Bioencapsulation of *Curcuma aromatica* extract in solid lipid nanoparticles (SLN)

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INTRODUCTION

Curcuma species such as turmeric or *Curcuma longa* Linn. (CL) and *Curcuma aromatica* Salisb. (CA) contains many pharmacologically active phenolic compounds, importantly curcumin. Many pharmacological actions of curcumin were reported such as anti-inflammatory, potent antioxidant, antimicrobial, antitumor, antiprotozoal, antivenom, and anti HIV properties (Ruby A.J., 1995). A major problem of curcumin is the very poor stability as can be seen from their color change upon exposure to light or storage under alkaline conditions but they are moderately stable upon heat exposure (TØnnsen H.H., 1986). Consequently, having curcumin in pharmaceutical or cosmetic products is quite problematic due to it exhibited the instability during usage or storage. *C. aromatica* contains curcumin as *C. longa*. Its special properties for skin treatment such as anti-rash, anti-infection, revival skin, anti-acne and whitening skin have been reported (Pushpangadan P., 1984; John D., 1984 ; Velayudhan K.C., 1990). Besides these benefits, its paler yellow color than that of turmeric powder makes the extract from *C. aromatica* more favorable to use in the cosmetic products. To overcome the instability problem of curcumin and other curcuminoids, stability of an extract from *Curcuma aromatica* in the form of solid lipid nanoparticles (SLN) as well as *C. aromatica* SLN in a skin-nourishing cream formulation was investigated. Solid lipid nanoparticles (SLN) are particles made from solid lipids with a mean diameter between approximately 50 and 1000 nm (Müller R.H., 2000). SLN have been introduced as a potent carrier system for various pharmaceutical drugs and cosmetic active ingredients (Müller R.H., 2000). Therefore, the aim of this study was to prepare a simple SLN and a cream containing the SLN of *Curcuma aromatica* extract in order to increase the stability of active curcuminoid compounds under the exposure to light.

MATERIALS AND METHOD

Fresh rhizomes of *Curcuma aromatica* (raw materials) were collected from Chiang Dow garden, Chiang Mai Province, Thailand in 2006. Whole plant materials were samplinged out and grew in a medicinal plant garden of the Faculty of Pharmacy, Chiang Mai University and identified by Dr. Charun Maknoi (Botanist, Queen Sirikit Botanical Garden, Thailand). The voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy, Chiang Mai University, Chaing Mai, Thailand. Standard curcuminoids were purchased from Fluka (Lot No.28260, India). Palmitic acid, stearyl alcohol, Tween 80 and Span 80 were commercial grade for cosmetic production. Acetonitrile and methanol used in this study were a HPLC grade and the other chemicals and solvents were of analytical grade.

Extraction and determination of curcuminoids from C. aromatica : Fresh rhizomes (10 kg) of *Curcuma aromatica* were dried, ground to fine powder and continuously extracted with 95% ethanol using a soxhlet apparatus for 12 hour. The ethanolic extract was filtered and then the

solvent was removed by using a rotary evaporator at 45°C until dry. Chromatographic fingerprint of *C. aromatica* and their three major curcuminoid components were analyzed by using HPLC. (Model HP1100, Agilent Technologies, HP, U.S.A.)

Preparation of Curcuma aromatica loaded SLNs : Curcuminoids extracted loaded SLNs were prepared by high pressure homogenization technique. An aqueous phase was consisted of 0.5% (w/w) curcuminoids extracted, tween 80 and deionized water. The water phase was heated to 70-75°C before added to an oil phase. The oil phase consisted of span 80, palmitic acid and stearyl alcohol was heated to 65-70 °C. The warm pre-emusion was dispersed in cold water at the ratio 1:10, under high speed stirring. The SLNs suspensions were passed through high pressure homogenizer (Model C3, Avestin, Canada) and centrifuged (Model SORVALL® SUPER T21, Dupont, USA.). Then precipitated SLNs were washed one time with the deionized water.

Characterization of Curcuma aromatica loaded SLNs : Morphologies of the empty and *C. aromatica* loaded SLNs were determined using a transmission electron microscopy (TEM), (Jeol, Model JEM 2110, Japan). The SLNs were redistributed, dropped onto a copper grid and stained with 2% potassium phosphotungstic acid. Mean particle size and particle size distribution were analyzed by photon correlation spectroscopic (PCS) techniques (Malvern Nano-ZS360, England).

Entrapment efficiency (%EE) : The suspension *C. aromatica* containing SLNs were centrifuged. The supernatant containing free *C. aromatica* was analyzed by HPLC. The percentage of curcuminoids entrapment efficiency was calculated with an indirect method. All samples were prepared in triplicate.

Photostability study of curcuminoids in SLNs and SLNs in cream : The curcuminoids loaded SLNs were aliquoted and put into clear glass bottles. Stability study upon light-exposure was performed in a black box with the 650 watts of fluorescent light at a room temperature for 8 hours. Stabilities of curcuminoids, curcuminoids in SLNs, curcuminoids in cream and curcuminoids SLNs in cream were studied. The contents of curcumin, demethoxycurcumin were determined by HPLC. All experiments were done in triplicate.

RESULTS AND DISCUSSION

Extraction C. aromatica : Percent yield of curcuminoids from a *C. aromatica* ethanolic extract was 15.3 % from rhizome-powder weight. HPLC chromatograms of the *C. aromatica* exhibited curcuminoids' peaks with demethoxycurcumin (DMC) as the highest content among all three major compounds. Percentages of curcumin, demethoxycurcumin and bisdemrthoxycurcumin in the extract were 28.5 %, 70.0 %, and 1.5 %, respectively. While the curcuminoids content from Fluka (> 95.0 %) were 67.4 %, 25.7 %, and 6.8 %, respectively. The chromatograms were shown in Figures 1 and 2.

