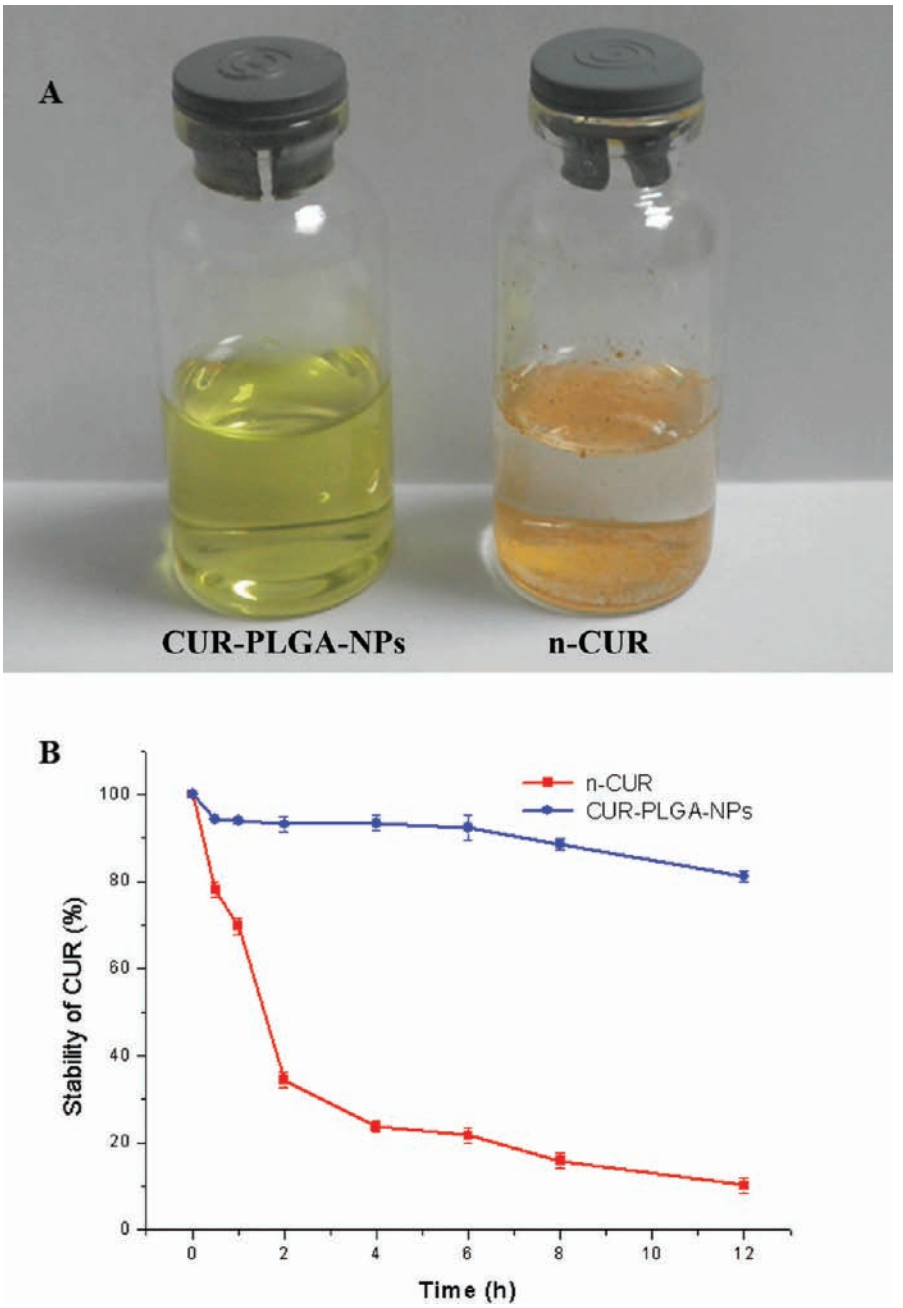

Figure 6. (A) Solubility of CUR (n-CUR and CUR-PLGA-NPs) in PBS (pH 7.4). n-CUR (5 mg) dissolved in PBS (pH 7.4) was insoluble in aqueous solution. An equivalent quantity of CUR-PLGA-NPs was fully soluble in aqueous solution. (B) Stability of n-CUR and CUR-PLGA-NPs in PBS (pH 7.4) at 37 °C (mean ± SD, $n = 3$).

