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Original Article

Formulation and Characterization of Bitter Melon (*Momordica charantia* Linn.) Fruit Fraction Loaded Solid Lipid Nanoparticles

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ABSTRACT

Background: The main active compound of bitter melon (*Momordica charantia* Linn.) fruit is charantin, which is believed to have important role on antihyperglycemic effect. However, charantin compound has a large molecular weight and is easily hydrolysed when given orally. Therefore, a colloidal drug delivery system, such as solid lipid nanoparticles (SLN), is required to provide a suitable and effective delivery of charantin, which is contained in a bitter melon fraction (BMF). **Objective:** This study aimed to prepare and evaluate SLN containing BMF with an appropriate characteristic for transdermal delivery. **Methods**: Bitter melon fruits were extracted with ionic liquid of [BMIM]BF_4 using ultrasound-assisted extraction (UAE) and fractionated with dichloromethane. Four formulas of BMF loaded SLN were prepared with various ratio of BMF to surfactant and various ratio of lipids using high-shear homogenization followed by ultrasonication method. The obtained SLN were characterized, including morphology, particle size distribution, zeta potential, and entrapment efficiency. Furthermore, the stability study of BMF-loaded SLN was also conducted. **Result**s: The result showed that BMF was a dry powder and brownish fraction with a specific smell. The BMF loaded SLN showed a spherical shape with the SLN F1 formula as a selected formula. The SLN F1 showed a particle size (Z-average) of 98.3±1.98 nm, polydispersity index of 0.26±0.01, zeta potential of -39.53±0.15 mV, and entrapment efficiency of 82.96±1.42 %. According to the stability study, it revealed that the BMF loaded SLN F1 had an acceptable stability, which the charantin content in the SLN was 96.52% after 3 months storage at 25°C ± 2°C. **Conclusion**: The BMF loaded SLN F1 with 1:12 ratio of BMF to surfactant and 1:2 ratio of capric caprylic triglyceride to glyceryl monostearate was selected as the best formula with the appropriate characteristics for transdermal delivery.

Key words: Bitter melon, Charantin, *Momordica charantia* Linn, Solid lipid nanoparticles.

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INTRODUCTION

Bitter melon is a tropical plant used for traditional medicine. Bitter melon has several activities as antioxidants, antimicrobials, antiviral, antihepatotoxic, anti-ulcer, anti-inflammatory, anti-tumor, hypocholesterolaemia, and antidiabetic. It has used for antidiabetic treatment in Asia, India, Africa, United States, Mexico, and the Caribbean.[1,2]

Bitter melon has several major constituents such as triterpenoids, saponins, polypeptides, flavonoids, alkaloids and sterols. One of the main active compounds in bitter melon (*Momordica charantia* Linn.), which is widely researched and believed to have antihyperglycemic activity, is the compound charantin. Charantin is a steroid glycoside (steroid saponin) group consisting of a mixture of stigmasterol and β-sitosterol glycosides in a ratio of 1:1.[3] Charantin extracted by alcohol, is a hypoglycaemic agent composed of mixed steroids that is more potent that the drug tolbutamide.[4] Charantin is a steroid glycoside group that is easily degraded in the digestive tract when giving orally.[5] The glycoside bonds between aglycones and glycones can be degraded by acids, enzymes and under alkaline conditions.[6-8] However, Harinantenaina and colleagues (2006) reported that aglycones from charantin do not have a significant effect on blood glucose levels. It shows that the hypoglycaemic effect occurs because of steroid glycosides of charantin.[6,9] Thus, it is necessary to find other alternative routes to solve the problem.

Transdermal drug delivery system is the delivery system of therapeutic agents through the skin into the blood stream for systemic effect. The transdermal route has several advantages, which is to avoid the degradation of drugs by acids and enzymes in the digestive tract. To be able penetrate, the active substance must have a molecular weight of less than 500 Da.[10] However, charantin has a molecular weight of 1151.68 g/mol, so modification in size and lipophilicity is needed.

The problem of drug penetration can be overcome with formulating the drug into solid lipid nanoparticles (SLN). The development of SLN system was carried out to increase loading capacity, solubility and stability. SLN is an alternative carrier system can use increase the bioavailability of drug. It is expected that bitter melon fraction loaded SLN has the shape of the particle size, large surface area, high loading dose of interaction at the interface, and the potential to improve the pharmaceutical performance. Drug delivery system via topical route is an option for using drugs that are local and faster effects because of direct application to target.[11-13]

The objective of this study was to develop SLN containing bitter melon fraction with appropriate characteristics for transdermal delivery. SLNs were prepared in various formulas. The produced solid lipid nanoparticles were characterized including morphology, particle size distribution, polydispersity index, zeta potential, entrapment efficiency and stability study.

