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The effect of rice bran wax on physicochemical properties of curcuminoid-loaded solid lipid nanoparticles

Tangkhajornchaisak P[1], Rojsitthisak P[2], Tiyaboonchai W[3], Wattanaarsakit P[1,*]

[1] *Department of Pharmaceutics and Industrial Pharmacy and* [2]*Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand.*

[3] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Pitsanulok 65000, Thailand.*

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Objectives: The aim of this study was to explore the potential of using rice bran wax as a natural lipid composition for curcuminoid-loaded solid lipid nanoparticles.
Methods: Blank solid lipid nanoparticle (SLN) formulations of three lipid types and concentrations; rice bran wax (RB), glyceryl behenate (GB) and cetyl palmitate (CP) at 2.5, 5 and 7.5%w/w were studied. SLN were prepared by a high pressure homogenization technique and characterized on their particle size, polydispersity index and zeta potential. SLN physical stability was also observed during storage. Curcuminoid-loaded SLN (C-SLN) were prepared and determined their physical characteristics and percentage of drug entrapment.
Results: The results showed that particle size of all SLN formulations ranged in nanosize with narrow size distribution and low zeta potential which indicating a good physical stability. SLN with RB had smaller size compared to GB and CP. The mean particle size of RB-SLN was slightly increased when a lipid concentration was increased from 2.5 to 7.5%w/w. Entrapment efficiency of the loaded-SLN with RB showed higher of loading amount than the loaded-SLN with GB or CP.
Conclusion: Curcuminoid-loaded solid lipid nanoparticles using rice bran wax was successfully prepared. This rice bran lipid carrier had better physical properties and entrapment efficiency. Therefore, the rice bran wax has a pharmaceutical potential usage as a natural lipid composition for SLN delivery systems.

* Corresponding author: Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand; Tel. +66(0)2-2188276; Fax. +66(0)2-218-8266 E-mail address: phanphen.a@chula.ac.th

Introduction

Solid lipid nanoparticles (SLN) are considered as an attractive lipid colloidal carrier system. This lipid nanocarrier is generally spherical in shape with an average diameter between 10-1000 nm. The system consists of a solid lipid matrix which stabilized by surfactants. Both of lipophilic and hydrophilic drugs can be incorporated into this system. SLN possess more advantages as their matrix has lipophilic nature lead to protect the incorporated active compounds against chemical degradation and high amount of lipophilic drug payload. It also has versatile properties such as drug targeting and controlled release. Manufacturing of SLN by a high pressure homogenization technique (HPH) is an easily process for scale up and large production scale. This technique can be used to prepare SLN without using organic solvents[1]. Quality control of the SLN preparation was studied on their physical properties such as particle size, polydispersity index and zeta potential which influence on their stability.

Lipid types and concentrations are important factor which affected on the property of SLN[2]. The lipid composition of SLN can be classified to glycerides, fatty acids, and waxes. Cetyl palmitate (CP) and glyceryl behenate (GB) are common lipids used for SLN preparations. CP, produced from spermaceti, composes of fatty acids mainly palmitic acid (C16) and hexadecyl ester. It has melting point of 42-50°C. GB composes of partial glyceride and fatty acids mainly behenic acid. It has melting point of 69-74°C. Rice bran wax (RB) is a natural vegetable wax (*Oryza sativa* L.) and is a value added by product received from rice bran oil refineries. It contains potent antioxidant of gamma oryzanol. The RB appearance is hard and non-tacky wax. It has melting point of 77-82°C. The chemical constituents of rice bran wax are mainly saturated monoesters (C-46 to C-60) of long chain fatty acids (C-22 to C-26) and long chain fatty alcohols (C-26 to C-30). Main esters are myricyl cerotate (43-45%), ceryl cerotate (21-22%) and isoceryl isocerotate (9-10%). Rice bran wax utilization in pharmaceuticals is worth investigating[3].

