A Porous Composites of Poly(ethylene glycol)-poly(ε – caprolactone) Nanomicelles and Chitosan for Curcumin Delivery

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As a multi-functional drug, curcumin has a great potential for various applications in medicine. However, the use of curcumin is still limited owing to its high hydrophobicity, poor absorption, rapid metabolism, and rapid systemic elimination. Recently, the utilization of biodegradable polymers for curcumin delivery presents a promising approach to overcome these disadvantages. This study developed a drug delivery system using curcumin-encapsulated methoxy poly(ethylene glycol)-block-poly(-caprolactone) nanomicelles, which were then submerged in chitosan networks (cur-mPEC-C). Fourier-transform infrared (FTIR) spectrometry was employed to investigate the chemical structures of both micelles and nanocomposites. Morphologies of the nanocomposites were obtained through scanning electron microscope (SEM) analysis. The average particle size of micelles between 144 to 182 nm was measured by DLS. Cur-mPEC with the average particle size of 144 nm (ratio of mPEC/Curcumin 8:1) was utilized as the optimized sample for incorporating chitosan. Curcumin-loaded mPEC/chitosan nanocomposites were produced by freeze-drying method and characterized. The drug release profile revealed that chitosan enabled avoidance of the burst release of curcumin.

Keywords: Chitosan; nanomicelle; curcumin; mPEG–PCL; drug delivery system

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Curcumin, a yellow pigment of turmeric (*Curcuma longa* L.), has been known as a bioactive compound to enhance effectiveness of drugs, such as antioxidants, antimicrobials, anticarcinogenics, anti-inflammatories, antiarthritics, enhance wound healing, and so on [1–4]. Curcumin also has therapeutic benefits for skin health, food, and biotechnological applications [5]. Additionally, curcumin has also been shown in recent studies to have other potentials in cancer treatment [6–8]. However, curcumin has some drawbacks that limit its utility in clinical settings. For instance, curcumin has low bio-availability and solubility (at acidic or neutral pH), and a short half-life in plasma [9]. Curcumin can be rapidly decomposed in an alkaline medium [10,11]. Most importantly, curcumin has been found to have poor absorption, rapid metabolism, high *in vivo* elimination rates, and inability to cross the stratum corneum to reach wounds [12,13].

Recently, nanosized drug delivery systems have proven to be crucial in delivering drugs in a targeted manner. Nanosized drug delivery systems improve efficacy, reduce side effects and clearance, increase cellular uptake, and prolong time in circulation. They have many advantages over free drugs, e.g., protecting drugs from premature degradation, preventing drugs from prematurely interacting with the biological environment, enhancing absorption of drugs into tumor tissues via the enhanced permeability and retention effect (EPR), controlling drugs' pharmacokinetic and tissue distribution profiles, and improving intracellular penetration [14].

Many effective techniques and approaches, such as encapsulation into nanoparticles, liposomes, micelles, phospholipid complexes, electrospraying, local drug delivery, and gelation, have been used to overcome the problems [15,16]. Among them, micelles can improve the gastrointestinal absorption of natural drugs, resulting in higher plasma levels and lower kinetic elimination, thereby improving bioavailability [12]. Letchford and colleagues discovered that polymeric formulations containing methoxy poly(ethylene glycol)-block-poly (-caprolactone) copolymers (mPEG-*b*-PCL) can increase the solubility of curcumin up to 13×10^5 folds [17], which attracted the enormous studies on these polymeric micelles. Nevertheless, the release of curcumin with mPEG-*b*-PCL occurs rapidly owing to the highly hydrophilic polymer segment of mPEG. Therefore, the development of hybrid micelles is considered a promising solution. Due to higher drug bioavailability, decreased drug cost, increased drug targeting, and decreased medication side effects, transdermal drug administration has shown the potential utility for curcumin delivery.

The objective of this study is to overcome the limitations of drug delivery by introduction of

curcumin into nanomicelles for drug delivery system; thus, a drug delivery system based on mPEG–PCL/ chitosan was developed. Curcumin was encapsulated in mPEG-PCL micelles, which was then hybridized with chitosan to control the drug release profile. It is well-known that chitosan is rigid, insoluble, and stable owing to strong intermolecular hydrogen bonds, making it a promising carrier material [18,19].