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MATERIALS AND METHODS

Materials

The dried simplicia of bitter melon fruits were obtained from the Research Institute of Spices, and Medicinal Plants (Bogor, West Java, Indonesia), and simplicia was determined at the Conservation Center of Bogor Botanical Gardens as well as Indonesian Institute of Sciences (Bogor, West Java, Indonesia), charantin (Chromadex, USA), dichloromethane (Merck, Jerman), methanol (Merck, Jerman), 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM]BF_4, glyceryl monostearate, capric caprylic triglyceride, Tween 80, Span 80, aqua demineralized (Brataco, Indonesia).

Extraction dan Fractionation

Bitter melon extract was obtained by ultrasound-assisted extraction (UAE) with a ratio of bitter melon dried powder to solvent of [BMIM]BF_4 was 1 to 10 for 47 minutes. After filtering the extract, the liquid extract of bitter melon was fractionated using a separating funnel with dichloromethane (DCM) and water as a solvent. The liquid extract of bitter melon was salting out with potassium hydrogen phosphate and shake for 5 minutes. Subsequently, water and DCM were added. The ratio between liquid extract and solvent was 1 to 1. Then, the mixture was put in for 10 minutes and allowed to stand until it formed two layers, a layer of water and dichloromethane. The dichloromethane layer was collected and evaporated using a water bath to obtain the dried bitter melon fraction (BMF).[14]

Charantin identification in a bitter melon fraction

Charantin was analyzed by reversed phase high performance liquid chromatography (HPLC) method. Analysis was performed on HPLC instrument with a C-18 column (250mm×4.6mm, 5µm), with a maximum wavelength (λmax) of 204 nm, a flow rate of 1 mL/min, and an injection volume of 20µL with a mobile phase of methanol 100%.[6,15] A calibration curve of standard solution, charantin was prepared in methanol medium. Twenty µL of standard solution with a concentration of 200 ppm was injected to find out the chromatogram of charantin. Bitter melon fraction as a sample with a concentration of 200 ppm in methanol medium was injected, and charantin content was analyzed. Analysis was carried out three times.[5,14]

Formulation of bitter melon fraction loaded Solid lipid nanoparticles

Solid lipid nanoparticles formulation was adapted from Wang et al. (2018) with modification and formulation is displayed in Table 1.

BMF-loaded SLN were prepared by high-shear homogenization and ultrasonication method. Glyceryl monostearate, Span 80, and capric caprylic triglyceride were added to a water bath at 80°C to form a lipid phase (homogeneous solution). The bitter melon fraction was put into the lipid phase at 50°C and homogenized. Tween 80 was dissolved in aqua demineralized for the aqueous phase and heated at 80°C in a water bath. Then, the lipid phase was put into the aqueous phase and homogenized with a homogenizer for 15 minutes at 50°C with speeds of 12,000 rpm to form an emulsion. The next step was particle size reduction with an ultrasonication probe for 10 minutes with a 55% amplitude with a vibration interval 10 seconds. Furthermore, the SLN was froze at -86°C for 4 hours. SLN was lyophilized for 48 hours and stored at 7°C.[16-18]

Solid lipid nanoparticles characterizations

Morphology of BMF loaded SLN

SLN morphology was observed using transmission electron microscopy (TEM, Microscope Tecnai 200 kV D2360 SuperTwin, Thermo Fisher Scientific, USA). A drop of SLN dispersion in water was placed onto the carbon-coated copper grid, dried at room temperature, then coloured using 1% solution of phosphotungstic acid. After drying, the sample was seen under a microscope with various magnifications.[12,16,19-20]

Particle size distribution, polydispersity index and zeta potential

Particle size distribution, polydispersity index and zeta potential of BMF loaded SLN were measured by Malvern Zetasizer with the dynamic light scattering (DLS) technique. One drop of SLN dispersion was dispersed by 10 ml of distilled water, one ml sample putting it in a cuvette. Sample measurements were carried out three times.[19-20]

Entrapment efficiency

The entrapment efficiency of the obtained SLN was determined by indirect method. The amount of charantin in BMF entrapped SLN was determined by HPLC. One millilitre of SLN dispersion was dissolved with 2 mL of methanol with ratio 1:2, then sonicated for 20 minutes and vortexed for 2 minutes. This solution was analysed as total charantin in SLN. The free charantin in the supernatant (1 mL) was analysed after centrifuged SLN dispersion at 6,000 rpm for 1 hour and 0.5 mL of supernatant was diluted with methanol ad 2 mL, sonicated for 20 minutes and vortexed for 2 minutes. The entrapment efficiency was calculated as the following formula:

Entrapment efficiency (%) = (Total charantin- Free charantin)/ (Total charantin) x 100

Stability study of bitter melon fraction loaded solid lipid nanoparticles

Stability evaluation of bitter melon fraction loaded solid lipid nanoparticles were performed by storing the suspension at high (40°±2°C), room (25°±2°C) and low (7°±2°C) temperature for 12 weeks. The appearances, pH, particle size distribution, zeta potential and charantin content were evaluated properly.

