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NANOEMULSIONS BASED ON SELECTED BERRY SEED OILS

NANOEMULSJE NA BAZIE WYBRANYCH OLEJÓW Z NASION OWOCÓW JAGODOWYCH

Abstract

The aim of this work was the preparation of O/W nanoemulsions based on supercritical CO₂ extracted (SC-CO₂) oils from seeds of strawberry and blackcurrant. The emulsion systems were obtained by the phase inversion composition method, at $T = 25^{\circ}\text{C}$. Polysorbate 80 and Natragem S150 were used as the surfactant and co-surfactant, respectively. Kinetic stability of the prepared formulations with varying surfactant: oil (S:O) and surfactant/co-surfactant (S/CoS) weight ratio, was analysed by measuring the droplet size (DLS method) in time. The obtained results show that a nanoemulsion based on blackcurrant seed oil was characterised by the smallest droplet size ($r = 15$ nm), a low polydispersity index (PDI = 0.246) and the longest kinetic stability (S:CoS = 8:2, S/CoS:O = 9:1).

Keywords: nanoemulsions, blackcurrant seed oil, strawberry seed oil supercritical CO₂ extraction

Streszczenie

Celem pracy było otrzymanie nanoemulsji O/W, w których rolę fazy olejowej pełnił ekstrakt z nasion truskawki lub czarnej porzeczki otrzymany w warunkach nadkrytycznego CO₂ (SC-CO₂). Układy emulsyjne otrzymano metodą składnikowej inwersji faz, w $T = 25^{\circ}\text{C}$. Jako surfaktant zastosowano Polisorbat 80, rolę kosurfaktantu pełnił Natragem S150. Kinetyczną stabilność otrzymanych formułacji badano poprzez pomiar wielkości cząstek nanoemulsji (technika DLS) w czasie, dla układów o różnym stosunku wagowym surfaktant: olej (S:O) oraz surfaktant/kosurfaktant (S/CoS). Otrzymane rezultaty wykazały, że najmniejszym rozmiarem cząstek fazy wewnętrznej ($r = 15$ nm), niskim indeksem polidispersyjności (PDI = 0.246) oraz najdłuższą kinetyczną stabilnością charakteryzowała się nanoemulsja na bazie oleju z nasion czarnej porzeczki (S:CoS = 8:2, S/CoS:O = 9:1).

Słowa kluczowe: nanoemulsje, olej z nasion czarnej porzeczki, olej z nasion truskawki, ekstrakcja nadkrytycznym CO₂

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Abbreviations

W	– water
O	– oil
S	– surfactant
CoS	– co-surfactant
S/CoS	– surfactant/co-surfactant
oil–S/CoS:O	– surfactant/co-surfactant
oil–S:O	– surfactant
O/W	– oil/water
W/O	– water/oil
NE	– nanoemulsion
SC–CO ₂	– supercritical carbon dioxide
PIC	– phase inversion composition
DLS	– Dynamic Light Scattering
OP	– blackcurrant seed oil
OT	– strawberry seed oil
OO	– olive oil
PDI	– polydispersity index

1. Introduction

Natural oils are a valuable source of active substances, e.g. unsaturated fatty acids and tocopherols. Unsaturated fatty acids are responsible for the proper construction of the intercellular cement of the *stratum corneum*. They reduce transepidermal water loss (TEWL), and thereby, assure the proper functioning of the epidermis. Tocopherols are natural antioxidative agents and intercellular membrane structures [1].

Nanoemulsions can be defined as ‘ultrafine’ or ‘submicron emulsions’ because of the small droplet size, in the range of 20 to 500 nm. They are used in personal care products as potential vehicles for the controlled delivery of active agents. The nanoemulsions are valued forms in skin care because of their good sensorial properties, i.e. rapid penetration, fluid textures and their biophysical properties [2, 3].

In studies concerning nanoemulsions, most authors used the following as an oil phase: mineral oil [4], decan [5], hexadecane [6], isopropyl mirystate [7, 8] or medium chain triglycerides [9–12]. Recently, there has been increasing interest in natural oils, such as castor oil [13], soybean oil [14, 15], canola oil [16], rice brain oil [17], grape seed oil [18] and olive oil [19].

