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Hydroxypropylmethyl cellulose-based sponges loaded self-microemulsifying curcumin: Preparation, characterization, and *in vivo* oral absorption studies

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ABSTRACT: Novel hydroxypropylmethyl cellulose (HPMC)-based sponges containing self-microemulsifying curcumin (SME-Cur) were prepared by a freeze drying method using different grades of HPMC (E5 LV, E15 LV, E50 LV, A15 LV, and A4C). The physical properties and drug release from these carriers were characterized and compared among the different formulations. The mean pore size values of the sponges from image analysis ranged from 43.36 ± 4.54 to 123.22 ± 8.19 nm. An increase in the concentration or viscosity of the HPMC, resulted in denser sponges and a slower drug release. The average microemulsion droplet size from the optimal sponge formulation was 34.80 ± 0.1 nm, and the curcumin was almost completely released within 120 min. The AUC after oral administration of the liquid and solid SME-Cur were 7- and 5-fold greater than that of the curcumin powder in the rabbit, respectively. The results demonstrated that the HPMC-based sponges loaded with SME-Cur could be efficiently used to enhance the oral bioavailability and might be useful as they could be administered at a lower dose compared to normal curcumin powder. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42966.

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INTRODUCTION

Curcumin, a polyphenolic compound, is the principal curcuminoids from the rhizome of the perennial herb turmeric or *Curcuma longa* Linn. (Zingiberaceae family). It is an orange-yellow crystalline powder that is practically insoluble in water. The aqueous solubility of curcumin was found to be 30 pmol/mL.[1] Curcumin has a variety of biological and pharmacological activities including anti-oxidative,[2] anti-inflammatory,[3,4] antimicrobial,[5] antiproliferative,[6] and anti-cancer.[7]

Among the approaches used to enhance the solubility and dissolution rate of drugs, self-microemulsifying drug delivery systems (SMEDDS) have been shown to improve the oral bioavailability of many highly lipophilic compounds.[8–10] Conventional SMEDDS formulations are generally prepared in a liquid dosage form and delivered in soft gelatin capsules. There are some limitations of this liquid form, for instance, a reduced amount of active ingredient due to its slow precipitation that causes less stability, also there can be interactions between the formulation components and the capsule shell, and maybe leakage from the capsules.[11] Solid dosage forms of SMEDDS could overcome some of the disadvantages of these liquid forms as well as to allow for better process control, reproducibility, and patient compliance.

Solidification of the liquid SMEDDS can be achieved by several techniques; such as adsorptions to solid carriers,[10] spray drying,[12,13] the extrusion/spheronization technique,[9,14] etc. Generally, solid SMEDDS comprise various components and require a complicated procedure to prepare. Balakrishnan and coworkers[10] used Aerosil 200 as an inert solid carrier to create a powder of dexibuprofen SEDDS by spray drying. The main limitations of the spray drying technique were the loss of product and the resulting environmental pollution from the production of a fine powder. In addition, the high-temperature process used could modify the properties of the product. In earlier

Table I. The Physical Properties of Selected METHOCEL™ Grade

METHOCEL™ premium products	E5 LV	E15 LV	E50 LV	K100 LV	A15 LV	A4C
Viscosity (mPa s, 2% in water at 20°C)	4.0-6.0	12-18	40-60	80-120	12-18	320-480
Methoxyl (%)	28.0-30.0	28.0-30.0	28.0-30.0	19.0-24.0	27.5-31.5	27.5-31.5
Hydroxypropyl (%)	7.0-12.0	7.0-12.0	7.0-12.0	7.0-12.0	0	0

Data in this table was modified from a technical review of the Dow Chemical Company.

studies,[10,14] on the preparation of SMEDDS pellets, they were prepared by the extrusion/spheronization technique. The production of these pellets was technically complex and time-consuming. In addition, there were many solid excipients involved to produce the pellets.

Biopolymer-based sponges are often used to help deliver to cells, genetic material and drugs into the body.[15,16] The advantages of these systems include their non-irritating, non-mutagenic, non-allergenic, and non-toxic products and they are biodegradable. Previously,[17] Nile red SMEDDS were entrapped in alginate sponges by a freeze-drying process, and the hydrophobic model drug showed a sustained release pattern from the sponges. This indicated that the hydrophilic polymer was able to adsorb the liquid SMEDDS and reduce the rate of the release. Therefore, the biopolymer-based sponges might be useful as a carrier containing SMEDDS for oral drug delivery.

Freeze drying appeared to be an interesting method to produce porous sponges without the organic solvent residue. Moreover, the number of components used in polymeric sponges is smaller than for other solid dosage forms. They are easy to fabricate and require a short preparation time. Lyophilized sponges could be prepared by freeze-drying aqueous gels of a polymer(s) to form a porous polymeric network.[18] During the whole process of freeze drying, the temperature stayed low and that was the main reason for producing high quality products. Hence, the production of sponges containing SMEDDS for oral delivery was a very attractive option and this method had not been previously developed.

In this study, the freeze drying technique was used to generate porous sponges that contained self-microemulsifying curcumin (SME-Cur) as a new oral delivery system. The drug loading, drug entrapment efficiency (DEE), *in vitro* drug release of the polymeric sponges produced from various grades of HPMC were determined. The *in vivo* oral absorption of the optimal sponge loaded SME-Cur formulation was also compared with unformulated curcumin in rabbits.