Table 1: Formulas of bitter melon fraction loaded solid lipid nanoparticles.

Materials	F1	F2	F3	F4
BMF to lipid	1:10	1:10	1:10	1:10
BMF to surfactant	1:12	1:12	1:9	1:9
Capric caprylic triglyceride to Glyceryl monostearate	1:2	1:1	1:2	1:1
BMF	0.5 g	0.5 g	0.5 g	0.5 g
Capric caprylic triglyceride	1.7 g	2.5 g	1.7 g	2.5 g
Glyceryl monostearate	3.3 g	2.5 g	3.3 g	2.5 g
Tween 80	2 g	2 g	1.5 g	1.5 g
Span 80	4 g	4 g	3 g	3 g
Aqua demineralized	90 mL	90 mL	90 mL	90mL

BMF: Bitter melon fraction

RESULTS AND DISCUSSION

Extraction and fractionation of bitter melon

Extraction of bitter melon fruits using UAE method with ionic liquid [BMIM]BF_4 solvent. IL-UAE was chosen because it has many advantages, it was very easy to perform, does not require complicated equipment, significantly reduces extraction time, and reduces solvent consumption. [BMIM]BF_4 was chosen because it contains the BMIM cation, which is commonly used for the extraction of semipolar and nonpolar compounds. BF_4 anion is amphiphilic, which can used to attract polar or nonpolar compounds. It makes possible for [BMIM] BF_4 to extract charantin from bitter melon fruits because charantin consists of aglycone parts that tend to dissolve in nonpolar solvents and glycones, which make it slightly soluble in polar solvents.[5-6,21-22]

The fractionation process of the bitter melon extract was done by the liquid-liquid partition method. Dichloromethane solvent was used to fractionated bitter melon extract because charantin has high solubility on semipolar solvents like dichloromethane and chloroform.[23]

Characterization of bitter melon fraction

Bitter melon fraction had brownish dry powder and a specific smell. HPLC analysis of BMF reveals that markers were present in this fraction with a retention time of charantin at 6.340 min. The retention time on charantin standard solution at 6.607 min. Based on analysis, the area obtained was then calculated based on calibration curve, the content of charantin in bitter melon fraction was 2.36 ± 0.62% (w/w). The yield of the dichloromethane fraction was 79.24%.

Formulation of bitter melon fraction loaded solid lipid nanoparticles

In this study, the BMF loaded SLN were prepared by high-shear homogenization, then followed by ultrasonication method. The principle of this method is to mix melted lipids containing the active substance with the surfactant solution phase, using high-speed mechanical stirring.[24] This method was easiest, does not require large amounts of surfactants and does not require organic solvents in the manufacturing process. The SLN emulsion which formed was a white colloid solution like milk, with a specific smell. The drying process of SLN was conducted by freeze-drying method to produce a more practical dosage form for storage and packaging. After drying for 48 hours, the resulting is a slightly yellowish-white solid, slightly wet and stored in a tightly closed container.[24-25]. Lyophilization by freeze-drying was used to prevent hydrolysis reactions.[26]

Morphology of solid lipid nanoparticles

TEM microphotographs of BMF loaded SLN are presented in Figure 1, which appeared a spherical shape and the particle size was in nanometric range.

Particle size distribution, polydispersity index and zeta potential

Particle size, polydispersity index (PDI) and zeta potential of BMF loaded SLN were summarized in Table 2, and particle size distribution of BMF loaded SLN was presented in Figure 2.