EXPERIMENTAL

Chemicals and Materials

All reagents used were of analytical grade and used without further purification. Curcumin 95% was purchased from Marven Bio Chem (India); chitosan (≥75%, deacetylated); and glutaraldehyde (50%) (GA) were obtained from Sigma Aldrich, Singapore. All the chemicals used for synthesizing mPEG-PCL, including ε-caprolactone (CL), tin(II) 2- ethylhexanoate $Sn(Oct)_2$, and methoxy polyethylene glycol (mPEG), were purchased from Sigma Aldrich, Singapore.

Preparation of Curcumin-loaded Methoxy Poly (ethylene glycol)-block-poly(ε-caprolactone) (cur-mPEC) Micelles

The mPEG–PCL diblock copolymer ($M_{n\,PCL}$ = 22,000) was successfully synthesized and characterized elsewhere [20]. In brief, the mPEG–PCL diblock copolymers were synthesized by ring opening polymerization of ε-caprolactone in the presence of mPEG-OH as a macro-initiator with $Sn(Oct)_2$ serving as a catalyst. Predetermined amounts of mPEG-OH, ε-caprolactone, and $Sn(Oct)_2$ were added into a 250 mL three-necked flask under a nitrogen atmosphere and mechanical stirring. The resulting mixture was refluxed for 4 h at 130°C. After the reaction was completed, the mixture was dissolved in DCM and precipitated in diethyl ether: hexane (9:1, v/v). The product was filtered and dried in vacuum for 24 h to deliver mPEC copolymer. The critical micelle concentration value of mPEC was 5.1×10^{-4} mg/mL. A modified solvent extraction method using acetone as the solvent was used to prepare curcumin-loaded micelles [21]. Firstly, 10 mg of curcumin was dissolved in 6 mL of acetone (the amount of curcumin was kept constant throughout the study). Next, a calculated amount of mPEC (Table 1) was added to the curcumin solution, followed by 25 mL of deionized water. The organic solvents were removed by rotary evaporation and filtered through a Millipore 0.45 µm filter to obtain the self-assembled micelles of the curcumin-encapsulated mPEC. The mass ratios of curcumin to mPEC are listed in Table 1. The particle size of prepared nanomicelles was measured using a particle size analyzer (LB-550, Horiba, Japan).

Preparation of Curcumin-loaded mPEG-PCL/ Chitosan (cur-mPEC-C) Nanocomposites

Chitosan is able to protect drugs from harsh environments and slows down the drug release rate [22]. Therefore, different amounts (0.1, 0.2, 0.7, and 2.5 g) of cur-mPEC micelles at optimum mass ratios were used to prepare cur-mPEC-C nanocomposites. Typically, chitosan solution 1.5% was obtained by dissolving 10 g of chitosan in 600 mL of CH_3COOH 1%. A prepared micelle was poured into 120 mL of chitosan solution under a continuous stirring of 600 rpm, and the mixture was stirred for further 1 h. Afterwards, 4 mL of glutaraldehyde (GA) 5% was added to the solution. The mixture was continuously stirred for 15 min. The product was transferred into a 50 mL Falcon tube and placed in a -40°C deep freezer for 24 h. The frozen samples were lyophilized for 48 h under a freeze dryer. The samples were then stored at room temperature for further experiments. The quantification of curcumin was measured by UV-Vis spectrophotometric method [23].

To confirm the presence of interactions between drug and polymer, and chitosan as well, the Fourier-transform infrared (FTIR) spectroscopic spectra of pure curcumin, diblock copolymer, chitosan, and the nanocomposites were recorded from 500 cm^{-1} to 4000 cm^{-1}. The morphologies of the micelles, as well as that of the nanocomposites, were measured by scanning electron microscope (SEM) technique.

Drug Encapsulation Efficiency

Drug encapsulation efficiency is defined as the weight percentage of curcumin in micelles relative to the initial feeding amount of curcumin. The amount of curcumin loaded in the micelles was determined by the absorption at 420 nm using UV-Vis spectrometry [23]. Curcumin solutions of various concentrations were prepared, and the absorptions of the solutions were measured to obtain a calibration curve.