EXPERIMENTAL

Materials

Curcumin was obtained from Sigma Aldrich (Buchs, Switzerland). Capryol 90™ (propylene glycol monocaprylate), Labrafac PG™ (propylene glycol caprylate/caprate), and Labrasol™ (caprylocaproyl macrogol-8 glycerides) were from Gattefossè (Saint-Priest, France). Cremophor EL™ (polyoxyethylene castor oil derivatives) was from BASF (Ludwigshafen, Germany). All types of hydroxypropylmethyl cellulose were from Colorcon (Indianapolis, USA). Hard gelatin capsules (size 00) were from Capsugel (Bangkok, Thailand). Acetonitrile and methanol (HPLC grade) were from RCI Labscan (Bangkok, Thailand). All other chemicals were of analytical grade.

Preparation of Self-Microemulsifying Curcumin (SME-Cur)

The SME-Cur was prepared according to our previous study.[9] In brief, the liquid components of surfactants and oils (Cremophor EL 315 mg, labrasol 315 mg, Capryol 90 135 mg, and Labrafac PG 135 mg) were mixed homogeneously. The curcumin powder (40 g) was added to the liquid mixture and dispersed by continuous stirring until a homogenous solution was formed. The SME-Cur was stored in tightly sealed glass bottles at room temperature until used.

Preparation of HPMC-Based Sponges Loaded SME-Cur

HPMC E5 LV, E15 LV, E50 LV, K100 LV, A15 LV, and A4C were used to prepare sponges. The physical properties of each type of HPMC are presented in Table I. All grades of HPMC solution (1-4% w/w) were separately prepared in distilled water. Then, 5% w/w of the SME-Cur was added into each polymer solution to make 100 g mixtures. The mixture was homogenized continuously to get a stable o/w emulsion. The emulsion was poured into 96 well plates and then stored in a refrigerator overnight to remove air bubbles. The sponges were manufactured by freeze-drying (Christ, USA) and kept in a desiccator until used.

Morphological Observation of HPMC-Based Sponges Loaded SME-Cur

Morphological examination of the external surface and cross-sectional structure of the sponges were studied with a scanning electron microscope (SEM). Mean pore diameters were determined by analysis of digital SEM images from sectioned samples. Six images (100 different pores) of each sponge were taken and utilized for the mean pore size computation.[19,20] Transmission electron microscopy (TEM) (JEOL, Japan) was used to observe the morphology of the microemulsion droplets formed after reconstitution with 0.1 *N* HCl. Sponges (equivalent to 1 g of SME-Cur) were introduced into 100 mL of 0.1 *N* HCl (100-fold dilution) at room temperature. The content was gently stirred for 2 h and was then filtered. Sample after dilution was placed on copper grids. The excess fluid was carefully removed using a filter paper. The samples were stained in 2% phosphotungstic acid for 10 min. The excess fluid was then drawn off, and the grid surface was air dried at room temperature.[8,21,22]

Weight Variation[23]

Twenty sponges were randomized from each formulation, and weighed individually using a digital balance (Denver instrument, USA). The average weight of each formulation was calculated. Not more than two of the individual weights should deviate from the average weight by more than 5%.

Emulsion Droplet Size Analysis[8]

HPMC-based sponges containing SME-Cur (equivalent to 100 mg of SME-Cur) were introduced into 20 mL of 0.1 *N* HCl at room temperature. The content was gently stirred using a magnetic stirrer for 2 h and the content was then filtered through a 0.45 μm filter. The droplet emulsion sizes and polydispersity index of the resultant microemulsion were determined using the Zetasizer Nano ZS, the Zeta potential, and particle size analyzer (Malvern, UK). Light scattering was achieved at a fixed angle of 90° and at a temperature of 25°C. The measurement time was 1 min, and each run had 10 subruns.

Determination of Drug Content and Drug Entrapment Efficiency

After freeze-drying, accurately weighed quantities of 100 mg HPMC-based sponges containing SME-Cur were placed into 50 mL of methanol, which could dissolve completely the active compound. Then, the mixture was sonicated and filtered. The absorbance of curcumin was determined with an ultraviolet–visible spectrophotometer (Spectronic genesys 5, USA) at 425 nm, and methanol was used as a blank. The actual amount of curcumin within the sponge was determined by back calculating from the data obtained against a predetermined calibration curve of curcumin in methanol.[24,25] The determinations were made in triplicate. Then, the encapsulation efficiency was calculated from the eq. (1):

$$EE = A/B \times 100\% \quad (1)$$

where *EE* was the entrapment efficiency; *A* was the actual amount of curcumin in the sponges and *B* was the initial amount of curcumin.[21,22]

In Vitro Drug Release

In vitro drug release studies were carried out using the USP XXIII Dissolution Apparatus II (paddle type). The sponges were dropped into 450 mL of 0.1 *N* HCl buffer pH 1.2, maintained at a temperature of 37 ± 0.5°C, and stirred at a speed of 50 rpm.[26,27] At different time intervals, a 5 mL aliquot was withdrawn at 5, 10, 15, 30, 45, 60, 90, and 120 min, and this volume was replaced with an equivalent amount of fresh dissolution medium kept at 37°C. The collected samples were filtered and analyzed at λ_{max} 425 nm using the ultraviolet–visible spectrophotometer against the methanol used as the blank.[28,29] The amount of curcumin released from the sponge was determined by back calculating from the found data against a predetermined calibration curve of curcumin in methanol. The determinations were made in triplicate for each formulation, and the data was presented as a mean value ± S.D. The data were plotted as a cumulative % release of curcumin against time to illustrate the drug release profiles.