Table 2 shows that particle size of BMF loaded SLN varies between 98-134 nm. With this size, BMF loaded SLN corresponds to the particle size for transdermal delivery. In addition, a low PDI value of less than 0.5 could be mean that all formulas are sufficiently monodisperse. PDI is dimensionless with the numerical value of PDI ranges from 0.0 (for a perfectly uniform sample with respect to the particle size) to 1.0 (for a highly polydisperse sample with multiple particle size populations). In drug delivery applications using lipid-based carriers, a PDI of 0.3 and below is considered to be acceptable and indicates a homogenous population of phospholipid vesicles.[27]

The particle size in the dispersion system can influence by the zeta potential (ZP) value. The zeta potential describes the properties of the electrostatic potential near the surface of a particle. Zeta potential was one of the important parameters that affect stability of dispersion because, from ZP value, it could be seen whether a suspension undergoes aggregation or flocculation. A break will become unstable, and aggregates will soon form if zeta potential was not guarded above 25 mV (positive or negative). All BMF loaded SLN formula have a zeta potential value above -25 mV, which ranges from -35 to -40 mV, it can predict that BMF loaded SLN will tend to be stable. The ZP values from the smallest to the largest are F3> F1> F4> F2, with a ZP value that is greater, F3 will be relatively more stable than the F1, F2, and F4 formulas.[24,28]

Entrapment efficiency

Table 2 shows that F1 has the highest entrapment efficiency value, 82.96%, which means 82.96% of charantin in bitter melon fraction was encapsulated by SLN. The increase in the lipid phase of the drug will increase the absorption efficiency value because of the large number of lipids in trapping the drug. Increasing the lipid to drug ratio can provide more expansive space for the drug can encapsulate with the addition of surfactants in the aqueous phase. Increasing tween 80 increases the thickness of the hydrophilic layer on the surface solid so that more drugs are dispersed on it.[28-30]

Stability study of solid lipid nanoparticles

The stability test of BMF loaded SLN was carried out on the four formulas stored at 7°C, 25°C and 40°C. In addition, a stability test was carried out on SLN that had been freeze-dried. Based on organoleptic observation, all formulas BMF loaded SLN did not change color significantly.

Based on pH measurement using pH meter, every formula gave pH value between 5.8 – 6.05. Every formula gave pH that was higher at week 0 than the other weeks. At weeks 4, 8 and 12, there was a decrease

Table 2: BMF loaded SLN characteristics.

Parameter	Solid lipid nanoparticles formula			
	F1	F2	F3	F4
Morphology	Spherical	Spherical	Spherical	Spherical
Z-average (nm)	98.39 ± 1.98	134.80 ± 3.46	106.16 ± 3.30	121.26± 1.35
Dv_{mean} (nm)	85.32 ± 8.87	96.06 ± 24.72	95.11 ± 43.70	116.92 ± 41.76
Dv_{10} (nm)	44.77 ± 2.01	49.53 ± 2.69	26.33 ± 16.78	53.03 ± 3.83
Dv_{50} (nm)	84.83 ± 3.16	122.43 ± 7.08	48.4 ± 39	113.77 ± 10.10
Dv_{90} (nm)	150.67 ± 6.42	240.67 ± 57.49	124 ± 20.22	218 ± 10.53
PDI	0.26 ± 0.01	0.42 ± 0.01	0.26 ± 0.03	0.23 ± 0.01
Zeta potential	-39.53 ± 0.15	-35.4 ± 2.16	-40.13± 1.15	-37.70 ± 1.13
Entrapment efficiency (%)	82.96 ± 1.42	77.83 ± 0.07	78.45 ± 0.68	70.91 ± 1.90

All value was represented as the mean ± SD (n = 3)

