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Research Article

Use of Novel Polyurethane Microspheres in a Curcumin Delivery System

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Despite having a wide range of beneficial pharmacological effects, curcumin is characterized by poor water solubility and absorption. In this study, novel polyurethane microspheres containing curcumin (Cur-PUMs) were prepared using carboxymethyl cellulose sodium to improve the bioavailability and prolong the retention time of curcumin. The prepared Cur-PUMs were characterized by Fourier transform infrared spectroscopy, scanning electron microscope, and ultraviolet spectrophotometer. The sustained-release effects of Cur-PUMs were demonstrated using stability tests *in vitro* and *in vivo* pharmacokinetic studies following oral administration. We found that the stability of Cur-PUMs was strongly affected by pH variation. Further, compared with free curcumin, Cur-PUMs showed significantly improved maximum concentration and half-life.

1. Introduction

Curcumin (diferuloylmethane, MW = 368.37), the main component of turmeric, possesses a wide range of pharmacological activities including anti-inflammatory, anticancer, antioxidant, wound healing, and antimicrobial effects and has been widely used for centuries in indigenous medicine for the treatment of inflammatory conditions and other diseases (Figure 1) [1]. Further, epidemiological studies suggest that curcumin consumption may reduce the risk of cancer as well as having other protective biological effects in humans [2].

Raw curcumin products contain a mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin [3]. Currently, there are few methods to determine the amount of curcumin in food. Although alkaline extraction regimes have been used previously to determine levels of annatto in food, these methods are unsuitable for the extraction of curcumin as it is unstable in alkaline conditions [4–6]. Owing to this instability, it is unsurprising that the relative alkalinity of the intestines promotes the decomposition of curcumin, preventing its absorption. This effect has been demonstrated in rats via oral administration of different doses of 3H-curcumin [7]. Following oral administration, only trace amounts of curcumin were found in urine. Rather, approximately 75% of curcumin was excreted in the feces because of its rapid metabolism and low aqueous solubility [8].

Several strategies have been developed to circumvent the limitations of curcumin, including encapsulation in liposomes, biodegradable microspheres, cyclodextrin, hydrogels, polymeric nanoparticles, and lipid-based nanoparticles [9]. Recently, Sun et al. showed that the anti-inflammatory activity of curcumin is enhanced when curcumin is encapsulated in exosomes [10]. Their work indicated that exosomes, but not lipids alone, are required for the enhanced effect. Further, Akhtar et al. [11] found that curcumin bound to chitosan nanoparticles did not degrade as rapidly as free curcumin when the particles were incubated in mouse plasma *in vitro* at room temperature. These results suggested that the binding of curcumin to chitosan nanoparticles increased its chemical stability and enhanced its bioavailability when fed to mice. Souguir et al. made a nanoencapsulation of curcumin in polyurethane and polyurea shells using an emulsion-diffusion method and investigated parameters affecting

Figure 1: The structure of curcumin.

the mean diameter and size distribution of the particles [12]. Finally, Cassano et al. successfully prepared a novel curcumin-based microsphere for the oral delivery of azathioprine [13].

Understanding the effects of pH on curcumin may help achieve drug delivery in a manner that promotes a more concentrated release of curcumin and/or the protection of curcumin from rapid degradation. The progress of pH targeting nanotechnology has been reviewed previously [15]. In addition, the theory behind pH targeting has also been discussed by Filippov et al. [16]. A number of groups have published work reporting pH responsive drug delivery. Wang et al. recently prepared temperature- and pH responsive nanoparticles of biocompatible polyurethanes for the delivery of doxorubicin [17]. In addition, Hong et al. published PLGA-PEG-PLGA thermo- and pH responsive copolymer micelles [18]. Finally, Zhang et al. have developed pH responsive microspheres and evaluated them in different pH conditions [19].

In this paper, carboxymethylcellulose sodium (Na-CMC) polyurethane microspheres (PUMs) containing curcumin were prepared using isophorone diisocyanate (IPDI), polyethylene glycol (PEG), and Na-CMC. The primary metabolite of IPDI is an ester ring diamine, which is considered nontoxic to humans and is not known to cause inflammation. Moreover, pure CMC has undergone stringent biological research and toxicological testing and has been approved by the World Health Organization (WHO) for food applications. PEG is a nonimmunogenic, biodegradable molecule with many beneficial physical and biochemical properties. For example, PEG is nontoxic and miscible in many solvents. Moreover, PEG itself is not adsorbed by platelets or proteins.

