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Olive oil nanoemulsion preparation using high-pressure homogenization and D-phase emulsification – A design space approach

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ABSTRACT

Olive oil has been extensively applied in the pharmaceutical and cosmetic industries due to its biological properties. These are attributed to monounsaturated fatty acids like oleic acid and other minor components, such as phenolics and triterpenic acids. Oil-in-water nanoemulsion may enhance the solubility of poorly water-soluble drugs, which comprise about 40% of the currently marketed ones. However, the development of vegetable oil nanoemulsions is challenging due to their complex composition. In this study, olive oil nanoemulsions were prepared using high-pressure homogenization (HPH) and D-phase emulsification (DPE), as high- and low-energy processes, respectively. DPE has the potential to overcome the drawbacks of conventional Phase Inversion Methods. Aiming to achieve a deeper understanding of HPH and DPE processes, a design of experiment approach was successfully applied. This approach allowed identifying and understanding the relationship between input factors and their associated output response, at the stage of the nanoemulsion development. Moreover, in a specific range of critical process parameters and compositions, within the design space, nanoemulsions with similar mean particle sizes of 275 nm were achieved with equal composition, regardless of the use of the HPH or DPE process.

1. Introduction

Nanoemulsions are oil-in-water (O/W) or water-in-oil (W/O) emulsions on the nanometer scale. The oil phase of nanoemulsion is composed of liquid lipids, allowing in their core high concentrations of vegetable oils. Nanoemulsions have several benefits, including solubilization of highly lipophilic drugs and active compounds, increasing their bioavailability, drug carrier property and increased stability [1]. Moreover, the physicochemical properties of nanoemulsions can be conveniently tailored by several processes and component selections, as well as the surface modification for specific targeting organs [2]. Therefore, the right combination of process and composition selection is the key to the successful development of multiple purpose nanoemulsions [3].

Two processes are used for preparation of nanoemulsions: high and low energy processes. The first one, which is attributed to the mechanical method, including high-pressure homogenization (HPH), ultrasonication and microfluidization, generates ultrafine droplets by mechanical fracturing of the oil phase by intensive disruptive forces like collision, compression, and cavitation [4–7]. The second one, also known as the physical-chemical method, includes phase inversion temperature (PIT), phase inversion composition (PIC), spontaneous emulsification, and the less known D-phase emulsification (DPE) methods. Low energy processes produce nanoemulsions by spontaneous shift of the interfacial curvature of the oil and water phase, under specific conditions [8]. The advantage of the high-energy process is the non-dependence of the hydrophilic-lipophilic balance (HLB) of the components for the formation of nanoscale emulsions, although the high-cost of the equipment can be considered a disadvantage. The advantage and disadvantage of the low-energy over high-energy process is usually the low cost of the equipment and the strict adjustment of the HLB, respectively [9]. Nevertheless, the DPE process has unique properties, enabling the preparation of nanoemulsions without strict adjustment of HLB, and the incorporation of a high content of vegetable oils, which were considered limitations in the conventional phase inversion methods (PIT and PIC). The presence of the isotropic D phase enables easy dispersion of the oil phase to provide the final nanoemulsion [10].

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There are few studies in the literature correlating the efficacy of high- and low-energy processes in the same composition system for nanoemulsion preparation. Yang et al. [11] compared the microfluidization and spontaneous emulsification methods in the system composed of food-grade oils and surfactants (Tweens). Kotta et al. [12] used Capryol 90 (Propylene glycol monocaprylate) and Transcutol HP (Diethylene glycol monoethyl ether) as the oil phase and Tween 20 as surfactant to compare HPH and PIC methods.

Olive oil (*Olea europaea* L.) is a mixture of fatty acids, triterpenoids, carotenoids, phenolic compounds (such as flavonoids, phenolic alcohols, secoiridoids and lignans), but the phenolic composition and concentration in virgin olive oil have variations that can be explained by several factors such as cultivation, geographic region, fruit maturation and fruit-processing methods to obtain oil [13]. Its antioxidant [14] and anti-inflammatory properties [15,16] were mainly attributed to carotenoids and phenolics. In addition, it may be highlighted that virgin and refined olive oils have some difference in fatty acid composition, but oleic acid (C18:1) is the main (60–80%) component [15,17].

A study demonstrated the anti-inflammatory activity and the possible antiatherogenic activity of olive oil due to the presence of polyphenols oleuropein and hydroxytyrosol, which, at a thousand times lower concentrations, presented antioxidant activity equivalent to *N*-acetylcysteine [18]. Another study showed an effective reduction of UVB-induced tumors in the murine skin after the topical application of olive oil, possibly due to its antioxidant action, reducing DNA damage [19]. Additionally, olive oil is applied to reduce the risk of developing chronic diseases such as diabetes, atherosclerosis, cancer, and cardiovascular disease. Recent studies indicate that hydroxytyrosol and other minor components of olive oil are potential therapeutic candidates to prevent neurodegenerative disorders, such as Alzheimer's disease [20]. Furthermore, olive oil has been used as a drug carrier in nanoemulsions - the bioavailability of active component pterostilbene, a natural component found predominantly in blueberries and several grape types, has increased significantly due to increased *trans*-enterocyte transport, using olive oil as a carrier compared to flaxseed oil [21].

