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Solubility of Core Materials in Aqueous Polymeric Solution Effect on Microencapsulation of Curcumin

Hesham Abdul Aziz, Kok Khiang Peh, and Yvonne Tze Fung Tan
School of Pharmaceutical Sciences, University of Science Malaysia, Penang, Malaysia

Curcumin, the main active constituent of turmeric herb (*Curcuma longa L.*) have been reported to possess many medicinal values. The application of curcumin in dermatological preparations is limited by their intense yellow color property, which stains the fabric and skin. The objectives of this study were to reduce the color staining effect and enhance the stability of curcumin via microencapsulation using gelatin simple coacervation method. As for curcumin, ethanol and acetone were used as coacervating solvents. Curcumin was dispersed in ethanol while dissolved in acetone. Irrespective of the types of coacervating solvents used, microencapsulation resolved the color-staining problem and enhanced the flow properties and photo-stability of curcumin. Nevertheless, it was found that more spherical curcumin microcapsules with higher yield, higher curcumin loading, and higher entrapment efficiency were obtained with acetone than ethanol. The in vitro release of curcumin after microencapsulation was slightly prolonged. Further evaluation of the effects of solubility of core materials in coacervating solvent or polymeric aqueous solution using six different drug compounds, namely, ketoconazole, ketoprofen, magnesium stearate, pseudoephedrine HCl, diclofenac sodium, and paracetamol, suggested that the solubility of core materials in aqueous polymeric solution determined the successful formation of microcapsules. Microcapsules could only be formed if the core materials were not dissolved in the aqueous polymeric solution while the core materials could either be dissolved or dispersed in the coacervating solvent. In summary, microencapsulation not only circumvents the color-staining problem but also improved the stability and flowability of curcumin. The solubility of core material in aqueous polymeric solution plays a pivotal role in determining the successful formation of microcapsules.

Keywords curcumin; gelatin-simple-coacervation; microencapsulation; staining effect; flowability; photo-stability

INTRODUCTION

Curcumin was used as a formulation aid to protect light-sensitive drugs in soft gelatin capsules. The half-lives of some photolabile drugs such as nifedipine, chloramphenicol, furosemide, and clonazepam were increased by 20% when incorporated in capsules mixed with curcumin than in plain gelatin capsules (Tonnesen & Karlsen, 1987). Thoma (1996) highlighted the stabilizing effect of curcumin on certain photolabile drugs in solution and in topical preparations. Curcumin can be used as natural colorant to replace the artificial ones found in commercial jellies (Calvo & Salvador, 2000). Moreover, the stability of gelatin gels (commercial jellies) during storage was improved by adding curcumin as a colorant. Curcumin has been sold as commercial products in the forms of tablets (Healthspan, 2005; iherb.com, 2005; Natural Health Consultants, 2005), soft-gels (also contain turmerones) (iherb.com, 2005), and capsules (Sahelian, 2005). Curcumin has also been formulated as transdermal film (Purohit, Gupta, Pokharkar, & Verma 2002). Recently, Jang, Hahm, Kim, Byun, Suh, Lee, Kim, Park, Kim, and Gwon (2004) reported the development of curcumin-coated stent. The stent was coated by dip-coating method in ethanol containing curcumin. Curcumin underwent degradation under acidic, basic, light and oxidation conditions (Ansari, Ahmad, Kohli, Ali, & Khar, 2005). In another study, curcumin was reported to undergo photo-degradation when exposed to light in solution as well as in solid form (Tonnesen & Karlsen, 1985). Furthermore, its high color intensity stained fabrics and skin. Tonnesen, Masson, and Loftsson (2002) formulated curcumin with cyclodextrin complexes to improve curcumin water solubility, hydrolytic, and photochemical stability. The objectives of this study were to reduce the color staining effect and enhance the stability of curcumin via microencapsulation. A simple coacervation method with gelatin as a coating material was employed while ethanol and acetone were used as coacervating agents. Curcumin was dispersed in ethanol but dissolved in acetone. The curcumin microcapsules were characterized by determining curcumin yield, loading, entrapment efficiency, wall thickness, morphology, particle size, flowability properties, staining intensity, and in vitro release of curcumin microcapsules. Moreover, the stability of curcumin microcapsules to light was evaluated. Lastly, the effect of solubility of core material in coacervating solvent and/or aqueous polymeric solution on

Address correspondence to Hesham Abdul Aziz, School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia. E-mail: heshamaziz424@yahoo.co.uk

microencapsulation was examined using six different drug compounds, namely, ketoconazole, ketoprofen, magnesium stearate, pseudoephedrine HCl, diclofenac sodium, and paracetamol.

MATERIALS AND METHODS

Materials

Gelatin (Type B, bloom 225) was purchased from Sigma (Missouri, US), Curcumin was purchased from Acros Organic, New Jersey. Acetone and glacial acetic acid were purchased from R & M Chemicals, UK. Ethanol 95% and methanol were obtained from Fisher Scientific, UK. Formaldehyde was purchased from Merck, Germany. Ketoconazole, magnesium stearate, pseudoephedrine HCl and diclofenac sodium were purchased from Nutech, India. Ketoprofen was purchased from Wuxue Xunda Pharm, China. Paracetamol was purchased from Vangfou Pharmaceutical, China.

Determination of Coacervated Layer

Known amount of gelatin was dissolved in hot distilled water at 50°C using a magnetic hot plate stirrer (Heidolph, Germany) in a 50 mL beaker. The coacervating agent was added with a constant stirring for 5 min. The mixture was cooled to room temperature and the coacervated layer formed was transferred into a 25 mL measuring cylinder containing 5 mL of distilled water. The volume of the coacervated layer was taken as the increase in volume in the measuring cylinder. Two different types of coacervating solvents were investigated, namely, ethanol and acetone. The experiment was carried out in triplicates (Table 1).