The objective of this study was to prepare Cur-PUMs and optimize an encapsulation process based on the emulsion-diffusion method. Further, the drug release profile and pharmacokinetic parameters of Cur-PUMs were investigated *in vitro* and *in vivo* following oral administration to evaluate its sustained-release and pH responsive properties.

2. Experimental

2.1. Materials. PEG (MW = 1000.800 Da), IPDI (99%), Na-CMC (99%), N-(2,3-dimercaptopropyl)phthalamidic acid (DMPA, 99%), 1,4-butanediol (BDO, 99%), dibutyltin dilaurate (DBTDL, 99%), ethyl acetate (EA, 99%), triethylamine (TEA, 99%), and ethylenediamine (EDA, 99%) were purchased from Xi Reagent Co., Ltd. (Sichuan, China). Curcumin (Cur) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). PEG, DMPA, and Na-CMC were dehydrated in a vacuum oven at 110°C for 3 h. Curcumin must be dehydrated before placement in a nitrogen atmosphere.

2.2. Preparation of Cur-PUMs. Polyurethane was synthesized by a condensation reaction, via coupling reactions between the terminal hydroxyl groups (–OH) of PEG and Na-CMC and the isocyanate groups (–NCO) of IPDI and DMPA. BDO was used as a chain extender, DBTDL was used as a catalyst, and TEA and EDA were used as neutralization agents in this reaction.

First, IPDI, EA (as solvent), the requisite percent of Na-CMC, and 10 g PEG were added into three-neck round-bottom flask with a stirrer, reflux condenser, and thermometer. DBTDL was added dropwise (4-5 drops) as a catalyst. The flask was then heated to 70–90°C and stirred for 1–3 h. The flask was cooled to 50–70°C and DMPA was added. The reaction was incubated for 3 h. BDO was added and the reaction mixture was incubated for an additional 0.5 h. Next, the mixture was cooled to 40°C and 5 g of curcumin was added. TEA was added dropwise to the reaction for 30 min. This was followed by addition of EDA and incubation for 30 min. Finally, the flask was cooled to 30°C, and deionized water was added with stirring for 1–3 h until emulsified. The mixture was distilled at 65°C by reducing pressure.

2.3. Characterizations. Synthesized PUMs, curcumin, and Cur-PUMs were analyzed by Fourier transform infrared spectroscopy (FTIR) using a Jasco 4200 instrument. The test samples were prepared as described previously [12].

A standard curve was plotted to determine the concentration of curcumin versus ultraviolet spectrophotometer (UV) absorbance. To develop this curve, the absorbance of known concentration of curcumin dissolved in methanol (0.612 ng/mL, 1.244 ng/mL, 1.836 ng/mL, 2.448 ng/mL, 3.060 ng/mL, and 15.30 ng/mL) was determined via Nanodrop 1000 spectrophotometer.

Encapsulation efficiency (EE) is defined as the curcumin contained within the Cur-PUMs (m_E) divided by the initial amount of curcumin used (m_I). m_F is the curcumin left in solution during the loading study, as given by the following equation:

$$\mathrm{EE}\,(\%) = \frac{m_E}{m_I}, \quad \left(m_E = m_I - m_F\right). \tag{1}$$

Drug loading efficiency (DL) is defined as the contained curcumin amount (m_E) divided by the amount of microspheres (m_M). The m_M could be obtained via filtration and distillation after the PU shells were dissolved and cleaned with DMF:

$$\mathrm{DL}\,(\%) = \frac{m_E}{m_M}. \tag{2}$$

All the samples were deposited with gold before scanning with a scanning electron microscope (SEM) using JEOL JSMT 300A instrument, just as previously described [11].

2.4. Influences of Process Parameters on EE and DL during Synthesis. Several parameters were taken into account and should be defined first briefly as follows:

$$R = \frac{n(-\mathrm{NCO})}{n(-\mathrm{OH})} \quad (3)$$

$$M_{\text{Na-CMC}} = \frac{m(\text{Na-CMC})}{m_1} \times 100 \quad (4)$$

$$C_{\text{Cur}} = \frac{m(\text{curcumin})}{v(\text{solvent})}. \quad (5)$$

n(−NCO) and n(−OH) are the molar contents of the –NCO group and –OH group of all the components. m(Na-CMC) and m(curcumin) are the mass content of Na-CMC and curcumin. m_1 is the mass of all components except Na-CMC and curcumin. The influence of C_{Cur} on EE and DL was studied when R was 3.0 and $M_{\text{Na-CMC}}$ was 2.0. The influences of $M_{\text{Na-CMC}}$ were assessed when R was 3.0 and C_{Cur} was 0.3. The influences of R were assessed when $M_{\text{Na-CMC}}$ was 0.3 and C_{Cur} was 2.0. Finally, the influences of R were assessed again in the optimized process, as were the effects of both $M_{\text{Na-CMC}}$ and C_{Cur} on EE and DL.

