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Formulation and characterization of curcuminoids loaded solid lipid nanoparticles

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Abstract

Curcuminoids loaded solid lipid nanoparticles (SLNs) have been successfully developed using a microemulsion technique at ~75 °C. It was found that variation in the amount of ingredients had profound effects on the curcuminoid loading capacity, the mean particle size, and size distribution. At optimized process conditions, lyophilized curcuminoids loaded SLNs showed spherical particles with a mean particle size of ~450 nm and a polydispersity index of 0.4. Up to 70% (w/w) curcuminoids incorporation efficacy was achieved. *In vitro* release studies showed a prolonged release of the curcuminoids from the solid lipid nanoparticles up to 12 h following the Higuchi's square root model. After 6-month storage at room temperature in the absence of sunlight, the physical and chemical stabilities of the lyophilized curcuminoids loaded SLNs could be maintained, i.e. the mean particle size and the amount of curcuminoids showed no significant changes ($P > 0.05$) compared to the freshly prepared SLNs. In addition, the chemical stability of curcuminoids incorporated into SLNs was further investigated by dispersing them into a model cream base. The results revealed that after storage in the absence of sunlight for 6 months, the percentages of the remaining curcumin, bisdemethoxycurcumin and demethoxycurcumin were 91, 96 and 88, respectively.

Keywords: Curcuminoids; Solid lipid nanoparticles; Microemulsion; Lyophilization

1. Introduction

Curcuminoids are one of the best recognized antioxidants isolated from the rhizome of *Curcuma longa* Linn. (Ahsan et al., 1999; Khopde et al., 1999; Sreejayan and Rao, 1994). Curcuminoids are obtained as a yellowish pigment and consist primarily of three phenolic compounds: curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Fascinatingly, they can prevent lipid peroxidation at a significantly higher degree than e.g. pine bark extract, grape seed extract and synthetic antioxidants like butylated hydroxytoluene (BHT) (Kim et al., 1997) and show therefore also excellent properties in retarding skin ageing. Unfortunately, they are degraded by acidic and alkaline hydrolysis, oxidation and photodegradation (Bernabe-Pineda et al., 2004; Pfeiffer et al., 2003; Price and Buescher, 1996; Tonnesen et al., 1986; Wang et al., 1997). Many studies showed that curcuminoids decompose in a pH-dependent manner, with faster reactions at neutral to basic conditions. They are known to be stable at a pH below 6.5.

Recently curcuminoids have gained special interest in cosmeceutic products. However, stability issues of curcuminoids have not been successfully overcome in such preparations. Tonnesen found that micellar solubilization could stabilize curcumin against hydrolytic reaction, but the half-life of curcumin in such a system was only 2 months. Moreover, curcumin stabilized by a micellar system showed a higher photodecomposition rate compared to curcumin in aqueous solution (Tonnesen, 2002). A preliminary study of the authors revealed that the percentage of curcuminoids remaining intact in a model cream formulation was less than 50% after 3 months' storage. Therefore, further investigation with suitable cosmetic formulations has to be carried out in order to prolong the curcuminoids stability.

Among modern drug delivery carriers solid lipid nanoparticles (SLNs) seemed to be a promising colloidal carrier system. Solid lipid nanoparticles made from biodegradable solid lipids exist in the submicron size range and can be prepared by several

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methods. The advantages of SLN are as follows: possibility of controlled drug release and drug targeting, protection of incorporated compound against chemical degradation, no biotoxicity of the carrier, avoidance of organic solvent and no problems with respect to large scale production (Marengo et al., 2000; Mehnert and Mäder, 2001; Müller et al., 2000).

Thus, in this study, the curcuminoids stability in a model cream preparation was improved by developing curcuminoids loaded SLNs using a microemulsion technique at moderate temperature as developed by Gasco (1997). The primary goal here was to characterize the processing factors affecting the characteristics of the curcuminoids SLNs, including the optimal conditions for their preparation as well as their stability during storage. Additionally, the size and morphology of the SLNs were studied, as well as the efficiency of drug incorporation. The pattern of drug release was also investigated in relation to the stability of curcuminoids in cream formulations containing curcuminoids SLNs.

2. Materials and methods

2.1. Materials

Curcuminoids extract was purchased from Thai-China Flavours and Fragrances Industry Co., Ltd. (Ayutthaya, Thailand). Stearic acid was obtained from Srichand United Dispensary Co., Ltd. (Bangkok, Thailand). Dioctyl sodium sulfosuccinate (AOT) was purchased from Fluka Chemie (Buchs, Switzerland). Poloxamer 188 was a gift from BASF (Ludwigshafen, Germany). Glyceryl monostearate (GMS) and Arlacel 165 were obtained from Numsiang Trading Co., Ltd. (Bangkok, Thailand). Standard curcuminoids were purchased from Sigma (Lot No. 69-s3457, MO, USA). All other chemicals and solvents were of analytical grade. Polycarbonate membrane, diameter 25 mm, with a 0.22 μm pore size (Isopore™) and polyethersulfone membrane, diameter 76 mm, with a molecular weight cut off (MWCO) of 100,000 (Millipore® YM100) were purchased from Millipore Corporation (MA, USA).

