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Evaluation of the influence of some polymers on the physical stability of lipid self-double-emulsifying systems with Alendronate Sodium

Ivaylo Pehlivanov[a,*] **, Krastena Nikolova**[b] **and Velichka Andonova** [a,b]

[a] *Department of Pharmaceutical Technologies, Medical University of Varna, Tsar Osvoboditel blv, 84, Varna, Bulgaria*

[b] *Department of Physics and Biophysics, Medical University of Varna, Tsar Osvoboditel blv, 84, Varna, Bulgaria*

E-mail: ivaylo.pehlivanov@mu-varna.bg, kr.nikolova@abv.bg, velichka.andonova@mu-varna.bg

Abstract

The Self-emulsifying drug delivery systems (SEDDS) containing lipids, surfactants, and co-surfactants are a promising oral platform for drugs with problematic solubility and/or permeability. However, those systems presenting a liquid phase may have certain shortcomings, such as in vivo drug precipitation, limited lymphatic transport, and storage problems. Including some polymers in their composition would increase the system's stability during storage and dispersion in the gastrointestinal tract. For example, alendronate sodium (NaALD), a Biopharmaceutical Classification System (BCS) class III drug, is characterized by low permeability and good solubility. Its biopharmaceutical characteristics could be improved by inclusion in w/o/w SEDDS formulation.

The present study aimed to investigate the effect of the natural polymer gelatine and co-emulsifier soybean phosphatidylcholine on the physical stability of Alendronate Sodium-loaded coconut oil-based w/o/w self-double-emulsifying systems (w/o/w SDEDDS-NaALD).

Pseudoternary phase diagrams were used for the determination of the excipient ratios of self-emulsification. We prepared the model self-emulsifying systems by a two-stage emulsification technique and a high-speed homogenizer at 65°C. Four models were developed and were physically and thermodynamically characterized by sedimentation analysis and spectrophotometric analysis, self-emulsification time determination, and Dynamic Light Scattering (DLS).

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*Ivaylo Pehlivanov

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Abreviations

SEDDS – self-emulsifying drug delivery systems;

NaALD – Sodium Alendronate, Alendronate sodium;

BCS – Biopharmaceutical Classification System;

SDEDDS-NaALD – w/o/w self-double-emulsifying drug delivery system with Sodium Alendronate;

DLS – Dynamic light scattering;

SM – sorbitan monooleate, SPAN 80;

PS – polyoxyethylene 20 sorbitan monooleate, polysorbate 80, TWEEN 80;

PCh – phosphatidylcholine, L-α-lecithin from soybean;

PE – primary w/o emulsion;

F – experimental formulation, model formulation;

SDEDDS-NaALD-F – w/o/w SDEDDS-NaALD formulation;

SET – self-emulsification time

1. Introduction

The self-emulsifying drug delivery systems (SEDDS) are defined as isotropic mixtures of one or more oils, hydrophilic solvents, surfactants, and surfactants/co-solvents, which form fine o/w or w/o/w emulsions following gentle agitation (gastrointestinal peristalsis) followed by dilution in an aqueous medium such as gastrointestinal fluids. These formulations can be modified in various ways to meet a wide range of product requirements per the disease condition, route of administration, biopharmaceutical shortcomings, product cost, stability, toxicity, and efficacy. The lipid-based carriers are safe and efficient since they have been proven to be an attractive platform for the delivery of pharmaceuticals, vaccines, diagnostics, and nutraceuticals [1,2,3,4,5].

The self-double-emulsifying drug delivery systems (SDEDDS) are sub type of SEDDS that form double emulsions such as w/o/w or o/w/o. They have the advantage to incorporate hydrophilic active ingredients [6]. They are suitable platform for drugs that are characterized by low permeability and good solubility. In many cases such drug has to be injected in order to achieve a therapeutic plasma concentration because of their poor oral bioavailability [7]. Different approaches have been employed to address this issue such as absorption enhancers, chemical modifications and other pharmaceutical means. Incorporating a hydrophilic drug into SDEDDS can potentially ameliorate the permeability profile and thus it seems to be a suitable formulation strategy [8].