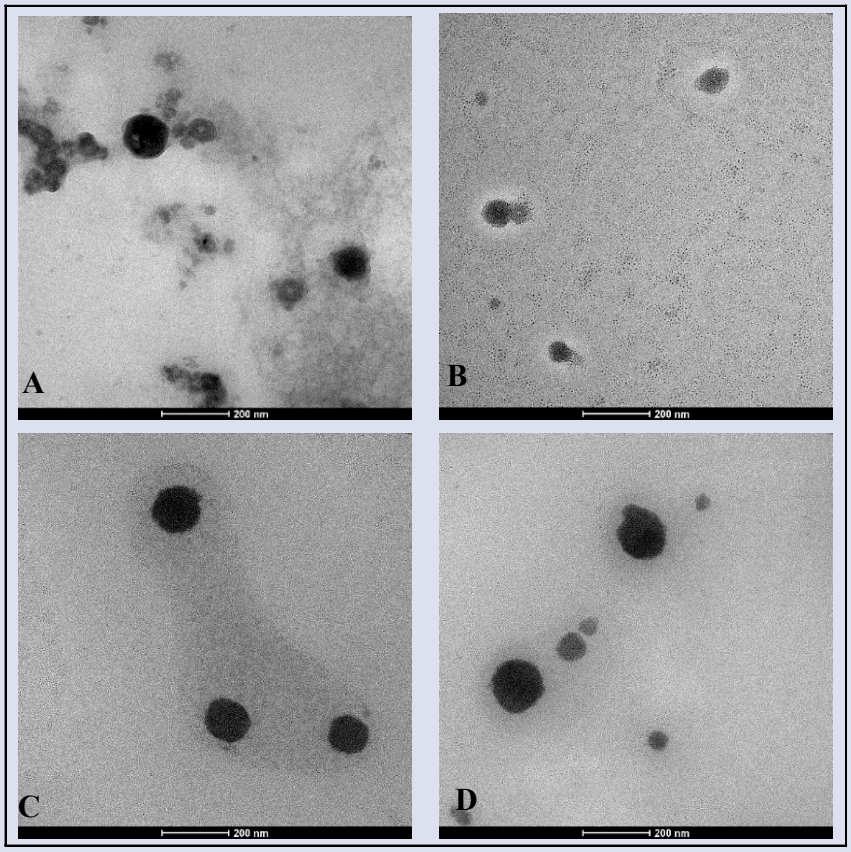

Figure 1: TEM micrographs of BMF loaded SLN at 38000 magnifications, (A) F1, (B) F2, (C) F3 and (D) F4.

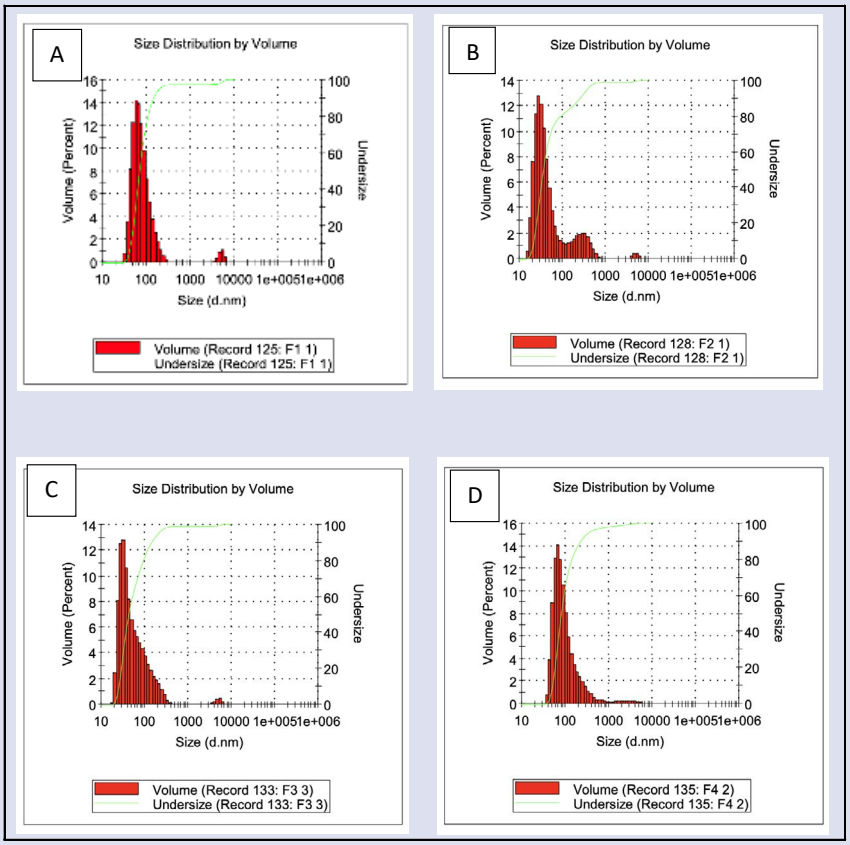

Figure 2: Particle size distribution of BMF loaded SLN of (A) F1; (B) F2; (C) F3 and (D) F4.