Drug Release Profile

The release of curcumin from the nanocomposites was carried out at pH = 7.4 and 5. These two pH values were used to mimic the pH gradient from the stomach to the intestine [24]. 100 mg of freeze-dried nanocomposite was dissolved in 10 mL of PBS solution and incubated at 37°C. Then, at determined time intervals, 3 mL of the solution was taken out and replaced by 3 mL of fresh PBS. The concentration of the released curcumin was measured using an ultraviolet-visible (UV-Vis) spectrophotometer at the wavelength of 420 nm.

Statistical Analysis

All experiments were conducted in triplicate. Statistical analyses were performed by the Student's test. Statistical differences were considered to be significant when *P*-value was less than 0.05. Results are calculated and presented as mean ± standard deviation. The curcumin calculation method was validated by our laboratory before being applied to the

analysis of curcumin. In detail, correlation coefficient (R^2), relative standard deviation (RSD, %), and recovery (%) are 0.99, 2.85% and 94-102%, respectively.

RESULTS AND DISCUSSION

Preparation of Cur-mPEC Micelles

The amphiphilic mPEC with the longer hydrophobic segment (PCL) was used to encapsulate curcumin due to its hydrophobicity [12]. As seen in Figure 1, cur-mPEC micelles only formed at the mass ratio range of 8:1 – 12:1. The reason behind this is curcumin could be physically encapsulated into copolymeric nanoparticles due to the hydrophobic interaction between the drug and the PCL core [22]. For cur-mPEC-4 and cur-mPEC-6 samples, the mPEC distributed throughout the solutions and acted as a surfactant. It adsorbed at the aqueous-organic solvent interface (DI water-acetone in this study) instead of interacting with curcumin to form micelles due to the insufficient number of copolymer chains. With increasing amounts of mPEC, the adsorbed mPEC at the interface increased, resulting in the interaction between mPEC and curcumin molecules to produce the micelles (cur-mPEC-8, cur-mPEC-10, and cur-mPEC-12). As the amount of mPEC reached the saturated point (cur-mPEC-15), the micelles became unstable and resulted in disassembling, releasing free chains that could be adsorbed again at the interface [25]. Similar explanations for the average size and that of the particle size distribution of cur-mPEC micelles were considered. The curcumin encapsulation efficiency increased with the increase of mPEC amount. The curcumin encapsulation efficiencies were 33.6, 38.3, and 41.2 for Cur-mPEC-8, Cur-mPEC-10, and Cur-mPEC-12, respectively (Table 1). The high encapsulation efficiency of curcumin into SEDDS and α-tocopherol nanoemulsion has also been reported [26,27]. From the results in Table 1 and Figure 2, the particle size increased with the increase in the mass ratio of mPEC. Cur-mPEC-8 obtained the minimal average particle size of 144 nm, which was chosen as the optimum micelles since there was no precipitation. The *in vitro* stability of Cur-mPEC-8 was investigated at 37°C. The size of Cur-mPEC-8 was evaluated via DLS measurements for a period of up to 7 days. The result was, Cur-mPEC-8 retained its size over the 7 days incubation period. This could be attributed to the high CMC value of mPEC.

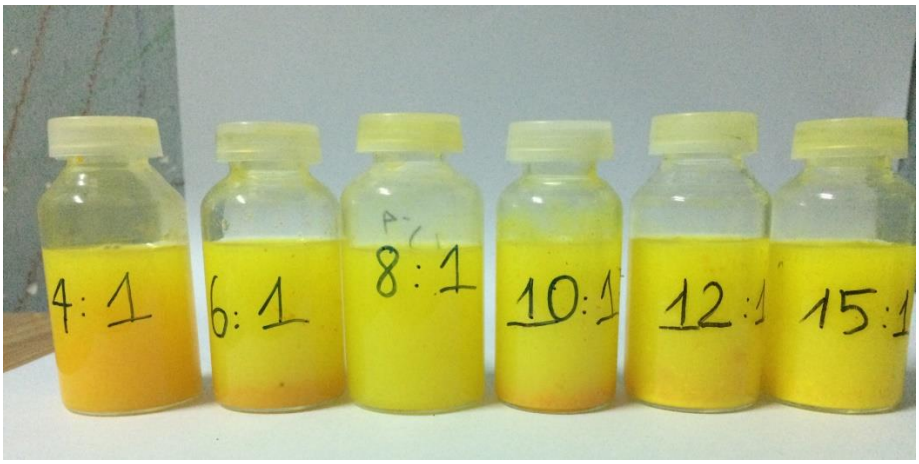

Figure 1. Image of micellar formulations with mass ratios of mPEC to curcumin of 4:1 to 15:1 (left to right).