Alendronate sodium (NaALD), a BCS class III drug, is characterized by low permeability (2.5% from Caco-2 cells) and good solubility (23.7 $mg.mL^{-1}$) [9]. The low permeability of the NaALD is due to its low lipophilicity, and it is the rate-controlling step in the absorption process. Hence, this shortcoming could be improved by formulating NaALD in w/o/w-SDEDDS. Being solubilized in the internal water phase, NaALD will be encapsulated in a lipidic shell that could increase its lipophilicity. For the multiple w/o/w emulsions, the volume fraction of the aqueous phase has a significant effect on its stability as well as the nature and concentration of the surfactant/co-surfactant system [10].

A w/o/w double emulsion is a complex multiphase system in which smaller water droplets are dispersed in oil phase forming w/o emulsion. This primary w/o emulsion is

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dispersed in water phase, in the form of lipid droplets and thus a double w/o/w emulsion is produced. Such system can be very unstable, and this is limiting their pharmaceutical application [11]. The stability of w/o/w is dependent on various processing parameters such as type of emulsifier, emulsifier to co-emulsifier ratio, and way of preparation. Previous studies have reported that the hydrophobic emulsifier could cause water transport in double emulsions and lead to the swelling /shrinkage of the emulsion droplets, thus decreasing the stability of emulsion samples. This phenomenon can be regulated by inclusion of osmotically active agent in both internal and external phases in order to counteract the Laplace pressure related to the curvature of the droplet surface. Another approach in this direction is to add a biopolymer, such as gelatin, to the inner water phase. Interactions between surfactants and the biopolymer forming a viscoelastic barrier could be an explanation for the improved stability in addition to the aforementioned osmosis regulation [8, 11, 12]. Gelatin could undergo thermo-reversible gel-sol transition and it is dependent on various factors such as temperature, dosage form composition, aging, and others [13].

Lecithin has already been used in combination with Span 80 as an emulsion component by Matsuzawa [14]. As an additive, lecithin results in the stabilization of multiple W/O/W emulsions. The increased stability is related to a change in the diffusion coefficient of water and water-soluble substances subject to the oil-phase composition. The lower the diffusion coefficient, the more stable is the emulsion [15]. The addition of lecithin to multiple emulsions resulted in greater stability due to the forming of bilayer on the w/o/w interphases. This bilayer is much stronger than a monolayer formed by a single surfactant [16,17].

Since NaALD is very hydrophilic, in the present study the lipid excipients were selected according to other characteristics that differ from their solubilizing capacity. Coconut oil is usually present in the human diet and is rich in medium-chain triglycerides (MCT), such as caprylates and caprates. Therefore, its presence can stimulate both the enteric lymphatic transport and bile salts secretion, which, combined with the presence of a large amount of MCTs, potentially makes this excipient a great permeation enhancer [18-22]. The other excipients were selected according to their digestibility, low toxicity, and their ability to stabilize multiple emulsions at relatively low concentrations. The emulsifier pair sorbitan monooleate / polysorbate 80 was frequently used by other authors in both w/o and o/w emulsions and was chosen as a benchmark [23-25].

The present study aimed to investigate the effect of the natural polymer gelatin and co-emulsifier soybean phosphatidylcholine on the physical and the thermodynamic stability of Alendronate Sodium-loaded coconut oil-based w/o/w self-double-emulsifying drug delivery systems (w/o/w SDEDDS-NaALD).

2. Materials and methods

2.1. Materials

The surfactants Span 80 (sorbitan monooleate, SM) and Tween 80 (polyoxyethylene 20 sorbitan monooleate, polysorbate 80, PS) were of analytical grade (Sigma-Aldrich Chemie GmbH, Germany) and L-α-lecithin (soybean), granular (>97% phosphatidylcholine, ARCOS-ThermoScientific, Portugal) were used without further purification; Gelatine was of 120 bloom (95%, Sigma-Aldrich Chemie GmbH, Germany); Raw coconut oil (sertificate# BG-Bio-18, origin: Philippines) was purchased by BioBalev LTD – Bulgaria; Alendronate Na trihydrate (99.7%, Polpharma S.A. - Poland) was of Pharmacopeial grade.

