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Preparation of *Zingiber Officinale* Extract Loaded Solid Lipid Nanoparticles

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Abstract. Solid lipid nanoparticles (SLNs) loaded ginger extract were prepared by microemulsion technique. The nanoparticles were composed of stearic acid as solid lipids, Cremophor RH 40 as surfactant and ethanol as co-surfactant. It was found that variation in the amount of surfactant and co-surfactant had profound effects on the mean particle size, the drug entrapment efficiency and loading capacity. Transmission electron microscope (TEM) revealed the spherical nature of the particles. The mean particle size of SLNs ranging between 453.1 and 551.7 nm were measured by dynamic light scattering (DLS). The entrapment efficiency (EE) and drug loading capacity (LC) determined by high performance liquid chromatography (HPLC) found to be in the range of 85.23–90.07% and 1.41–1.49%, respectively.

Introduction

Solid lipid nanoparticles (SLNs) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. SLNs are produced by replacing the liquid lipid (oil) of the emulsions by a solid lipid, which means lipids being solid at room temperature and also at body temperature. During the past several years, SLNs were used in topical formulations, not only for pharmaceutical but also for cosmetic products [1]. Compared with innovative carrier systems, such as liposomes and nanoemulsions, SLNs combined their advantages, such as protection of incorporated compound against chemical degradation, possibility of controlled drug release [2,3], occlusion effect, favor drug penetration into the skins and drug targeting, UV blocker [1,4], high drug payload, incorporation of lipophilic and hydrophilic drugs feasible, no biotoxicity of the carrier, avoidance of organic solvent and no problems with respect to large scale production [2].

Recently, the research activities on SLNs has gradually focused on the cosmetic and topical product. SLNs as a topical carrier were used for topical delivery of several drugs, e.g. isotretinoin [4] vitamin A [5] curcuminoids [6] and tea polyphenols [7]. Ginger extract as one of the best recognized antioxidant and anti-inflammatory [8], has gained special interest in cosmetic products. Ginger extract was isolated from the rhizome of *Zingiber officinale* Roscoe. Ginger mainly contains essential oil and oleoresin. Oleoresin is the non-volatile pungent components. It's major component is gingerols, mainly 6-gingerol. However, the gingerols are unstable in the presence of light, heat and air, so incorporation of such active ingredient in SLNs can overcome the chemical stability.

This study focused on the preparation in the ginger extract-loaded SLNs by a microemulsion technique and the characterization of the ginger extract-loaded SLNs. The effect of surfactant and co-surfactant concentration on characteristics of ginger extract-loaded SLNs such as mean particle size, size distribution, entrapment efficiency, loading capacity, physical and chemical stability were investigated.

Experimental procedure

Ginger extract was prepared by maceration method. The fresh ginger was washed by water, sliced and dried in oven at 45 °C for 48 hrs. 100 grams of the dried ginger was minced by the blender and extracted by 500 mL of acetone in 1000 mL erlenmeyer flask, then stirred at 500 rpm on a magnetic stirrer at room temperature for 8 hrs, left to stand overnight at room temperature. After maceration, two flasks were collected and mixed together. The ginger extract was separated from solid plant by filter paper (Whatman no.1). The solvent was evaporated by rotary evaporator at 35 °C. Ginger extract was kept at - 20 °C until used. Ginger extract-loaded SLNs were prepared by a microemulsion technique. The formulations were shown in Table 1. Briefly, stearic acid was melted at 70 °C then ginger extract was dissolved in the melted lipid. A warm water solution of Cremophor RH 40 and ethanol was, then, added to the melted lipid. The warm microemulsion was immediately dispersed in cold water at 2 °C (warm microemulsion:cold water ratio of 1:20) under high-speed homogenization at 4000 rpm for 15 min. The mean particle size and size distribution of ginger extract-loaded SLNs were measured by dynamic light scattering (DLS) at 165 ° angle and 25 °C using a Delsa^{TM} Nano C particle analyzer. The measurement was repeated three times for each sample. The entrapment efficiency and loading capacity of ginger extract-loaded SLNs were directly determined by ultrafiltration method using centrifugal filter tubes with a 10 kDa molecular weight cut-off. Briefly, 2 mL of ginger extract-loaded SLNs dispersion was placed into a centrifugal filter tube which was centrifuged at 10,000 rpm for 1 h at 25 °C. The 6-gingerol is a major component in oleoresin, therefore it was used as a marker indicating an existence of the extract in SLNs. The amount of free 6-gingerol in the aqueous phase after isolation of the system was detected by HPLC. The amount of incorporated drug was calculated as a result of the initial drug minus free drug. The entrapment efficiency and loading capacity of 6-gingerol in SLNs were calculated according to the following equations :

$$EE = \frac{W_{initial\ drug} - W_{free\ drug}}{W_{initial\ drug}} \times 100 \quad (1)$$

$$LC = \frac{W_{initial\ drug} - W_{free\ drug}}{W_{lipid}} \times 100 \quad (2)$$

where $W_{initial\ drug}$ is the amount of drug used for the assay and the $W_{free\ drug}$ is the amount of free drug detected in the aqueous phase after isolation of the dispersion. The W_{lipid} is the total weight of the lipid in the formulation. The morphology of the ginger extract-loaded SLNs were observed under transmission electron microscopy (TEM). SLNs dispersions were placed on copper grid and dried at room temperature. Then, the sample was negatively stained with a 2% solution phosphotungstic acid and examined using TEM. All dispersion samples were kept in well-closed amber glass vials for stability study. Samples for physical stability of ginger extract-loaded SLNs were stored at 4 °C for 3 months then mean particle size and size distribution were determined. To study chemical stability, samples were stored at 4 °C and 30±2 °C for 3 months then 6-gingerol entrapment efficiency was determined. Statistical data were analyzed using the pared t-test with $p<0.01$ as the minimal level of significance.