2.2. Methods

2.2.1. Preparation and lyophilization of curcuminoids loaded SLNs

Curcuminoids loaded SLNs were prepared by a microemulsion technique at moderate temperature according to Gasco (1997). The water phase consisted of 0.1% (w/w) curcuminoids extract, 5–15% (w/w) poloxamer 188, 5–15% (w/w) AOT, 5–20% (w/w) ethanol, and deionized water added to 100% (w/w). The water phase was heated to ∼75 °C before added to the oil phase. The oil phase, consisting of 5–12.5% (w/w) stearic acid and 4% (w/w) GMS, was heated to ∼75 °C. The obtained warm microemulsion was dispersed in cold water, ∼2 °C, under high-speed homogenization (Model SL2, Silverson, Chesham Bucks, England) at 8000 rpm for 15 min. The volume ratio of the warm microemulsion to the cold water was 1:20. Then, the SLNs dispersion was washed two times with deionized water using an ultrafiltration cell system fitted with a membrane molecular weight cut off of 100,000 Da. Finally, 4% (w/v) mannitol was added to the SLNs dispersion before it was shock-frozen in liquid nitrogen and lyophilized at 0.40 mbar and −30 °C for 24 h.

The SLNs were prepared under different processing parameters to study the effect of a number of variables on their physicochemical properties. Processing parameters were varied as follows: the concentration of stearic acid was varied from 5% to 12.5% (w/w); the GMS emulsifier concentration was varied from 5% to 15% (w/w); ethanol concentration was varied from 5% to 20% (w/w); and the mannitol concentration was varied from 1% to 4% (w/w). The selection of these variables was based on preliminary experiments. Empty nanoparticles were prepared using the same procedure variables. All samples were prepared in duplicate.

2.2.2. Physicochemical characterization of the curcuminoids loaded SLNs

The morphology of the lyophilized empty and curcuminoids loaded SLNs was determined using a scanning electron microscopy (Model LEO 1455VP, LEO, Cambridge, England). The lyophilized SLNs were evenly distributed onto a conductive tab on a stud and then sputter coated with gold in a cathodic evaporator.

The mean particle size and particle size distribution were analyzed by photon correlation spectroscopy (PCS) employing a Zetasizer (Model Nano ZS90, Malvern Instrument, Worcestershire, England). This instrument was fitted with a 4 mW He–Ne diode laser operating at 633 nm. An aliquot of lyophilized curcuminoids SLNs was resuspended in deionized water. Measurements were performed at a fixed angle of 90° to the incident light and data were collected over a period of 3 min. The particle size analysis data were evaluated using the hydrodynamic diameter and the measurements were repeated three times for each sample.

2.2.3. Determination of curcuminoids incorporation efficiency

Ten milligrams of lyophilized curcuminoids containing SLNs were accurately weighed and dissolved in 10 ml of methanol. The dispersion was centrifuged at 18,000 × *g* for 30 min. Then the amount of curcuminoids in the supernatant was determined from its absorption at 420 nm using UV–vis spectrophotometer (Model Cary-1E, Varian, MA, USA).

The percentage of curcuminoids incorporation was then calculated using a calibration curve from the range of 0.8–4.0 μg/ml of standard curcuminoids which were firstly dissolved in methanol. Dilutions were made with 50% ethanol (v/v).

2.2.4. Preparation of a model cream containing curcuminoids SLNs

The aqueous phase of the model cream was composed of 5% (w/w) glycerol, 3% (w/w) propylene glycol, 0.1% (w/w) methyl paraben and deionized water to 100% (w/w). The oil phase contained 3% (w/w) mineral oil, 1% (w/w) stearic acid, 2% (w/w) cetyl alcohol, 3% (w/w) Arlacel 165 and 0.05% (w/w) propyl paraben. The oil phase and water phase were separately

heated to 70–75 °C. Then, the water phase was gradually added to the oil phase. The resulting mixture was cooled down to room temperature while stirring.

The cream containing curcuminoids SLNs was prepared by dispersing the lyophilized curcuminoids SLNs in the cream base. Curcuminoids SLNs composed of stearic acid 5% (w/w), poloxamer 5% (w/w), AOT 5% (w/w), and ethanol 15% (w/w) were chosen as they showed being the optimal formulation giving small particles with high entrapment efficacy. The final cream contained 0.01% (w/w) curcuminoids.