2.2. Methods

2.2.1.Pseudoternary phase diagrams

We used the water titration method to construct pseudoternary phase diagrams to assess the appropriate excipient ratios for self-emulsification [26] (Fig. 2).

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Two primary W/O emulsions (PE) composed of water, coconut oil, and an emulsifier/co-emulsifier (HLB 7.5) system were prepared. For PE1, the emulsifier/co-emulsifier system was composed of SM/PS; for PE2, the emulsifying system was composed of SM/ phosphatidylcholine. To each primary emulsion (PE1 and PE2), the hydrophilic surfactant (PS) was added in suitable volume ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9). To the obtained mixtures distilled water was added dropwise under agitation. The addition of each water portion was followed by visual inspection, and the appearance of milky, opalescent, or clear dispersion was noted. The amount of water added to each sample was from 5% to 95%.

2.2.2.SDEDDS-NaALD model formulations preparation

The SDEDDS-NaALD model formulations were prepared by a two-stage emulsification technique (IKA T25 Ultra Turrax Homogenizer, IKA RCT basic hotplate, Germany) (Fig. 1). Briefly, the first stage of the emulsification consisted in preparing the primary w/o emulsion by phase inversion. In the second stage, the hydrophilic surfactant was added to the formulations after equilibrating the primary emulsions for 24 hours. The composition of the formulations (F) is presented in Table 1.

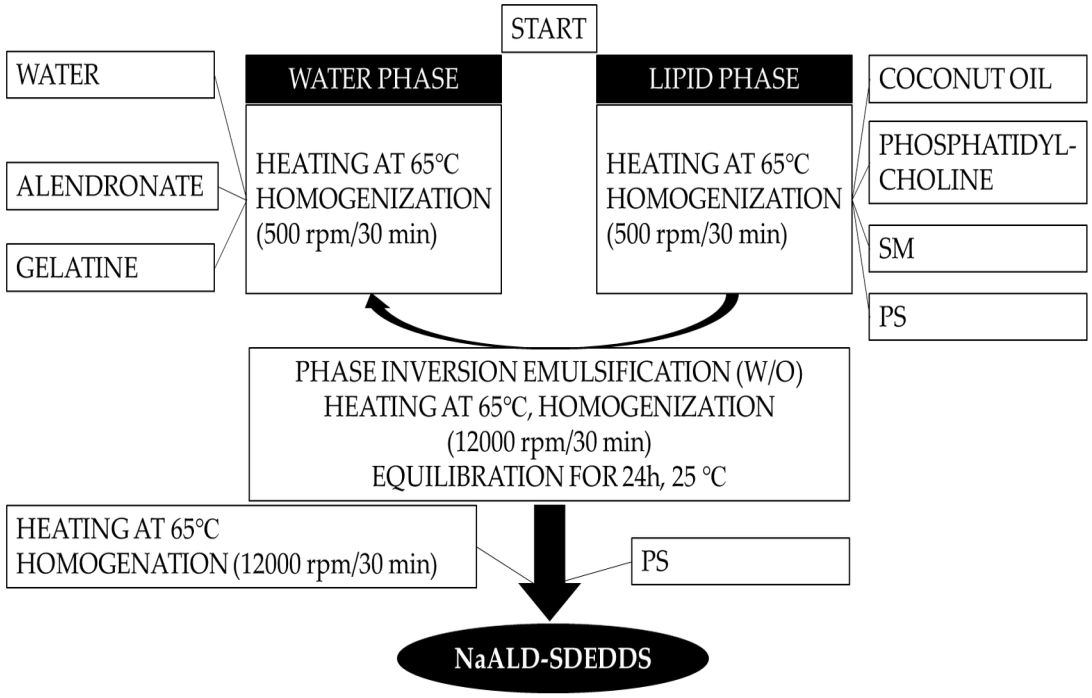

Figure 1: Technological scheme for preparing of the self-emulsifying systems.