As it appears from the literature review, there is no information about nanoemulsions containing strawberry seed oil or blackcurrant seed oil as the oil phase. From the cosmetic point of view, they are very valuable raw materials. Strawberry seed oil is a natural active ingredient, which is characterised by good oxidative stability, high biological activity and a gentle anti-wrinkle active. It consists mainly of C18:1 (15%), C18:2 (45%), C18:3 (35%) [20–22].

Blackcurrant seed oil is a valuable nutritional oil and a rich source of GLA. It is composed of C18:0 (3%), C18:1 (40%), C18:2 (45%) and C18:3 (12%) [21]. Due to its special features, it can promote healthier-looking skin and act as a moisturiser [20].

The studies on obtaining oil from the seeds of berries (including strawberry, blackcurrant, blackberry), through the extraction with supercritical carbon dioxide (SC-CO₂), have been conducted for several years. Carbon dioxide is non-toxic, non-explosive, readily available and easily removed from the extracted products. Supercritical CO₂ extraction is often carried out at a mild temperature, in the absence of oxygen. Due to this fact, it is possible to avoid thermal and oxidative damage of the bioactive compounds in the extract. Moreover, SC-CO₂ extraction is an environment-friendly process. The advantages of oils obtained under supercritical fluids over oils separated by other methods are: reproducible and stable quality of the oils, and high process efficiency [23, 24].

The aim of this work was to prepare (by low energy emulsification method) and characterise highly dispersed emulsion systems, containing oil phase supercritical CO₂ (SC-CO₂) extracted from the seeds of strawberry and blackcurrant, for a potential cosmetic application.

2. Materials and methods

2.1. Materials

The raw materials used in the studies are shown at Table 1. Distilled water was used as the aqueous phase of the emulsions. The applied surfactants Polysorbate 80 (P80) and Natragem S150 (N150) were selected for the studies because of their high HLB value (HLB = 15.0). Ostertag and co-workers [25] found that, in the case of nanoemulsions based on natural oils, the smallest particle size was achieved when the surfactant had an intermediate HLB value of around 15,0 [25]. Additionally, both of the used emulsifiers are non-ionic surfactants known from their good dermatological characteristics, and in the case of Natragem S150, for its natural origin.

Berry seed oils were applied as the oil phase in the prepared systems; similarly to olive oil, which was used for comparison purposes.

Table 1

Raw materials used in the studies

Raw material	INCI name	Role	Producer
Polysorbate 80	Polysorbate 20	Emulsifier (HLB = 15.0)	Caesar&Loretz GmbH
Natragem S150	Polyglyceryl-4 Laurate/Sebacate (and) Polyglyceryl-4 Caprylate/Caprata (and) Water	Emulsifier (HLB = 15.0)	Croda Poland
Blackcurrant seed oil	Ribes Nigrum (Blackcurrant) Seed Oil	Oil phase	INS Puławy
Strawberry seed oil	Fragaria Ananassa (Strawberry) Seed Oil	Oil phase	INS Puławy
Olive oil	Olea Europaea (Olive) Fruit Oil	Oil phase	Ol'Vita

2.2. Formation of emulsion systems

Nanoemulsions were prepared using the phase inversion composition (PIC) method, by the gradual addition of water (W) into the mixture of oil (O) and surfactant (S)/co-surfactant (CoS) or to the mixture of oil and surfactant (S), at ambient temperature (25°C). The weight ratios of surfactant or the blends of surfactants to oils were varied as 9:1, 8:2, 7:3, 6:4 and 5:5. Also, the weight ratios of S surfactant to co-surfactant S/CoS used in the compositions were different as 9:1, 8:2 and 7:3.

2.3. Construction of a phase diagrams

Pseudo-ternary phase diagrams were constructed in the following way: mixtures of surfactant:oil (S:O) or surfactant/co-surfactant:oil (S/CoS:O) were titrated by water, at 25°C. The compositions were considered as nanoemulsions, when they were transparent and translucent, showing a bluish shine. It was confirmed that they were not microemulsions, as their properties depended on the preparation method and the storage temperature.