2.2.5. *In vitro dissolution studies*

In vitro release of curcuminoids SLNs was studied using vertical Franz diffusion cells (Model No. 57-951-061, Meditron, Völklingen, Germany) at 37 ± 0.5 °C. The area for diffusion was 1.77 cm^2 and the receptor chamber volume was 7.8 ml. Polycarbonate hydrophilic membrane (Isopore™ Membrane 0.22 μm, 25 mm) was fitted between donor and receptor compartment. The receptor medium consisting of 50% (v/v) ethanol was continuously stirred. The vertical Franz diffusion cell was allowed to equilibrate for 15 min before applying the sample. An amount of cream containing 40 μg curcuminoids was evenly spread on the membrane surface. Half a milliliter of the receptor medium was taken at predetermined time intervals of 10, 30 min, 1, 3, 5, 8 and 12 h. The amount of curcuminoids released was determined using its absorbance at 420 nm. In addition, *in vitro* release of cream containing free curcuminoids was investigated.

2.2.6. *Stability studies of lyophilized curcuminoids solid lipid nanoparticles*

Lyophilized curcuminoids SLNs were stored at room temperature in a desiccator and protected from light for 6 months. An aliquot of sample was taken at predetermined time intervals of 3 and 6 months to investigate the particle size and curcuminoids content. The mean particle size and polydispersity index (PI) were determined by PCS as described in Section 2.2.2. The percentage of curcuminoids incorporation was determined by UV–vis spectrophotometer as described in Section 2.2.3. Finally, the contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin were determined by a HPLC method which was modified from Jayaprakasha et al. (2002). An HPLC apparatus (Model LD10A, Shimazu, Kyoto, Japan) equipped with a 250 mm × 4.6 mm (i.d.) reversed-phase C18 column with 5 μm particle size (Luna, Phenomenex, CA, USA) and a UV–vis detector was used. The mobile phase consisted of acetonitrile and acetate buffer in a ratio of 1:1 (v/v), the pH of mobile phase was adjusted to 3.1 with glacial acid. A flow rate of 1.2 ml/min and detection wavelength of 425 were employed. Ten milligrams of lyophilized curcuminoids SLNs were accurately weighed and dissolved in 1 ml of methanol. The system was centrifuged at 18,000 × *g* for 60 min. The supernatant was decanted and then further centrifuged at 18,000 × *g* for 5 min before injecting into the column. The amounts of curcumin, demethoxycurcumin and bisdemethoxycurcumin were calculated by the peak areas using a calibration curve constructed from 1 to 100 μg/ml of standard curcuminoids. Unloaded SLNs were used as a reference. All experiments were done in triplicate.

2.2.7. *Stability studies of a model cream containing curcuminoids solid lipid nanoparticles*

The stability of the curcuminoids in the model cream containing curcuminoids SLNs was studied in the absence and presence of light. The cream containing curcuminoids SLNs was stored in a well-closed white glass and amber glass container and was kept at room temperature for 6 months. The content of curcumin, demethoxycurcumin and bisdemethoxycurcumin was determined by HPLC as described above. Two hundred milligrams of cream were accurately weighed and dissolved in 1 ml of methanol. The dispersion was centrifuged at 18,000 × *g* for 60 min. The supernatant was decanted and then further centrifuged at 18,000 × *g* for 5 min before injecting into the column.

3. Results and discussion

3.1. *Physicochemical properties*

3.1.1. *Morphology*

Curcuminoids loaded SLNs were successfully prepared by a microemulsion technique at a temperature range of 70–75 °C. An oil-in-water microemulsion was spontaneously obtained as recognized by a clear solution after adding the heated water phase into the oil phase of the same temperature. The SLNs were obtained immediately when dispersing the warm microemulsion into cold water with the aid of a homogenizer. The cold water facilitated rapid lipid crystallization and prevented lipid aggregation (Mehnert and Mäder, 2001). Yellow soft curcuminoids containing SLNs were obtained after freeze-drying. They could be easily redispersed in water and in the model cream base. Scanning electron micrographs revealed that lyophilized unloaded and curcuminoids loaded SLNs were spherical in shape with smooth surfaces and uniformly distributed throughout the mannitol flake (Fig. 1A and B). The morphology of particles was found to be independent of the processing conditions (data not shown).

3.1.2. *Mean particle size and size distribution*

The mean particle size and size distribution of the lyophilized curcuminoids loaded SLNs were determined by PCS. The results showed that the amount of lipid, emulsifier (poloxamer 188) and co-emulsifier (AOT) were critical parameters for the size of the nanoparticles.