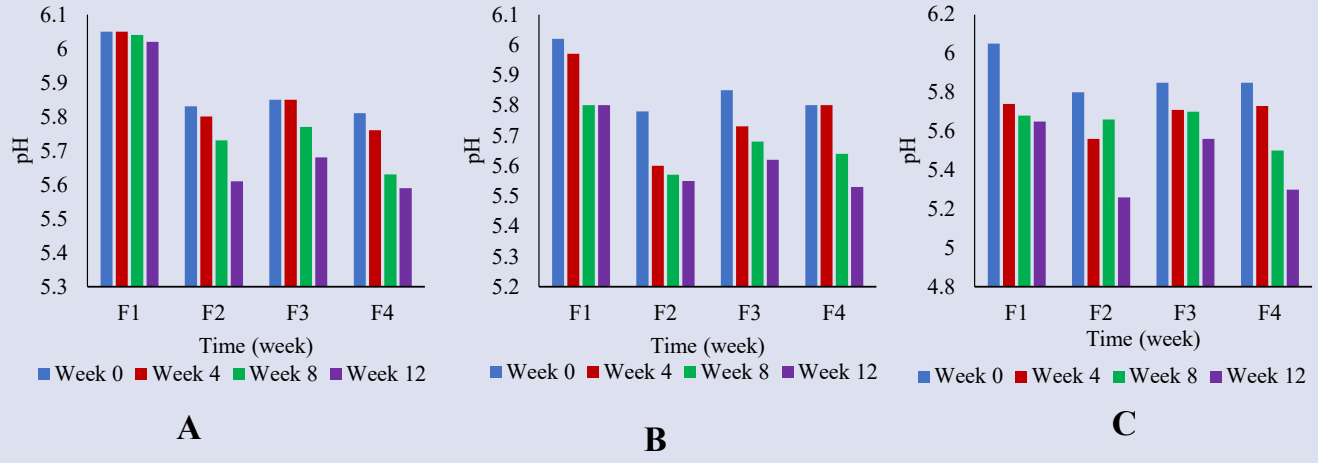

Figure 3: pH value of BMF loaded SLN under storage (A) 7°C; (B) 25°C; (C) 40°C for 12 weeks.

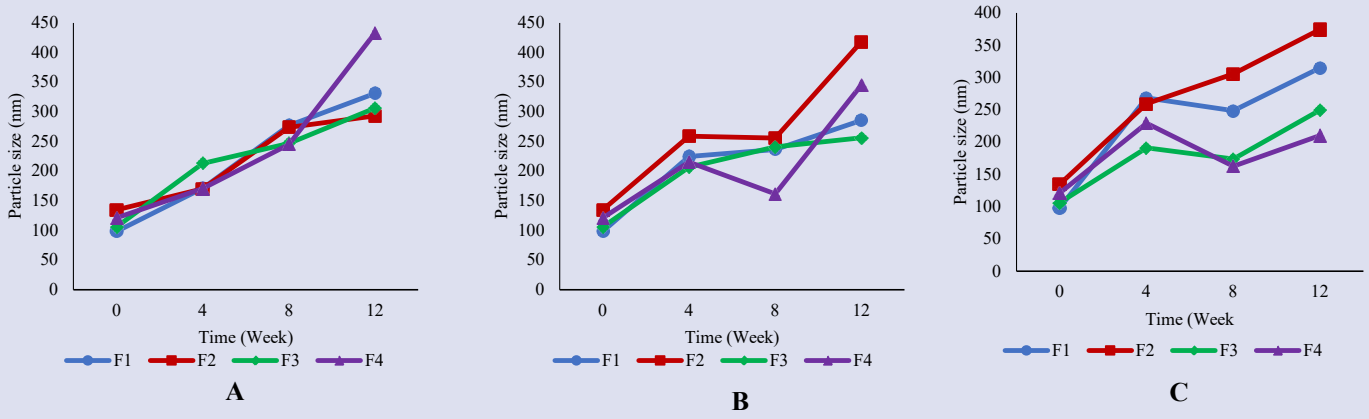

Figure 4: Particle size of BMF loaded SLN under storage (A) 7°C; (B) 25°C; (C) 40°C for 12 weeks.

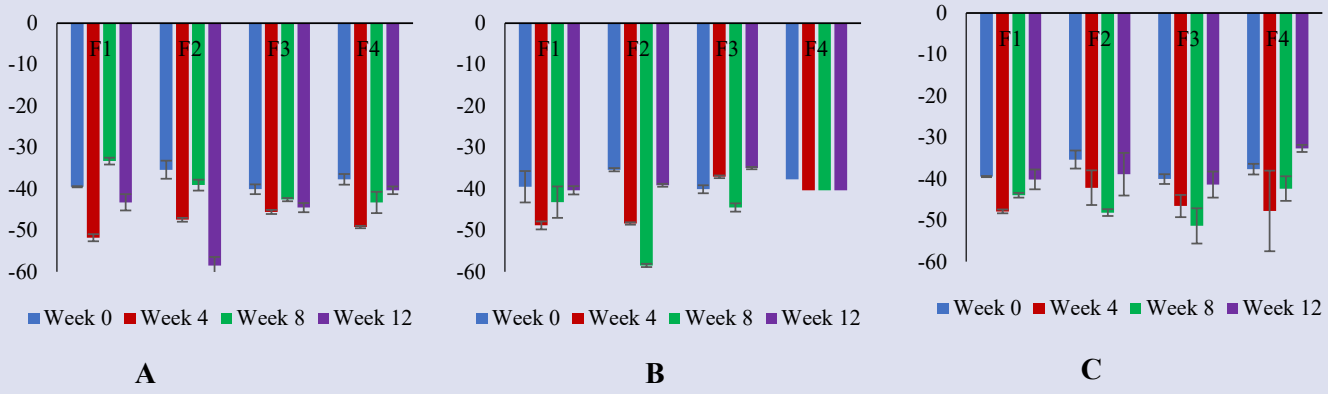

Figure 5: Zeta potential of BMF loaded SLN under storage (A) 7°C; (B) 25°C; (C) 40°C for 12 weeks.