2.4. Droplet size determination

The average internal phase droplet size of the emulsions was measured by the Dynamic Light Scattering (DLS) method, using Malvern Zetasizer Nano ZS device. The measurements of particle size, which ranged from 0.3 nm to 10 µm, were based on scattering photons from a sample and determined by the change in the diffracted light intensity. The scattering angle was 173°.

2.5. Kinetic stability measurement

The emulsions stability was assessed by measuring droplet size as a function of time at a constant temperature (25°C). Moreover, the samples were stored at ambient temperature for 48 hours, for one week and for one month. The stability of the formulations was also assessed visually.

3. Results and discussion

In the first stage of the studies, blackcurrant (OP) and strawberry seed (OT) oils, obtained by the supercritical CO₂ extraction method, were tested in order to obtain stable nanoemulsion systems. The olive oil (OO) was also used at the studies as the reference attempt. All formulations were prepared by low energy emulsification method, by dropwise addition of water to the mixture of surfactant:oil or surfactant/co-surfactant:oil. The ratios of surfactant to oil (S:O) or surfactant/co-surfactant to oil (S/CoS:O) were varied as 9:1, 8:2, 7:3, 6:4 and 5:5. Surfactant and co-surfactant (S/CoS) were also blended at different weight ratio (9:1, 8:2, 7:3). The compositions and characteristics of some of the obtained systems are shown at Table 2.

Table 2

Composition and characteristic of the natural oil-based formulations with the constant water concentration (90%)

Sample name	S:O	S:CoS	S/CoS:O	Z-Ave d [nm]	PDI
Olive oil nanoemulsions					
NE/P80/OO/9:1	9:1	–	–	16 ± 1	0.308
NE/P80/OO/8:2	8:2	–	–	–	–
NE/P80/N150/9:1/OO/9:1	–	9:1	9:1	264 ± 66	0.595
NE/P80/N150/8:2/OO/9:1	–	8:2	9:1	133 ± 7	0.643
NE/P80/N150/7:3/OO/9:1	–	7:3	9:1	–	–
Strawberry seed oil nanoemulsions					
NE/P80/OT/9:1	9:1	–	–	43 ± 11	0.371
NE/P80/OT/8:2	8:2	–	–	77 ± 15	0.654
NE/P80/N150/9:1/OT/9:1	–	9:1	9:1	27 ± 10	0.273
NE/P80/N150/8:2/OT/9:1	–	8:2	9:1	128 ± 18	0.576
NE/P80/N150/7:3/OT/9:1	–	7:3	9:1	201 ± 3	0.251
NE/P80/N150/9:1/OT/8:2	–	9:1	8:2	201 ± 1,5	0.484
NE/P80/N150/8:2/OT/8:2	–	8:2	8:2	1455 ± 294	0.966
Blackcurrant seed oil nanoemulsions					
NE/P80/OP/9:1	9:1	–	–	11 ± 1	0.111
NE/P80/OP/8:2	8:2	–	–	18 ± 1	0.259
NE/P80/N150/9:1/OP/9:1	–	9:1	9:1	16 ± 1	0.341
NE/P80/N150/8:2/OP/9:1	–	8:2	9:1	15 ± 3	0.246
NE/P80/N150/7:3/OP/9:1	–	7:3	9:1	35 ± 3	0.277

As it is shown in table 2, the increase of oil content in the formulations causes the increase of droplet size of the internal phase. In other words, when the surfactant:oil ratio decreased, the particle droplet size became larger. This observation is compatible with the results of other research groups [19, 26, 27]. Li and co-workers [26] investigated the composition and stability of natural oils based nanoemulsions containing D-limonene as an active. They used Tween 80 as a surfactant, deionised water as the aqueous phase and a number of different oils (olive oil, corn oil, sunflower oil and soybean oil), used separately as an oily phase. Researchers

found that, with an increase in the concentration of olive oil, the mean particle diameter of the emulsions increased as well. The nanoemulsions were obtained only when the concentration of olive oil was less than 15% w/w, however, the droplet size distribution was bimodal for the entire range of oil concentrations. Comparable results were obtained for the rest of plant oils.