Stability Studies

The stability test was achieved according to the ICH guidelines on the topic of Q 1 A (R2): stability testing of new drug substances and products.[30] The stability testing conditions were intermediate conditions (30 ± 2°C/65 ± 5% RH, 6 months), and included accelerated conditions (45 ± 2°C/75 ± 5% RH, 6 months). Samples were kept in a constant climate chamber (Memmert HPP 260, Germany) with control of both humidity and temperature. The appearance of the emulsion droplet size and drug content of the SMEDDS sponges were evaluated.

In Vivo Absorption Studies[8,9]

Male New Zealand white rabbits (mean body weight of 4.0 ± 0.2 kg) from the Animal House, Faculty of Science, Prince of Songkla University were used in this experiment. The animals were kept in the animal room (22°C) under a 12 h-light/dark cycle and fed with standard diet and free access to distilled water prior to the experiment. The investigational processes were supported by the Committee on Animal Care and were in accordance with the Guiding Principles for the Care and Use of Research Animals promulgated by Prince of Songkla University (MOE 0521.11/1288).

The overnight fasted rabbits were divided into three groups with three rabbits per each group. Either curcumin aqueous suspension (equivalent to 50 mg/kg of curcumin), liquid SME-Cur (equivalent to 12.5 mg/kg of curcumin), or HPMC-based sponges loaded SME-Cur (equivalent to 12.5 mg/kg of curcumin) was orally administered as a single dose.[31] The curcumin powder, liquid SME-Cur or HPMC-based sponges loaded SME-Cur was each suspended in 25 mL of distilled water and mixed homogeneously prior to oral administration. Blood samples (1 mL) were collected via the auricular artery[8,32] at 0, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min after oral administration and were immediately transferred to a heparinized microcentrifuge tube and centrifuged at 4000g for 20 min at 4°C. The plasma samples were separated. Acetonitrile was added to each plasma sample (acetonitrile: plasma was 1:1 v/v), vortexed, sonicated, and allowed to stand for 5 min for deproteinization. The protein precipitate was removed by centrifugation at 4000g for 20 min at 4°C. The supernatant was pipetted into a tube and then diluted with methanol (supernatant: methanol was 1:0.5). The solution was filtered through a 0.45 μm membrane filter and subjected to the validated HPLC. The external standard was used for quantitative determination of curcumin in the plasma samples, and the method used will be described. The pharmacokinetic parameters including maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), and the area under the concentration–time curve (AUC_{0-6h}) were determined.

Quantification of Curcumin in the Plasma Samples by HPLC Analysis

The quantitative determination of curcumin was performed using an Agilent HPLC system (HP 1100, Agilent, USA) with a VertiSep™ UPS C18 5 μm column (4.6 × 250 mm) and a guard VertiSep™ UPS C18 5 μm column (4.6 × 10 mm) (Ligand Scientific, Bangkok, Thailand). The mobile phase was composed of acetonitrile-2% acetic acid (70 : 30, v/v) at a flow rate of 1.0 mL/min.[9] The injection volume was 20 μL, and

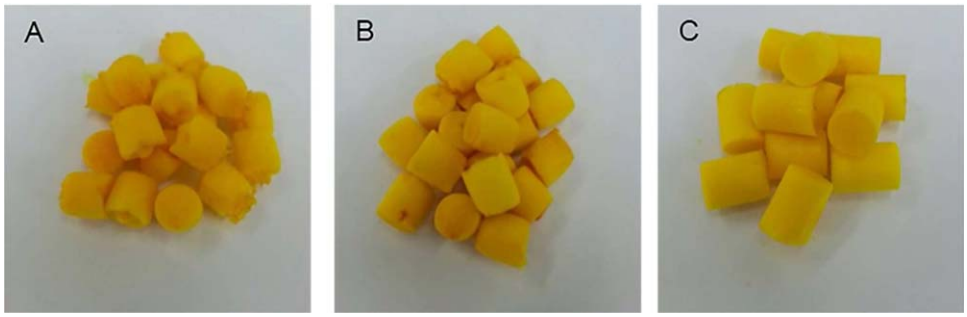

Figure 1. Morphology of HPMC sponges containing SME-Cur prepared from different concentrations of HPMC E50 LV (A: 2% w/w, B: 3% w/w, and C: 4% w/w). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

detection was at a wavelength of 425 nm. The curcumin stock solution ranged from 0.01 to 5 μg/mL and achieved a good linearity with a correlation coefficient (r^2) of 0.9997. The intraday accuracy and precision of the assay were defined by repeated three replicates for each concentration of samples. The intraday percentage relative standard deviation (%RSD) was from 0.86 to 2.62. The interday precision of the analytical gave a %RSD that ranged from 0.98 to 3.48. The recovery percentage of the method was between 92.22 and 102.42.

RESULTS AND DISCUSSION

Morphological Observation

The HPMC-based sponges containing SME-Cur were yellow in color, soft, and highly porous in structure as shown in Figure 1. HPMC is the cellulose ether product and the HPMC products are categorized by the first letter of the product label (Table I). At different substitution levels, methylcellulose products are labeled as A; HPMC products are labeled as E, F, K or J. Only A, E, and K were selected to use in this work. The viscosity of HPMC were rearranged respectively – beginning with E5 LV, E15 LV/A15 LV, E50 LV, K100 LV, and A4C. The methylcellulose sponges (A15 LV and A4C) were prepared and showed similar physical properties. HPMC (E5 LV, E15 LV, and E50 LV) could be transformed to the sponges; nevertheless, it required an adequate content to provide a strong network. Sponges could not be produced from 1% w/w HPMC E15 LV while 1% w/w HPMC A15 LV could be transformed to sponges at a comparable viscosity. These indicated that the sponge formation not depended on only viscosity but also depended on the HPMC substitution of the polymer.

