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Preformulation, Characterization, and *In Vitro* Release Studies of Caffeine-Loaded Solid Lipid Nanoparticles

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Synopsis

Encapsulation of active agents in solid lipid nanoparticles (SLNs) is an alternative to other controlled release systems for topical delivery. In this study, caffeine was encapsulated in SLNs to produce a delivery system with controlled release. Caffeine-loaded SLNs (Caf-SLNs) were prepared using the double emulsion method with homogenization and ultrasonication. The characterization studies were performed using dynamic light scattering (DLS), zeta potential, scanning electron microscopy (SEM), and differential scanning calorimetry (DSC) analyses. The encapsulation efficiency tests were performed using UV spectrophotometry. *In vitro* release studies were conducted using a dialysis bag technique and high-performance liquid chromatography (HPLC) for the quantification of caffeine (Caf). The results from the DLS analysis showed that all formulations had a polydispersity index <0.3 with particle sizes <210 nm. The DSC and SEM results showed that Caf was dispersed in the SLNs. The encapsulation efficiency was 49.22%. The release studies indicated that after an initial burst at 3 min, the SLNs released Caf in a controlled manner over a 6-h period. Taken together, the SLNs can be used as a carrier for the topical delivery of Caf.

INTRODUCTION

Delivering active agents across the stratum corneum is difficult because of their physicochemical structures such as lipophilicity and molecular weight. Thus, scientists have focused on topical delivery systems to carry active agents with hydrophilic properties (partition coefficient values or LogP between one and four) and high molecular weights (>500 g/mol) (1,2). Caffeine (Caf) is a water-soluble active agent with low molecular weight (194.2 g/mol) and favorable LogP value (–0.07) (1). It is topically used for reduction of cellulite appearance and UVB-induced skin cancer treatment (3,4). Several groups have used colloidal particulate carrier systems for the topical delivery of Caf (5–7). Solid

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lipid nanoparticles (SLNs), a colloidal delivery system, consist of a high melting point solid lipid forming a lipid matrix surrounded with surfactant (5,8). SLNs have several advantages including high tolerability, rapid biodegradation, high bioavailability, high drug-loading capacity, good production scalability, and a lack of organic solvents in the preparation process. SLNs can also provide a controlled release profile for many active agents and increase the skin penetration rates of the active agents (3,8). Homogenization, solvent emulsification or evaporation, ultrasonication, and solvent diffusion methods are used to prepare SLNs (9).

The aim of the present study was to prepare SLNs containing Caf with controlled release and to evaluate the formulations with *in vitro* experiments.

MATERIALS AND METHODS

MATERIALS

Softisan 100® (Hydrogenated Coco-Glycerides) was obtained from Cremer Oleo GmbH & Co. KG (Hamburg, Germany). Tween 20® (Polysorbate 20), Span 20® (Sorbitan Laurate), and Caf were purchased from Merck KGaA (Darmstadt, Germany). All organic solvents and other chemicals were analytical grade and obtained from Merck KGaA.

PREPARATION METHOD FOR CAF-SLNS

Caf was dissolved in 80°C water. The aqueous solution was added into the oil phase which comprised a Softisan 100® and Span 20® mixture at the same temperature. It was mixed under constant stirring (27,000 rpm) with a Silent Crusher M homogenizer (Heidolph®, Schwabach Germany) at 80°C for 2 m to obtain a primary emulsion (w/o). Finally, Tween 20® dissolved in hot water (80°C) was slowly added into the emulsion with constant stirring (27,000 rpm) at 80°C for 10 m to produce a double emulsion (w/o/w). To decrease the particle size, the emulsion was sonicated using an Bandelin SonoPlus HD 2070 probe type sonicator (Bandelin®, Berlin, Germany) at 70% amplitude level for 2 min to obtain a nanoemulsion (6).

FREEZE-DRYING OF SLN DISPERSIONS

The nanoemulsions were frozen at −20°C for 5 h and lyophilized in a Alpha 2-4 LSCplus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 48 h. The SLNs were collected for use in differential scanning calorimetry (DSC) analyses (8).

PARTICLE SIZE AND ZETA POTENTIAL MEASUREMENTS

The mean diameter, polydispersity index (PI), and zeta potential of each sample were obtained using a Malvern Zetasizer Nano ZS (Malvern Instruments, U.K.) at 25°C. Before all measurements, SLNs were diluted with distilled water (10).