The characteristic peaks of n-CUR presented in Figure 4 can be inferred from the crystalline structure of n-CUR, whereas this characteristic was not exhibited in CUR-PLGA-NPs. The phenomenon possibly suggested the formulation of an amorphous complex with the intermolecular interaction between n-CUR and PLGA polymers, which is in agreement with the FTIR and DSC results.

SEM Study. To directly observe the CUR-PLGA-NPs formed, SEM was carried out to determine the morphology of polymeric nanoparticles. Figure 5 shows the SEM image of CUR-PLGA-NPs. CUR-PLGA-NPs appeared as round, homogeneous shapes with a smooth surface. The dimensions of CUR-PLGA-NPs are <200 nm, which confirms that the obtained CUR is converted to near-nanoform. The small difference in size between Mukerjee et al. and our research may be attributed to the molecular weight of PLGA, organic phase, and PVA concentration. On the other hand, the sonication time is an important factor in nanoparticle size during preparation.[20,24]

In summary, we have successfully encapsulated CUR on the PLGA polymer as determined by FTIR, DSC, and XRD. Meanwhile, the SEM study directly certified that the obtained CUR had changed to nanoform compared with n-CUR. The reduction in particle size of CUR can lead to improvement in its solubility and bioavailability.

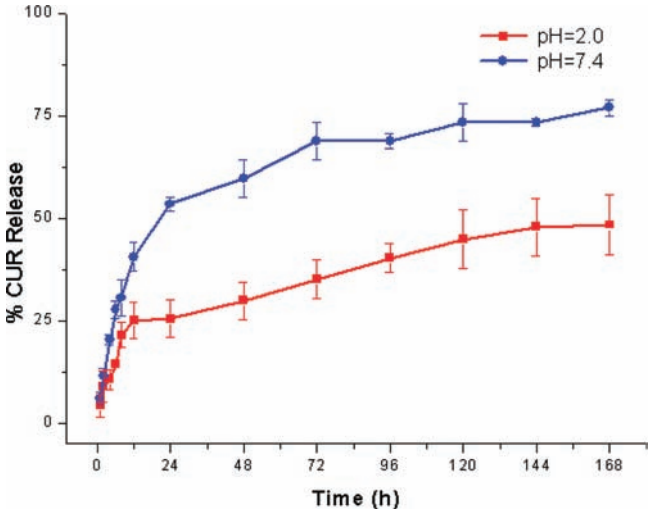

Figure 7. Release rates of CUR from PLGA nanoparticles in vitro in PBS (in artificial gastric juice at pH 2.0 and in artificial intestinal juice at pH 7.4) over a period of 7 days (mean ± SD, $n = 3$).

Loading and Encapsulation Efficiency. The loading and encapsulation efficiency was calculated using eqs 1 and 2 described above. Loading and encapsulation efficiency were found to be 5.75 and 91.96%, respectively, and were similar to those in the previous study.[20] It is generally recognized that the encapsulation efficiency of a chemical compound on PLGA polymer, an important parameter for drug delivery system, is correlated to PLGA concentration, molecular weight, and volume ratio.[16,24] PLGA polymer with a low molecular weight ($M_w = 30000$) may result in high CUR loading and encapsulation efficiency. The higher value of CUR entrapment into the low molecular weight polymer is due to the favorable interaction between the phenolic hydroxyl of CUR and the carboxylic groups of PLGA.