Table 1 Composition of ginger extract-loaded SLNs (value given as %w/w)

Formulation code	Stearic acid	Cremophor RH 40	Ethanol	Ginger extract	Water add
A	10	15	15	0.7	100
B	10	20	20	0.7	100
C	10	25	25	0.7	100

Results and discussion

Measurement of mean particle size and size distribution. The mean particle size and size distribution of the ginger extract-loaded SLNs were determined by DLS. As shown in Table 2, the results showed that when increasing surfactant and co-surfactant concentration from 15% to 25% (w/w), the mean particle size decreased from 551.7 to 453.1 nm, respectively. The increasing of

surfactants content in SLNs formulations could reduce the interfacial tension between lipid matrix and dispersion medium (aqueous phase), consequently favor the formation of SLNs with smaller particle size [3]. The size distribution or polydispersity index (PI) was also considered when evaluating the nanoparticles. Low PI values are desirable whereby high PI usually underlines broader particle size distribution. In the present study, it was found that the PI of the SLNs ranged from 0.15 to 0.33 (Table 2). The observed low PI (<0.35) values for the nanoparticles indicate mono-modal and narrow size distribution, suggesting nanoparticle monodispersity.

Table 2 The effect of surfactant and co-surfactant concentration on characteristics of ginger extract-loaded SLNs (mean ± SD, n=3)

Formula	Surfactant	Co-surfactant	Characteristics			
	Cremophor RH 40	Ethanol	Size (nm)	Polydispersity Index (PI)	Entrapment efficiency (EE%)	Loading capacity (LC%)
A	15	15	551.70 ± 13.64	0.30 ± 0.05	85.23 ± 0.14	1.41 ± 0.0058
B	20	20	466.00 ± 4.30	0.15 ± 0.01	88.14 ± 0.20	1.46 ± 0.0058
C	25	25	453.10 ± 2.93	0.33 ± 1.73	90.07 ± 0.59	1.49 ± 0.0082

Determination of encapsulation parameters. The 6-gingerol entrapment efficiency and loading capacity of ginger extract-loaded SLNs were shown in Table 2. The results showed that when increasing surfactant and co-surfactant concentration from 15% to 25% (w/w), the 6-gingerol entrapment efficiency increased from 85.23% to 90.07% and the loading capacity increased from 1.41% to1.49%, respectively. The surfactant and co-surfactant played the important roles to improve the 6-gingerol incorporation. This finding was in agreement with the previous work by Liu et al. who reported that increasing the surfactant concentration in formulation resulted in higher entrapment efficiency [4]. That is due to the high concentration of surfactant reduces the particle size, increases thickness of lipid matrix, disperse and dissolves more active ingredients, thus results in higher entrapment [9].

Morphology. The TEM image (Fig.1.) revealed that ginger extract-loaded SLNs had a spherical shape and that the particle size was in nanometric range.

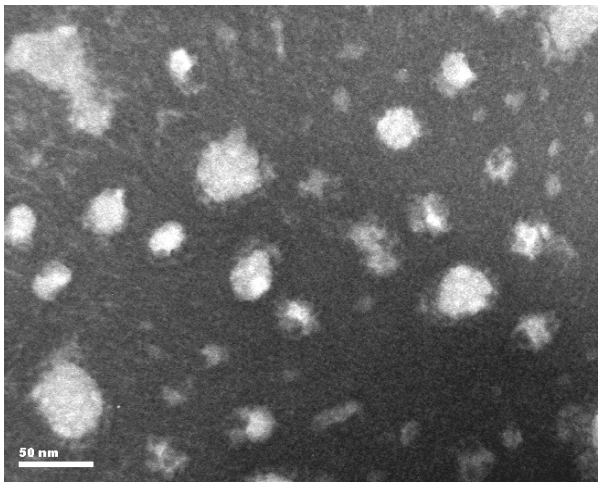

Fig.1. TEM image of ginger extract-loaded SLNs for formula B

Stability studies. The mean particle size and size distribution of ginger extract-loaded SLNs were evaluated by DLS before and after storage at 4 °C, protected from light for 3 months in order to determined physical stability of preparations. The mean particle size of all formulations did not change significantly within 3 months (p<0.01) (data not shown). Also, low PI values was observed with time. To study the effect of temperature on chemical stability of 6-gingerol, the SLNs

dispersions were kept separately at 4 °C and 30±2 °C, protected from light for 3 months. The 6-gingerol entrapment efficiency was evaluated by HPLC for freshly prepared samples and after 1, 2, and 3 months of storage. The 6-gingerol entrapment efficiency of all formulations did not significantly differ from those determined when the nanoparticles were freshly prepared ($p<0.01$) (data not shown). It was clearly concluded that the temperature did not have an effect on chemical stability of 6-gingerol encapsulated in SLNs.

Conclusions

Ginger extract-loaded SLNs were successfully prepared via a microemulsion technique. The amount of surfactant and co-surfactant were the crucial factors for the resulting mean particle size, the 6-gingerol entrapment efficiency and loading capacity. The mean particle size of ginger extract-loaded SLNs decreased when increasing the surfactant and co-surfactant concentration. On the other hand, the entrapment efficiency and drug loading capacity were increased. The best formulation of ginger extract-loaded SLNs consisted of 25% Cremophor RH 40 and 25% ethanol. Moreover, the SLNs formulations showed good physical and chemical stability up to 3 months.

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