Microencapsulation of Curcumin Using Ethanol and/or Acetone as Coacervating Solvent

Curcumin was dispersed in the gelatin solution with a constant stirring using a magnetic hot plate stirrer (Heidolph, Germany). Ethanol was added to the curcumin dispersion using a syringe pump (Argus 600, Switzerland) at a feeding rate of 1 mL/min and a stirring speed of 500 rpm. The mixture was stirred continuously at 500 rpm for an additional hour at 10°C to ensure a complete deposition of gelatin onto the curcumin. Formaldehyde solution (37% v/v) was added to rigidize the gelatin coating. The volume of formaldehyde solution added was equivalent to the volume of coacervated layer obtained (1:1, v/v). The microcapsules collected were washed three times with ethanol, followed by cold water (5°C), redispersed in water, kept frozen at −70°C for 24 hr, dried by lyophilization (Labconco, Missouri, US) and finally sieved through a 100-mesh sieve (150 μm). On the other hand the experiment was repeated but using acetone as the coacervating agent, which could dissolve curcumin. Curcumin was dissolved in acetone and added to the gelatin solution using a syringe pump at a feeding rate of 1 mL/min. Washing was performed using acetone followed by cold water after the addition of formaldehyde solution (Table 2).

Determination of Microcapsule Yield, Drug Loading, and Entrapment Efficiency

20 mg of curcumin-loaded microcapsules was triturated and ruptured using mortar and pestle. 10 mL of methanol was added to the powdered sample and stirred for 1 hr at 750 rpm using a magnetic hot plate stirrer (Heidolph, Germany). The mixture was centrifuged at 5000 rpm for 30 min. The supernatant was filtered using 0.45 μm PTFE filter, and the amount of

TABLE 1
Composition of Coacervate Samples Used for Preparing Phase Diagram

Formulation		Coacervated Layer (mL)		Gelatin Solution	Percentage w/w Composition					
					Gelatin B		Water		E/A	
E	A	E	A	(%)	(g)	%(w/w)	(g)	%(w/w)	(g)	%(w/w)
E1	A1	1.3 ± 0.15	0.8 ± 0.10	2.50%	0.25	1.4	10	54.4	8.13	44.2
E2	A2	3.5 ± 0.14	2.6 ± 0.10	5.00%	0.50	2.7	10	53.7	8.13	43.6
E3	A3	6.6 ± 0.31	4.0 ± 0.17	10.0%	1.00	5.2	10	52.3	8.13	42.5
E4	A4	4.5 ± 0.20	3.1 ± 0.21	10.0%	1.00	3.7	10	36.7	16.26	59.6
E5	A5	2.7 ± 0.15	2.2 ± 0.21	10.0%	1.00	2.8	10	28.3	24.39	68.9
E6	A6	1.4 ± 0.23	1.0 ± 0.12	1.25%	0.25	0.7	20	54.8	16.26	44.5
E7	A7	3.7 ± 0.15	2.8 ± 0.12	2.50%	0.50	1.4	20	54.4	16.26	44.2
E8	A8	7.1 ± 0.25	4.3 ± 0.10	5.00%	1.00	2.7	20	53.7	16.26	43.6
E9	A9	4.9 ± 0.30	3.3 ± 0.21	5.00%	1.00	1.9	20	37.4	32.52	60.8
E10	A10	2.9 ± 0.21	2.2 ± 0.25	5.00%	1.00	1.4	20	28.7	48.78	69.9

E = Ethanol and A = Acetone. Note: all experiments were performed using fixed stirrer and beaker dimension: Stirrer length = 4 cm, Stirrer width = 7.5 cm, Beaker height = 15.5 cm and Beaker diameter = 5.5 cm.

TABLE 2
Preparation of Curcumin-Loaded Microcapsules

Formulation		Curcumin	Gelatin	Water	E/A	*C : G	Gelatin	
E	A	(g)	(g)	(mL)	(mL)	Ratio	Solution	Observation
C1	D1	0.5	1	10	10	1 : 2	10%	Agglomeration
C2	D2	0.5	1	20	20	1 : 2	5%	Agglomeration
C3	**D3**	**1**	**1**	**10**	**10**	**1 : 1**	**10%**	**Microcapsules**
C4	**D4**	**1**	**1**	**20**	**20**	**1 : 1**	**5%**	**Microcapsules**
C5	D5	2	1	10	10	2 : 1	10%	No coacervation
C6	D6	2	1	20	20	2 : 1	5%	No coacervation

*C: G Ratio = curcumin to gelatin ratio.

curcumin was quantified using HPLC method. The percentage of microcapsule yield, drug loading and entrapment efficiency of the curcumin-loaded microcapsules were calculated using the following equations (Hu, Jiang, Ding, Zhang, Yang, Zhang, Chen, & Yang, 2003);

$$Yield\,(\%) = \frac{Weight\ of\ microcapsules}{Weight\ of\ polymer\ and\ drug\ fed\ initially} \times 100\% \quad (1)$$

$$Loading\,(\%) = \frac{Weight\ of\ drug\ in\ microcapsules}{Weight\ of\ microcapsules} \times 100\% \quad (2)$$

$$*EE\ (\%) = \frac{Weight\ of\ drug\ in\ microcapsules}{Weight\ of\ drug\ fed\ initially} \times 100\% \quad (3)$$

*EE = Entrapment efficiency.

Light Microscopy

Selected curcumin-loaded microcapsules were examined using a light microscope (Leica DMLB, Cambridge, UK) connected to a camera (Leica DC 300, Cambridge, UK) and a compact workstation (Leica Compact, UK).

Scanning Electron Microscopy

The surface morphology of selected curcumin-loaded microcapsules was examined using a scanning electron microscope (Leica Cambridge S360, UK). The samples were first sputter coated with gold under argon atmosphere (Emitech K750, Kent, UK).