Table 1. Compositions and particle sizes of prepared curcumin/mPEC.

No.	Abbreviation	mPEC (mg)	Curcumin (mg)	mPEC/ Curcumin mass ratio	Encapsulation efficiency (%)	Average diameter (nm)	PDI	Zeta potential (mV)
1	Cur-mPEC-4	40	10	4:1	-	-	-	
2	Cur-mPEC-6	60	10	6:1	-	-	-	
3	Cur-mPEC-8	80	10	8:1	33.6	144	1.38	-11.3
4	Cur-mPEC-10	100	10	10:1	38.3	158	1.21	-11.8
5	Cur-mPEC-12	120	10	12:1	41.2	182	1.18	-12.4
6	Cur-mPEC-15	150	10	15:1	-	-		

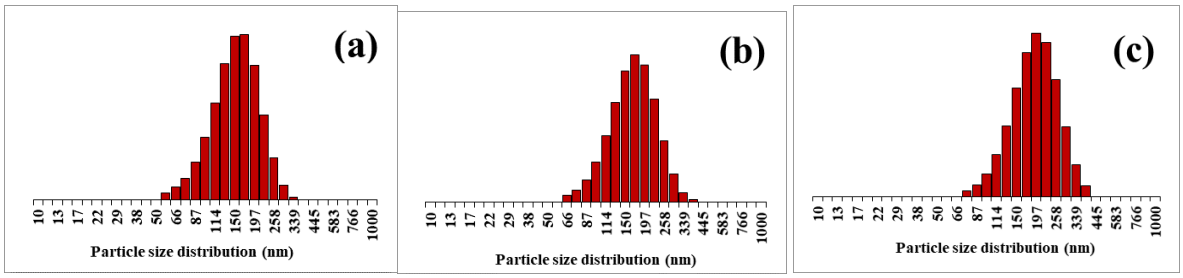

Figure 2. Particle size distributions of (a) Cur-mPEC-8, (b) Cur-mPEC-10, and (c) Cur-mPEC-12.

Table 2. Compositions of Cur-mPEC-C composites.

No.	Chitosan (g)	Cur-mPEC micelle (g)	% Curcumin	Abbreviation
1	2	0.1	0.005	0.5-cur-mPEC-C
2	2	0.2	0.01	1-cur-mPEC-C
3	2	0.7	0.03	3-cur-mPEC-C
4	2	2.5	0.05	5-cur-mPEC-C

Characterization of Cur-mPEC-C Nanocomposites

Cur-mPEC polymeric micelles have a PCL core that can incorporate hydrophobic drugs and release them by means of either dilution-induced collapse or degradation of micelle-forming polymers. mPEC is known as a highly hydrophilic polymer that can limit drugs' delivery efficiency [17]. Hence, chitosan is incorporated into drug-polymeric micelles to maintain the cur-mPEC micelles as much as possible. In this study, the amount of chitosan was kept constant, while the weight percentage of curcumin was changed by increasing the weight of micelles (mPEC/curcumin mass ratio was 8:1) (Table 2). This current composite membrane contained 0.005, 0.01, 0.03, and 0.05% curcumin, corresponding to 10, 20, 60, and 100 mg of curcumin in 2 g of chitosan. The findings are consistent with a previous study.