Table 1. SDEDDS-NaALD formulations' composition

Ingredients, %	F I	F II	F III	F IV
Alendronate Sodium	6.42	6.42	6.42	6.42
Coconut oil	18.24	18.24	18.24	18.24
Soybean phosphatidylcholine	4.59	0.00	4.59	0.00
Gelatin	0.00	0.00	0.63	0.63
Sorbitan monooleate	2.96	2.96	2.96	2.96
Polysorbate 80	45.00	49.59	48.92	53.51
Distilled water	22.79	22.79	18.24	18.24

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2.2.3.Assessment of physical stability by centrifugation

The physical stability of SDEDDS-NaALD formulations was evaluated by analytical centrifugation cycles (DLAB D2012 plus high-speed mini microcentrifuge, Scientific Co LTD). Briefly, aliquots (1 mL) of the SDEDDS-NaALD-Fs were subjected to cycles of heating (15 min at 45°C), cooling (15 min at -20°C), and centrifugation (1 min at 3000 rpm). After each centrifugation cycle, a visual inspection was performed to assess phase separation phenomena. The centrifugation was repeated until complete phase separation of each sample was reached. The time to reach phase separation was determined. After homogenization, the sample was subjected to the next temperature cycle.

2.2.4.Assessment of thermodynamic stability

Water dispersions of different emulsion concentration (v/v) were used as standards. The dilutions were made as following: 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL of the emulsions were diluted with distilled water in 50 mL volumetric flask. The optical density of the obtained dispersions was investigated in the visible spectrum. The measurements were carried out by using a spectrophotometer (Genesys 10UV Thermo Scientific, Massachusetts, USA). The absorption spectrum of the sample with the highest concentration was measured to determine the wavelength at which the sample absorption is strongest. The research was carried out with a cuvette of 1 cm path length at λ= 230 nm. The equilibrium constant of the process was determined by the dilution method [27 - 29].

Theoretical background for determination of thermodynamic parameters

Enthalpy, entropy, and Gibbs energy are thermodynamic parameters and indicators of the stability of a system. The stability of an emulsion is influenced by various factors – composition of the emulsion, size of the emulsion drops (colloidal particles), etc.

Enthalpy was determined using the Clausius and Clapeyron equation (1), and Gibbs energy and entropy were determined using classical thermodynamic equations (2-3).

$$\frac{d \ln K}{d(1/T)} = \frac{- \Delta H}{R} \quad (1)$$

where: ΔH is enthalpy (kJ mol^{-1}), R is universal gas constant (R = 8.314 J K^{-1} mol^{-1}), K is equilibrium constant.

$$\Delta G = - RT \ln K \quad (2)$$

The entropy was calculated by using the classical equation after the determination of the enthalpy and Gibbs energy:

$$\Delta S = \frac{(\Delta H - \Delta G)}{T} \quad (3)$$

where: ΔS is an entropy (kJ K^{-1} mol^{-1}).

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2.2.5.Self-emulsification time

The self-emulsification time (SET) or the time for an SDEDDS preconcentrate equivalent to 35 mg NaALD to form a homogeneous mixture upon dilution was detected. The process was monitored by visual observation of the dispersion's opalescent or clear appearance. The experiments were done in triplicate. The formation of an opalescent to clear dispersion indicated that micro or nanoemulsion was formed [30]. In our experiments, a gelatin capsule prefilled with a model SDEDDS-NaALD was introduced in 200 mL of simulated gastric fluid, pH=1.2 (0.1 N HCl), maintained at (37±1)°C and under mild agitation (75 rpm, magnetic stirrer IKA RCT basic hotplate, Germany). 1 mL aliquots of the obtained dispersions were used to assess the particle size distribution.