The effect of stearic acid concentration on the mean particle size was evaluated by varying the concentration from 5%, 7.5%, 10% to 12.5% (w/w) while maintaining the amount of poloxamer at 5% (w/w), AOT at 5% (w/w) and ethanol at 15% (w/w). The results showed that when increasing lipid concentration from 5% to 12.5% (w/w), the monomodal size distribution with the mean particle size increased from 447 to 1198 nm and the polydispersity index increased from 0.4 to 0.70, respectively (Table 1). This finding was in agreement with Mehnert et al. who reported that increasing the lipid content over 5–10% (w/w) in most cases resulted in larger mean particle sizes and broader size distributions (Mehnert and Mäder, 2001). Therefore, 5% (w/w) stearic acid was found

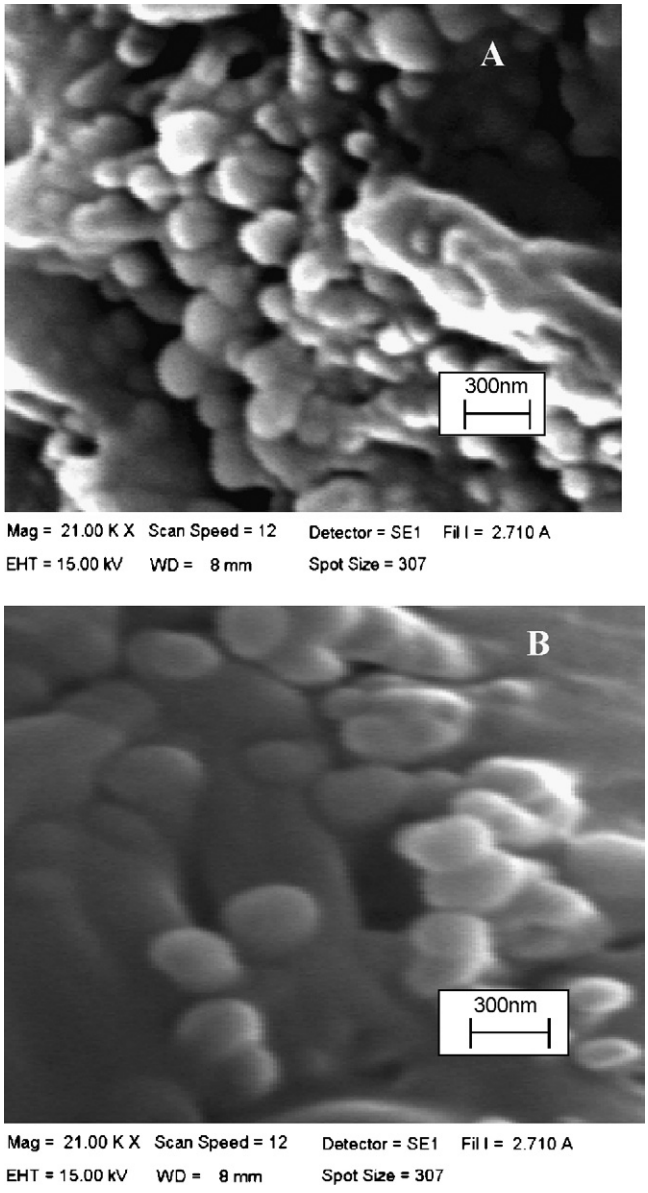

Fig. 1. Scanning electron micrographs of (A) unloaded solid lipid nanoparticles and (B) curcuminoids loaded solid lipid nanoparticles consisting of stearic acid 5% (w/w), poloxamer 5% (w/w), AOT 5% (w/w), ethanol 15% (w/w) and lyophilized with 4% (w/v) mannitol.

Table 1
Effect of stearic acid concentration on the mean particle size and polydispersity index of curcuminoids loaded solid lipid nanoparticles

Stearic acid (%, w/w)	Area intensity (% ± S.D.)	Mean particle size (nm ± S.D.)	PI ± S.D.
5.0	100	447 ± 55	0.46 ± 0.02
7.5	100	703 ± 55	0.68 ± 0.18
10.0	100	1305 ± 109	0.51 ± 0.02
12.5	100	1118 ± 368	0.67 ± 0.05

Composition of the SLNs: poloxamer 188, 5% (w/w); AOT, 5% (w/w); ethanol, 15% (w/w).

Table 2
Effect of poloxamer concentration on the mean particle size and polydispersity index of curcuminoids loaded solid lipid nanoparticles

Poloxamer (%, w/w)	Area intensity (% ± S.D.)	Mean particle size (nm ± S.D.)	PI ± S.D.
5.0	100	447 ± 55	0.46 ± 0.02
7.5	95 ± 12; 5 ± 1	672 ± 52; 254 ± 36	0.58 ± 0.02
10	84 ± 5; 16 ± 3	964 ± 88; 236 ± 43	0.75 ± 0.04
12.5	81 ± 7; 17 ± 4	917 ± 101; 205 ± 151	0.77 ± 0.13
15	78 ± 2; 12 ± 2	863 ± 147; 101 ± 57	0.67 ± 0.07

Composition of the SLNs: stearic acid, 5% (w/w); AOT, 5% (w/w); ethanol, 15% (w/w).

to be an optimum concentration for the formulation of the SLNs.