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

The nanostructure of the samples was observed using SEM (EVO 40 Carl Zeiss AG, Dresden, Germany). After drying the samples in the oven, they were coated with 15 mm gold-palladium and analyzed using SEM (11).

DIFFERENTIAL SCANNING CALORIMETRY (DSC) ANALYSIS

DSC (Setaram DSC 131 evo, Caluire, France) analysis was performed to study thermal behavior of the materials. Approximately 6 mg of sample was used for each measurement. The raw materials were analyzed at a speed of 5°C/min in a 20–100°C temperature range. Free caffeine, unloaded (without caffeine) SLNs, and Caf-SLNs were scanned at the same rate speed at a scanning temperature ranging from 20 to 300°C (6).

CAFFEINE ENCAPSULATION EFFICIENCY

Caf encapsulation efficiency was measured using an ultrafiltration method. Caf-SLNs (4 ml) were placed in the upper chamber of an ultracentrifuge tube (Amicon® Ultra-4, 10kDa, Merck Millipore, Darmstadt, Germany). The filtered phase containing Caf was freeze-dried and then dissolved in dichloromethane, which was analyzed for Caf amount by using UV spectrophotometry at 273 nm. A calibration curve was generated using six solutions with a concentration range of 10–100 mg/ml, and the correlation coefficient was 0.99 [6]. Caf loading efficiency was calculated using Equation 1 given by:

$$\text{Loading Efficiency (\%)} = \text{(Actual amount of Caffeine in the SLNs/Theoretical amount of Caffeine in the SLNs)} \times 100 \quad (1)$$

IN VITRO RELEASE STUDY

Caf release from nanoparticles was performed using the dialysis bag technique (12,13). The dialysis bags (MWCO: 3,5 kDa, Spectrum Laboratories, Inc., CA) were soaked and preconditioned before the experiment. The required amount of formulation (2 ml) was placed into the preconditioned dialysis bag. Then, the dialysis bag was put in 200 ml phosphate buffered saline (pH: 5.5) and incubated in a thermostatic reciprocating shaker maintained at 37 ± 0.5°C and continuously shaken at 100 rpm. An aliquot of 1 ml of release medium was withdrawn at predetermined time intervals (0.08, 0.25, 0.50, 1, 2, 3, 6, 12, and 24 h) and replaced immediately with the same volume of fresh medium to maintain the sink conditions. The concentration of Caf in the aliquot was quantified using high-performance liquid chromatography (HPLC).

HPLC ANALYSIS

A pharmacopeia method was used for the HPLC analysis of Caf during release studies (14). For this purpose, an Agilent 1260 HPLC system (Agilent Technologies, Waldbronn,

Germany) consisting of a G1311B model quaternary pump, a G1329B model auto injector, a G1316A model thermostat column compartment, and a G4212B model diode array detector (DAD) was used. The chromatograms were monitored and integrated using Agilent ChemStation software. Chromatographic separations of analytes were achieved on 5 µm Inertsil ODS-3 (4.6 × 250 mm) and the column was thermostated at 25 ± 1°C during analysis. The detection wavelength was 275 nm and injection volume was 20 ml. Elution of Caf from the column was performed isocratically with a mobile phase mixture of water:methanol (60:40) at a flow rate 1.0 ml/min.

DATA ANALYSIS

All experiments were repeated three times and the results were presented as mean ± SD. Release profiles were evaluated using different kinetic models including the zero order ($Qt = Q_0 + K_0t$), first order ($\ln Qt = \ln Q_0 + K_1t$), Higuchi ($Qt = KH_t^{1/2}$), Hixson–Crowell ($Q_0^{1/3} - Q_1^{1/3} = K_St$), and Korsmeyer–Peppas ($Qt/Q\infty = Kt^n$) models (15,16).