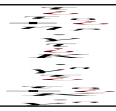
2.2.6.Assessment of particle size

Particle size (expressed as Z-average) and particle size distribution of SDEDDS-NaALD (expressed as PDI) were determined using ZetaSizer Serial number MAL 1106241 (Malvern Instruments, British) at 25°C. All measurements were performed in triplicate, and the results were reported as mean values ± SD of 3 replicates (n=3) for each formulation.

2.2.7.Rheological studies

The rheological measurements were performed at (20±1)°C and (70±1)°C using Thermo Scientific HAAKE Viscotester 550 (Germany). The analyses were carried out in an SV DIN coaxial cylinder sensor at shear rates ranging from 0.0123 s^{-1} to 1000 s^{-1}. The range of shear rates was selected based on a literature reference for rheological studies of emulsions with PS and coconut oil as the main components. The total measuring time was t = 200 seconds; this corresponds to 2 seconds for each measuring point.Thepoint. The measurement time is too short for structural changes to occur in emulsions. Such can be observed in rheological studies at a constant velocity gradient for a long time [31,32]. The data for the shear stress as a function of the share rate for each model were investigated. Three linear and nonlinear models were used to obtain the main rheological parameters, presented in Table 2. The mathematical modeling was done in the special application software of the instrument**.**

Table 2. Mathematical models for rheological properties of samples

Type of model	Mathematical equation
Bingham Plastic Model (BPM)	
Power Law Model (PLM)	
Herschel-Bulkley Model (HBM)	

Note: τ is shear stress (Pa), γ is the shear rate (s^{-1}), τ_0 is yield stress, K is consistency index (Pa.s), n is power law index (shear-thickening n>1 or shear-thinning n<1), and η_p is plastic viscosity;

All the determinations were done in triplicate. The best model was selected based on the maximum determination coefficient (R^2).

2.2.8.Statistical analysis

The data on the three parallel measurements of investigated parameters were processed to obtain the mean value and the standard deviation (SD). The analysis of dispersion was used to compare the mean values with a significance level of $p < 0.05$. Nonlinear models were obtained by the IBM SPSS Statistic 26 computer program, USA.

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3. Results and discussion

3.1. Excipient selection. Pseudoternary phase diagrams

The self-formation of micro-sized w/o/w double emulsions required the addition of a secondary (hydrophilic) emulsifier. The minimum amounts of PS to add for PE1 and PE2 were in the range 45-53.51% (v/v), as determined by the pseudoternary phase diagrams (Fig. 2). Slightly opalescent dispersions were obtained (Fig. 3) as confirmation during SETs determination. To the internal water phase were added NaALD and gelatin (Tab.1) during the preparation stage of the SDEDDS model formulations to be studied.

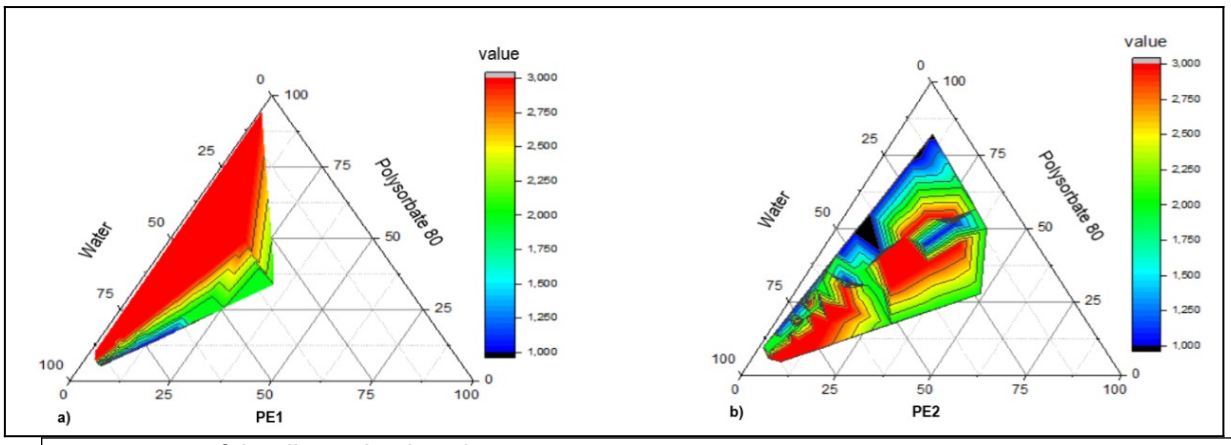

Figure 2: Pseudo-ternary phase diagrams: a) PE1 – primary W/O emulsion, SM/PS**; b)** PE2 – primary W/O emulsion, SM/PCh