Solubility and Stability Study. To verify the solubility of CUR before and following the nanometer process, we dissolved n-CUR and CUR-PLGA-NPs in aqueous solution. The results showed that CUR-PLGA-NPs dissolved provided a clear, well-dispersed formulation with CUR natural color; in contrast n-CUR is hydrophobic and hardly dissolved in aqueous media with a slightly soluble compound visible in the solution. Furthermore, the highest solubility was achieved for CUR-PLGA-NPs dried powder, which was 4.35 mg/mL, much higher than that of n-CUR, which was 6.79 μg/mL. The solubility of CUR-PLGA-NPs in water was approximately 640-fold that of n-CUR. Dry and lyophilized powder of CUR-PLGA-NPs was found to have good solubility in water (Figure 6). The oral bioavailability of a drug is associated with its molecular physical and chemical properties. In general, the prime limitation of poor bioavailability is CUR's insolubility in aqueous solutions. Therefore, investigators may be interested in how to improve CUR's solubility. To improve the water solubility of CUR, Marcolino et al. designed cyclodextrin complex inclusion,[11] and Manju and his colleagues prepared the conjugation of CUR onto hyaluronic acid.[25] We employed PLGA as the carrier because PLGA can increase the water solubility of hydrophobic drug distinctly with encapsulating drugs into the pore of PLGA.

One of the major challenges of drug delivery to cancerous tissue is its instability and biodegradation in physiological pH.[26]

Table 1. Pharmacokinetic Parameters of CUR with n-CUR, ig or CUR-PLGA-NP Administration Following Intravenous Injection (iv) or Intragastrical Administration (ig) in Rats

administration[a]	units	n-CUR, iv	n-CUR, ig	CUR-PLGA-NPs,[b] ig
dose	mg/kg	10	100	100
$t_{1/2}$	min		74.2 ± 5.9	135 ± 45**
C_{max}	μg/mL	8.82 ± 0.11	1.55 ± 0.21	6.75 ± 1.54**
t_{max}	min	3 ± 0	102 ± 16	120 ± 0**
AUC_t	min μg/mL	776 ± 146	367 ± 21	2066 ± 332**
F_{abs}	%		4.73	26.5
F_{rel}	%			563

[a] $t_{1/2}$, plasma half-life; C_{max}, maximum concentration; t_{max}, time to reach C_{max}; AUC_t, area under the blood concentration vs time curve; F_{abs}, absolute bioavailability; F_{rel}, relative bioavailability. mean ± SD, $n = 5$. [b] (**) $p < 0.01$ vs n-CUR, Student t test.

To study the biodegradation and instability properties of CUR, we incubated n-CUR and CUR-PLGA-NPs in PBS (0.01 M, pH 7.4) and calculated their concentrations with time by UV−vis spectrophotometer. It was suggested that n-CUR went through high-speed degradation in PBS. However, CUR-PLGA-NPs were stable under the same condition. Consequently, our nanometer process has increased the stability of CUR in PBS by guarding the encapsulated CUR against hydrolysis and biotransformation. Physical and chemical stabilities were readily dispersible in water and could be stored at room temperature for over 72 h without any decomposition or aggregation.

Release Kinetics in Vitro. In our study, pH 2.0 and 7.4 phosphate buffer solutions were selected to imitate the environment of gastric juice and intestinal juice, respectively. The release kinetics of CUR from PLGA polymer was taken out in PBS (pH 2.0 and 7.4) at 37 °C for 7 days. The cumulative percentage release of CUR from PLGA polymer is shown in Figure 7. We observed that there was a fast rate of CUR release within the initial 8 h both in artificial gastric juice (pH 2.0) and in intestinal juice (pH 7.4). This could be attributed to CUR desorption and release from the surface of nanoparticles. A sustained CUR release to a total of approximately 77% was discovered from the nanoparticles in the intestinal juice, whereas the release was only about 48% in artificial gastric juice during the whole period of our study.

The release rate of CUR from the nanoparticles in the intestinal juice was higher than in artificial gastric juice, which could be explained by the main absorption of CUR happening in the intestine, not in the stomach. Slow CUR release from nanoparticles in intestinal juice will result in sustained and effective treatment.