The products obtained from each formulation were not the same size because the content of the polymer changed. The size of the dry sponges was larger when the content of the polymer was increased. A higher viscosity of the polymer resulted in a more dense and dry sponge (HPMC A4C). In the case of using the lowest viscosity of HPMC (E5 LV), the sponges presented as a fragile network; so, these were unsuitable for further study. An increasing HPMC concentration, resulted in larger, denser, and with more solids on the sponge as seen in Figure 1.

SEM Observation of HPMC Sponges Containing SME-Cur

According to the SEM micrographs, the surface of the sponges presented a porous network structure, and the cross-sectional structure shown in Figure 2 presented a position of a polymeric network. SEM observation proved that sponges manufactured from different HPMC concentrations had different pore sizes. The average pore diameter of the sponges ranged from 43.36 ± 4.54 to 123.22 ± 8.19 nm (Figure 3). The morphology of the microemulsion droplets produced from sponges after reconstitution with 0.1 *N* HCl observed by TEM were almost spherical, as shown in Figure 4.

The presence of higher concentrations of HPMC seemed to create a more tightly formed polymeric network since higher concentrations of HPMC produced denser porous structures and created fewer holes. The structural conformation of the pore that was created by the lower concentrations of HPMC were larger. Many small holes could be seen on sponges made from high amounts of polymer. In this case, water could permeate throughout the structure. The pore diameters play an important role in that they allowed for water diffusion into and out of the pores so that the drug could diffuse out of a solid structure. Josef *et al.*[17] produced Nile red SMEDDS sponges by a different freeze-drying schemes that included liquid nitrogen (N_2), freezing at -18°C (FRZ), or they were placed in isopropanol for 15 min followed by freezing at -18°C (IPRO), then freeze-dried at -25°C. The release of the FRZ-sponges showed the largest burst effect. The N_2-sponges showed the slowest release profile as a result of their smaller pore size. In addition, bovine serum albumin loaded into the freeze-dried pectinate gel beads was released faster than the air-dried beads due to the higher porosity.[33] In general, a more porous structure implies a higher surface area so that water might be able to permeate better.

Weight Variation

The weight variation of sponges is presented in Table I. Almost all prepared sponges showed consistent weight uniformity. The weight was increased when the concentration of HPMC was higher. The concentrations of 2-4% w/w of HPMC E5 LV, HPMC E15 LV, and HPMC E50 LV were suitable to produce sponges whereas 1% w/w of these polymers produced too weak structures. Moreover, HPMC K100 LV solution was very viscous, and the concentration suitable to make the sponges was 1-3% w/w.

Drug Content and Drug Entrapment Efficiency (%DEE)

The drug content and percent drug entrapment efficiency were decreased when the amounts of the HPMC were increased (Table II). These revealed that some part of the drug contained

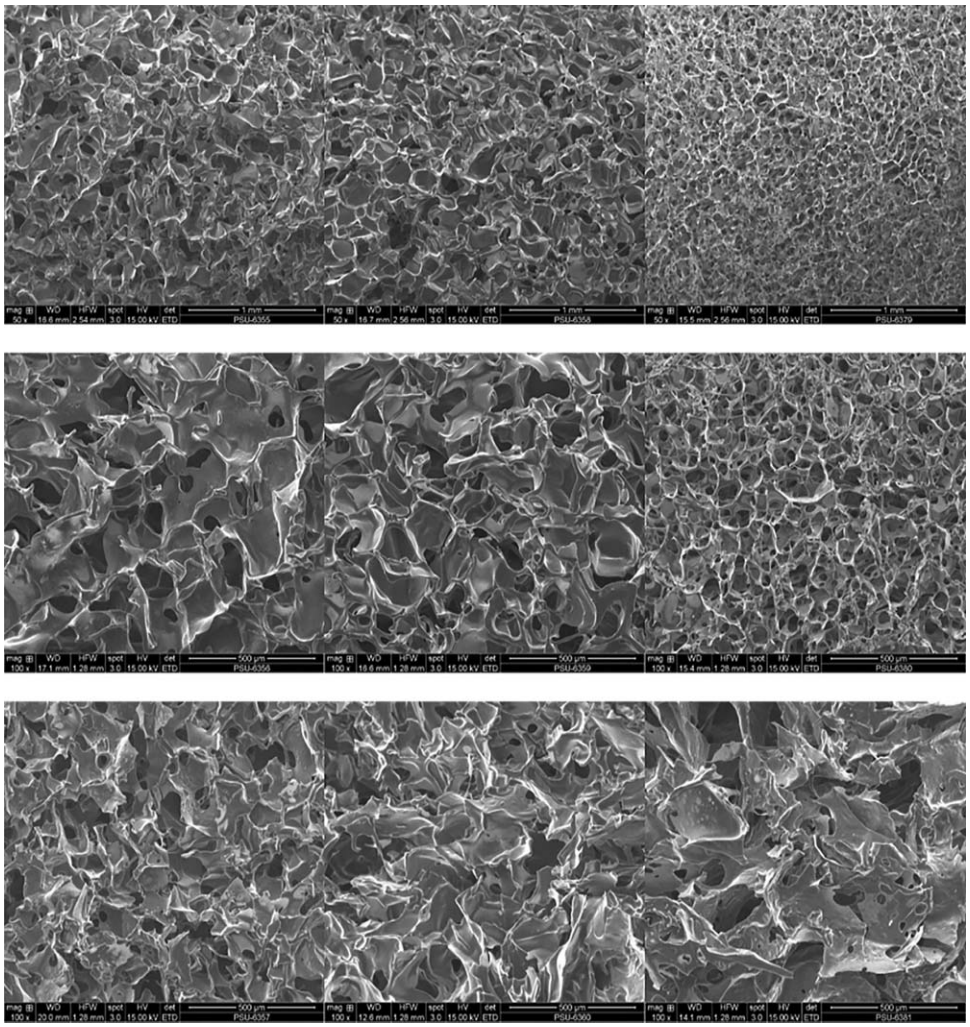

Figure 2. Upper (×50) and middle (×100) row surface morphologies of the sponges made from 2, 3, and 4% w/w HPMC E50 LV, respectively. Lower row, cross-sectional morphologies of the sponges prepared from 2, 3, and 4% w/w HPMC E50 LV, respectively (×100).