The effect of the amount of emulsifier and co-emulsifier, poloxamer 188 and AOT, on the mean particle size was studied by varying the amount of each emulsifier from 5%, 7.5%, 10%, 12.5% to 15% (w/w) while maintaining the amount of stearic acid at 5% (w/w) and ethanol at 15% (w/w), respectively. An optimal particle size with narrow size distribution was obtained with 5% (w/w) of each emulsifier. When the amounts of poloxamer 188 and AOT were increased, the mean particle size and polydispersity index were also increased (Tables 2 and 3). The results revealed that only formulation with 5% (w/w) poloxamer and 5% (w/w) AOT showed a monomodal size distribution with a mean particle size of 447 nm whereas SLNs formulated with poloxamer 188 higher than 5% displayed a bimodal size distributions. Approximately 80% of particles were in the range of 700–900 nm and approximately 20% were in the range of 100–250 nm. The same trend was observed when the amount of AOT was higher than 5% (w/w). An explanation for the observed aggregation may involve an intrinsic thermodynamic instability of the nanoparticle system with dispersed molecules of the surfactant and co-surfactant in the lipid matrix but finally resulting in an adsorption of the emulsifier to the particle surface. At very low concentration, the emulsifier is adsorbed directly onto the surface of the particles. However, at high concentration of the emulsifier, compression of the emulsifier molecules at the particles surface with formation of loops and tails became prominent and finally leading to the bridging between the primary nanoparticles (Freitas and Müller, 1999; Goppert and Müller, 2005).

Table 3
Effect of AOT concentration on the mean particle size and polydispersity index of curcuminoids loaded solid lipid nanoparticles

AOT (% w/w)	Area intensity (% ± S.D.)	Mean particle size (nm ± S.D.)	PI ± S.D.
5.0	100	447 ± 55	0.46 ± 0.02
7.5	93 ± 11; 7 ± 2	805 ± 30; 249 ± 112	0.76 ± 0.05
10.0	91 ± 8; 9 ± 3	1295 ± 141; 209 ± 77	0.80 ± 0.16
12.5	85 ± 7; 15 ± 6	1184 ± 62; 236 ± 55	0.71 ± 0.09
15	76 ± 6; 24 ± 11	1420 ± 209; 212 ± 43	0.48 ± 0.04

Composition of the SLNs: stearic acid, 5% (w/w); poloxamer 188, 5% (w/w); ethanol, 15% (w/w).

Table 4
Effect of ethanol concentration on the mean particle size and polydispersity index of curcuminoids loaded solid lipid nanoparticles

Ethanol (%, w/w)	Area intensity (% ± S.D.)	Mean particle size (nm ± S.D.)	PI ± S.D.
5.0	100	972 ± 61	0.70 ± 0.20
10.0	100	583 ± 199	0.50 ± 0.11
15.0	100	447 ± 55	0.46 ± 0.02
20.0	79 ± 12; 21 ± 13	814 ± 105; 130 ± 52	0.74 ± 0.07

Composition of the SLNs: stearic acid, 5% (w/w); poloxamer 188, 5% (w/w); AOT, 5% (w/w).

The effect of ethanol on the mean particle size was studied by varying the amount of ethanol from 5%, 10%, 15% to 20% (w/w) while maintaining the amount of stearic acid, poloxamer and AOT at 5% (w/w) each. Ethanol acts as a co-emulsifier and curcuminoids co-solvent. As expected, the results revealed that when increasing the concentration of ethanol from 5% to 15%, the mean particle size decreased from 972 to 447 nm, respectively (Table 4). However, the increase of the ethanol concentration to more than 15% (w/w) resulted in a larger mean particle size, 814 nm, and a broad size distribution. In addition, only formulations with 5–15% ethanol showed monomodal size distributions. On the contrary, formulation with 20% (w/w) ethanol showed a bimodal particle size distribution with ∼79% of the nanoparticles having a mean size of 814 nm while ∼21% showed a mean particle size of 135 nm. Therefore, ethanol induced bridging of primary formed nanoparticles by an accumulation of the surfactant and co-surfactant at the particle surface may be again a reasonable explanation for the formation of the larger particles as described before.

It is well known that a lyoprotectant is necessary to decrease nanoparticle aggregation during the lyophilization process. In this study, mannitol was chosen as a lyoprotectant. The lyoprotective effect of mannitol was tested at different concentrations ranging from 1% to 4% (w/v). The results showed that the mean particle size and size distribution decreased with increasing amounts of mannitol (Table 5). Formulation with 4% (w/v) mannitol resulted in the smallest mean particle size, ∼447 nm, with a monomodal size distribution. In addition, they could be easily redispersed in aqueous media. On this basis, 4% (w/v) mannitol was selected as the optimal concentration for the lyophilization process.