Pharmacokinetics and Bioavailability in Vivo. The main pharmacokinetic parameters of n-CUR and CUR-PLGA-NPs in rat are shown in Table 1, and the plasma concentration−time profile is shown in Figure 8. After n-CUR by oral dose of 100 mg/kg, or by iv dose of 10 mg/kg, in rats, the maximum concentrations (C_{max}) were 1.55 ± 0.21 and 8.82 ± 0.11 μg/mL and AUC_t values were 367 ± 21 and 776 ± 146 min, respectively. The main pharmacokinetics parameters for CUR-PLGA-NPs by a single oral dose of 100 mg/kg in rat were as follows: t_{max} was 120 ± 0 min; C_{max} was 6.75 ± 1.54 μg/mL; AUC_t was 2066 ± 332 μg/mL·min; and $t_{1/2}$ was 135 ± 45 min. The result showed that our CUR-PLGA-NPs had high plasma concentration, low clearance,

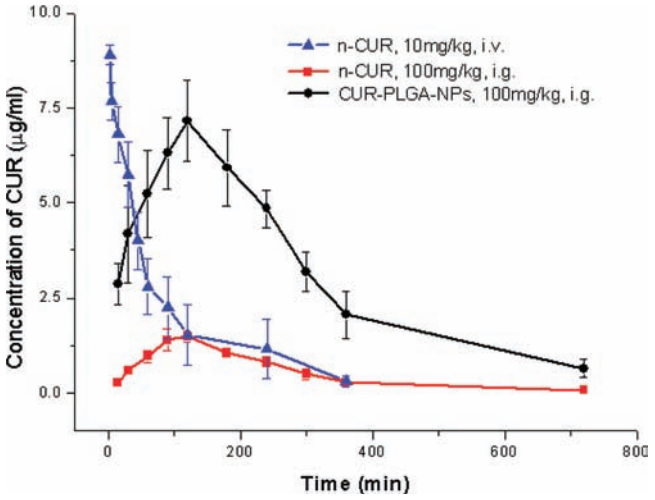

Figure 8. Mean plasma concentration−time profiles of CUR in rats: (▲) n-CUR (iv, intravenous administration; 10 mg/kg); (■) n-CUR (ig, oral administration, 100 mg/kg); (●) CUR-PLGA-NPs (ig, oral administration, 100 mg/kg). Each point represents the mean ± SD, $n = 5$.

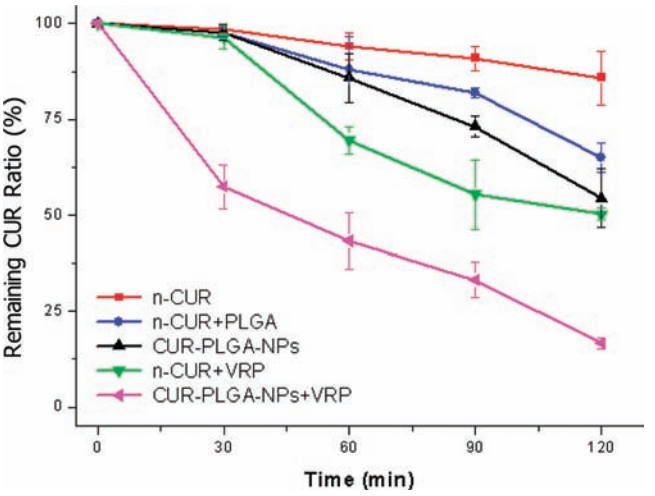

Figure 9. Mean absorption of CUR with and without P-gp inhibitor VRP in the jejunum by in situ single-pass intestinal permeability test in rats (mean ± SD, $n = 3$).

and long half-life as compared with n-CUR in rats. The absolute bioavailability of CUR was a significant increase from 4.73 to 26.5%. The relative bioavailability of CUR-PLGA-NPs is about 563% compared to that of n-CUR. CUR-PLGA-NPs showed about the 5.6-fold increase in apparent bioavailability and had a longer half-life than n-CUR.