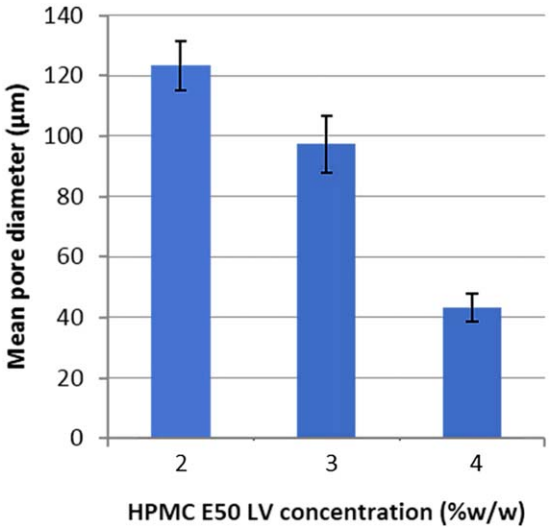

Figure 3. Mean pore diameter of the sponges containing SME-Cur prepared from different concentrations of HPMC E50 LV. Values represent means ± S.D. (n = 100). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

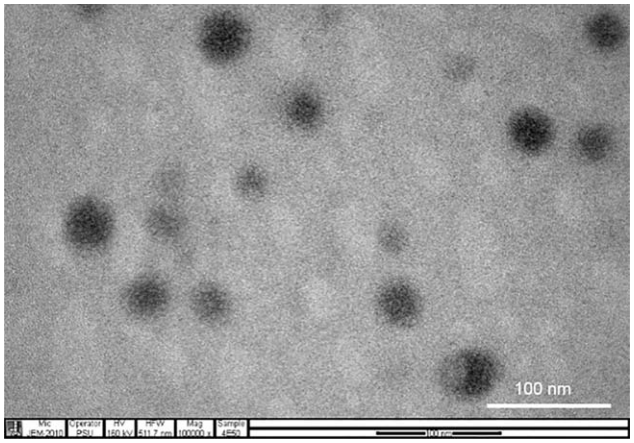

Figure 4. TEM micrographs (×100,000) of the optimal sponges containing SME-Cur (4% HPMC E50 LV).

Table II. Drug Content and % DEE of Sponges Loaded with SME-Cur Prepared by Different Types of HPMC

HPMC	Concentration of polymer (% w/w)	Weight (mg±SD)	Drug content (mg±SD)	%DEE±SD
E5 LV	1	-	-	-
	2	16.40 ± 0.72	2.70 ± 0.04	88.72 ± 1.33
	3	18.18 ± 0.44	2.23 ± 0.03	83.73 ± 1.10
	4	18.95 ± 0.60	1.98 ± 0.01	83.59 ± 0.32
E15 LV	1	-	-	-
	2	16.07 ± 0.53	2.64 ± 0.05	86.99 ± 1.76
	3	18.65 ± 0.24	2.19 ± 0.03	82.16 ± 1.28
	4	20.47 ± 0.29	2.02 ± 0.00	81.35 ± 0.42
E50 LV	1	-	-	-
	2	15.15 ± 0.74	2.71 ± 0.04	89.05 ± 1.47
	3	18.89 ± 0.23	2.35 ± 0.01	88.50 ± 0.46
	4	20.14 ± 0.43	2.01 ± 0.02	84.95 ± 1.01
K100 LV	1	12.88 ± 0.57	2.88 ± 0.02	89.17 ± 0.52
	2	16.48 ± 0.25	2.70 ± 0.02	88.95 ± 0.71
	3	19.54 ± 0.45	2.26 ± 0.02	84.81 ± 0.66
	4	-	-	-
A15 LV	1	12.71 ± 0.24	3.09 ± 0.03	87.25 ± 0.83
	2	16.05 ± 0.31	2.54 ± 0.03	83.47 ± 1.13
	3	18.14 ± 0.36	2.19 ± 0.04	82.41 ± 1.46
	4	20.06 ± 0.26	1.89 ± 0.04	80.00 ± 1.70
A4C	1	9.78 ± 1.80	2.41 ± 0.02	68.01 ± 0.63
	2	16.47 ± 0.21	2.66 ± 0.02	87.55 ± 0.66
	3	19.38 ± 0.43	2.23 ± 0.02	83.91 ± 0.61
	4	20.86 ± 0.28	1.99 ± 0.02	82.99 ± 0.80

in the sponges was replaced by an amount of HPMC. The percent drug entrapment efficiency of all formulations except A4C was greater than 80% indicated that this technique was suitable for encapsulation of SME-Cur.