SEM micrographs showed more aggregated particles at lower amounts of mannitol (data not shown) resulting in a bigger particle size and a higher polydispersity index. Thus, the action of this mannitol as lyoprotectant is probably best explained by the particle immobilization hypothesis (Carpenter et al., 1999) which suggests that mannitol protects the particles against aggregation by providing spatial separation and immobilization of the particles in a glassy matrix during dehydration. Mannitol may interact with the polar head group of the surfactant molecules and thus prevents contact between discrete nanoparticles through stearic hindrance (Mehnert and Mäder, 2001; Müller et al., 2000).

Table 5
Effect of mannitol concentration on the mean particle size and polydispersity index of curcuminoids loaded solid lipid nanoparticles

Mannitol (%, w/w)	Area intensity (% ± S.D.)	Mean particle size (nm ± S.D.)	PI ± S.D.
1.0	100	696 ± 19	0.52 ± 0.02
2.0	100	656 ± 69	0.60 ± 0.03
3.0	100	527 ± 9	0.40 ± 0.08
4.0	100	447 ± 55	0.46 ± 0.02

Composition of the SLNs: stearic acid, 5% (w/w); poloxamer 188, 5% (w/w); AOT, 5% (w/w); ethanol 15% (w/w).

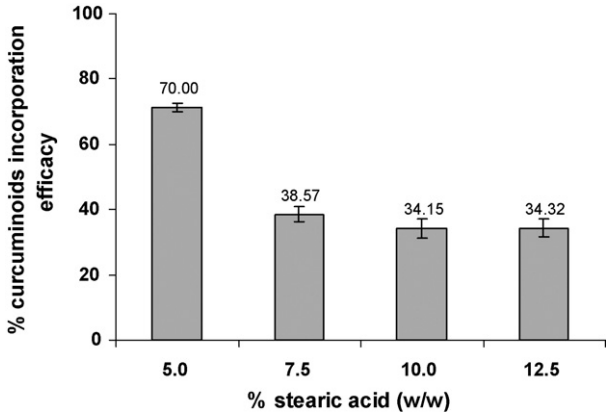

Fig. 2. Incorporation efficacy of curcuminoids on the surface of SLNs formulated with different stearic acid concentrations.

3.2. Determination of curcuminoids incorporation efficacy

The formulations studied demonstrated moderate to high curcuminoids incorporation efficacy in the range of 35–70% (w/w). The experimental results indicate that the concentration of lipid, emulsifier and co-emulsifier had critical effects on the curcuminoids incorporation efficacy (Figs. 2 and 3). Curcuminoids are poorly soluble in water and in lipids. They are soluble in alkaline medium and ethanol. Their solubility in the aqueous phase, however, could be enhanced by the addition of surfactant and co-surfactant. The effect of the amount of lipid on the entrapment efficacy was studied by maintaining the amount of surfactant while varying the amount of lipid. The result showed that the entrapment efficacy decreased as the amount of lipid increased due to the insolubility of the curcuminoids in stearic acid. Thus, the curcuminoids are incorporated in the surfactant layer at the surface of the SLNs leading to a high entrapment efficacy. On the contrary, at high lipid concentration, more SLNs were produced with insufficient amounts of (co)surfactants to solubilize the total amount of curcuminoids at the particle surface resulting in low entrapment efficacy.

It was found experimentally that the incorporation efficacy was optimal at 5% lipid, 5% (w/w) poloxamer 188, 5% (w/w) AOT and 15% (w/w) ethanol, respectively. The curcuminoids incorporation efficacy was significantly decreased with increasing the amounts of poloxamer 188, AOT and ethanol in the formulations, respectively (Fig. 3A–C). As the amount of emulsifier and co-emulsifier increased at a constant amount of lipid, the surface of the formed SLNs is too small to adsorb all surfactant and co-surfactant molecules, which will result in the