The poor bioavailability of CUR has been proven in animal and clinical trials.[7] A study by Yang et al. showed that the AUC of CUR was 7.2 ± 1.2 and 3.6 ± 0.6 min μg/mL for intravenous (10 mg/kg) and oral (500 mg/kg) doses in rats, respectively. The absolute bioavailability was only about 1%.[27] Some of the possible ways to overcome poor bioavailability have been studied. Takahashi et al. reported that the AUC_{0-120} value of CUR after oral administration of LEC, a liposome-encapsulated CUR, was 26502.8 μg min/L, which was 4.96-fold greater than that after n-CUR administration.[9] The pharmacokinetics in vivo revealed that CUR-entrapped nanoparticles demonstrate at least a 9-fold increase in oral bioavailability when compared to n-CUR administered with piperine as an absorption enhancer.[28] The oral administration of Theracurmin was found to be >40-fold higher than that of n-CUR in rats. Then, the AUC of Theracurmin was 27-fold higher than that of n-CUR administered orally at 30 mg to human volunteers.[29] PLGA used for CUR delivery by oral administration can increase bioavailability at different levels. Tsai et al. developed the CUR-PLGA-NPs with size about 158 nm, for which the oral bioavailability was 22-fold higher than that of n-CUR.[30] Anand and his colleagues reported that PLGA-NPs of CUR were more bioavailable and had a longer half-life than n-CUR in mice.[21] These findings demonstrated that PLGA nanoformulation could potentially be applied to increase the bioavailability of hydrophobic polyphenols. Our results were consistent with these previous results. However, the previous studies had not researched the mechanism of increasing bioavailability.

As stated above, our CUR-PLGA-NPs showed the longer half-life of 135 min compared to n-CUR (74.2 min). Anand et al. reported that their CUR-PLGA-NPs had a substantially longer half-life in mice, although they did not present pharmacokinetics parameters, the properties of drug concentration−time curve showed that t_{max} of CUR-NP was attained at 1.0 h, whereas t_{max} of CUR was at 0.25 h, and were consistent basically with our results.[21] Ling and his coauthors researched the bioavailability of vincristine sulfate (VCR)/PLGA nanoparticles (VCR-DPNs) by oral administration in rats and found that pharmacokinetic parameters $t_{1/2}$ and t_{max} of VCR-DPN increased about 7- and 1.9-fold compared to those of VCR.[31] Therefore, from the literature and our results, we consider that the longer half-life of CUR-PLGA-NPs is principally associated with the prolonged absorption phase. The conjecture coincides with the release rate of CUR from the nanoparticles in the intestinal juice being higher than in gastric juice (Figure 7). Because we had not detected the level of PLGA in blood plasma, we cannot be sure whether the polymer-coated CUR gets intact into circulation. If so, because of its biodegradability and biocompatibility, PLGA is a safe therapeutic device approved by the FDA. More studies are required to determine whether this may affect the potential biological and pharmacological activities of CUR-PLGA-NPs.

Mechanisms of Improving Oral Bioavailability with CUR-PLGA-NPs. In recognition of increased bioavailability of CUR-PLGA-NPs, we have revealed the mechanism of enhancement on oral bioavailability. The permeability to the membrane, P-gp-mediated efflux, metabolism by CYP_{450}, and residence time are the major determining factors for drug absorption by oral administration. To demonstrate further and help interpret the absorption of CUR in vivo, an in situ single-pass intestinal permeability test was conducted to assess the potential effect of CUR-PLGA-NPs on the above factors.