In Vitro Drug Release

The release of curcumin from sponges produced by HPMC E50 LV showed an immediate release and nearly completed release within 120 min which higher level of polymer provided a slower drug release rate as shown in Figure 5. Further, the HPMC E5 LV, E15 LV, and K100 LV also revealed a fast release within a similar duration (Figure 6). In the same period, the methylcellulose sponges (A15 LV, A4C) gave only a slight release of curcumin and an incomplete release. From the results obtained with the same type of HPMC, curcumin was released from the sponges with low level of polymer at higher rate compared to that of high level of polymer. It indicated that the drug release was correlated with the size of the pore diameter as described above.

From Figure 6, HPMC A4C had the highest viscosity and presented the slowest drug release at an equal concentration. On the other hand, HPMC E5 LV showed a more rapid release

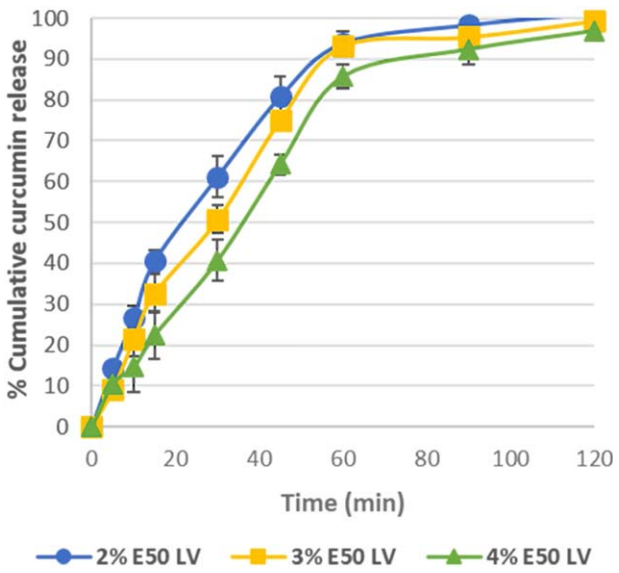

Figure 5. Effect of the concentrations of HPMC E50 LV on the release of curcumin from the sponges in 0.1 *N* HCl. Data represents the mean values ±SD (n=3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

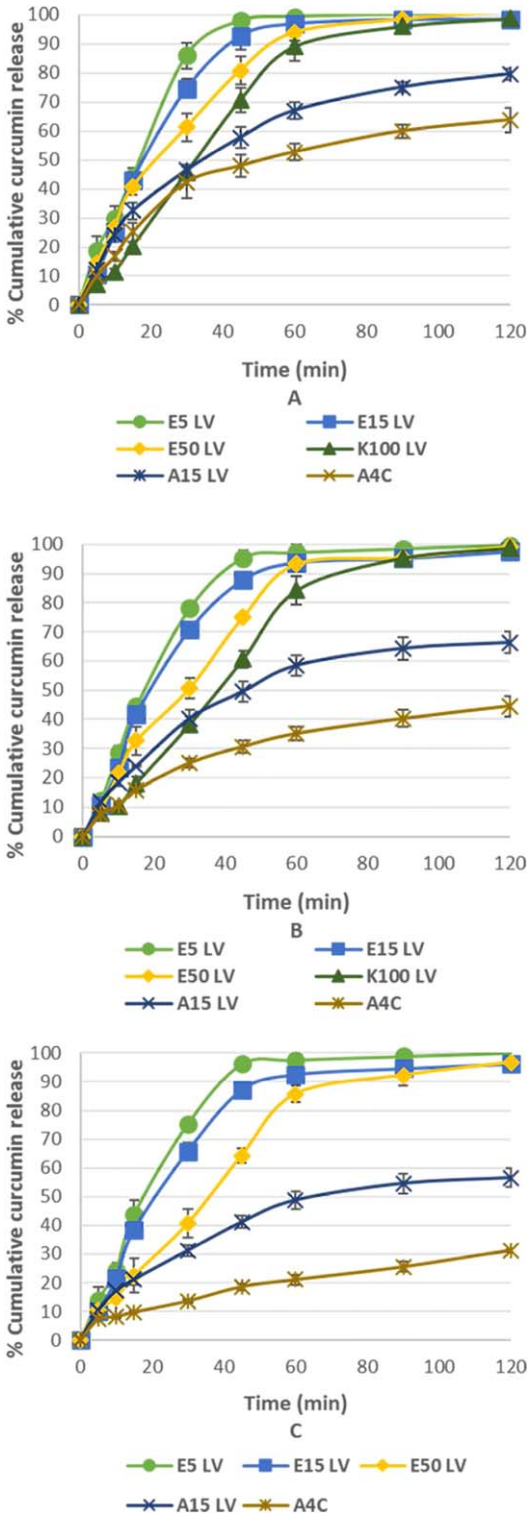

Figure 6. Release profiles of curcumin from the sponges prepared with different types of HPMC. A, B, and C were the release profiles of 2, 3, and 4% w/w of HPMC sponges, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

than the others because of its lower viscosity. The tendency of the drug release was dependent on the viscosity of the polymers at an equal concentration.

