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Formulation and Characterization of Surfactin-Containing Self-Microemulsifying Drug Delivery Systems (SF-SMEDDS)

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Introduction

Surfactin (SF), which has an amphiphilic and cyclic character with a molecular weight of 1036 Da, is a very powerful biosurfactant that is obtained from Bacillus Subtilis (Figure 1). Surfactin owes its amphiphilic character to the polar amino acid head group and the non-polar hydrocarbon chain in its structure. Its activity is not only to lower the surface tension; SF also plays an important role in biological activities, such as antimicrobial[1], antibacterial[2], antifungal[3], antiviral[4], cytolytic activity[5] These properties imply that SF could be used as a surfactant or an active substance in a suitable pharmaceutical formulation, such as self-(micro/nano)emulsifying drug delivery systems (S[M/N]EDDS).

S(M/N)EDDS are mixtures of oils and surfactants, ideally isotropic, sometimes including cosolvents, which emulsify under conditions of gentle agitation, similar to those which would be encountered in the gastrointestinal tract[6-8]. When compared with emulsions, which are sensitive and metastable dispersed forms, S(M/N)EDDS are physically stable formulations that are easy to manufacture[9]. An additional advantage of

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S(M/N)EDDS over simple oily solutions is that they provide a large interfacial area for partitioning of the drug between oil and water[10].

In this study, stable SMEDDS formulations were designed and characterized, and the bioactive SF compound was successfully incorporated into these formulations.

Materials and Methods

Materials

SF (≥ 98% purity) was purchased from Sigma (USA). Gelucire 44/14 and Labrasol were kind gifts from Gattefossé (France). Vitamin E was a kind gift from Roche Pharmaceuticals (İstanbul, Turkey). Poly ethylene glycol 3000 (PEG 3000) was purchased from Fluka (USA). Nanopure water was used throughout the experiments.

Preformulation Studies

In preformulation studies, 4 main formulation systems were designed. The oil phase and the surfactants were weighed and mixed on a water bath at 40°C. After dilution with water, the formulations were then evaluated according to their physical appearance as a function of time. The compositions of the formulations are shown in Tables 1, 2, 3 and 4. The ternary phase diagrams of the formulations can be seen in Figures 2, 3, 4 and 5.

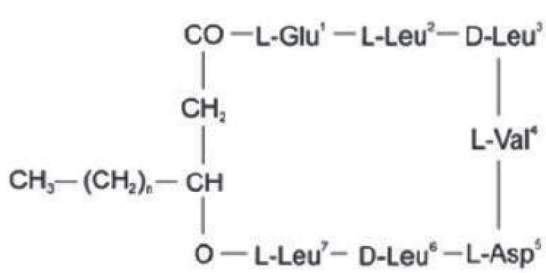

Figure 1
Primary structure of surfactin[11]

TABLE I

The Compositions of the Formulations

	Ternary Phase Diagram Values of the Components			
Formulation No/ Component	PEG 3000	Gelucire 44/14	Labrasol	Vitamin E
F 1.1	10	80	10	5
F 1.2	20	60	20	5
F 1.3	30	40	30	5
F 1.4	40	30	30	5
F 1.5	30	30	40	5
F 1.6	60	20	20	5
F 1.7	40	20	40	5
F 1.8	20	20	60	5
F 1.9	80	10	10	5
F 1.10	10	10	80	5

At a later stage, the points around F1.10 were chosen for the main formulation studies. Table 5 shows the composition of these formulations. The ternary phase diagram also is also shown in figure 6.

Preparation of Formulations

In the preformulation studies, optimum surfactant, co-surfactant and oil combinations were determined with ternary phase diagrams. The formulations composed of different surfactants with varying ratios were determined. The optimum SMEDDS formulation contained Vitamin E, Gelucire 44/14, Labrasol and PEG 3000.

In the SMEDDS formulations, the amount of Vitamin E had a constant value at 5% w/w. The oil, co-solvent and surfactants were weighed individually into a tube. The excipients were stirred on a water bath at 40 °C. At the end, the formulations were diluted to 10 ml with pH 7.0 buffer. The formulations were analyzed as a function of time (days).