RESULTS AND DISCUSSION

In this study, Caf-SLNs were successfully prepared using double emulsion methods with the mixture of Softisan 100®, Span 20®, Caf, and Tween 20® (T20) at different ratios (Table I). The average particle size and size distribution of the formulations are shown in Table 2. Inclusion of Caf increased the particle size. The smallest average size (175.10 nm) was observed in the F4 formulation with 5.0% T20. According to several studies, SLNs with particle sizes ranging from ≈170 to 500 nm make a film on the skin and provide an occlusive effect, which can increase the penetration rate of active agents across the stratum corneum (5,17,18). In this study, because of the low particle size and occlusive property of the SLNs, it is thought that Caf can easily pass through the stratum corneum (5,17–20). The PI values of the formulations were lower than 0.3. Kazemi et al. reported that PI ≤ 0.3 indicates great homogenous distribution (9). The zeta potential of the formulations was positive and in the range 0 to + 2 mV. Although zeta potentials of the particles were very low, increasing the T20 concentration resulted in smaller particles with good PI values. However, this behavior was not linear and further increase of T20 above 5.0% in F5 and F6 decreased the particle size. Higher T20 concentrations limit the dispersal of the primary emulsion phase (w/o emulsion) in the external phase and the second emulsification process. These data clearly show that the T20 optimum concentration

Table I
Preparation of Caf-SLNs Formulations

Ingredients	F1 (Unloaded, %)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
Softisan 100®	3.00	3.00	3.00	3.00	3.00	3.00
Tween 20®	5.00	1.00	3.00	5.00	7.00	10.00
Span 20®	0.20	0.20	0.20	0.20	0.20	0.20
Caffeine	0.00	0.10	0.10	0.10	0.10	0.10
Water	91.80	95.70	93.70	91.70	89.70	86.70

Table II
Particle Size and PI (polydispersity index) Values of the Formulations

Formulation code	Particle size (nm)	PI
F1 (unloaded)	161.27 ± 2.00	0.24 ± 0.02
F2	195.52 ± 9.77	0.26 ± 0.06
F3	181.68 ± 3.14	0.25 ± 0.03
F4	175.10 ± 5.68	0.22 ± 0.05
F5	196.80 ± 11.47	0.23 ± 0.01
F6	209.43 ± 10.05	0.28 ± 0.14

was 5.0% (Figure 1). In addition, the SLNs were coated with the nonionic surfactant, which prevented their coalescence into larger particles (21). Shah et al. developed SLNs using T20 and reported that T20 coated the SLN surface and remained stable despite having a lower zeta potential (22).

Figure 2 shows SEM images of free Caf, Caf-SLNs, and unloaded SLNs. The particles were roughly spherical with a homogenous distribution confirming the size distribution results of the DLS measurements (Table 2). The crystallization of Caf-SLNs, unloaded SLNs, Caf, and Softisan 100® were analyzed using DSC. A DSC thermogram of Caf-SLNs, unloaded SLNs, Caf, and Softisan 100® is shown in Figure 3. The Caf at 236.76°C and Softisan 100® at 40.35°C peaks dissapeared in the Caf-SLNs thermogram at 38.44°C and there was a slight change between Caf-SLNs and unloaded SLNs (36.72°C) melting point peaks. The DSC analysis results show that Caf was introduced into the lipid matrix of the SLNs. In a previous study, Caf was found to be dissolved in Softisan 100®, similar to our results (6).

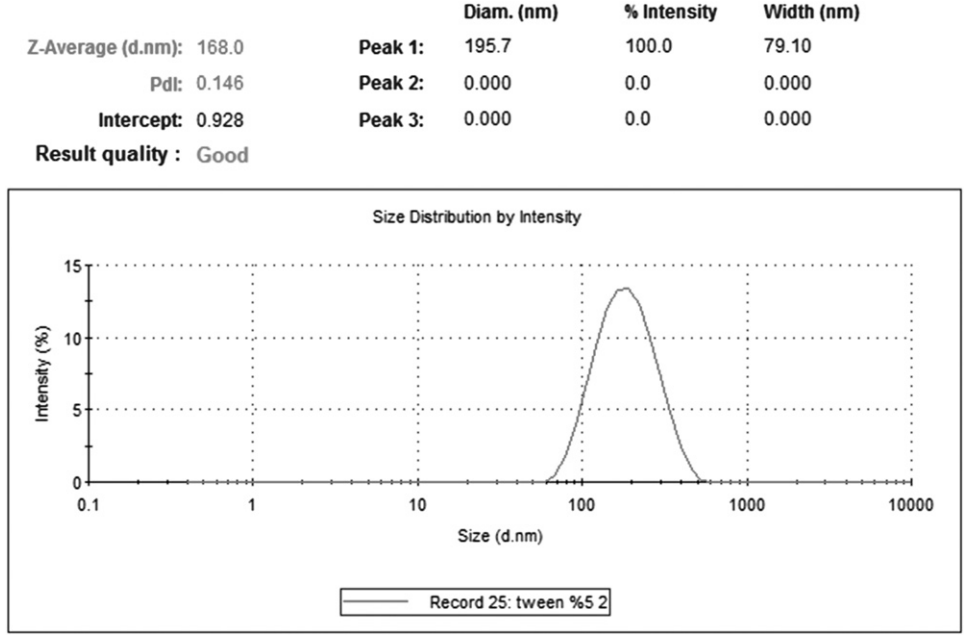

Figure 1. Particle size diagram of the F4 formulation.