2.5. Analysis of Curcumin Concentration In Vitro and In Vivo. A standard curve was established for use in both *in vitro* and *in vivo* studies. For the *in vitro* study, 0.5 mg/mL curcumin in acetonitrile was diluted to 5–50 μmol/L with PBS (pH 7.4). Absorbance was measured with a spectrophotometer at 420 nm. For the *in vitro* assay, the standard curve was developed by diluting 0.5 mg/mL curcumin with plasma collected from Sprague Dawley rats. The samples were mixed with emodin and centrifuged to remove protein in the plasma. The samples were then detected by UV-HPLC with a C_{18} column and at a wavelength of 420 nm, as previously reported by Sun et al. [10]. PBS solutions were prepared as previously reported [19].

Cur-PUMs were placed in an Eppendorf tube with 0.5 mg curcumin, and 5 mL of releasing medium was added. All samples were incubated at 37°C under gentle agitation in the dark. The absorbance of the samples was detected at various time points (0, 0.5, 1.0, 2.0, 3.0, and 20 h). The extractions were centrifuged at 10000 rpm for 15 min and the supernatant was discarded. The solution was then diluted and detected, as mentioned above.

To study the releasing properties of Cur-PUMs *in vivo*, Sprague Dawley rats were divided into two groups. Group A received an oral administration of curcumin (200 mg/kg), whereas Group B received Cur-PUMs (200 mg/kg). Blood (0.5 mL) was extracted at various time points (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, and 12 h) after feeding and frozen at −80°C. All measurements were made as described above, using plasma at room temperature.

3. Results and Discussion

3.1. Characterization of Synthetic Cur-PUMs Chemical Structure. The assignments of adsorption peaks for Na-CMC polyurethane are presented in Figure 2(a). The peaks at 1703 cm^{-1}, 1573 cm^{-1}, and 3400 cm^{-1} correspond to the C=O band, N–H deformation vibration band, and N–H stretching vibration band of urethane, respectively. The absence of a peak at 2250 cm^{-1} indicates that no isocyanate groups remain in the obtained polymer. These records confirm the formation of polyurethane. The sharp peak at 1624 cm^{-1} corresponds to C=O of –CH_2COONa, indicating the Na-CMC incorporated into the polyurethane successfully. However, few changes were observed in the encapsulated curcumin particles (Figure 2(c)). Nevertheless, two thin shoulders can be observed at 1630 cm^{-1} and 1680 cm^{-1}. The peak at 1680 cm^{-1} is attributed to the enol groups in curcumin (Figure 2(b)). Thus, we can confirm that curcumin was incorporated into the PUMs. Our data indicates that Cur-PUMs were prepared successfully without new chemical bonds emerging, suggesting that there are no chemical interactions between polyurethane and curcumin.

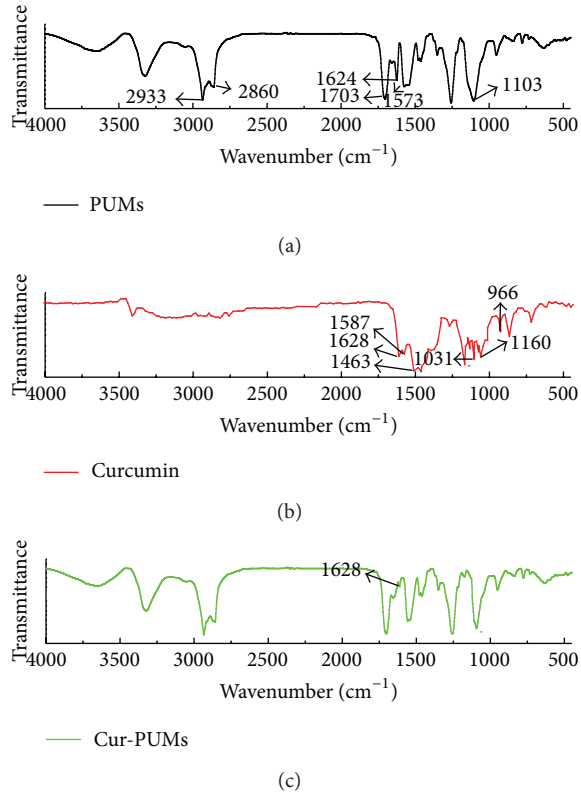


FIGURE 2: FTIR spectroscopy of PUMs (a), Cur (b), and Cur-PUMs (c).