In the formulation studies; the active substance, SF was added to the optimum formulations. The formulations were analyzed both macroscopically and microscopically. Characterization studies were performed by measuring pH, droplet size distribution, zeta potential and thermal properties of the stable microemulsions.

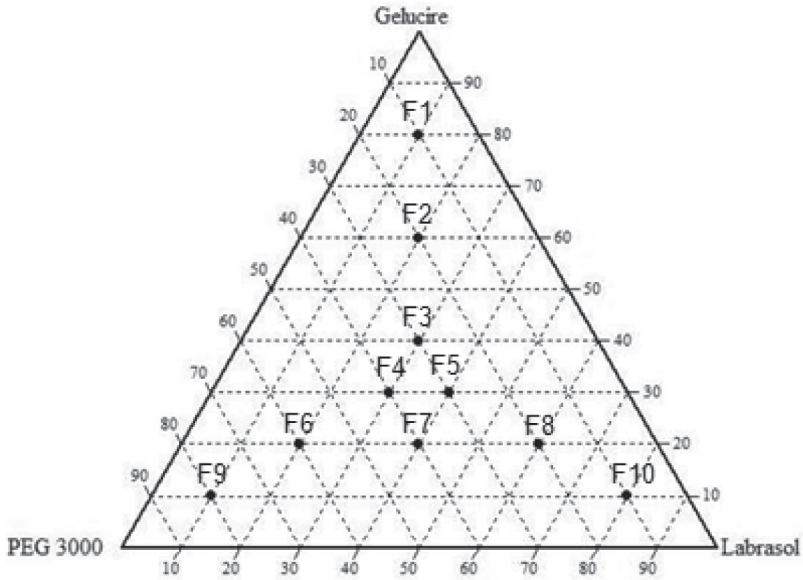

Figure 2
Ternary Phase Diagram for the formulations shown in Table I

TABLE II

The Compositions of the Formulations

	Ternary Phase Diagram Values of the Components			
Formulation No/ Component	PEG 3000	Gelucire 44/14	Tyloxapol	Vitamin E
F 2.1	10	80	10	5
F 2.2	20	60	20	5
F 2.3	30	40	30	5
F 2.4	40	30	30	5
F 2.5	30	30	40	5
F 2.6	60	20	20	5
F 2.7	40	20	40	5
F 2.8	20	20	60	5
F 2.9	80	10	10	5
F 2.10	10	10	80	5

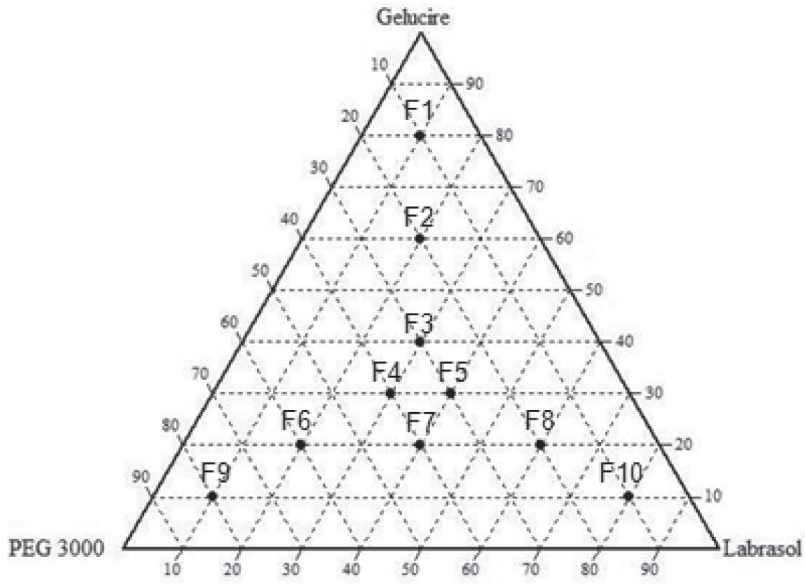

Figure 3
Ternary Phase Diagram for the formulations shown in Table 2

TABLE III

The Compositions of the Formulations

	Ternary Phase Diagram Values of the Components			
Formulation No/ Component	PEG 3000	Tyloxapol	Labrasol	Vitamin E
F 3.1	10	80	10	5
F 3.2	20	60	20	5
F 3.3	30	40	30	5
F 3.4	40	30	30	5
F 3.5	30	30	40	5
F 3.6	60	20	20	5
F 3.7	40	20	40	5
F 3.8	20	20	60	5
F 3.9	80	10	10	5
F 3.10	10	10	80	5