Particle Size Determination

The particle size determination was carried out using Mastersizer S (Malvern Instruments, MAM5005, UK) fitted with a small sample dispersion unit (MS1) connected to a dispersion unit controller. A beam length of 2.4 mm and 300 RF lens (range 0.05–900 um) were used. Curcumin-loaded microcapsules were first sonicated in distilled water for 1 min before loaded into the small sample dispersion unit and stirred at a speed of 1000 rpm until an obscuration value between 12–17% was obtained. Before each sample run, the system was aligned and a background measurement was taken with either distilled water or ethanol (filtered through 0.45 um cellulose nitrate membrane), which were used as the dispersing solvents. Measurement of the sample was performed at 12000 sweeps and a run number of 10. The determination was carried out in triplicate for each sample.

Determination of Mean Wall Thickness

The mean wall thickness of microcapsules was determined using the equation proposed by Si-Nang, Carlier, Delort, Gazzola, and Lafont (1973) and Ueda, Nakamura, and Makita (1993).

$$h = \frac{r}{3} \times \frac{(1-p)\,d_1}{pd_2 + (1-p)\,d_1} \quad (4)$$

where h = wall thickness, r = radius of microcapsules, p = drug content in microcapsules, d_1 = density of core material (curcumin), and d_2 = density of wall (gelatin).

Evaluation of Flow Property

The flow properties were studied using the angle of repose method reported by Chan and Heng (1998). The angles were averaged and for each sample, at least five replicates were carried out and the mean value calculated. A smaller angle of repose indicates a better flowability.

Color Staining Study

White adhesive paper labels (4 x 4 cm) were pasted onto an aluminium tray. Known amount of curcumin powder and

curcumin-loaded microcapsules were evenly spread onto the paper labels and kept in an enclosed container for 12 hr, after which the tray was inverted and lightly tapped to remove the powder or microcapsule samples.

Effect of Ethanol and Water on Microcapsule Swelling

100 mg of curcumin-loaded microcapsules were transferred into a graduated test tube and the volume of the microcapsules (V1) was recorded. After adding a known volume of either ethanol or water to the test tube, it was left undisturbed for 2 hr. The volume of the microcapsules (V2) was recorded again after 2 hours. The difference in the volume of the microcapsules (V2 – V1) was taken as a measurement of the extent of swelling of the microcapsules.

In Vitro Curcumin Release Study

The in vitro release profiles of curcumin powder and curcumin-loaded microcapsules were investigated using a modified Franz diffusion cell. 25 mg of curcumin powder or known amount of microcapsules (contained equivalent amount of curcumin) were carefully transferred onto the cellulose nitrate membrane. The dissolution medium consisted of ethanol and water (7:3, v/v) was stirred at 50 rpm and maintained at a temperature of 32°C. At preset time intervals of 10, 20, 30 min, 1, 2, 4, 8, 12 and 24 hr after the commencement of the study, 0.2 mL of sample was withdrawn and immediately replaced with the same volume of fresh dissolution medium. The amount of curcumin released was quantified using the HPLC method.

Determination of Curcumin by HPLC Method

The high performance liquid chromatography (HPLC) system was comprised of a pump (Model 307, Gilson), 6-valve injection port (Rheodyne,), a UV detector (Model 115, Gilson, France) and an integrator (D-2500 Chromato-Integrator, Hitachi, Japan). The detector was operated at a detection wavelength of 375 nm. A reversed phase column (Luna C18 (2), 5 μm, 150 × 4.5 mm ID, Phenomenex) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, Wisconsin) was used for chromatographic separation. The mobile phase consisted of acetonitrile, methanol and water (35:10:55, v/v) and adjusted to pH 3.0 with glacial acetic acid. The analysis was run at flow-rate of 1.3 mL/min. Sample of 20 μL was injected into the column.

Light Stability Study

The stability of curcumin powder and microcapsules to light was investigated by exposing 200 mg of curcumin powder and 400 mg of curcumin microcapsules of formulations C4 and D4 containing equivalent amount of curcumin in enclosed glass petridish to sunlight for one month. The time of light exposure for samples was 12 hr/day with a total of 360 hr. Samples were collected at predetermined time interval of 0 (pre-expose), 5, 10, 15, 20, 25, and 30 days. The curcumin content for curcumin powder and microcapsules was analyzed using HPLC method.

Preparation of Drug Loaded Microcapsules

Six different drug compounds, namely, ketoprofen, ketoconazole, magnesium stearate, pseudoephedrine HCL, diclofenac sodium, and paracetamol were used in the study. The ratio of drug to gelatin was set at 1:1. For each gram of gelatin, 20 mL of water was used and coacervated with 20 mL of ethanol. The method of preparation was similar to that described under section 3.3. The drug loaded microcapsules were evaluated in terms of morphology and particle size.

Statistical Analysis

The results were treated statistically using SPSS software (Version 13, USA). One-way analysis of variance was employed for the comparison of the results. When there was a statistically significant difference, post-hoc Tukey Honestly Significant Difference (Tukey-HSD) test was applied. A statistically significant difference was considered when $p < .05$.