3.2. Influences of the Investigated Processing Parameters on EE and DL. As shown in Figure 3, the optimal wavelength for measuring the concentration of curcumin in methanol is 415 nm. The absorbance measurements for the curcumin standard curve are shown in Table 1.

The standard curve was drawn with the peak absorbance as the vertical axis (A) and the concentration of curcumin (ng/mL) as the horizontal axis (C), as shown in Figure 4.

Table 1: Absorbance measurements for the curcumin standard curve.

C (ng/mL)	6.12	12.24	18.36	24.48	30.6
A	0.003761	0.006890	0.009953	0.013397	0.017074

Table 2: EE and DL values obtained with optimized parameters.

Sample	EE (%)	DL (%)
1	86.91	26.43
2	84.95	25.68
3	85.65	25.85
Average	**85.84**	**25.99**

EE: encapsulation efficiency; DL: drug loading efficiency ($M_{\text{Na-CMC}} = 2.0$, $C_{\text{cur}} = 0.3$).

The regression equation for curcumin was determined as follows:

$$A = 5.4145C + 2.754, \qquad R = 0.9979. \tag{6}$$

The optimized encapsulation parameters were determined ($M_{\text{Na-CMC}} = 2.0$ and $C_{\text{Cur}} = 0.3$), as shown in Figure 5. Using these parameters, the microspheres were prepared and characterized with variations in R. The effect of this variation on EE and DL is shown in Table 2. Morphology was then determined by SEM, as shown in Figure 6.

As shown in Table 2, the values of EE and of DL are stable and feasible using the optimized processes (Table 2). Further, Cur-PUMs are morphologically spherical and heterogeneous in size (Figure 6). The diameters of Cur-PUMs prepared with an R value of 1 ranged from 7.74 to 67.62 μm (mean diameter 28.26 ± 18.46 μm). The diameters of Cur-PUMs with an R value of 3 ranged from 8.35 to 47.5 μm (mean diameter 19.61± 2.50 μm). Based on these results, the optimized parameters with a maximum EE and DL ($M_{\text{Na-CMC}} = 2.0$, $C_{\text{Cur}} = 0.3$, $R = 3$) were selected to prepare Cur-PUMs for the following experiments.

3.3. The Study of Stability In Vitro and Pharmacokinetics In Vivo. The optimal wavelength for measuring the absorbance of curcumin in PBS (pH 7.4) was 420 nm (Figure 7). This value was used to determine the concentration of curcumin in the following experiments.

We determined the time points at which 50% residual curcumin from the Cur-PUMs remained, using PBS with varying pH values. At pH 1.0, 4.0, 6.8, and 7.4, 50% of the residue among negatively charged groups. Swelling of the shell thereby promotes drug release. In contrast, the lower media pH will prevent the dissolution of the shell via a shielding effect, as the nonionized carboxylic acid group becomes more hydrophobic. This leads to the formation of a more compact surface structure. This mechanism will be beneficial to release curcumin in a more concentrated manner with a lower dosage. 50% of residual curcumin remained at 3.25, 1.24, 0.51, and 0.35 h, with pH 7.4, 6.8, 4.0, and 1.0, respectively (Figure 8). These results are consistent with the mechanism proposed (Figure 9) by Huang et al. [14]. Because carboxylic acid groups will obtain a negative charge as the pH increases, the shell will easily swell or decompose due to the electrostatic interaction.