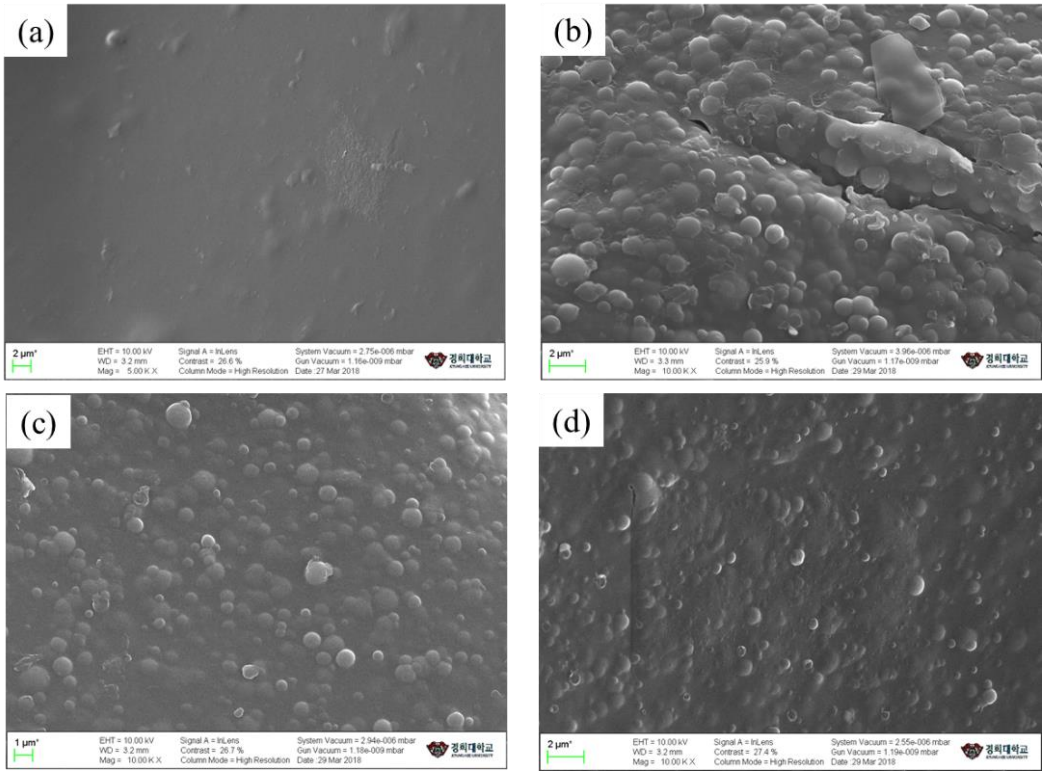

Figure 3. SEM images of (a) chitosan, (b) 0.5-cur-mPEC-C, (c) 1-cur-mPEC-C, and (d) 3-cur-mPEC-C.

Figure 3 exhibits the morphologies of chitosan and chitosan-containing cur-mPEC micelles. As shown, the surface of chitosan was smooth and there were no cracks on the surface (Figure 3a). Meanwhile, the presence of cur-mPEC caused changes in the surface of composites. Obviously, the surface of chitosan became rougher and more micelles were mixed (Figures 3b-d).

Figure 4 shows the FT-IR analyses of the prepared samples. The characteristic bands of curcumin are shown in Figure 4a. The peak at 3508 cm^{-1} was assigned to the O-H stretching of a phenolic hydroxyl group. The specific peaks of C=C stretching and the C=C bend of benzene were shown at wavelengths 1602 cm^{-1} and 1512 cm^{-1}, respectively [21]. The FT-IR spectrum of mPEC (Figure 4b) demonstrated that the peak of C–H stretching (~2944 cm^{-1}) and C=O stretching band (~1722 cm^{-1}) were attributed to the PCL segments in the copolymer. Moreover, a peak at 1106 cm^{-1} was assigned to the C–O–C bond of the repeated $–OCH_2CH_2$ units of methoxy poly(ethylene glycol) (mPEG) [20,28]. The strong broad peaks between 3200 and 3570 cm^{-1} were attributed to stretching of the O-H bond that formed the hydrogen bridges between water and chitosan (Figure 4d). The peak at 1650 cm^{-1} corresponded to the stretching mode of the amide I group in chitosan, which agrees with previous reports [17,29]. It is clear that all the specific peaks of micelles and chitosan appeared in the FT-IR spectrum of the cur-mPEC-chitosan composite (Figure 4b). It was noted that when the amount of chitosan was much larger than that of the micelles, the peaks at 3546 and 3508 cm^{-1}, as well as the peak at 2944 cm^{-1}, were not observed. Notably, the shift of the C=O vibration from 1722 to 1512 cm^{-1} evidenced that the drug was successfully loaded onto the mPEC micelles. As shown in Figure 4, the peaks at 1000-1300 cm^{-1} became stronger and sharper due to the incorporation of cur-mPEC in chitosan.