Curcuminoid is an interesting active component extracted from rhizomes of turmeric *(Curcuma longa* L.*)* which widely used as a traditional herb. It is insoluble in water but dissolved well in polar organic solvents such as ethanol or acetone. The polyphenolic structure of curcuminoid decomposes rapidly under the influence of light. It was also degraded quickly in alkaline aqueous environment (pH 9-10) to ferulic acid and feruloylmethane[4]. Therefore, curcuminoid was selected as

a model of anti-inflammatory drug for topical use. The aim of this study was to explore the potential of using rice bran wax as a natural lipid composition for curcuminoid-loaded solid lipid nanoparticles.

Methods

***Drugs and chemicals*:** Curcuminoid (Thai china flavours & fragrances industry Co., Thailand). Rice bran wax (Thai agrifoods Co., Thailand), Glyceryl behenate (Compritol® 888 ATO from Gatefossé, France), Cetyl palmitate and Tween® 80 (Thai sanguanwat Co., Thailand).

***Preparation of curcuminoid loaded solid lipid nanoparticles*:** Blank nanoparticles were prepared using three lipid types and concentrations; rice bran wax, cetyl palmitate and glyceryl behenate at 2.5, 5 and 7.5%w/w by the high pressure homogenization technique[5]. Both lipid and aqueous phase were separate melted at temperature of a lipid melting point plus 10°C in order to complete pre-emulsification. The hot aqueous phase containing 1%w/w tween 80 as surfactant was dispersed in hot lipid phase. Pre-emulsion was homogenized using a high speed homogenizer (Ultra-Turrax T-25 Basic, IKA-Werk) at 10,000 rpm for 5 minutes. Then, the emulsion was reduced droplet size to nano range using a high pressure homogenizer (HPH, EmulsiFlex-C5, Avestin Inc.) at 1,000 bars, 3 cycles and 85°C. The nanodispersion was rapidly cooled using an ice bath to solidify the nanoparticles and avoided of particle fusion during the process. The system of 7.5% lipid and 1% tween 80 were chosen for curcuminoid loading. C-SLN was prepared similar to the blank SLN. Curcuminoid was dispersed in hot aqueous containing surfactant and ethanol.

***Characterization and Physical stability of SLN*:** The mean particle size, polydispersity index (PdI) and zeta potential (ZP) of the SLN and C-SLN were determined by photon correlation spectroscopy (Zetasizer Nano-ZS, Malvern). The dispersion samples were diluted with ultrapure water at ratio of 1:20 to ensure that the light scattering intensity. Triplicated samples were studied at 25°C[6]. Physical stability of blank SLN was observed on their particle size, PdI and ZP change for 60 days storage in sealed amber glass vials at room temperature.

Entrapment efficiency: Curcuminoid entrapment efficiency was determined by ultrafiltration method using centrifugal filter device of Amicon® cellulose membrane, MWCO 100K equivalent to 30-90 nm[7-8]. Samples were centrifuged at 12,000 rpm 25°C for 30 minutes. The supernatant was collected for free drug analyzed at 420 nm using UV-vis spectrophotometer[9-10]. Then precipitated SLN containing curcuminoid were lyzed by adding methanol and centrifuged. The supernatant of lyzed-lipid matrix was analyzed for amount of drug entrapment. Triplicate sample were studied. The percentages of entrapment efficiency was calculated by the following equation[11]

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of entrapped curcuminoid}}{\text{Amount of initial curcuminoid added}} \times 100 \frac{\text{Amount of entrapped curcuminoid}}{\text{Amount of initial curcuminoid added}} \times 100$$

Results

Characterization and Entrapment efficiency: Particle size comparison of the blank SLN has shown in Figure 1. The result showed that all of blank SLN had size range from 167.7±2.40 to 410.5±3.60 nm with low polydispersity index indicating narrow of particle size. Blank SLN with RB had smallest of particle size compared to the CP and GB. Increasing lipid concentration of RB from 2.5 to 7.5%w/w, the mean particle size was increased and broader size distribution. GB SLN showed largest of particle size and significantly increased as the lipid content increase.