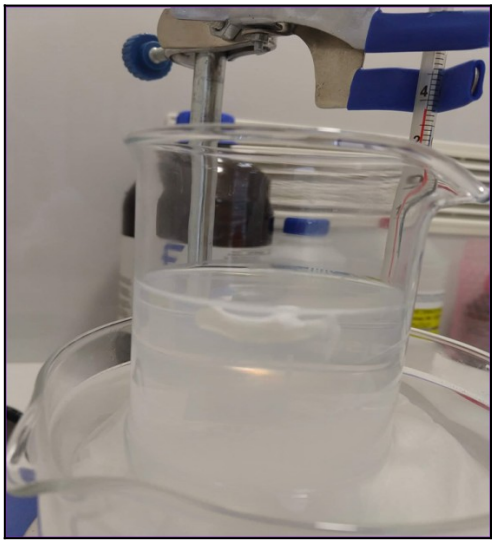

Figure 3: Self – emulsification time experimental setup

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3.1. Self – emulsification time (SET)

According to J. O'Grady *et al*. and J. Worsøe *et al*. [33,34], the medium gastric transit time at fasted state varies between 20 min to 2 hours, being 56 min to 57.5 min the median time. The self-emulsification time at the selected conditions varied between 69 and 110 min. F III and F IV were characterized by a faster SET (70 min and 69 min, respectively) compared to F I and F II (110 min and 71 min, respectively). These results suggest that F III and F IV could be more suitable delivery platforms since they self-emulsify faster than the other model SDEDDS.

3.2. Physical stability by centrifugation

The centrifugation time to complete phase separation was evaluated after each temperature cycle (Fig. 4). The four SDEDDS-NaALD exhibited different times to complete phase separation after centrifugation. F I and F II showed sedimentation after 2 min at 3000 rpm. Four cycles (1 min at 3000 rpm) of centrifugation were necessary for F III and F IV. Therefore, F III and F IV were more physically stable than F I and F II.

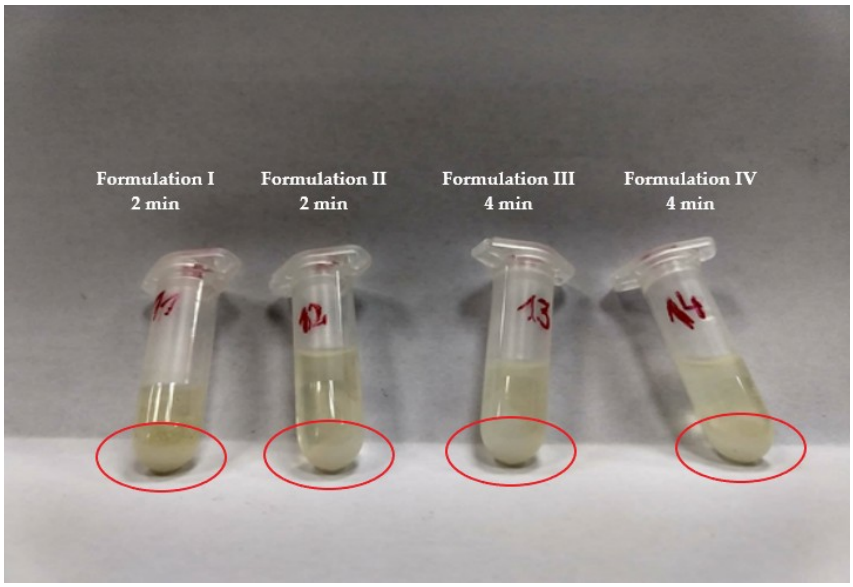

Figure 4: Complete phase separation after centrifugation at 3000 rpm.