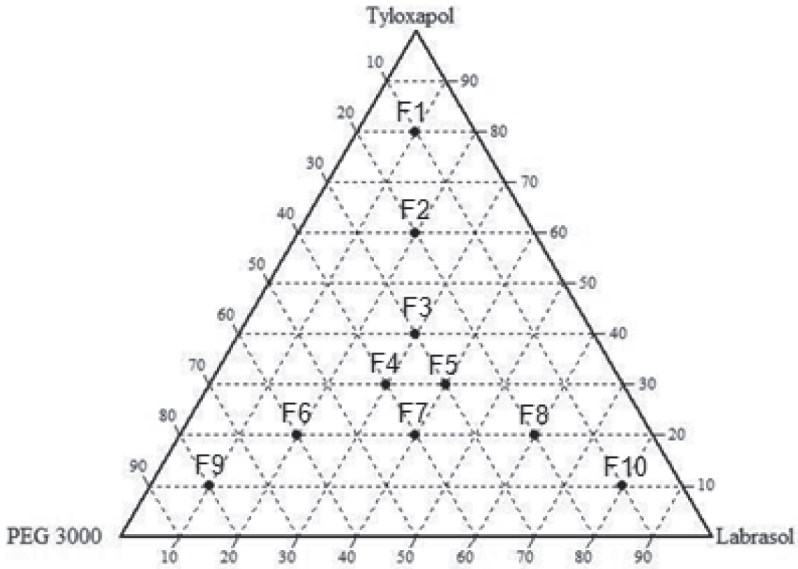

Figure 4
Ternary Phase Diagram for the formulations shown in Table 3

TABLE IV

The Compositions of the Formulations

	Ternary Phase Diagram Values of the Components			
Formulation No/ Component	Propylene Glycol	Gelucire 44/14	Labrasol	Vitamin E
F 4.1	10	80	10	5
F 4.2	20	60	20	5
F 4.3	30	40	30	5
F 4.4	40	30	30	5
F 4.5	30	30	40	5
F 4.6	60	20	20	5
F 4.7	40	20	40	5
F 4.8	20	20	60	5
F 4.9	80	10	10	5
F 4.10	10	10	80	5

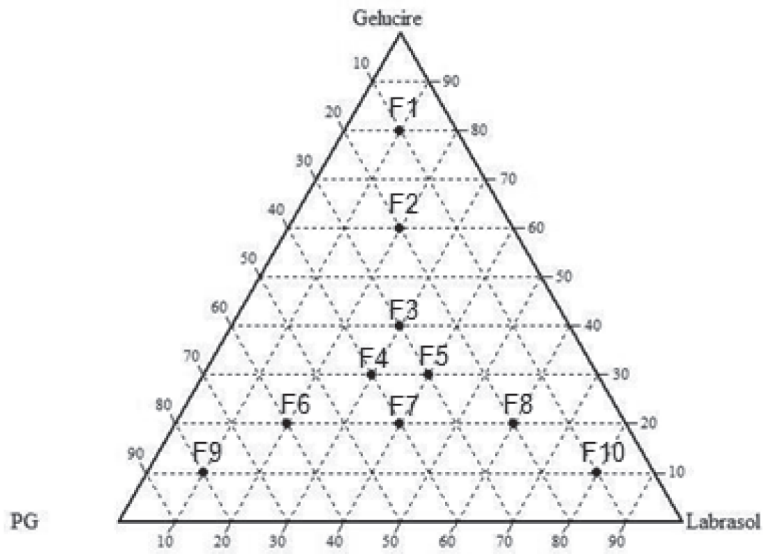

Figure 5
Ternary Phase Diagram for the formulations shown in Table 4

TABLE V

Compositions and the components formulations around F1.10

	Ternary Phase Diagram Values of the Components			
Formulation No/ Component	PEG 3000	Gelucire 44/14	Labrasol	Vitamin E
F 1.10	10	10	80	5
F 1.12	35	0	65	5
F 1.14	20	5	75	5
F 1.25	25	5	70	5
F 1.27	15	10	75	5
F 1.29	15	5	80	5
F 1.31	10	5	85	5
F 1.32	5	5	90	5