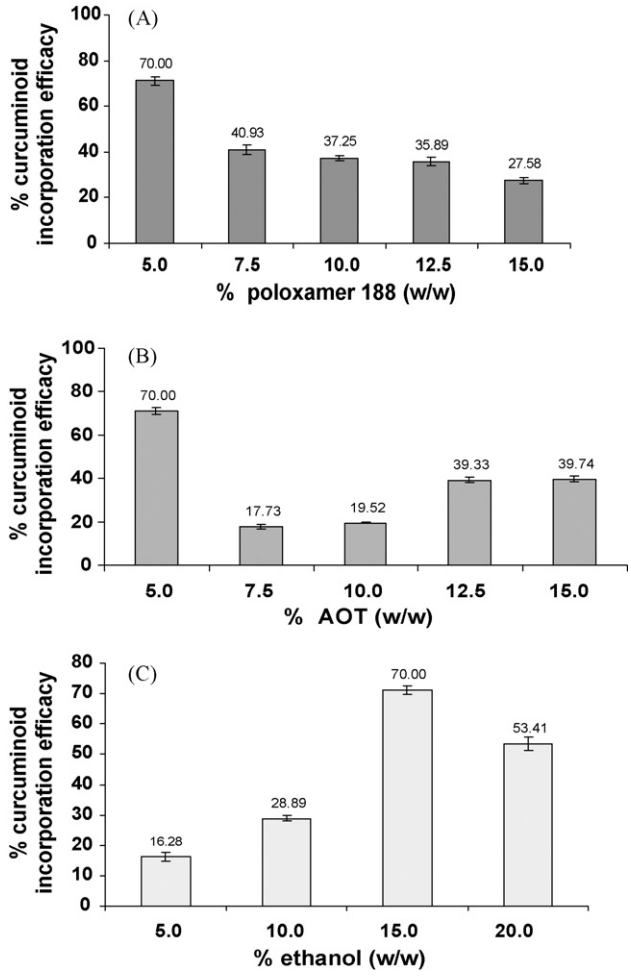

Fig. 3. Incorporation efficacy of curcuminoids in SLNs formulated with different amount of (A) poloxamer; (B) AOT; (C) ethanol.

formation of micellar solutions of the curcuminoids. Hence the solubility of the curcuminoids in the water phase will be increased. Therefore, curcuminoids could partition from the SLNs into the formed micelles in the water phase during the washing procedure by ultrafiltration technique thus reducing the final incorporation efficacy on the surface of the SLNs (Gohel et al., 2000; Yonezawa et al., 2001)

3.3. In vitro dissolution studies

Curcuminoids possess very poor aqueous solubility. Thus, to provide sink conditions 50% (v/v) ethanol was chosen as a receptor medium. The *in vitro* release studies from both model cream containing curcuminoids SLNs and cream containing free curcuminoids demonstrated a prolonged release characteristic following Higuchi's square root model (Fig. 4). However, a more rapid release of curcuminoids from cream containing free curcuminoids was observed, ~90% of curcuminoids were release within 8 h. In the contrary, 70% of curcuminoids were released from SLNs within 12 h. Therefore, the results indicated that most of the curcuminoids were incorporated at the surface of the SLNs and diffused into the cream matrix until a steady state was reached and finally from the cream into the

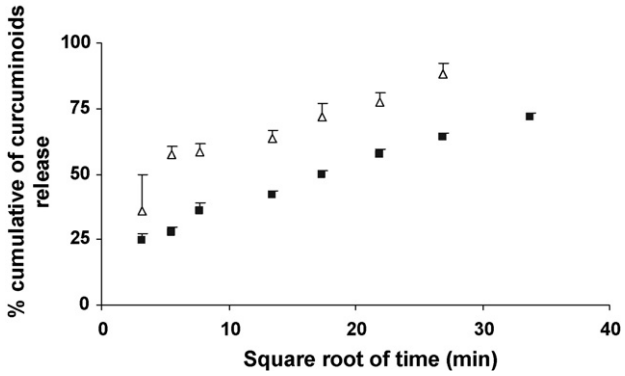

Fig. 4. Release profile of curcuminoids from (△) a model cream containing free curcuminoids and (■) a model cream containing curcuminoids SLNs. Curcuminoids SLNs consisting of stearic acid 5% (w/w), poloxamer 5% (w/w), AOT 5% (w/w), and ethanol 15% (w/w).

dissolution medium. Many studies reported that drug loaded SLNs provide a controlled release pattern following Higuchi's square root model (Gohel et al., 2000; Yonezawa et al., 2001). However, when incorporated in our cream system a 25% burst release of the curcuminoids within 10 min was observed, suggesting the partition of the curcuminoids from the SLNs into the cream base. From a therapeutic standpoint a burst release profile can be considered as an advantage as a sufficient amount of curcuminoids is rapidly released from the cream to the skin to exert an initial therapeutic effect followed by a controlled release (square root type) of the remaining curcuminoids from the SLNs.

3.4. Stability studies

Curcuminoids are notorious as light and oxygen sensitive substances. Thus, it is important to maintain their stabilities during storage. The lyophilized curcuminoids SLNs demonstrated physical and chemical stabilities for at least 6-month storage in the absence of sunlight under room temperature. The mean particle size of SLNs after preparation and after 6-month storage were 447 and 471 nm, respectively. The percentage of remaining curcumin, bisdemethoxycurcumin and demethoxycurcumin after 6-month storage were 99, 97 and 95, respectively. These results show that lyophilized SLNs have the same particle size and curcuminoids content as the initial preparation with a 95% confidence level. These findings are in agreement with the results of other researchers suggesting that the transformation of SLNs into a dry powder can prevent the aggregation of nanoparticles and improve the stability of light and oxygen sensitive substances (Mehnert and Mäder, 2001; Müller et al., 2000).