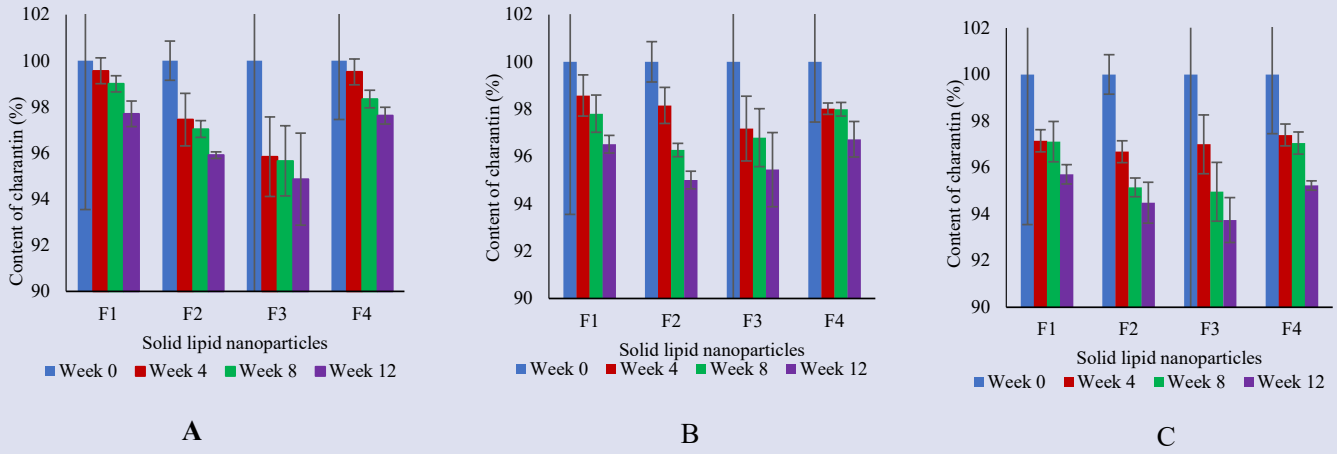

Figure 6: Charantin content of BMF loaded SLN under storage (A) 7°C; (B) 25°C; (C) 40°C for 12 weeks.

in pH at low temperature, room temperature and high temperature but it was still within the skin pH range with a pH value range of 5.26-5.80. It was caused by the oxidation of fraction that increased the acidity.[14] The decrease in pH can also be caused by temperature, the higher the storage temperature, the lower the pH of the preparation. The higher the weather, the more collisions between particles will increase, which causes the ability of water to ionize and form more hydrogen.[14] Therefore, the pH decreases. The results showed that pH value of BMF loaded SLN as shown in Figure 3.

The stability of particle size and zeta potential for 12 weeks are presented in Figure 4 and 5, respectively. Zeta potential is a measure of the magnitude of the electrostatic or charges repulsion or attraction between particles in a liquid suspension. Zeta potential is a fundamental parameter for describing the stability of a dispersion system, as it provides detailed insight into the causes of dispersion, aggregation, or flocculation. A sample can be said to be stable if it has a value of zeta potential is more positive than of +30 mV or more negative than -30 mV. The results showed that the zeta potentials of all formulas were negative charge with the range of 32.77 mV to 41.4 mV, which indicated an excellent particle stability.[31]

The particle stability of BMF loaded SLN was also measured. The results showed that the particle size of SLNs F1, F2, F3 and F4 were increased to the range of 209 nm - 432 nm after 12 weeks storage. However, the particle sizes were still in the range of satisfactory values of particle size.