As shown in Figure 9, the RCR of n-CUR in the jejunum during 120 min was about 85.8%, which was higher than that of other groups. This showed that n-CUR had not been absorbed or metabolized by the epithelium cell. Recently, on the basis of its poor aqueous solubility and permeability in intestinal epithelial cells[9,14] and the Caco-2 cell line,[15] CUR was classified as a BCS Class IV molecule. Our results showed that n-CUR is poorly permeable by in situ single-pass perfusion method. However, the RCRs of n-CUR with PLGA (1:9, w/w) and CUR-PLGA-NPs were about 64.9 and 54.4%, respectively. The obvious reduction of RCR reflected that PLGA and CUR-PLGA-NPs promoted the

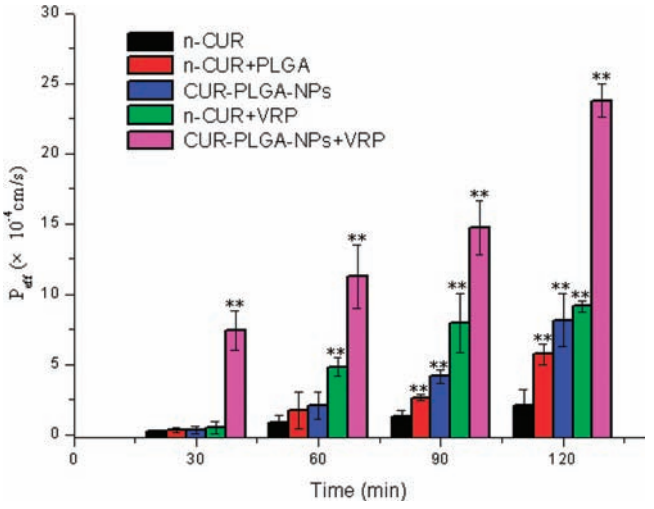

Figure 10. Permeability coefficients of CUR (50 μg/mL) with or without VRP (50 μM) transport across the jejunum by in situ single-pass intestinal permeability test in rats (mean ± SD, $n = 3$). (*) $P < 0.05$ amd (**) $P < 0.01$, compared with n-CUR.

permeability of CUR. To explore whether the effect of CUR-PLGA-NPs in improving the intestinal absorption of CUR was connected with P-gp inhibition, VRP was chosen to compare the influence on permeability of CUR. The RCRs of n-CUR with VRP and CUR-PLGA-NPs with VRP were about 50.2 and 16.7%, respectively. Figure 10 illustrates the permeability of different CUR formulations with and without VRP in the jejunum. The mean permeabilities of n-CUR with PLGA (1:9, w/w) and CUR-PLGA-NPs, n-CUR with VRP, and CUR-PLGA-NPs with VRP were 2.77, 3.95, 4.42, and 11.51 times compared to that of n-CUR at 120 min, respectively. The permeability of two groups treated with VRP (n-CUR, CUR-PLGA-NPs) was higher than that of another three groups without VRP (n-CUR, n-CUR + PLGA, CUR-PLGA-NPs). This phenomenon demonstrated that VRP as a P-gp inhibitor could improve the absorption of CUR, which was consistent with the previous study.[13] However, there was no notable difference ($P > 0.05$) between the abilities of PLGA and P-gp inhibitor VRP in assessing the permeability coefficient at 120 min. In contrast, CUR-PLGA-NPs with VRP showed significant differences in the permeability coefficient at different times realtive to n-CUR ($P < 0.01$). Therefore, we speculate that CUR-PLGA-NP coencapsulation of P-gp inhibitor VRP in the drug delivery system might provide greater bioavailability for CUR.