Komaiko and McClement [27] tested four main variables: surfactant-to-oil ratio (SOR), type of surfactant, type of oil and the surfactant location. Researchers used various oil phases to prepare the emulsions: medium chain triglycerides, orange oil, mineral oil, lemon oil, fish oil, grapeseed oil, canola oil, sesame oil, peanut oil and virgin olive oil. The formulations were stabilised by non-ionic surfactants: Tween 20, 40, 60, 80, 85 and Span 20. They have observed that, at lower surfactant-oil-ratios (SORs), larger droplets of the oil phase were formed and smaller droplets were formed at higher SORs. The emulsions appeared less turbid with the increase of SORs. The nanoemulsions at higher SORs were monomodal, with a narrow particle size distribution. Similar results were obtained by Ostertag and co-workers [25]. They rely on the discovery of Anton and Vandame [28] that if there is insufficient surfactant concentration to stabilise the droplets and to cover all of the droplet surfaces formed, then coalescence will occur. Moreover, they pointed out that, if the surfactant concentration is too high, the particle size may increase due to the formation of liquid crystals. Davidov-Pardo and McClements [18] supposed that a decrease in the droplet size is related to a reduction in the interfacial tension caused by an increase of the surfactant concentration. On the other hand, an increase in the polydispersity of the sample at high surfactant level can be due to the formation of bimodal distribution.

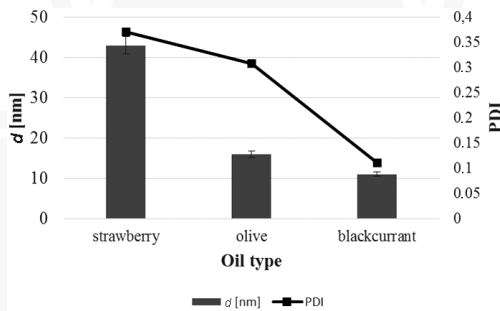


Fig. 1. Mean particle droplet size of nanoemulsions stabilised by Polysorbate 80 with constant S:O ratio (9:1) based on three tested oils

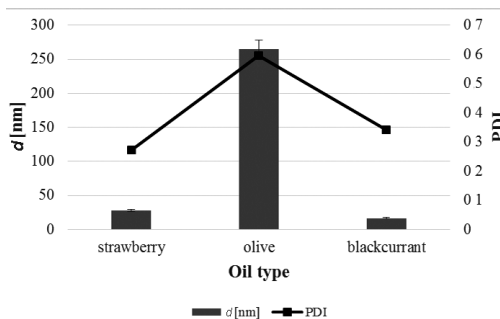


Fig. 2. Mean particle droplet size of nanoemulsions stabilised by the mixture of Polysorbate 80/Natragem S 150 (S/CoS = 9:1) based on three tested oils

Figure 1 shows that nanoemulsions stabilised by Polysorbate 80, based on blackcurrant seed oil, are characterised by the smallest droplet size ($d = 11$ nm). Olive oil-based nanoemulsions have an average droplet size. In the case of emulsion systems stabilised by the mixture of surfactants (Polysorbate 80 and Natragem S150), olive oil-based nanoemulsion is characterised by the lowest dispersion degree (Fig. 2). According to the results of other research groups [25, 27], which also used polyethoxylated esters of sorbitan and fatty acid as emulsifiers and low-energy emulsification method, particle size of the prepared nanoemulsions depended on kind of oil used. Ostertag et al. [25] obtained the smallest particles for olive oil systems and the largest ones for canola oil. On the other hand, Kamaiko&McClements [27] found that the smallest particles was had by the grapeseed oil-emulsion, and the largest ones by the olive oil-based emulsion. These results confirmed that there is no simple correlation between the mean droplet diameter of nanoemulsions and the physicochemical properties of the used oils (such as: refractive index, density, interfacial tension and viscosity) [27]. The nature of the surfactant used in the studies had a major impact on the size of the obtained formulations [25].