The increase in particle size during storage is likely due to the transformation of lipid melt to lipid crystals, and lyophilization changes the properties of the surfactant layer due to removal of water and increases the particle concentration which favor particle aggregation.[24]

Charantin content in BMF loaded SLN was also measured for the stability study. The result showed a decrease in charantin content in BMF loaded SLN for 12 weeks of storage in Figure 6. The percentages of charantin contents in BMF loaded SLN F1-F4 for 12 weeks storage at 7°±2°C, 25°±2°C and 40°±2°C were 94.87-97.90%, 95.01-96.73% and 93.74-95.71%, respectively. Although there was a decrease in charantin content during storage for 12 weeks, they still met a content requirement, which is more than 90% content. It was caused by oxidation of sterol on charantin structure. Interactions between lipid in BMF loaded SLN formulas and temperature made a drastic effect on total content charantin oxides formed and on the reaction pathways of oxidation. The oxidation of charantin was slower when the storage temperature was lower.[32]

CONCLUSION

The BMF loaded SLN F1 with the 1:12 ratio of BMF to surfactant and the 1:2 ratio of capric caprylic triglyceride to glyceryl monostearate was selected as the optimal formula, which had good characteristics as SLN in terms of particle shape and size, zeta potential, polydispersity index, and entrapment efficiency. Moreover, the BMF loaded SLN F1 had an acceptable stability, which the charantin content in the SLN was 96.52% after 3 months storage at 25°C ± 2°C.

These results indicate that the BMF loaded SLN F1 has potential applicability for transdermal delivery.

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CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest regarding the publication of this article.

ABBREVIATIONS

BMF: Bitter melon fraction; DCM: Dichloromethane; DLS: Dynamic light scattering; HPLC: High performance liquid chromatography; IL: Ionic liquid; PDI: Polydispersity index; SLN: Solid lipid nanoparticles; TEM: Transmission electron microscopy; UAE: Ultrasound-assisted extraction; ZP: Zeta potential.

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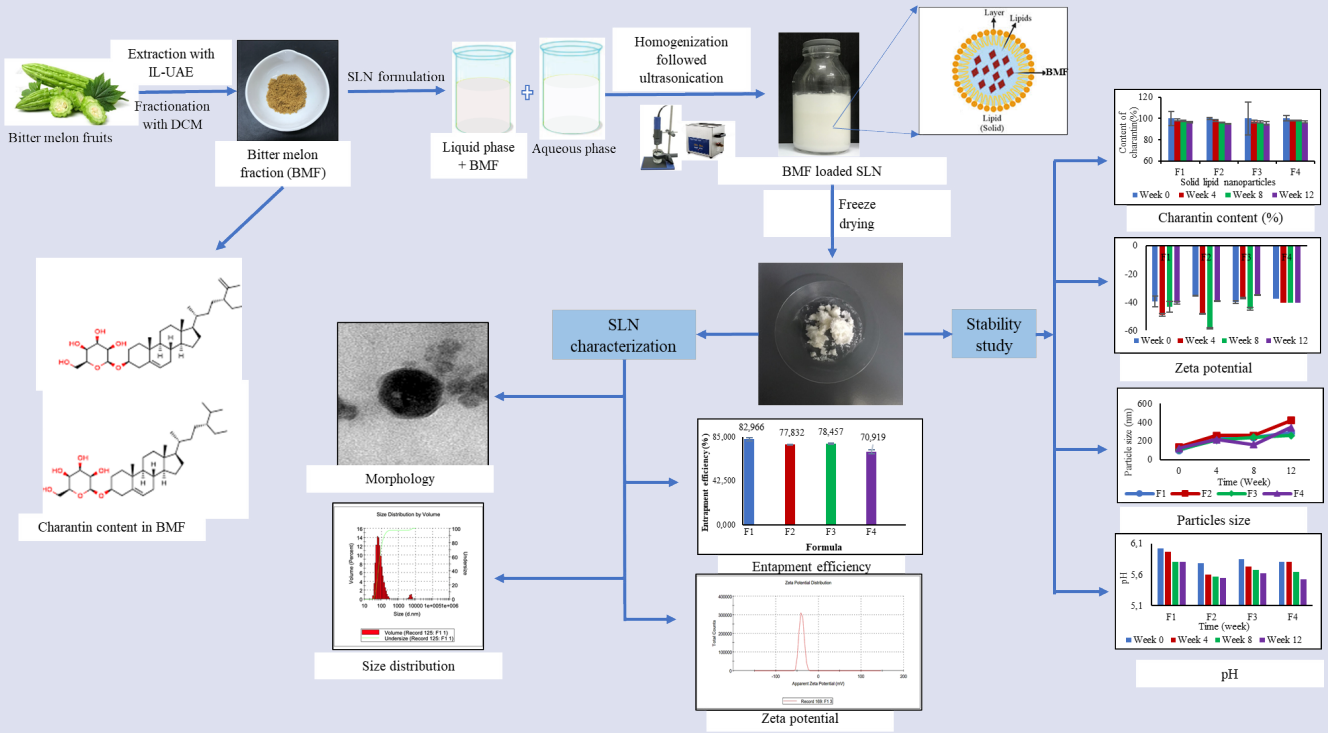
GRAPHICAL ABSTRACT



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