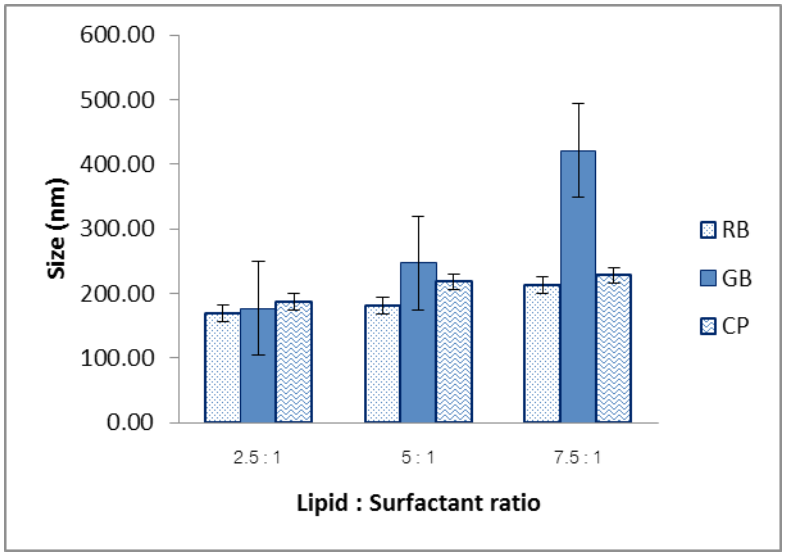

Figure 1. Comparison of particle size of SLN containing various lipid types and concentrations: RB, GB and CP at 2.5, 5.0 and 7.5%w/w.

The mean particle size, PdI and zeta potential of the blank and curcuminoid loaded SLN were studied as showed in Table 1. Particle size of the curcuminoid loaded SLN was larger than the blank SLN. At 7.5%w/w lipid concentration, curcuminoid loaded SLN with RB and GB had similar of particle size and larger than the CP. The RB loaded SLN had

larger of particle size. This result was related to their entrapment efficiency which had highest amount of curcuminoid loaded compared to GB-SLN and CP-SLN. Physical appearance of freshly prepared curcuminoid loaded SLN was shown in Figure 2.

Table 1. Particle size, polydispersity index and zeta potential of SLN and curcuminoid loaded SLN and their entrapment efficiency (*n*=3).

Formulation	Blank SLN			C-SLN			
	Mean particle size (nm) ± SD	PdI	Zeta Potential (-mV)	Mean particle size (nm) ± SD	PdI	Zeta Potential (-mV)	EE (%)
RB2.5	167.70 ± 2.40	0.40	29.20 ± 0.70	304.05 ± 5.89	0.43	28.21 ± 0.98	62.18% ± 0.33
RB5.0	178.30 ± 1.90	0.31	30.59 ± 3.82	412.55 ± 9.66	0.49	23.22 ± 0.83	65.27% ± 0.09
RB7.5	208.30 ± 3.00	0.26	22.86 ± 0.83	330.40 ± 8.74	0.40	23.41 ± 0.96	71.62% ± 0.01
GB7.5	410.50 ± 3.60	0.59	31.06 ± 19.41	330.66 ± 4.15	0.36	24.14 ± 1.50	53.37% ± 0.01
CP7.5	224.60 ± 4.60	0.22	24.99 ± 0.47	213.33 ± 3.56	0.18	26.49 ± 8.44	38.27% ± 0.01

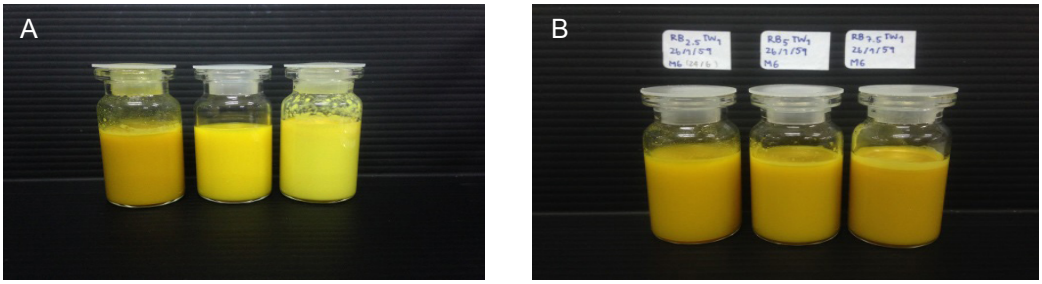

Figure 2. Physical appearance of freshly prepared curcuminoid loaded SLN:
(A) RB 2.5, 5.0 and 7.5%w/w; (B) RB, GB and CP 7.5%w/w (left to right)