Furthermore, most of the poorly bioavailable drugs are substrates for the biological transporters (including P-gp). P-gp is an ABC-transporter of the MDR/TAP subfamily, extensively distributed and expressed in the intestinal epithelium, hepatocytes, and renal proximal tubular cells. P-gp-mediated efflux for drug extensively influences the pharmacokinetics and bioavailability in the absorption, metabolism, and clearance of the body.[7,32] The intestinal P-gp efflux pump has been proposed to contribute a major barrier to the oral bioavailability for a number of compounds. Inhibition of P-gp efflux pump is an effective concept to enhance oral bioavailability of substrates for the biological transporters. P-gp inhibitors affect the binding sites of the P-gp with substrates and interrupt the functions of P-gp by competitive inhibition or decrease P-gp expression in the intestinal epithelium. VRP is an L-type calcium channel blocker of the phenylalkylamine class and has been used in the treatment of hypertension, angina pectoris, and cardiac arrhythmia. On the other hand, VRP also is an inhibitor of P-gp.[13] Several studies have reported that the coencapsulation of anticancer agents and VRP was highly effective in overcoming MDR in tumor cells and improving oral bioavailability.[31,33] Usually, the doses of VRP used as a P-gp inhibitor are much higher (2−6 mM) than those for antiarrhythmic indications (0.4−1.2 mM). However, some short-comings have been reported accompanying these effects, including toxicity, adverse effect by their pharmacology activities, and interaction with other drugs by coadministration.[34] To reduce these side effects, more attention has been paid recently to a number of excipients that can modulate P-gp and thus potentially enhance drug absorption. These excipients, such as vitamin E TPGS,[35] Cremophor EL,[36] β-cyclodextrin derivatives,[37] and chitosan derivatives,[38] moderate P-gp efflux pump by other ways directly or indirectly, enhance the intestinal absorption of the P-gp substrates, and, therefore, improve the bioavailability of these substrates. From the results, we infer that PLGA polymer can increase the residence time of CUR in the intestine and inhibit P-gp-mediated efflux for CUR.

It has been reported that PLGA as a drug carrier moderates the P-gp effect and MDR reversal activity.[33] In our study, the mean permeability of CUR coperfusion with PLGA (1:9, w/w) was 3.16 times that of n-CUR without PLGA in the jejunum at 120 min (Figures 9 and 10). Therefore, it can be inferred from the rat intestinal circulating perfusion test that PLGA may enhance the absorption of CUR by influencing P-gp. It is possible that the effect of improving oral absorption and bioavailability of CUR might be due to bypassing the P-gp-mediated efflux induced by PLGA polymer. Thus, encapsulating hydrophobic drugs on PLGA polymer is a promising method for sustained and controlled drug delivery with improved bioavailability. However, the P-gp inhibition mechanism of PLGA, which mainly involves changing the fluidity of the cellular membrane, inhibiting P-gp ATPase, and reducing P-gp expression, remains unclear. Therefore, further study is needed to illustrate fully the mechanism.

Moreover, much attention was recently given to bioadhesive delivery systems to enhance the drugs' bioavailability by increasing the residence time, which subsequently facilitated the absorption of drug through adhesion with the cellular surface.[39] CUR was released slowly from CUR-PLGA-NPs in the intestinal environment as described in Figure 7. In contrast with n-CUR, there was a pronounced time prolongation of CUR to reach the maximum concentration by oral administration as demonstrated in Table 1. These results showed that PLGA may have bioadhesive properties and bind with the mucosa of the gastrointestinal tract. This may increase the residency time and enhance drug absorption due to intimate contact with epithelium cells.

PLGA is an emulsifier and flavoring agent in the food industry and a pharmaceutical excipient and has many advantages compared to P-gp inhibitors. Overall, our results suggest that CUR-PLGA-NPs are likely to have great potential as therapeutic or functional foods, but more studies are required.

In conclusion, to improve the oral bioavailability of CUR, our study focused on the development of PLGA-loaded CUR nanoparticles of <200 nm in average size. The oral relative bioavailability of CUR-PLGA-NPs was significantly enhanced to about 5.6-fold compared with that of n-CUR in rat. The effect in improving the oral bioavailability of CUR may result from improving the water solubility, a higher release rate in the intestinal juice, enhancing the absorption by improving the

permeability, inhibiting P-gp-mediated efflux, and increasing the residence time in the jejunum. Thus, encapsulating hydrophobic drugs on PLGA polymer is a promising method for sustained and controlled drug delivery with improved bioavailability of BCS Class IV, such as CUR.

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