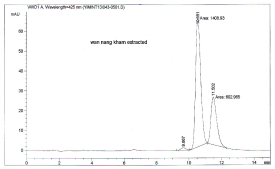

Fig.1. Chromatogram of curcuminoids from *C. aromatica*.

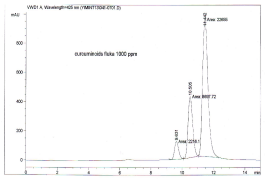

Fig.2. Chromatogram of standard curcuminoids (Fluka)

Preparation of C. aromatica loades SLNs and Entrapment efficiency (%EE) : *C. aromatica* loaded SLNs were successfully prepared by high pressure homogenization technique. Firstly, *C.aromatica* extract was dispersed homogeneously in a melted lipid phase. High speed stirring was performed to obtain a pre-emulsion, which was subsequently dispersed in cold water prior to passing to a high pressure homogenizer. In order to select a suitable lipid for loading the *C.aromatica* extract into the SLNs, several lipids such as palmitic acid, cetyl palmitate, cetyl alcohol and stearyl alcohol were employed at different ratios. The most suitable nanoparticles were derived from palmitic acid and stearyl alcohol mixture with appropriate amount of surfactants. The suspension of the prepared nanoparticles was concentrated by centrifugation. Then, the extract loaded SLNs were added in a model cream base. Transmission electron micrographs (TEM) revealed that the shape of the *C.aromatica* extract unloaded and loaded SLNs were a spherical in shape. (Fig. 3 and 4). The means particle size of them were 236.8 ± 2.8 and 353.8 ± 7.6 and the polydispersity index were 0.25 ± 0.01 and 0.29 ± 0.04, respectively. The %EE was measured by HPLC. The *C.aromatica* extract loaded SLNs were found to have the %EE of curcumin, demethoxycurcumin, and bisdemethoxycurcumin of 52.2%, 44.5%, and 37.0%, respectively.

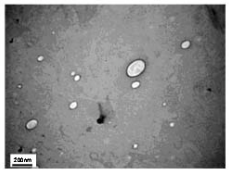

Fig.3 TEM photograph of unloaded solid lipid nanoparticles (60,000X)

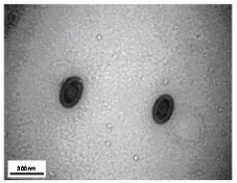

Fig.4 TEM photograph of curcuminoids loaded solid lipid nanoparticles (60,000X)

Photostability of curcuminoids loaded SLNs : After an exposure of the fluorescent light for 8 hours, the stability of curcumin and demethoxycurcumin SLN was monitored at predetermined time-intervals. Greater stability of the curcuminoids in SLN was observed in both SLNs and SLNs in cream. At 8 hours, curcumin extract (WC1) remained 58.91%, curcumin in SLNs (SLNWC1) remained 74.27%, curcumin in cream (WC1cr) remained 74.29% and the curcumin SLN in cream (SLNWC1cr) remained 84.76%. Therefore the preparation of the photolabile curcuminoids in

theSLNs could enhance their stability in both SLNs and in cream formulation significantly. Similar observation was revealed when the demethoxycurcumin contents were determined (Fig 5 and 6).

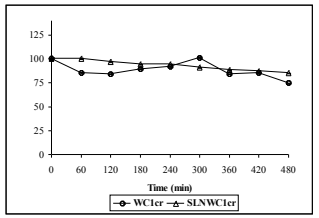

Fig.5 Influence of light on curcumin and SLN loaded curcumin in cream

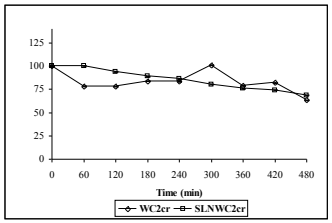

Fig.6 Influence of light on demethoxycurcumin and SLN loaded demethoxycurcumin in cream

CONCLUSION

The stability of curcuminoids was successfully improved by preparing in the form of SLN. The stability was increased in both free SLN and SLN in cream. Suitable combination of lipids and surfactants for the preparation of SLN is the key factors for the stable SLNs with appropriate size and size distribution. This SLNs technique yielded mean particle size of *Curcuma arom*atica loaded SLNs of 353 nm with sufficiently high % entrapment efficiency.

BIBLIOGRAPHY

Ruby, A. J. et al. (1995), *Anti-tumor and antioxidant activity of natural curcuminoids.* Cancer Lett. 94, 79-83

TØnnsen, H. H. et al. (1986), *Studies on curcumin and curcuminoids. VIII. Photochemical stability of curcumin. Z.* Lebensm.-Unters. Forsch. 183, 116-122

Pushpangadan, P. and Atal, C. K. (1984) *Ethno-Medico-Botanical Investigation in Kerala I.* J. Ethnopharmacol. 11, 59-77

John, D. (1984), *One Hundred Useful Raw Drugs of the Kani Tribes of Trivandrum Forest Division.* Int. J. Crude Drug Res. 22(1), 17-39

Velayudhan, K. C. et al. (1990), J. Econ. Tax. Bot. 14, 579-582

Müller, R. H. et al. (2000) *Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art.* Eur. J. Pharm. Biopharm. 50, 161-178