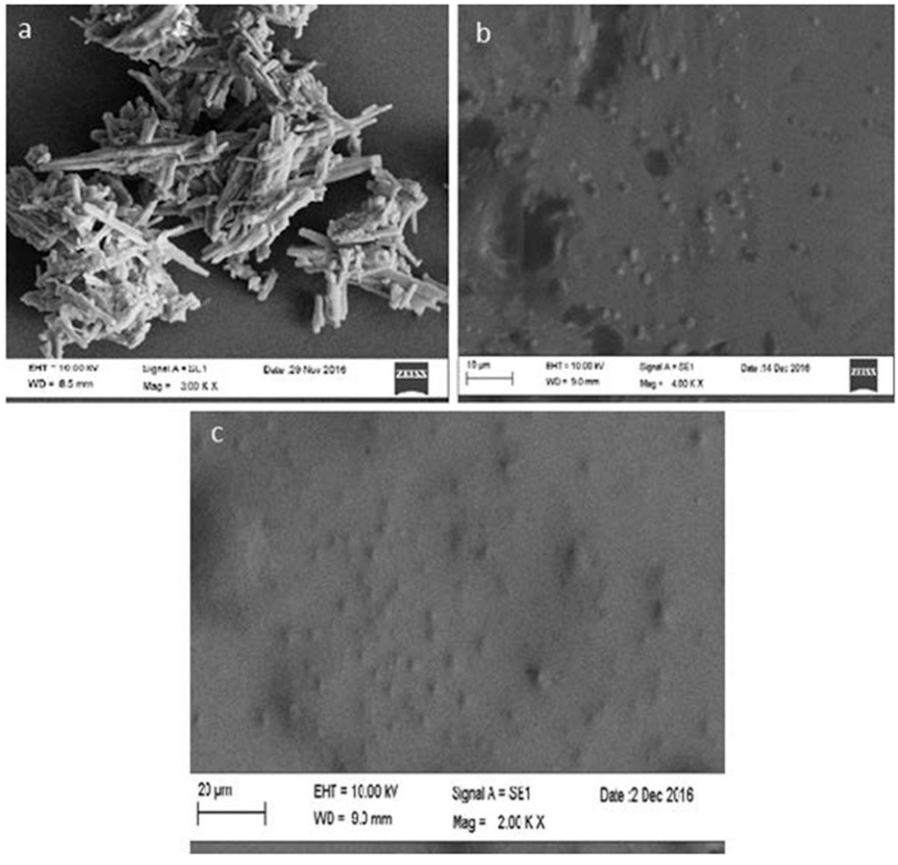

Figure 2. (A) SEM micrographs of caffeine, (B) caffeine loaded SLNs, and (C) unloaded SLNs.

At the end of the loading procedures, the encapsulation efficiency of the SLNs was 49.22%. Hydrophilic substances such as caffeine tend not to dissolve in the lipid matrix of SLNs. Therefore, such active agents are expected to be poorly encapsulated in SLNs (23). *In vitro* release studies were performed and Figure 4 shows the cumulative release profile of Caf from Caf-SLNs. The release profile demonstrated a two-step release behavior; an initial burst released without detectable lag time followed by a relatively slow release. The initial burst release in the first 5 min was nearly 20% of the total amount of Caf followed by a sustained release of the remaining Caf over nearly 6 h (Figure 4). The initial burst of Caf release from the Caf-SLNs may originate from the Caf molecules closest to the SLN surface. The sustained release phase may be related to the diffusion of Caf molecules from the lipid matrix. In addition, it may be because of the effect of decreasing Caf amounts. Such release behavior also has been observed in the release of Caf from niosomes by Khazaeli et al. (15). The release kinetics of the Caf-SLNs were evaluated using zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas equations. The results of the calculations indicated that the release profile of the Caf from Caf-SLNs best fit with the Korsmeyer–Peppas model release kinetics (Table 3). The n value was between 0.5 and 1.0, which indicated that the release mechanism was anomalous transport. The release kinetic model indicated both diffusion and erosion release patterns from polymeric