3.3. Thermodynamic stability

The calculated enthalpies of all emulsions have large negative values. The obtained results can be associated with endothermic solid processes, which shift the equilibrium to the direction of the formation of the products. Entropies in the system have minimal negative values tending to zero. F III has the highest total Gibbs energy, followed by F IV. The Gibbs free energy increases in modulus in the presence of gelatin and phosphatidylcholine in the emulsion. Therefore, F III is the most thermodynamically stable, followed by F IV, while F II is the most thermodynamically unstable (Table 3).

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Table 3. Thermodynamic stability evaluation results

Formu-lation	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	ΔS [kJ mol^{-1} K^{-1}]	K
F I	-1.89 ±0.20	-18.16 ±0.64	-0.06 ±0.01	2.14 ±0.10
F II	-0.20 ±0.03	-17.43 ±0.25	-0.06 ±0.01	1.08 ±0.09
F III	-6.37 ±0.13	-20.11 ±0.39	-0.05 ±0.01	13.06 ±0.12
F IV	-1.91 ±0.07	-18.17 ±0.61	-0.05 ±0.01	2.17 ±0.07

3.4. Particle size distribution

Since F III and F IV demonstrated a better physical and thermodynamic stability than F I and F II, they were selected for further investigations.

Aliquots of the dispersion obtained (as described in section 2.2.5) were analyzed for particle size distribution. According to the particle size, both F III (Fig. 5a) and F IV (Fig. 5b) self-emulsify to a microemulsion. Both models presented bimodal particle size distribution. F III presents one peak at 178.4 nm (82.4%) and one at 21.47 nm (17.6%), while F IV presents two peaks at 181.4 nm (54%) and 22.78 nm (46%), respectively.

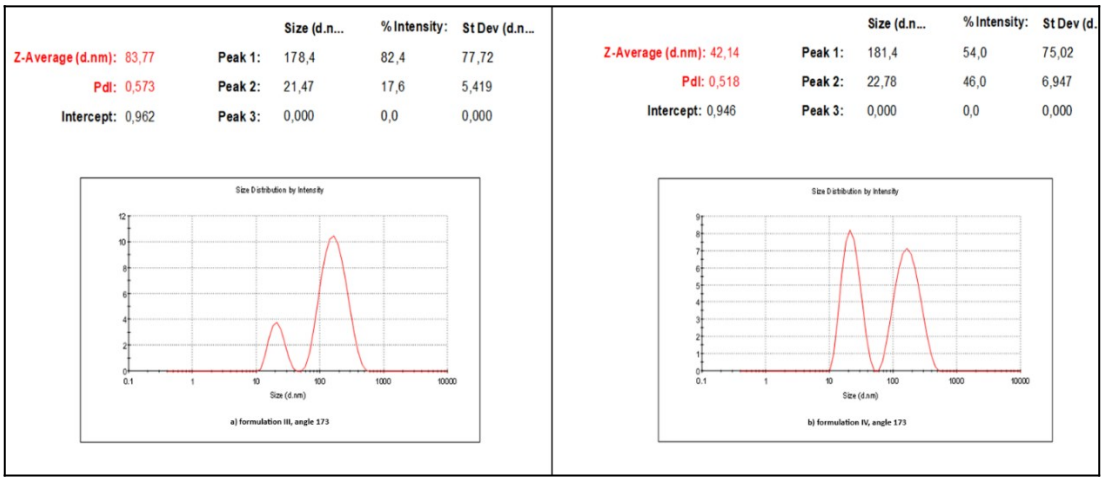

Figure 5: Particle size distribution after self-emulsification of F III and F IV.

3.6. Rheology studies

The rheological properties at different temperatures are essential characteristics, both during production and storage. SEDDS are usually filled in softgel capsules at 65-70°C and stored at room temperature.