RESULTS AND DISCUSSION

Determination of Coacervated Layer

Phares and Sperandio (1964) reported the use of phase diagram in the preparation of microcapsules using gelatin (Type A), ethanol and water by a simple coacervation phase separation method. They found that the concentration of gelatin solution had a distinct effect on the coacervated layer formed. In addition, they showed that 10% w/w of gelatin solution produced a comparatively higher coacervated layer (reaction region) than 2.5 and 5.0% w/w gelatin solutions. In the present study, although a different grade of gelatin (Type B) was employed, the concentrations of the gelatin solution used were close to the concentrations as recommended by Phares and Sperandio (1964) at 1.25, 2.5, 5, and 10%. Table 1 shows the results of coacervated layers formed from the formulations. In general, all the formulations (E1 – E10 and A1 – A10) produced two layers, namely, a clear upper layer and a coacervated lower layer. At 10 mL (8.13 g) of ethanol or acetone, the volume of coacervated layer increased with an increase in the amount of gelatin used in the preparation (E1–E3 and A1–A3). At 20 mL (16.26 g) of ethanol or acetone, similar trend of increase in coacervated layer was obtained with an increase in the amount of gelatin (E6–E8 and A6–A8). All these formulations produced easily separable microcapsules after drying of coacervated layers. However, at a constant amount of gelatin, an increase in ethanol or acetone caused a decrease in the volume of coacervated layer (E3–E5; E8–E10, and A3–A5; A8–A10). In addition, when the volume of ethanol or acetone was

increased to 20 mL and above, flocculated coacervated layers that were difficult to separate upon drying as exemplified by E4, E5, E9, E10, and A4, A5, A9, A10, were obtained. Figure 1 shows the schematic phase diagram indicating the optimum region of coacervated layers, which produced separable particles. In addition, it was also observed that at a constant amount of gelatin, an increase in the volume of water and ethanol/acetone did not have a significant effect ($p > 0.05$) on the coacervated layer as long as the ratio of water to ethanol/acetone was 1:1.

Preparation of Curcumin-Loaded Microcapsules

The effect of stirring speed on microencapsulation was reported by a number of researchers. Ueda et al. (1993) found that a stirring speed of 400–600 rpm resulted in spherical microcapsules, while at a stirring speed of less than 400 rpm, the encapsulated particles inclined to aggregate and coalesce due to the week agitation force. On the other hand, at a stirring speed of more than 800 rpm, strong collision of encapsulated particles with the baffles of the apparatus and the wall of vessel caused some polymer loss and deformation of microcapsule shape. Fekete (1992) reported the use of a coacervation method at a stirring rate of 400 rpm to encapsulate the core material with ethyl cellulose. Helliwell and Martin (1992) documented a complex coacervation method (gelatin-acacia) to encapsulate the drug at a stirring rate of 600 rpm. Mauguety, Legrandy, Brujes, Carnelly, Larrez, and Popineau (2002) reported a simple coacervation method to encapsulate gliadin at a stirring rate of 430 rpm. In the present study, the stirring speed was kept constant at 500 rpm in all the experiments, which was within the suitable range of stirring speed as reported by the above researchers. Curcumin is not soluble in water. Its solubility is approximately 1 g in 250 mL of ethanol and 1 g in 20 mL of acetone. Therefore, two different methods were employed in the preparation of curcumin-loaded microcapsules. Curcumin was either dispersed when ethanol was used or dissolved in acetone. Three different amount of curcumin were used, namely, 0.5, 1.0, and 2.0 g, while the amount of gelatin was fixed at 1.0 g, to produce the ratios of curcumin and gelatin at 1:2, 1:1, and 2:1. The ratio of water and ethanol or acetone was kept at 1:1, which was the best result obtained from the determination of coacervated layer study. The results showed that at curcumin and gelatin ratio of 1:2, agglomeration of the microcapsules occurred after rigidization with formaldehyde. At curcumin and gelatin ratio of 1:1, the microcapsules were separable. When curcumin and gelatin ratio was increased to 2:1, coacervation did not occur and microcapsules could not form (Table 2).

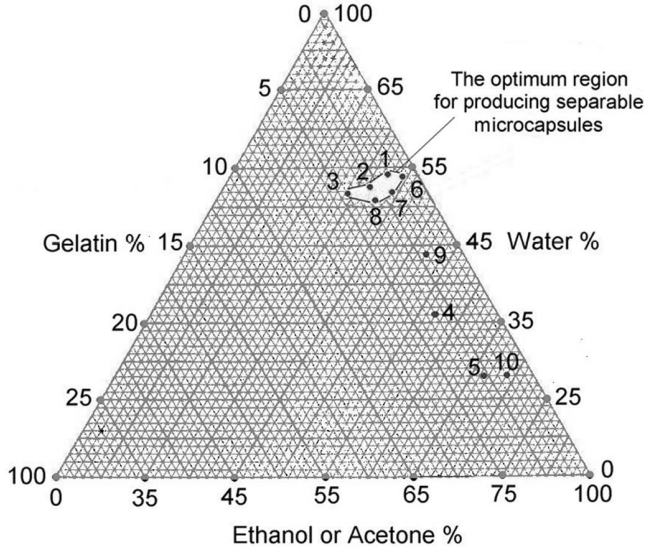

FIGURE 1. Phase diagram showing the compositions of coacervate samples with ethanol or acetone as coacervating solvents.

Microcapsule Yield, Drug Loading, and Entrapment Efficiency

The successfully formed microcapsules C3, C4, D3, and D4 were selected for this part of study. The results of the microcapsule yield, curcumin loading and entrapment efficiency are presented in Table 3. The results of microcapsule yield, curcumin loading and entrapment efficiency of C4 were significantly higher than C3 ($p < 0.05$). Similarly, the values of D4 were also comparatively higher than D3. The results showed that an increase in the volume of water and ethanol/acetone from 10 to 20 mL enhanced the entrapment efficiency of the microcapsules. In addition, when comparing the values of C3 with D3 and C4 with D4, microcapsules prepared by dissolving curcumin in acetone were found to have higher yield, curcumin loading and entrapment efficiency, indicating that the solubility of curcumin in a coacervating solvent played an important role in microencapsulation. Nastruzzi, Pastesini, Cortesi, Gambari, Menegatti, and Esposito (1995) encapsulated arabinofuranosylcytosine with gelatin using a simple coacervation method and the entrapment efficiency was about 58%. In the present study, the entrapment efficiency was markedly improved from 54.66 to 75.53% by replacing ethanol with