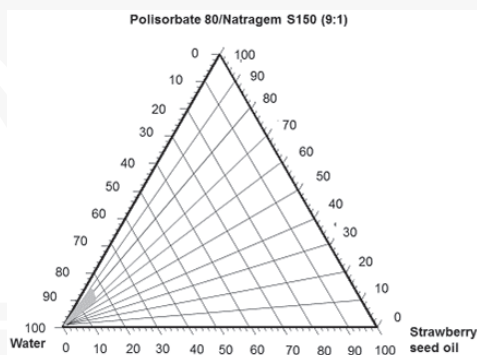


Fig. 3. Pseudo-ternary phase diagram for the Strawberry seed oil/Polysorbate 80/Natragem S150/Water system, at $T = 25^{\circ}\text{C}$

For each studied oil, the area of nanoemulsion occurrence was very narrow, despite the co-surfactant addition. Only for strawberry seed oil (Fig. 3), transparent or transparent-bluish liquid dispersions appeared after addition of approximately 85 wt. % of water to the surfactant/co-surfactant:oil (S/CoS:O) mixture of weight ratio 9:1 and 8:2. In the case of other oils, the region of nanoemulsion formation could not be extended at a S/CoS:O ratio higher than 9:1 (Table 2).

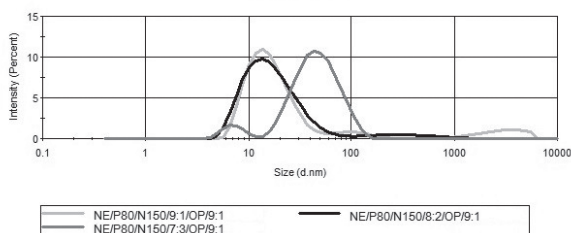


Fig. 4. Particle size distribution of blackcurrant seed oil nanoemulsion with different surfactant-to-co-surfactant (S/CoS) ratio

An addition of co-surfactant to the strawberry and blackcurrant oil-based systems resulted in an increase of their particle droplet size (Fig. 4). However, nanoemulsion based on blackcurrant seed oil, which was stabilised by both surfactants (Polysorbate 80 and Natragem S150), was characterised by a very small droplets size, (around 15 nm), and narrow distribution (PDI around 0.25).

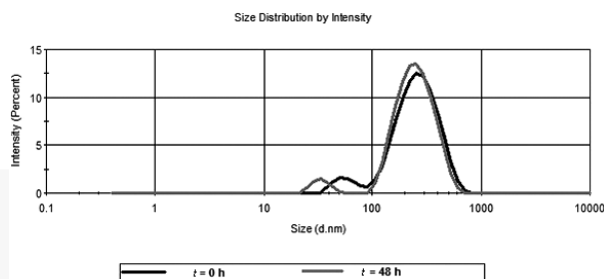


Fig. 5. Comparison of particle size distribution of nanoemulsion NE/P80/N150/7:3/OT/9:1 after preparation and after 48 hours, $T = 25^{\circ}\text{C}$

Moreover, formulations with co-surfactant were kinetically stable during 48 hours of storage. The average droplet size and polydispersity of index remained almost unchanged (Fig. 5).

4. Conclusions

The obtained results confirmed that an increase of natural oil content in the nanoemulsion formulations causes an increase of the droplet size of the internal phase. However, there is no simple correlation between the mean droplet diameter of the nanoemulsions and the physicochemical properties of the oils used. A major impact on the size of the obtained formulations is had by the nature of the applied surfactants. In the case of strawberry and blackcurrant oil-based systems, the addition of co-surfactant resulted in an increase of their droplets size, but a decrease of their kinetic stability. The nanoemulsions with the smallest droplets size ($d = 15$ nm), low polydispersity index ($\text{PDI} = 0.25$) and high kinetic stability, were obtained using blackcurrant seed oil and a mixture of surfactant/co-surfactant (Polysorbate 80/Natragem 150).

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