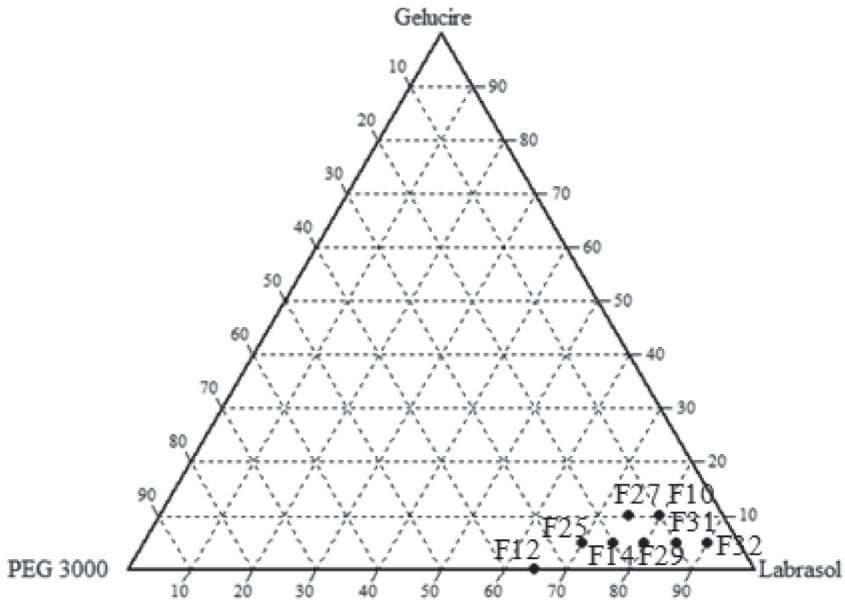

Figure 6
Ternary Phase Diagram for the formulations shown in Table 5

Characterization Studies

Macroscopic and Microscopic Analysis

Macroscopic properties of the formulations were determined in both preformulation and formulation studies. Physical stability was monitored by visual inspection for occurrence of phase separation and other organoleptic properties.

It was expected that liquid crystals would be formed in the formulations as a result of high surfactant concentration. Thus, the formulations were also analyzed for the presence of liquid crystal structures. Polarizing light microscopy technique was utilized for the evaluation of liquid crystal structures. The studies were performed by Leica DM EP polarizing microscope (Germany).

pH Measurements

pH measurements of the formulations were conducted as a function of time (days), before and after the addition of SF. These measurements were performed by Sartorius PP 20 pH meter (Germany).

Droplet Size Distribution Analysis

Droplet size distribution and zeta potential analyses were carried out in the formulation studies. Each measurement was repeated 3 times utilizing Malvern Nanosizer ZS2000 nanosizer (UK). The droplet size distribution analysis results are shown in Table 6. Table 7 also shows the zeta potential values of the formulations.

Differential Scanning Calorimetric (DSC) Analysis

Differential scanning calorimetry (DSC) measurements were performed with TA Instruments Q100 (USA). Each excipient, blank SMEDDS formulations (before and after dilution) and SF-SMEDDS formulation (before and after dilution) were scanned 3 times for the thermal analyses. Samples were placed in closed aluminum pans and heated from 25 °C to 140 °C at a rate of 10 °C/min. The flow rate of N_2 was constant at 50 ml/min. Calibration was made by Indium.