TABLE 3
The Results of Microcapsule Yield, Curcumin Loading, and Entrapment Efficiency

Formulation	Yield (%)	Drug Loading (%)	Entrapment Efficiency (%)
C3	75.21 ± 0.48	32.65 ± 0.29	48.97 ± 0.67
C4	**82.57 ± 0.41**	**41.61 ± 0.36**	**54.66 ± 0.32**
D3	91.08 ± 0.69	33.18 ± 0.63	60.39 ± 0.47
D4	**97.57 ± 0.41**	**44.25 ± 0.78**	**75.53 ± 0.15**

acetone as the coacervating agent. Formulation C4 was chosen together with D4 for further studies to examine the effect of coacervating solvent on the properties of curcumin microcapsules.

Morphology of Microcapsules

Figure 2 shows a photomicrograph of curcumin-loaded microcapsules C4 in the aqueous medium before drying. Although some of the microcapsules appeared to be spherical in shape, irregular and rod shaped microcapsules were also seen. After drying, the microcapsules shrank and turned into irregular shapes (Figure 3). Ueda et al. (1993) reported that the appearance of microcapsules prepared using phase separation method was influenced by the shape of the core drug. Since curcumin was dispersed in aqueous medium and also insoluble in ethanol, the morphology of the microcapsule was thus governed by the shape of curcumin, which was not spherical. Figure 4 shows the photomicrograph while Figure 5 shows the scanning electron photomicrograph of curcumin-loaded microcapsules D4 after drying. Drying did not alter the spherical morphology of D4. Spherical microcapsules of a wide size distribution could be noted.

Particle Size Analysis of Microcapsules

Since the curcumin-loaded microcapsules were to be eventually formulated in aqueous dermatological preparations, it is important to determine the particle size of the microcapsules in water. The results show that all the parameters of size analysis for both C4 and D4 were comparatively larger in water than in ethanol ($p < 0.05$). This may be explained by the swelling nature

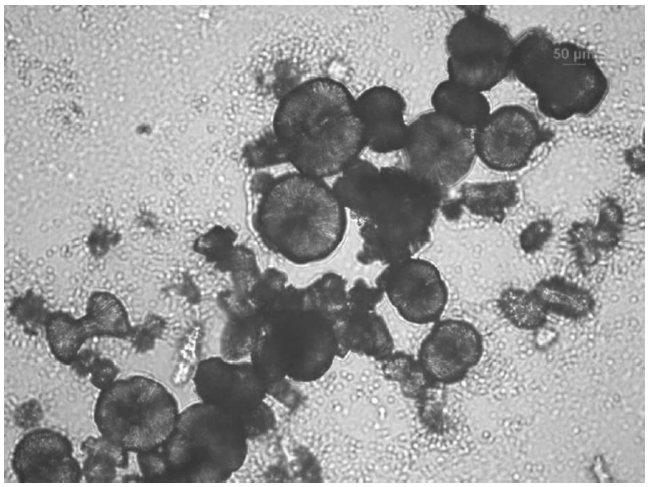

FIGURE 2. Photomicrograph of microcapsules (formulation C4) in aqueous medium before drying. Magnification: 200X.

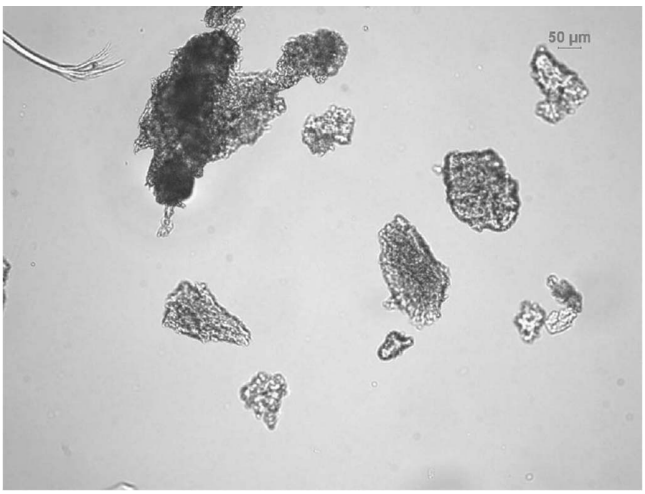

FIGURE 3. Photomicrograph of microcapsules (formulation C4) after drying. Magnification: 200X.

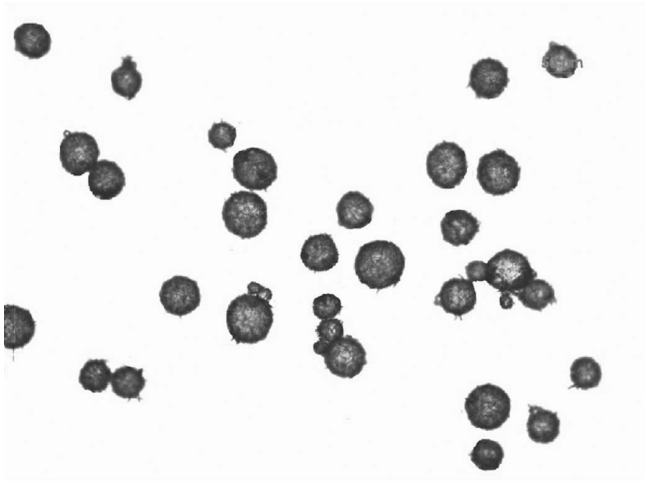

FIGURE 4. Photomicrograph of microcapsules (formulation D4) after drying. Magnification: 200X.