Formulations F III and F IV are non-Newtonian fluids with pseudo-plastic behavior at 20°C. F IV has the highest plastic viscosity. At 70°C, both F III (Fig. 6a) and F IV (Fig. 6b) exhibit Newtonian fluid behavior. These results suggest that the F III and F IV could be suitable for filling into the softgel capsules where high-temperature stability of the formulation is required [35].

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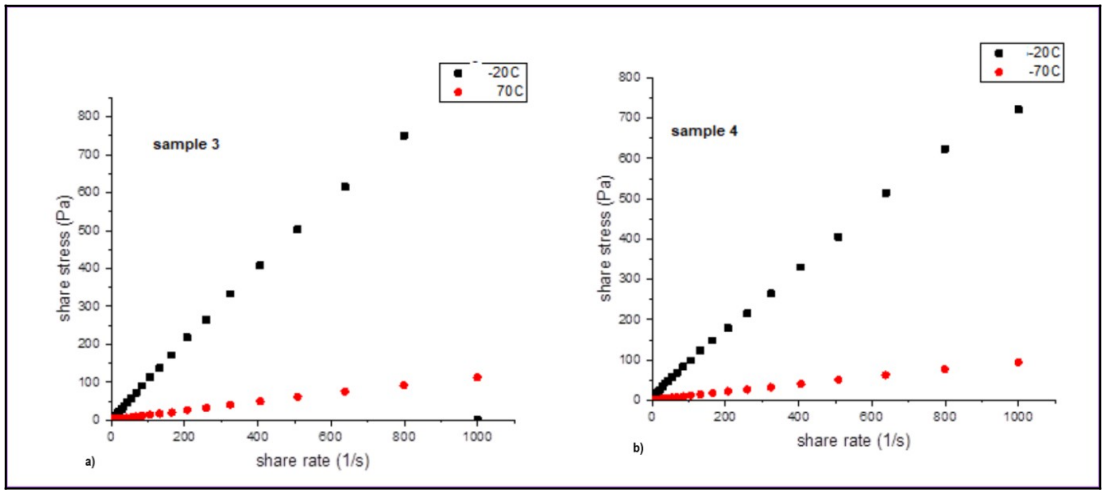

Figure 6: Rheology behavior of SDEDDS F III and F IV at 20 and 70℃.

The most appropriate rheological model for F III and F IV is the Herschel-Bulkey model. The results of flow curve data fitting using the rheological model at a temperature of 20℃ are presented in Table 4. The best fit was evaluated through the determination coefficient R^2 and the lowest value of the minimum square error (RMSE).

The determination of the yield stress gives information about the structural stability of the materials. At share stress below the yield stress, the substance deforms as an elastic solid body, and at share stress above the yield stress, it flows as a viscous fluid [36]. Formulation F III have no yield stress compared to Formulation F IV. The latter fact can relate to the presence of soy lecithin in the composition of F III. A similar result was reported by Bhattacharya et all. [37]. The authors noted that no yield stress was observed for lecithin emulsions with soybean oil.

Table 4. Rheological parameters at 20°C

Type	Herschel-Bulkey model				
	R^2	RMSE	n	K, Pa.s	τ_o, Pa
F III	0.85	0.1084	0.55 ±0.1	11.8 ±1.1	0±0.1
F IV	0.999	0.0895	0.89 ±0.1	1.6 ±0.1	1.41±0.10

4. Conclusion

Physical and thermodynamic stabilities are important issues to be faced for a successful and performing SDEDDS formulation. Of the developed SDEDDS-NaALD, F III and F IV emerged with the fastest SETs and were thermodynamically and physically stable. These results correlate with the finding that upon mild agitation, they form microemulsions, which are more thermodynamically stable systems than coarse and nanoemulsions [38] (F III – 87.3 nm, F IV – 42.14 nm, as Z-average).

The presence of soybean phosphatidylcholine and gelatin in the formulation conferred pseudo-plastic properties at room temperature and Newtonian fluid behavior at 70°C. These are important stability features regarding production (short term) and storage (long term). Potentially

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improved formulation stability and permeability of NaALD during *in vivo* application in the aggressive gastric environment can also be expected.

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