Results

Macroscopic and Microscopic Analysis

Several compositions of oils and surfactants were prepared using ternary phase diagrams as part of preformulation studies. Of the excipients tested, the composition of Gelucire 44/14, Labrasol, PEG 3000 and Vitamin E gave the most stable SMEDDS (Figure 7). Stable microemulsions were obtained as shown in region A, which exhibited Newtonian type rheological behavior upon aqueous dilution. In region B, unstable microemulsions which became cloudy as a function of time, were obtained. In region C, coarse emulsions were obtained.

The optimum SMEDDS formulations were examined utilizing a polarizing light microscope to observe the appearance of possible liquid crystal structures due to the presence of high amount of surfactants in the formulations. Due to the nano size range of the SMEDDS formulations, no image was observed under the polarizing light microscope, as expected. Moreover, no liquid crystal structures were observed, which are known to exhibit characteristic images under the polarizing light microscope.

TABLE VI

Droplet Size distributions of the formulations

Formulation No	Average Droplet Size, nm (± Standard Deviation, n=3)	PDI
F 1.10	18,15 (±0,04)	0,13
F 1.12	23,68(±0,10)	0,14
F 1.14	20,38(±0,16)	0,13
F 1.27	20,08(±0,16)	0,15
F 1.31	17,07(±0,24)	0,13
F 1.32	15,78(±0,09)	0,11
F 1.25	4386,67(±1089,49)	1,00
F 1.29	6999,67(±2970,61)	0,43

TABLE VII

Zeta Potential Values of the formulations

Formulation	Average Zeta Potential, mV
F 1.10	-0,45
F 1.12	-1,07
F 1.14	0,36
F 1.27	-0,28
F 1.31	-0,01
F 1.32	-0,42
F 1.25	-1,01
F 1.29	-1,03

pH Measurements

For the pH measurements, it appears that the pH (6.75±0.006) of the formulation did not vary over a period of time indicating sustained physical stability of SMEDDS formulation.

Droplet Size Distribution Analysis

The droplet size distribution and zeta potential of the SMEDDS formulation were determined as a function of time, before and after the addition of SF. The results are shown in table 8.

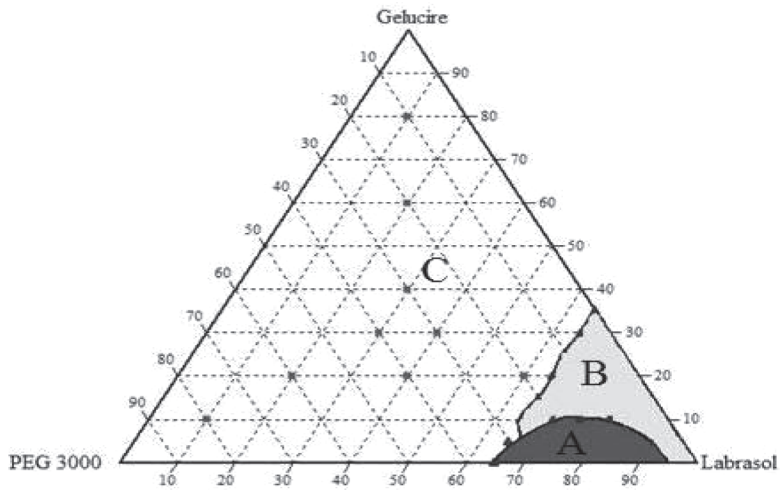

Figure 7
Ternary phase diagram of the SMEDDS formulations

TABLE VIII

Droplet size distribution, PDI and Zeta Potential measurements of a typical SMEDDS formulation before and after the addition of SF

SMEDDS Formulation	Average Droplet Size ± SD (nm)	Average PDI±SD	Average Zeta Potential ± SD (mV)
Before the addition of SF	8.80±0.03	0.127±0.005	0.18±0.248
After the addition of SF	9.46±0.02	0.120±0.01	-2.72±1.06

Differential Scanning Calorimetric (DSC) Analysis

Thermal analysis was performed on the excipients (Gelucire 44/14, Labrasol, PEG 3000 and Vitamin E), on SF, and on the SMEDDS before and after dilution.