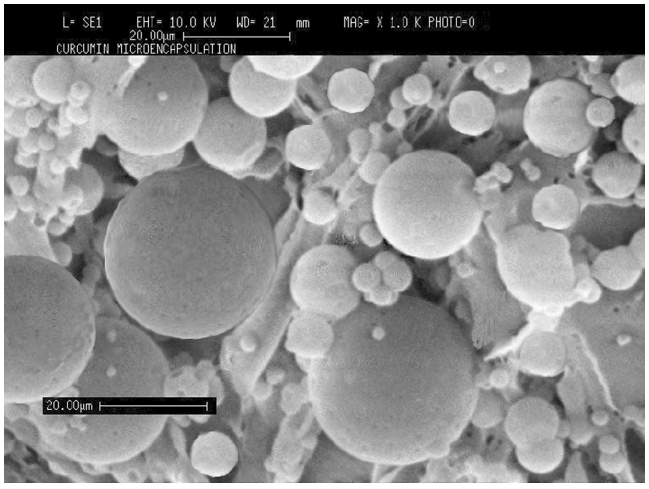

FIGURE 5. Scanning electron photomicrograph of microcapsules D4.

of gelatin in the presence of water, which caused an increase in particle size during measurement. In general, the particle size of curcumin powder in water was smaller than C4 and D4 (in water and in ethanol). However, curcumin powder showed a higher Span value indicating a wider size distribution than C4 and D4 (Table 4). In water, the volume mean diameter, D [4, 3] of curcumin, C4 and D4 were 28.69, 92.90, and 86.3 μm, while in ethanol, D [4, 3] of C4 and D4 were 79.09 and 83.86 μm, respectively. The results showed that the particle size of C4 was affected by water and a statistically significant difference was obtained between the particle size of C4 in water and ethanol. On the contrary, the particle size of D4 was not affected by water and no statistically significant difference was observed between the particle size of D4 in water and in ethanol ($p > 0.05$). Both ethanol and acetone are coacervating agents and anti-solvents for the aqueous gelatin solution. Nevertheless, the ability of acetone to remove water from the system was greater than ethanol, which could have facilitated deposition and mergence of gelatin droplets on the surface of curcumin particle with the production of a more rigid gelatin coat after treatment with formaldehyde as hardening/cross-linking agent. As such, the gelatin coat was less swellable in water.

Determination of Mean Wall Thickness

The mean density of curcumin and gelatin were 0.558 ± 0.05 g/cm^3 and 0.734 ± 0.02 g/cm^3, respectively. Using Eq. (4.1), the mean wall thickness of C4 in water and ethanol were 6.73 ± 0.04 and 5.72 ± 0.21 μm, while that of D4 were 4.18 ± 0.06 and 4.06 ± 0.06 μm, respectively. The mean wall thickness of D4 in both water and ethanol were significantly less than that of C4. This result further supported that microcapsules produced using acetone as the coacervating solvent had thinner and harder gelatin coat.

Evaluation of Flow Properties of Microcapsules

The values of angle of repose for curcumin, C4 and D4 were 64.80 ± 1.48°, 33.40 ± 1.14°, and 32.20 ± 1.64°, respectively. The results indicated that microencapsulation significantly improved the flow properties of curcumin as shown by the comparatively smaller values of angle of repose of C4 and D4 ($p < 0.05$). In addition, the values of angle of repose between C4 and D4 were not significantly different ($p > 0.05$). Wells (2002) reported a guideline of angle of repose used in the investigation of flow property of powders. Based on this guideline, the flow property of curcumin powder was considered as very poor, while the flow properties of curcumin microcapsules C4 and D4 were passable.

Staining Effect of Curcumin Microcapsules

Curcumin microcapsules C4 and D4 were easily removable and left light staining on the paper labels. On the contrary, curcumin powder tended to agglomerate and adhere to the paper labels, which left distinct dark yellow stain even after light tapping of the sample tray.

Effect of Ethanol and Water on the Swelling of Curcumin Microcapsules

The mean swelling volumes of C4 in water and in ethanol were 8.8 ± 0.84 and 5.0 ± 0.71 mL, respectively, while that of D4 in water and in ethanol were 6.4 ± 0.55 and 5.0 ± 0.71 mL, respectively. The results showed that the difference in volume between aqueous and alcoholic medium for D4 microcapsules was 1.4 ± 0.55 mL, which was significantly less than the difference between C4 microcapsules of 3.8 ± 0.45 mL. Therefore, D4 microcapsules were more suitable to be formulated in dermatological preparations such as cream or lotion as compared with C4 microcapsules.

In Vitro Curcumin Release from Microcapsules

As depicted in Figure 6 the in vitro release profiles of C4 and D4 were almost similar but at a slower rate when compared to that of curcumin powder. At the end of 12 hr, curcumin powder was released completely, while only about 85% of curcumin was released from C4 or D4 after 24 hr. The $T_{50\%}$

TABLE 4
Particle Size Analysis of Curcumin and Microcapsules C4 and D4

	Particle Size (μm)				
		C4		D4	
Parameters	Curcumin (In Water)	(In Water)	(In Ethanol)	(In Water)	(In Ethanol)
D (v, 0.1)	0.50 ± 0.05	25.64 ± 0.14	8.83 ± 2.05	39.01 ± 2.35	29.52 ± 0.41
D (v, 0.5)	16.61 ± 3.51	85.12 ± 0.19	69.66 ± 3.50	84.34 ± 0.43	82.92 ± 0.92
D (v, 0.9)	60.15 ± 12.04	171.5 ± 1.57	160.01 ± 3.92	143.53 ± 1.31	134.47 ± 1.44
D [4,3]	28.64 ± 5.64	92.90 ± 0.60	78.99 ± 2.89	86.30 ± 1.15	83.86 ± 1.18
Span	3.60 ± 0.12	1.71 ± 0.01	1.17 ± 0.06	1.35 ± 0.01	1.15 ± 0.03