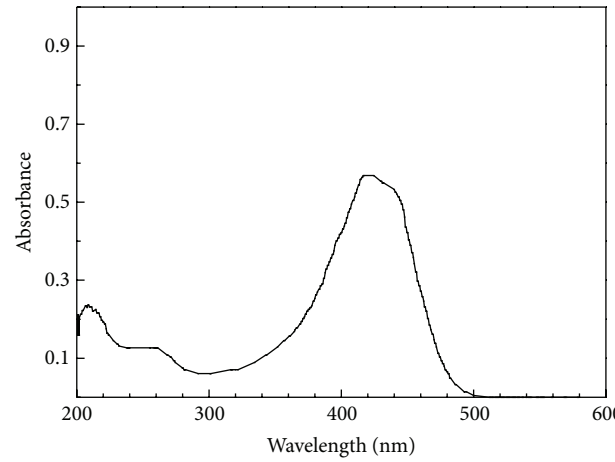


Figure 3: Scanning spectrogram for Cur in methanol.

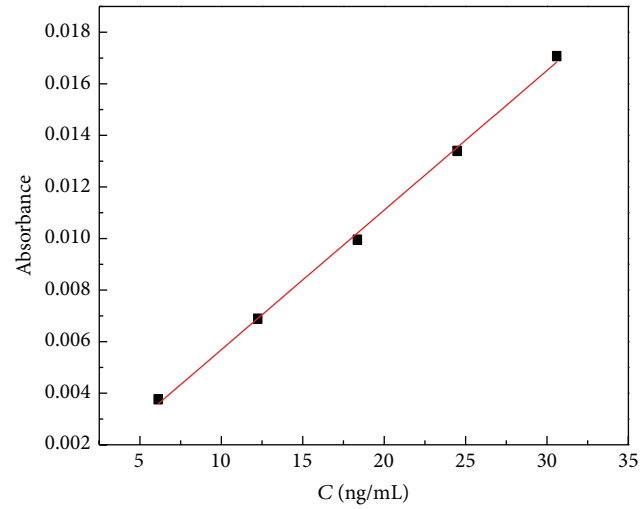


Figure 4: The standard curve obtained for use in the curcumin releasing profile.

The concentrations of curcumin in the plasma of rats demonstrated the sustained-release properties of Cur-PUMs (Figure 10). The T_{max} for Cur-PUMs treated rats (3 h) was six times higher than that of rats that received curcumin alone (0.5 h). Further, the C_{max} (803.27 ± 50.81 ng/mL) for Cur-PUMs treated rats was 4 times higher than those that received curcumin (194.02 ± 14.75 ng/mL) (Table 3). The pharmacokinetic parameters obtained reveal that Cur-PUMs enhanced the maximum curcumin concentration and prolonged the half-life of curcumin, resulting in a sustained-release effect.

4. Conclusion

A series of Na-CMC incorporated PUMs containing curcumin were synthesized and confirmed by FTIR. Optimized processing parameters were obtained through the assessment