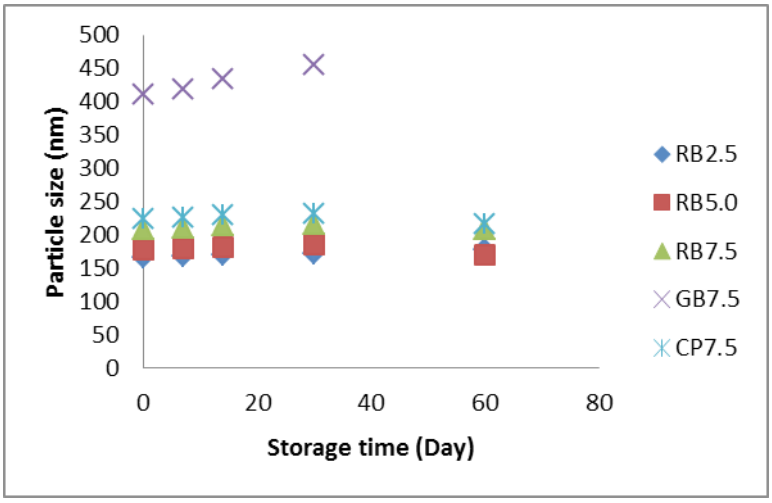

Figure 3. Particle size of SLN during 60 days storage.

Physical stability of SLN: Table 1, Zeta potential of all the SLN, ranged from 22.86±0.83 to 31.06±19.41 mV, indicated the systems have good stability. Physical stability from Figure 3, RB-SLN had smaller size with good stability compared to GB-SLN and CP-SLN. The particle size of SLN produced by GB was increased during storage time and shown unstable system with gel forming after 60 day storage.

Discussion

Lipid types and concentrations had significant affected on physical properties and entrapment efficiency of the SLN. Three types of selected lipid; rice bran wax, glyceryl behenate and cetyl palmitate have melting temperature between 54-82°C which suitable for a hydrophobic curcuminoid to be entrapped. Increasing of particle size may be associated with the viscosity of the sample. Waxes are plastic solid at room temperature[12]. RB and CP had wax appearance contrast to GB which composed of glycerides. At pre-emulsion preparation step, the GB showed viscous emulsion than RB and CP which resulted in larger particle size and broader PdI than RB and CP. This result related to further instability during storage time. Increasing of RB lipid content the particle size was also increased. This result similar to Schwarz, C. and Mehnert, W., 1999 who reported that increasing the lipid content resulted in a larger of mean particle

size and broader size distribution[7]. A higher lipid content which made the sample more viscous may decrease the efficiency of homogenization resulting in larger of particle size. Lower viscous lipid phase improves size reduction and enhances stability of SLN production[13]. Different structures and chain lengths of lipid types possibly affect particle size and entrapment of the SLN[14]. Rice bran wax composes of various saturated monoester of fatty acids and alcohols with different chain lengths. This mixture of various lipid constitutes might support their structure arrangement and enhance curcuminoid entrapment compared to glyceryl behenate and cetyl palmitate. Rice bran wax suitable for SLN preparation as its produced smallest size and highest entrapment efficiency. For glyceryl behenate and cetyl palmitate, the GB-SLN had largest size and instability and the CP-SLN had small size but low entrapment efficiency.

Conclusion

In this work, curcuminoid-loaded solid lipid nanoparticles using rice bran wax was successfully prepared by the high pressure homogenization technique. Lipid types and concentrations greatly affected physical properties and drug entrapment of the SLN. SLN with rice bran lipid had better physical properties and entrapment efficiency. Therefore, the rice bran wax has a pharmaceutical potential usage as a natural lipid composition for SLN delivery systems.

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