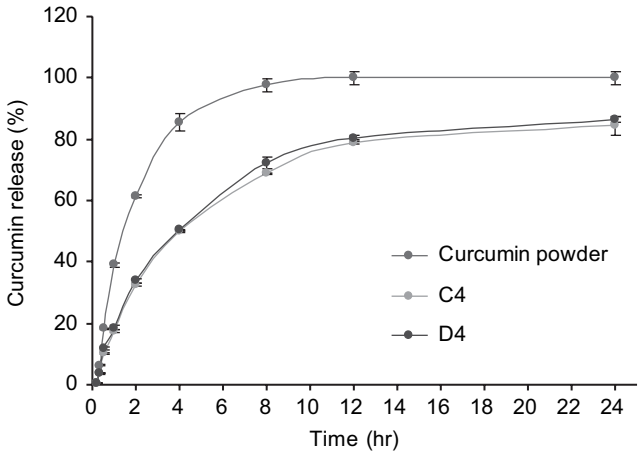

FIGURE 6. In vitro release of curcumin from curcumin powder and microcapsules C4 and D4. Mean ± *SD*, *N* = 3.

(time for 50% of curcumin release) values of curcumin powder, C4 and D4 were 1.49 ± 0.01, 4.03 ± 0.02, and 3.95 ± 0.1 hr. When analyzed statistically, the results showed that the $T_{50\%}$ value of curcumin powder was significantly different from C4 and D4 ($p < 0.05$), whereas the $T_{50\%}$ values of C4 and D4 were not significantly different ($p > 0.05$).

Stability of Curcumin Microcapsules

Figure 7 shows the photo-decomposition of curcumin over time for curcumin powder, C4 and D4. Microencapsulation clearly improved the stability of curcumin by protecting the curcumin from direct exposure to sunlight. The rate of decomposition calculated was 0.13 ± 0.04%/day for C4 and 0.08 ± 0.03%/day for D4, which were comparatively less than curcumin powder of 0.95 ± 0.10%/day, exhibiting significant improvement in photostability ($p < 0.05$).

Evaluation of Different Drug-Loaded Microcapsules

The results showed that among the six drugs studied, only ketoprofen, ketoconazole and magnesium stearate were successfully formulated as microcapsules. Figures 8, 9, and 10 show the drug-loaded microcapsules of ketoprofen, ketoconazole, and magnesium stearate in aqueous medium. The drug-loaded microcapsules appeared as discrete particles and were spherical in shape. In contrast, pseudoephedrine HCl, diclofenac sodium and paracetamol could not be microencapsulated to form microcapsules. When pseudoephedrine HCl and paracetamol were added to the aqueous gelatin solution at 40°C, clear solutions were obtained, but on cooling two turbid layers could be observed. When examined microscopically after freeze-drying, the drug particles could be vividly seen separated from the rubbery gelatin, which could be distinguished by the difference in color. As for diclofenac sodium, a mass was obtained after freeze-drying. When examined under microscope, drug particles could be seen on the surface of gelatin.

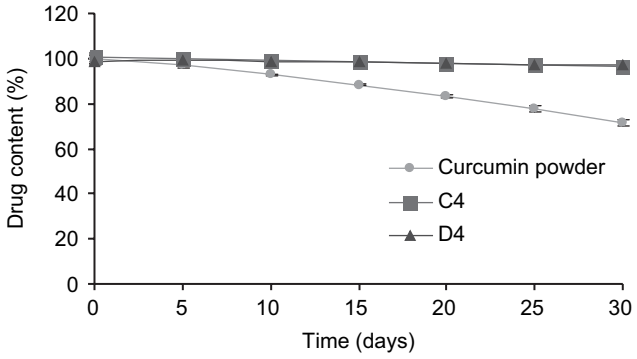

FIGURE 7. Photodecomposition of curcumin powder, C4 and D4.

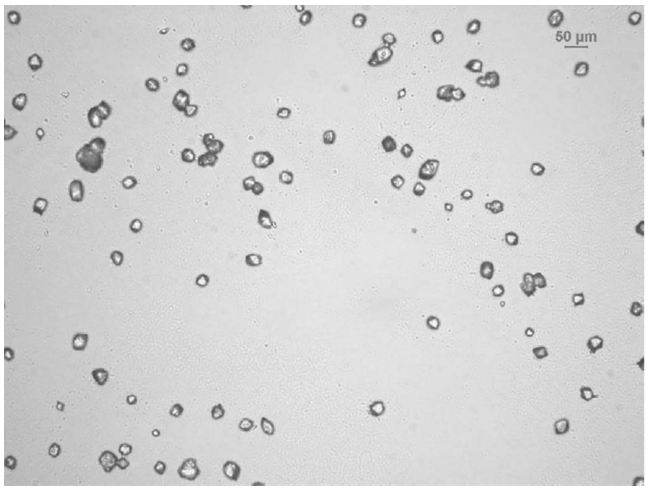

FIGURE 8. Ketoprofen-loaded microcapsules.

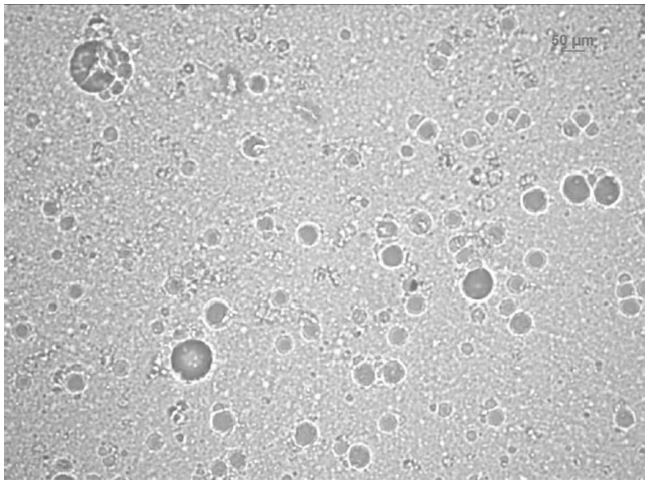

FIGURE 9. Ketoconazole-loaded microcapsules.