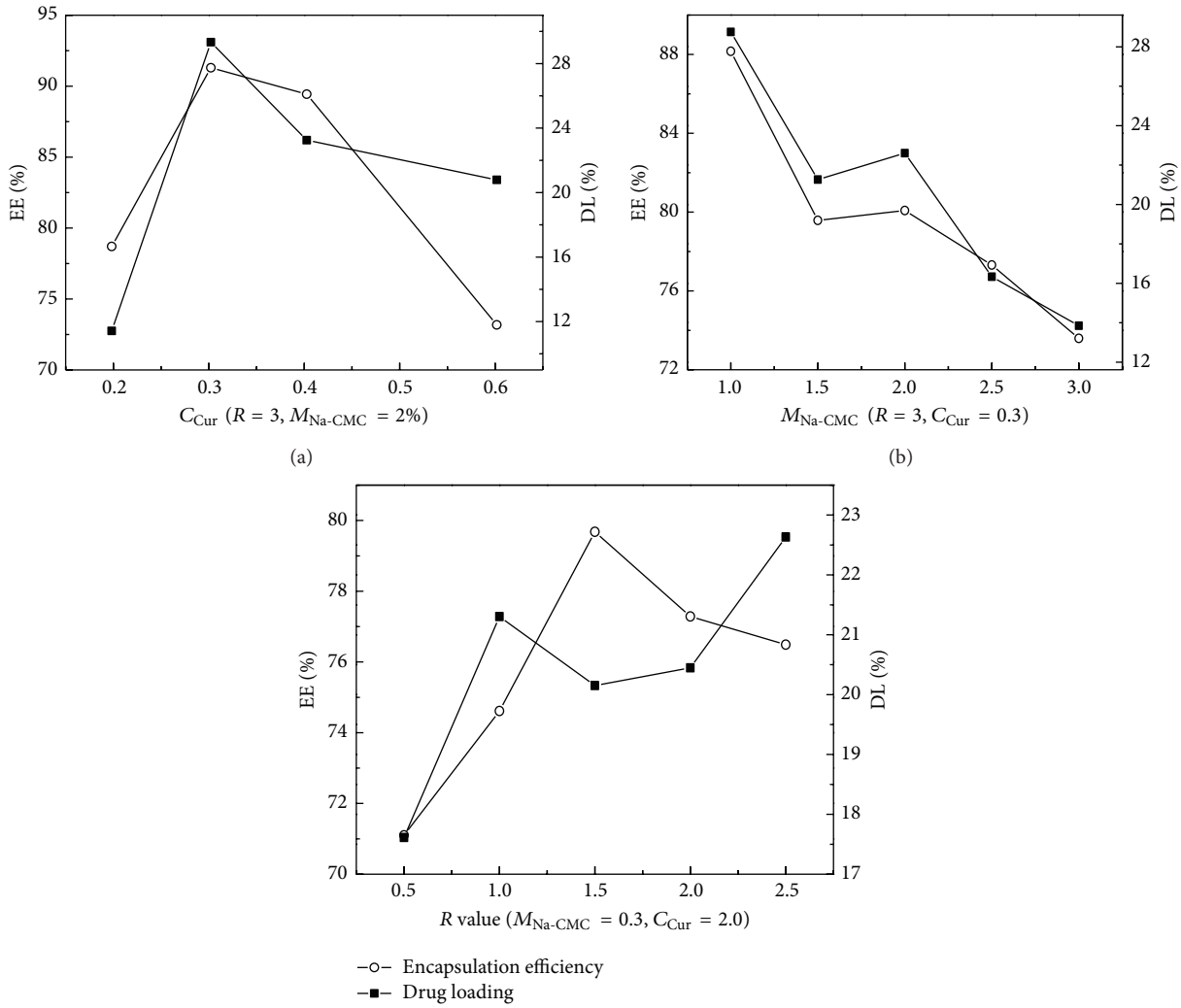
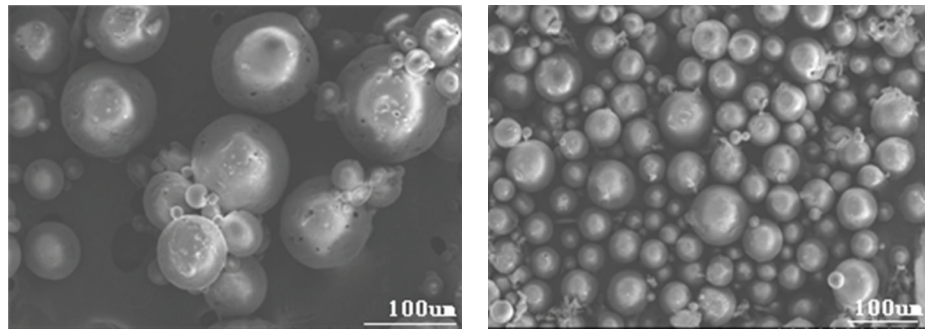


Figure 5: Influence on EE and DL at various concentrations of curcumin (a); at various concentrations of Na-CMC (b); at various values of R (c).



(a) (b)

Figure 6: SEM photographs of (a) R = 1; (b) R = 3.

Table 3: Pharmacokinetic parameters of different curcumin formulations.

Formulation	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\to\infty}$ (ng/mL h)	$T_{1/2}$ (h)
Curcumin	194.02 ± 14.75	0.5 ± 0.12	348.77 ± 44.83	0.91 ± 0.28
Cur-PUMs	803.27 ± 50.81	3.0 ± 0.23	3873.95 ± 265.22	5.61 ± 0.53

Values are reported as mean ± S.E.M. ($n = 5$). C_{max}: maximum concentration; T_{max}: time to reach peak concentration; AUC: area under the plasma concentration-time curve from 0 h to ∞; $T_{1/2}$: half-life.