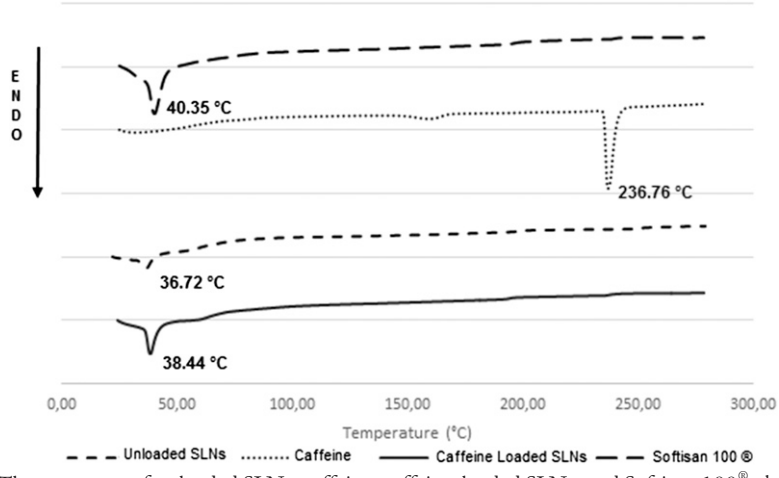

Figure 3. Thermograms of unloaded SLNs, caffeine, caffeine-loaded SLNs, and Softisan 100® obtained using DSC.

type carriers (24). Thus, it was named anomalous drug transport. The kinetic results show that Caf release was diffusion controlled and, because of lipid matrix degradation, lipid matrix erosion controlled.

CONCLUSIONS

A new delivery system using SLNs with Caf were successfully formulated and characterized for the first time. DLS and SEM measurements clearly indicated that Caf-SLNs had a spherical structure and nanometric particle size with good PI values. Caf was loaded into the SLNs with reasonable encapsulation efficiency. The release profile of Caf from the

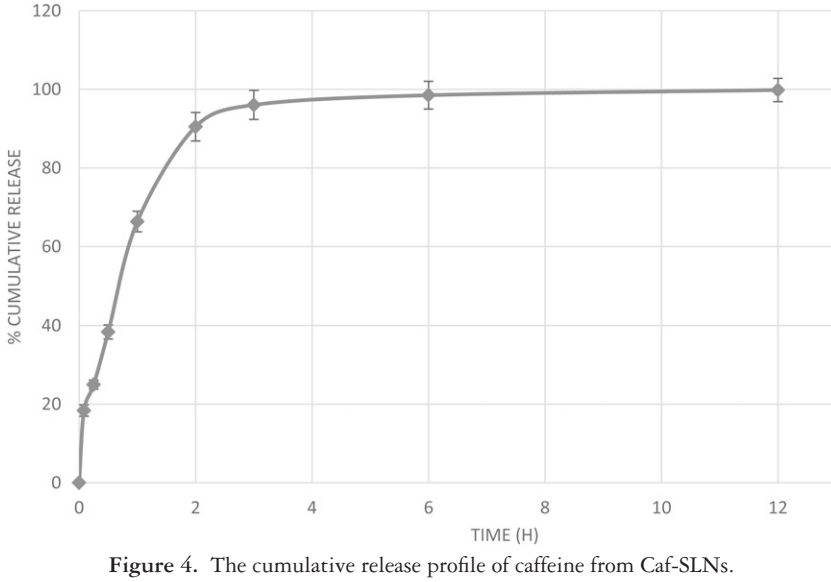

Figure 4. The cumulative release profile of caffeine from Caf-SLNs.

Table III
The Release Kinetics Results of Caf-SLNs

	Zero order	First order	Higuchi	Hixson–Crowell	Korsmeyer–Peppas
R	0.71	0.64	0.85	0.8584	0.97
R2	0.49	0.41	0.73	0.7369	0.95
m	5.95	0.05	27.38	0.3113	0.51 (n)
k	5.95	0.11	27.38	1.414	58.76

Caf-SLNs best fit the Korsmeyer-Peppas equation with the anomalous transport mechanism. The SLNs can be used potentially as a topical carrier for Caf with improved loading capacity and a controlled release profile.

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