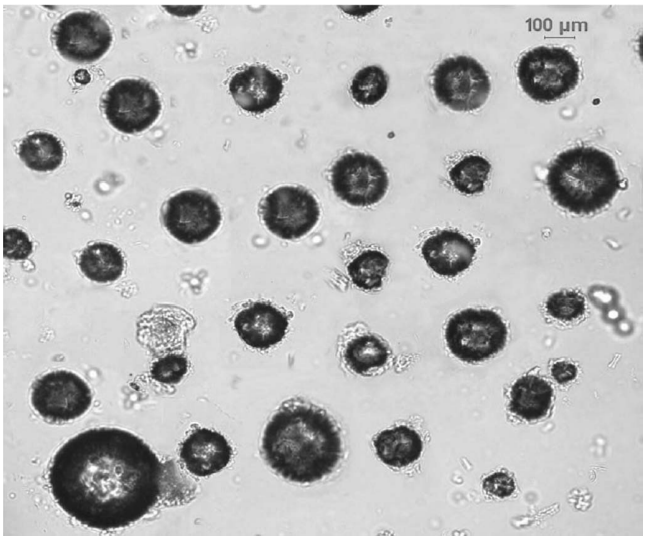

FIGURE 10. Mg-stearate-loaded microcapsules.

Pseudoephedrine HCl, diclofenac sodium and paracetamol are examples of drugs that dissolved in both water and ethanol. As such, when ethanol was added to the aqueous gelatin solution, coacervation did not occur, since the drug also dissolved in the aqueous environment. Hence, in order to achieve a successful microencapsulation, the drugs as core material have to be insoluble in the aqueous gelatin solution but can either be soluble or insoluble in the coacervating solvent. Table 5 shows the results of particle size analysis of ketoprofen, ketoconazole, and magnesium stearate powder. The particle size of ketoconazole powder was the smallest followed by magnesium stearate powder and lastly ketoprofen powder. Nevertheless, the difference between magnesium stearate powder and ketoprofen powder were not significantly different as the two were in the same subset while ketoprofen powder was in another subset. On the other hand, Table 5 shows the particle size of ketoprofen, ketoconazole, and magnesium stearate microcapsules. It can be seen that the particle size of ketoprofen microcapsules was smallest followed by ketoconazole and lastly magnesium stearate. The difference in the particle size among the microcapsules of the three drugs was statistically significant. In addition, the particle size of the drug loaded microcapsules was significantly larger than those of the drug powder ($p < 0.05$).

According to the solubility properties (British Pharmacopoeia, 2003), it can be seen that both ketoprofen and ketoconazole were soluble in the coacervating solvent, ethanol, while magnesium stearate was only dispersed in ethanol. This explains why the particle size of microcapsules with magnesium stearate was bigger than the particle size of ketoprofen and ketoconazole, since the particle size of microcapsules was highly affected by the solubility of the core material in the coacervating agent. In addition, magnesium stearate is also known as a metallic soap having anionic surface-active property. Due to its surface-active property, it could have also contributed to the bigger particle size of microcapsules containing magnesium stearate. In the case of curcumin microcapsules, the particle size of D4 was not significantly smaller than that of C4, even though D4 was curcumin dissolved in acetone, while C4 was curcumin dispersed in ethanol. This result contradicted with the result obtained from the above-mentioned three drugs. One possible explanation could be due to the limitation of the measuring instrument, Mastersizer, for samples with irregular and non-spherical morphology, such as C4.

CONCLUSION

In conclusion, curcumin microcapsules could be successfully prepared with ethanol and acetone as the coacervating solvents. When curcumin was dissolved in acetone, comparatively more spherical curcumin microcapsules with higher yield, higher curcumin loading and higher entrapment efficiency were obtained than dispersing curcumin in ethanol. Microencapsulation resolved the color staining effect and greatly enhanced the flow properties and photo-stability of curcumin. The in vitro release of curcumin was relatively slower after microencapsulation. The solubility of core materials in aqueous polymeric solution determined the successful formation of microcapsules. Microcapsules could only be formed if the core materials were not dissolved in the aqueous polymeric solution but could either be dissolved or dispersed in the coacervating solvent.

TABLE 5
Particle Size Results of Drug Powders and Microcapsules

	Ketoprofen (M.W. = 254.3)		Ketoconazole (M.W. = 531.4)		Mg-Stearate (M.W. = 591.3)	
Parameters (um)	Powder	Microcapsule	Powder	Microcapsule	Powder	Microcapsule
D [4,3]	19.03 ± 3.66	97.88 ± 0.60	7.03 ± 0.49	177.75 ± 7.55	16.09 ± 1.13	388.08 ± 2.98
D (v, 0.1)	1.33 ± 0.03	16.23 ± 0.19	0.27 ± 0.02	17.26 ± 0.40	1.22 ± 0.06	120.05 ± 0.87
D (v, 0.5)	11.19 ± 2.31	93.70 ± 0.49	4.53 ± 0.10	112.86 ± 4.49	9.93 ± 0.54	383.07 ± 4.31
D (v, 0.9)	47.84 ± 9.01	181.95 ± 1.36	12.99 ± 1.22	443.4 ± 20.93	39.96 ± 3.02	662.95 ± 3.54
Span	4.17 ± 0.07	1.77 ± 0.01	2.81 ± 0.28	3.78 ± 0.04	3.90 ± 0.10	1.42 ± 0.01

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