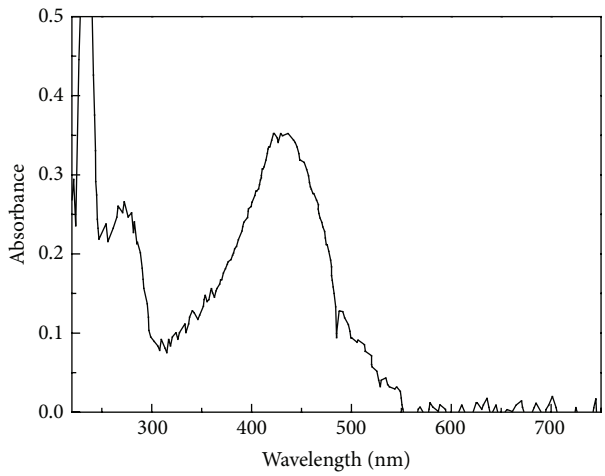


Figure 7: Scanning spectrogram of curcumin in PBS (pH 7.4).

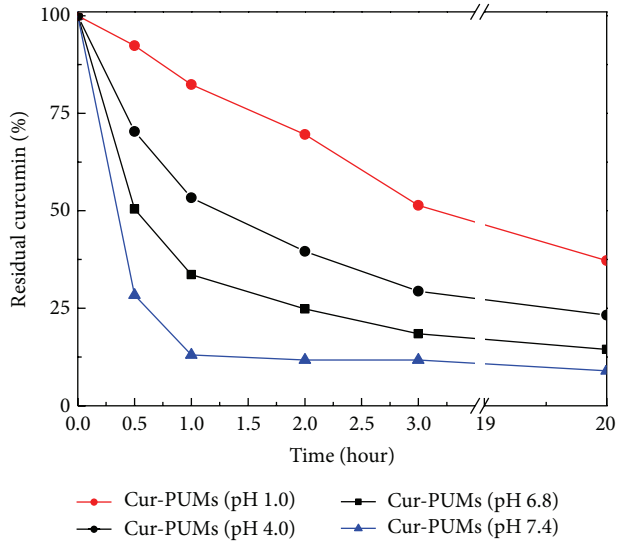


Figure 8: *In vitro* stability of curcumin from Cur-PUMs in different pH conditions ($n = 3$).

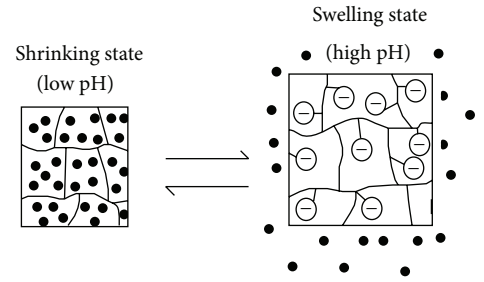


Figure 9: The mechanism of drug release in pH-sensitive hydrogels [14].

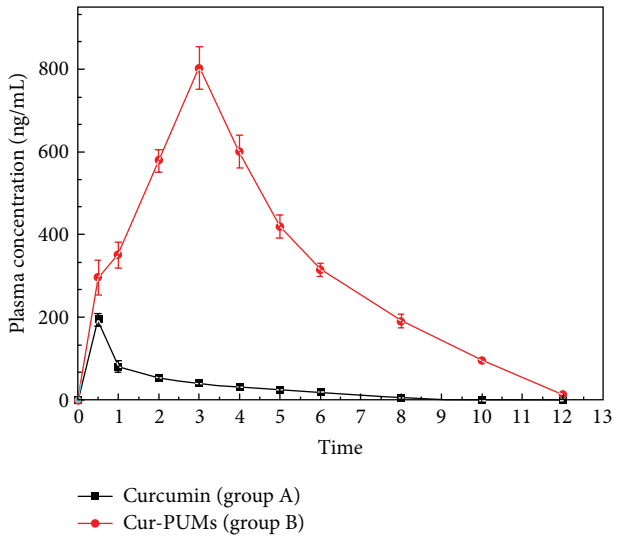


Figure 10: The concentration of curcumin in plasma versus time.

of drug loading and encapsulation efficiency. The optimized concentrations of Na-CMC and curcumin were 2 and 0.3, respectively, and R was defined as 3. The obtained Cur-PUMs had a spherical morphology. The pH responsive effects of Cur-PUMs were confirmed using *in vitro* stability tests. This analysis indicated that acidic conditions had a shielding effect, which prevented curcumin diffusion, whereas alkaline conditions disrupted the shield allowing for curcumin release. The sustained-release effects of Cur-PUMs were demonstrated directly *in vivo*. Cur-PUMs displayed a prolonged retention time and higher maximum concentration in plasma as compared to curcumin alone. Together, our data suggest that Cur-PUMs may provide an opportunity to utilize curcumin more efficiently with lower doses and improved efficacies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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