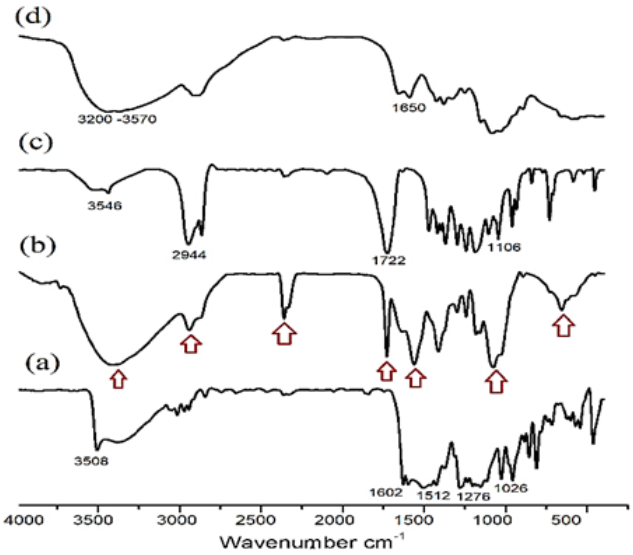

Figure 4. FT-IR spectra of (a) curcumin, (b) cur-mPEC-chitosan composite, (c) mPEC, and (d) chitosan. Arrows indicate the characteristic peaks of micelles and chitosan.

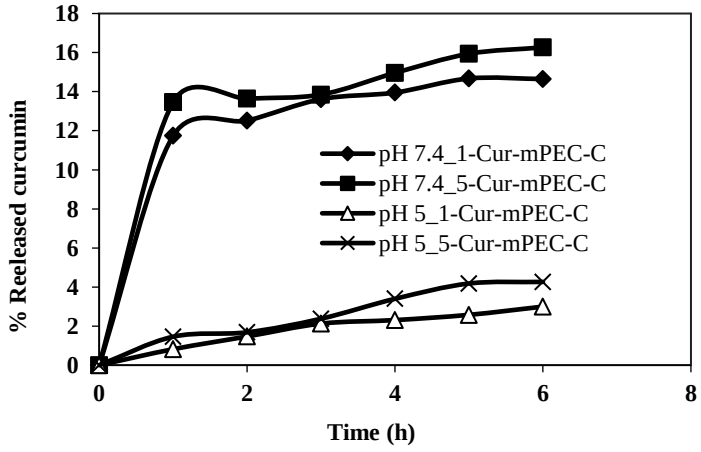

Figure 5. Release profiles of curcumin from cur-mPEC-C nanocomposites at pH = 7.4 and 5.0

Drug Release Profile

According to the literatures [21,30], chemical and biochemical factors strongly affect the release of curcumin from the carriers. As shown in Figure 5, the amount of curcumin released from the nanocomposite increased significantly as the pH increased from 5 to 7.4. This is because the solubility of curcumin increases as the pH value increases. It is worth noting that no burst release phenomena were observed at pH 5. This could be explained by the fact that chitosan solubility is limited at lower pH values [24], resulting in a longer release time of curcumin [31,32]. Furthermore, as the amount of micelles in nanocomposites increases, the amount of curcumin released increases.

CONCLUSION

The curcumin-loaded mPEC/chitosan nanocomposites were successfully synthesized by employing the freeze-drying method. The results indicated that curcumin was effectively encapsulated in the prepared micellar nanoparticles. The optimized copolymer/curcumin was 8:1, which had a particle size of ~144 nm. The *in vitro* drug release profile revealed that the release of curcumin was effectively controlled by adjusting the pH value, and there was no burst release of curcumin at pH = 5. These findings imply that the prepared mPEC/chitosan nanocomposite could be a promising curcumin delivery system.

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DECLARATION OF INTEREST

The authors declare they have no conflict of interest.

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