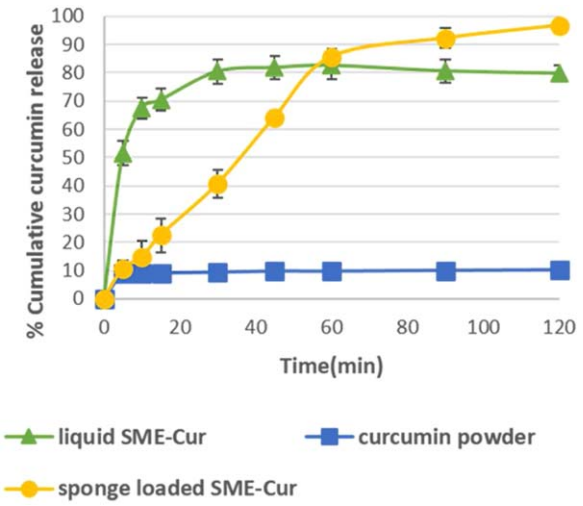

Figure 7. The release profile of curcumin from the sponge containing SME-Cur, liquid SME-Cur, compared with the dissolution of normal curcumin powder in 0.1 *N* HCl pH 1.2. Data are represented by a mean value ± SD (n= 3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

For the same viscosity and concentration, curcumin was released from the HPMC E15 LV sponges faster than from the HPMC A15 LV sponges (Figure 6). The difference of the two types was the substituent group, which was the major reason to describe this situation. According to these results, HPMC E and K grade that had a hydroxypropyl methylcellulose substitution showed the easier water permeation into the matrices more than the HPMC A grade. It was possible that the substituent group (hydroxypropyl substitution) was more soluble than the methyl substitution. In addition, the degree of substitution by the methoxy group showed a rational correlation to the hydrophobicity and the rate of hydration. Accordingly, methylcellulose products that had higher degrees of substitution were water insoluble.[34,35]

The HPMC E5 LV and E15 LV sponges were greasy after storage at room temperature; thus, they were not suitable for the next study. Sponges made from 4% HPMC E50 LV showed good physical properties, a percentage drug entrapment efficiency of over 80%, and nearly 100% release within 120 min. This clearly indicated that this formulation was appropriated for further studies.

The release profiles of curcumin from the optimized formulation (4% HPMC E50 LV) was determined and compared to the liquid SME-Cur and the unformulated curcumin (Figure 7). At the equivalent dose, the curcumin formulations as liquid and solid sponges gave the same tendency for drug release but was superior to that of the unformulated curcumin powder. Within 120 min, the total curcumin release from sponges nearly reached 100% and was greater than the curcumin powder by about 12-fold. Liquid SME-Cur showed an immediate release (total release of about 80%) although sponges loaded with SME-Cur produced a slightly lower release in the first hour, the release was complete in 2 h. The reason of this phenomenon might result from the ability of HPMC that could inhibit the crystallization and precipitation of curcumin in an aqueous situation and by maintaining supersaturation of the drug.[36]

Table III. Stability Data of the Sponge Loaded SME-Cur. Data Reported are Means±S.D. (n=3), Polydispersity index (PDI)

Time (month)	Droplet size (nm)	PDI	% Drug content
(A) 30°C/65% RH			
0	34.80 ± 0.1	0.122 ± 0.020	102.34 ± 1.26
3	32.93 ± 0.4	0.109 ± 0.056	99.32 ± 1.74
6	33.76 ± 0.2	0.098 ± 0.021	95.79 ± 1.89
(B) 45°C/75% RH			
0	34.80 ± 0.1	0.122 ± 0.020	102.34 ± 1.26
3	33.61 ± 0.2	0.087 ± 0.046	97.49 ± 1.51
6	33.57 ± 0.4	0.103 ± 0.012	94.81 ± 1.57

Measurement of the Droplet Size of the Resultant Microemulsion

When dispersed in 0.1 N HCl, the average droplet size and polydispersity index of the SME-Cur from the sponges (4% HPMC E50 LV) were 34.80 ± 0.1 nm and 0.122 ± 0.020, and from the liquid SME-Cur were 30.04 ± 0.2 nm and 0.173 ± 0.018, respectively. There were slight changes in the oil droplet size. The small polydispersity index value indicated that the size of the microemulsion droplets released from sponges was consistent. The droplet size of the resultant microemulsion was less than 50 nm, which confirmed the property of solid-self microemulsifying drug delivery system. The HPMC E50 LV structures seem to be good porous carriers.

Stability Studies

In order to assess the stability of the optimized preparation, the physical appearance, emulsion droplet size, and drug content determinations were carried out after 6 months of both the intermediate conditions (30 ± 2°C/65 ± 5% RH) and accelerated conditions (45 ± 2°C/75 ± 5% RH). The samples were kept in air-tight glass containers and protected from light. Under both storage conditions, the appearance, emulsion droplet size, and drug content were very similar to the freshly prepared formulation that showed little change (Table III). On the other hand, the content of curcumin in the sponges slightly decreased after storage (95.79 ± 1.89% and 94.81 ± 1.57%, respectively). It might be that the lyophilized formulation could potentially absorb the moisture and affect the chemical stability of curcumin. Furthermore, the containers in this work were not sealed and had no protection from moisture. For that reason, for this type of formulation there should be concern for special packaging like a moisture proof seal.

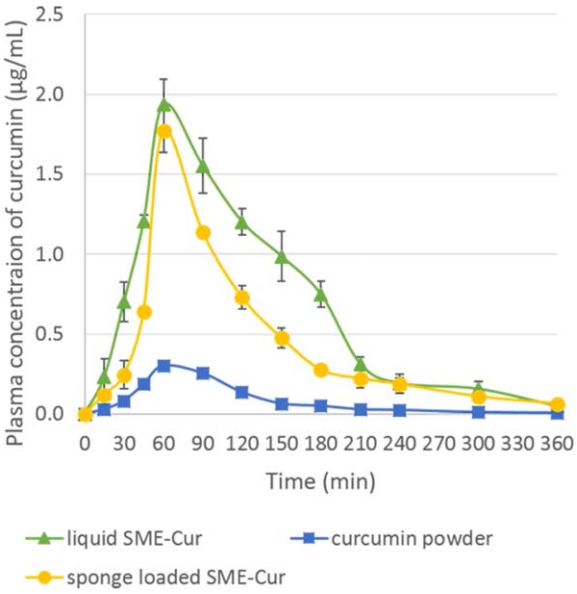

Figure 8. Plot of plasma concentration of curcumin versus time after oral administration of liquid SME-Cur (equivalent to 12.5 mg/kg of curcumin) and sponge loaded SME-Cur (equivalent to 12.5 mg/kg of curcumin) formulation, compared with an aqueous suspension (equivalent to 50 mg/kg of curcumin). Data reported are mean values ± SD (n = 3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In Vivo Absorption Studies