In addition, the chemical stability of curcuminoids incorporated into SLNs was further investigated by dispersing them into a model cream base. The lyophilized curcuminoids SLNs were uniformly dispersed within the cream base giving a yellowish cream color. The test products were kept in the absence and presence of sunlight in order to investigate the curcuminoids stability. The results revealed that after storage in the absence

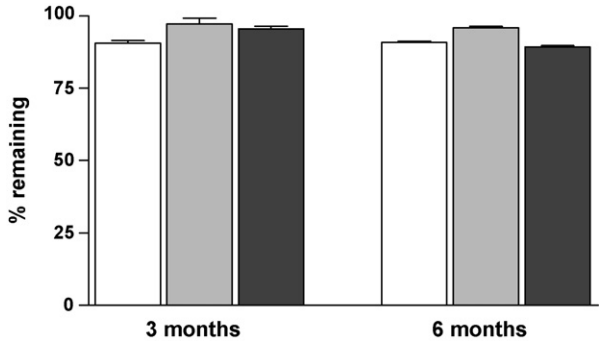

Fig. 5. Percentage of (□) curcumin, (▨) bisdemethoxycurcumin, and (■) demethoxycurcumin remaining in a cream formulation containing curcuminoids SLNs after 3 and 6 months storage under normal condition at room temperature in the absence of sunlight.

of sunlight for 6 months, the percentages of the remaining curcumin, bisdemethoxycurcumin and demethoxycurcumin were 91, 96 and 88, respectively (Fig. 5). In the presence of sunlight, the percentages of the remaining curcumin, bisdemethoxycurcumin and demethoxycurcumin were 71, 83 and 62, respectively (Fig. 6). The degradation of the curcuminoids during storage in the cream base in the presence of sunlight was a result of the curcuminoids released from the SLNs into the cream base. This amount of degraded curcuminoids was roughly consistent with the 25% burst release observed in the *in vitro* release studies. Thus, the findings indicate that SLNs could improve curcuminoids stability against light.

Furthermore in order to verify the quick degradation of free curcuminoids, we investigated the stability of non-encapsulated curcuminoids by incorporating them into the cream base. After storage of those curcuminoids creams both in the absence and presence of sunlight for 3 and 6 months, they showed 50% and 80% of curcuminoids loss, respectively (data not shown). Thus, these results strongly confirm that SLNs significantly improve the curcuminoids stability against light and oxidation reaction during storage when incorporated in a cream base. The viscosity and color of the cream formulations did not change after 6-month storage (data not shown). Additionally, no phase separation of the model creams was observed.

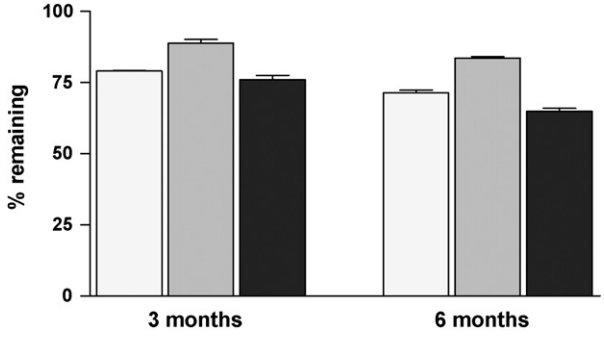

Fig. 6. Percentage of (□) curcumin, (▨) bisdemethoxycurcumin, and (■) demethoxycurcumin remaining in a cream formulation containing curcuminoids SLNs after 3 and 6 months storage under normal condition at room temperature in the presence of sunlight.

4. Conclusions

Curcuminoids loaded SLNs were successfully prepared by a microemulsion technique at moderate temperature. The process parameters, such as the amount of lipid and emulsifer, were crucial factors for the resulting mean particles size and the efficacy of drug incorporation. At optimal conditions, the mean particle size of curcuminoids loaded SLNs was 447 nm and incorporation efficacy of curcuminoids was 70% (w/w). Furthermore, this study demonstrates that the stability of curcuminoids in a cream containing curcuminoids incorporated into SLNs was significantly improved as compared to free curcumoids in the cream formulation. The light and oxygen sensitivity of curcuminoids was strongly reduced by incorporating curcuminoids into SLNs. Thus, SLNs are an attractive carrier system for light and oxygen sensitive substance. The release kinetics of curcumoids from a model cream base could be fitted with Higuchi's square root model and showed that approximately 70% of curcuminoids were released from cream SLNs within 12 h with a 25% burst release within the first 10 min, suggesting that most of the curcuminoids were still incorporated on the SLN surface stabilized by the (co)surfactants while about 25% (w/w) of the curcuminoids partitioned from the SLNs into the aqueous phase of the cream base.

Moreover, in an in vivo study with healthy volunteers, we found that creams containing curcuminoids loaded SLNs significantly reduced skin wrinkles, improved skin moisture and the firmness, elasticity, and viscoelasticity of the skin of the volunteers after application for 3 weeks with a 95% confidence level. Safety of the formulation was confirmed as skin irritation was not observed among the healthy volunteers (data to be published).

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