One of the most stable formulations from region A was tested for DSC experiments. The melting temperature of SF was found to be 131.54 °C (Figure 8). This data is in accordance with the results obtained by the capillary melting point method (132 °C) and the literature[12]. Table 9

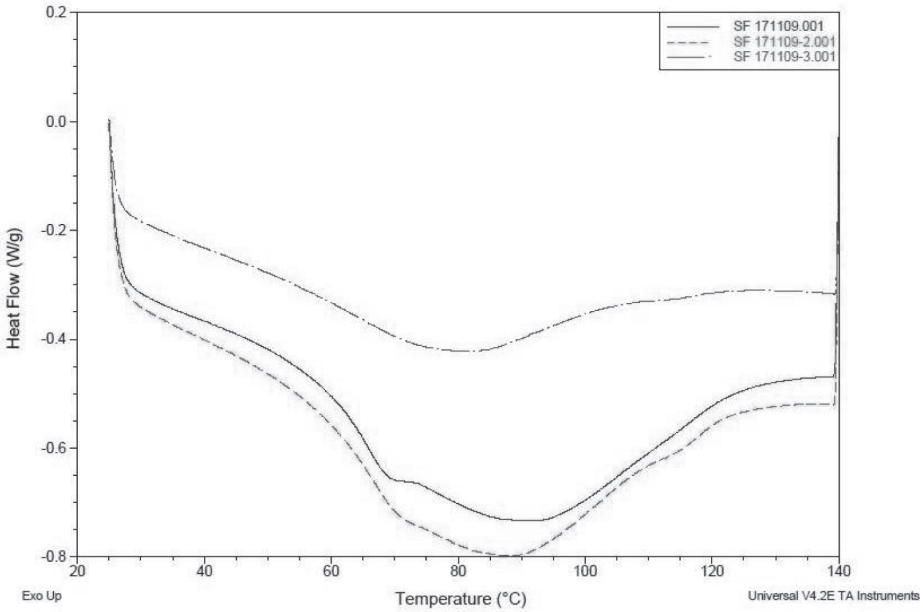

Figure 8
DSC Thermograms of SF (n=3)

TABLE IX

DSC data for the excipients and the SMEDDS formulation (n=3)

Component	Tm (°C)	ΔH (J/g)
Gelucire 44/14	47.88±2.09	60±7.12
PEG 3000	61.97±0.85	194.68±2.04
SMEDDS Before Dilution	39.48±0.79	12.81±4.31
SMEDDS After Dilution	70.50±3.51	291.60±21.35*

* n = 2

illustrates the melting temperature (T_m) and the enthalpy of transition (ΔH) for Gelucire 44/14, PEG 3000 and the SMEDDS formulation before and after dilution.

Discussion

Our aim in this project was to design and characterize self-microemulsifying drug delivery systems (SMEDDS) containing SF. SMEDDS formulations containing different compositions of excipients were prepared using ternary phase diagrams. Then, the physical stability of the SMEDDS formulations was monitored. Additionally, characterization studies were performed on the optimum formulations which were found physically stable.

Physically stable SMEDDS formulations comprised Vitamin E, Labrasol, Gelucire 44/14 and PEG 3000. In figure 2, a ternary phase diagram comprising Gelucire 44/14, Labrasol and PEG 3000 (most stable SMEDDS in Region A, unstable SMEDDS in Region B and coarse emulsions in Region C) is given.

The pH values of the blank and SF-containing SMEDDS formulations following 1:10 dilution with pH 7.0 phosphate buffer was 6.77±0.05 and 6.75±0.007, respectively. It has been reported that SF had nearly no surface activity at pH values from 2 to 4[13]. In a study, it was found that SF started to precipitate out of the production medium at pH≤5[14] and was dissolved completely when the pH returned to 6[15]. Thus, formulations were diluted with pH 7.0 phosphate buffer in the present study to sustain the surface activity of SF, which has surface activity above pH 5 with its maximum at pH 6[13].

The characterization studies were performed on both the blank formulations and SF-containing formulations. For the droplet size and zeta potential measurements, a monomodal droplet size distribution with a narrow range was obtained with stable SMEDDS formulations. The average droplet size of the blank SMEDDS formulation was found to be 8.80±0.03 nm. The addition of SF to the formulation increased the droplet size slightly. This could be due to the rearrangement of the surfactants upon insertion of SF at the oil-water interface.