In this work, the *in vivo* assessment of different dosage forms of curcumin was carried out in rabbits. The *in vivo* study was performed to define whether the developed sponges could improve the oral absorption of curcumin compared with unformulated curcumin. After oral administration of an aqueous suspension of curcumin, liquid SME-Cur or sponges loaded with SME-Cur, the pharmacokinetic parameters of curcumin were examined. The pharmacokinetic data are represented in Table IV. Figure 8 shows the plot of the plasma concentration of curcumin versus the time after oral administration of the curcumin suspension and the SME-Cur in the liquid and solid forms. The lowest average curcumin plasma concentration was observed after administration of an aqueous suspension of curcumin. At an equal dose equivalent to 12.5 mg/kg of curcumin, the $AUC_{0\text{-}6h}$ of both the liquid and solid SME-Cur were greater than the $AUC_{0\text{-}6h}$ of the full dose of the aqueous curcumin suspension. Curcumin administered as a liquid and as sponges had mean values for C_{max} of 1.93 ± 0.16 µg/mL and 1.77 ± 0.13 µg/mL, respectively i.e. much higher than the C_{max} achieved by the full dose of the unformulated curcumin (0.30 ± 0.03 µg/mL). The

Table IV. Pharmacokinetics Parameter of Curcumin after Oral Administration of an Aqueous Suspension of Curcumin (Equivalent to 50 mg/kg of Curcumin), Liquid SME-Cur (Equivalent to 12.5 mg/kg of Curcumin), and Sponge Loaded SME-Cur (Equivalent to 12.5 mg/kg of Curcumin). Data are Represented by a Mean Value±SD (n=3)

Formulation	C_{max} (µg/mL)	T_{max} (min)	$AUC_{0\text{-}6h}$ (ng h/mL)
Curcumin aqueous suspension	0.30 ± 0.03	60	509.49 ± 47.77
Liquid SME-Cur	1.93 ± 0.16	60	3983.38 ± 270.68
Sponge loaded SME-Cur	1.77 ± 0.13	60	2615.68 ± 97.79

time to attain the peak concentration (T_{max}) of each formulation was 60 min. From these results, the sponges loaded with the SME-Cur formulation produced a significantly enhanced oral absorption, compared to the unformulated curcumin.

The SME formulations in many studies have shown much better oral absorption over the unformulated bioactive compounds. Setthacheewakul *et al.*[9] have reported that the oral absorption of SME-Cur in both liquid and pellet forms, administered orally to mice (50 mg/kg of body weight), were significantly higher than for the unformulated curcumin. Sermkaew *et al.*[8] demonstrated that the half dose of an SME- *Andrographis paniculata* extract in liquid and pellet forms produced an enhanced oral absorption of andrographolide in rabbits compared to the full dose of *Andrographis paniculata* extract. The result showed that the AUC_{0-12h} of andrographolide in the SME liquid and pellets was 15-fold and 13-fold greater than the unformulated extract, respectively. Moreover, Balakrishnan *et al.*[10] produced solid dexibuprofen as a self-emulsifying drug delivery system (SEDDS) by spray drying with an inert solid carrier (Aerosil 200). After oral administration of the dexibuprofen powder or the solid SEDDS in capsules to rats at the dose of 10 mg/kg, the AUC and C_{max} of dexibuprofen in the solid form were significantly higher than for the dexibuprofen powder. The obtained results from the *in vitro* dissolution test confirmed that the release of curcumin from the liquid SME formulation was more rapid than from the solid SME. Furthermore, the HPMC in sponges could sustain the release of a drug. Accordingly, the AUC of the SME formulation was greater than that of the solid SME.

The enhanced oral absorption of curcumin in the SME formulation might be due to the high surface area produced by the microemulsion droplets after reconstitution of the system in the gastrointestinal contents. Curcumin dissolved in the SME system could be absorbed into the blood circulation as microemulsion droplets via the gastrointestinal tract.[37] In addition, Sha *et al.*[37] reported that a SME system containing Labrasol, which is one of the more commonly used surfactants, could open the tight junction for improving the mucosal absorption via the paracellular pathway and hence it enhanced the intestinal permeability.

CONCLUSIONS

The novel HPMC-based sponges loaded with SME-Cur produced using a relatively simple technology and a small number of ingredients, compared to other previous solid SME dosage forms were successfully prepared. The percentage release of curcumin from the HPMC-based sponges was significantly higher than for the unformulated curcumin, and provided a sustained release. The SME-Cur yielded a microemulsion with a globule size of 34.80 ± 0.1 nm. The *in vivo* oral absorption of curcumin given at a dose of 12.5 mg/kg was significantly enhanced by both the liquid and solid SMEDDS compared to the curcumin suspension itself provided at a dose of 50 mg/kg. This study has demonstrated that a new SMEDDS sponge could be utilized for improving oral absorption, and could be useful by reducing the dosage of the poorly water-soluble curcumin.

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