The average zeta potential values were in the range of -3.5–5.0 mV for the blank formulations. Since only nonionic surfactants were used in these formulations, zeta potential values close to neutrality were obtained. The average zeta potential of the blank and SF-containing formulations was 0.18±0.24 mV and -2.72±1.06 mV, respectively. The surface potential value of the microemulsion droplets was probably due

to the presence of the surfactant in its ionized form at the oil/water interface[16]. So, if the agent is cationic, the zeta potential value will be positive; if the agent is anionic, the zeta potential value will be negative. The surfactants used in these formulations were all nonionic. So, the zeta potential of the microemulsion droplets was about neutral. On the other hand, the zeta potential value of the SF-SMEDDS could be due to the presence of Glu/Asp residues which are in anionic form in the peptide chain of SF[17].

The main purpose of the DSC analysis was to determine the thermal behavior of SF and the formulation excipients. One of the most stable formulations from region A in the ternary phase diagram was tested for DSC experiments. The melting temperature of SF was found to be 131.54 °C. This data is in accordance with the results obtained by the capillary melting point method (132 °C) and the literature[12]. It can be inferred from the DSC data that excipients forming the SMEDDS behave differently before and after dilution. It appears that following aqueous dilution, the molecules exhibited structural rearrangements, so only the Gelucire melting endotherm appeared before dilution (39.48 °C) and only the PEG 3000 melting endotherm (70.50 °C) following dilution, both of them exhibiting a shift from their actual values. This may be due to the hydrophilicity of PEG 3000 which probably leads to more interaction with the water molecules. The melting endotherm of SF was not observed in the thermogram of the SF-SMEDDS formulation. This result may imply the presence of a strong interaction between SF and the excipients at the oil-water interface.

Conclusion

In this study, a novel and stable SMEDDS formulation containing a powerful biosurfactant, SF, was developed and characterized successfully. To the best of our knowledge, this is the first study incorporating SF into a pharmaceutical dosage form. It is worth investigating the bioactivity of SF in the newly developed SMEDDS formulation, which is actually the next step in our research.

Özet

Sürfaktin İçeren Kendiliğinden Mikroemülsifiye Olabilen İlaç Taşıyıcı Sistemlerin Formülasyonu ve Karakterizasyonu

Bu projenin amacı; çok güçlü bir biyosürfaktan ve aynı zamanda çeşitli terapötik etkileri olan sürfaktin (SF)'in, kendiliğinden mikroemülsifiye olabilen sistemler (SMEDDS) içinde formüle edilmesidir. Bunun için, üçgen faz diyagramları kullanılarak geliştirilen SMEDDS formülasyonları hazırlanmış ve karakterizasyon çalışmaları yürütülmüştür. Fiziksel görünüm, pH, damlacık büyüklüğü, zeta potansiyel, termal ve mikroskopik incelemeler; dayanıklı ve teknolojik açıdan uygun SF-SMEDDS elde edildiğini göstermiştir. Bilindiği kadarıyla, bu çalışma SF'in şimdiye kadar SMEDDS gibi bir farmasötik dozaj formunda formülasyonunu gösteren ilk çalışmadır.

Anahtar kelimeler: Sürfaktin, kendiliğinden-(mikro/nano) emülsifiye olabilen ilaç taşıyıcı sistemler, biyosürfaktanlar

Summary

The aim of this project was to formulate surfactin (SF), a powerful biosurfactant with several therapeutic properties, into self-microemulsifying drug delivery systems (SMEDDS). Thus, SMEDDS formulations were developed and prepared utilizing ternary phase diagrams, and characterization studies were carried out. The physical appearance, pH, droplet size, zeta potential, thermal and microscopic properties indicate that stable and technologically suitable SF-SMEDDS were obtained. To the best of our knowledge, this is the first study showing the successful incorporation of SF into a pharmaceutical dosage form such as SMEDDS.

Keywords: Surfactin; self-(micro/nano)emulsifying drug delivery systems, biosurfactants

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