To the best of our knowledge, no study has been reported presenting an optimized process for the preparation of a vegetable oil and highly hydrophilic surfactant system by HPH and DPE processes using a design space (DS) approach. DS is defined as the multidimensional combination and interaction of input variables (e.g., process and formulation parameters) that have been demonstrated to provide quality assurance and process understanding. 'Changing the parameters within the design space is therefore not considered a change and does not require any regulatory approval. Design-of-Experiment (DoE) methods, such as response surface methodology (RSM), can be used to establish the design space, which is one of the key elements of quality by design (QbD)' [22], and it is based on sound science and quality risk management. Distinct from the conventional one-factor-at-a-time method, the design of experiment (DoE) enables the evaluation of the multiple interactions between independent variables in the same experiments [23].

Đorđevića et al. [23] determined the design space for the nanoemulsion loaded with risperidone (RSP), a poorly water-soluble psychopharmacological drug. A general factorial experimental design was applied to evaluate the interactions of the nanoemulsion formulation and process parameters in their critical quality attributes (CQA). A nanoemulsion with a mean particle size of 160 nm and zeta potential around −50 mV were prepared by high-pressure homogenization. By *in vivo* test in mice, it showed a brain availability of risperidone 1.4–7.4 – fold higher compared to other nanoemulsions and the drug solution (all 1 mg/mL RSP). Using a similar approach, Amasya et al. prepared 5-Fluorouracil loaded lipid nanoparticle for treating non-melanoma skin cancer. Artificial neural network software allowed to establish the design space and formula optimization [24]. In addition, a design space was successfully established in the study for the optimization of preservatives and EDTA concentration in an emulsion cosmetic product. It was possible to reveal the synergistic and antagonistic combinations of preservatives as well as to determine the most effective preservative system for the microorganisms, simultaneously [25].

The purpose of this study was to develop an olive oil nanoemulsion prepared by HPH and DPE processes, containing the same composition, using a design space approach to determine the critical process parameters (CPP), aiming at an optimal region that offers a similar mean particle size, regardless of the use of the HPH or DPE process.

Table 1
Input factors and levels selected for Box-Behnken design in HPH process.

Input factor	Symbol	Coded levels		
		−1	0	+1
Pressure (bar)	X_1	250	350	450
Number of cycle	X_2	1	2	3
Surfactant (% w/w)	X_3	1.0	2.5	4.0
Olive oil (% w/w)	X_4	5.0	7.5	10.0
Glycerin (% w/w)	X_5	0.0	1.0	2.0

Table 2
Input factors and levels selected for Box-Behnken design in DPE process.

Input factor	Symbol	Coded levels		
		−1	0	1
Surfactant (% w/w)	Z_1	1.0	2.5	4.0
Olive oil (% w/w)	Z_2	5.0	7.5	10.0
Glycerin (% w/w)	Z_3	1.0	2.0	3.0
Initial water (% w/w)	Z_4	1.0	2.0	3.0
Temperature	Z_5	50	60	70

2. Materials

The material comprises oleth-20 (purchased from Croda, HLB = 15.3) as surfactant, olive oil (purchased from Sigma Aldrich - tested according to Ph. Eur., HLB = 7.0), glycerin (purchased from Sigma Aldrich), carbomer 940 (purchased from Mapric), and ultrapure water.

3. Methods

3.1. Nanoemulsion development using Box-Behnken statistical design

The nanoemulsions were prepared based on the same key components used by Endoo and Sagitani [26] and Yukuyama et at [27]. These components are: oleth-20, glycerin and olive oil.

3.1.1. HPH process

The nanoemulsions were obtained initially using the Ultra-Turrax (IKA T25) apparatus for 5 min (10 000 rpm, 50 ml volume) for the preparation of coarse emulsion at 50 °C. This coarse emulsion was subsequently subjected to a piston-orifice type homogenizer (Nano DeBEE, BEE International, Inc. USA) to obtain the final nanoemulsion.

The CPP, which influences the mean particle size (MPS) and polydispersity index (PdI) were determined by a design space approach. The process and formulation parameters were the pressure and number of cycle and the concentrations of olive oil, glycerin and surfactant (input factors), respectively. The independent variables or input factors are shown in Table 1. A total of 46 formulas with 5 central points was prepared in randomized order using statistical software Minitab 17 (State College PA, USA).

3.1.2. DPE process

The preparation by DPE process was based on the study of Endoo and Sagitani [26], with some modifications. Initial water, oleth-20 and

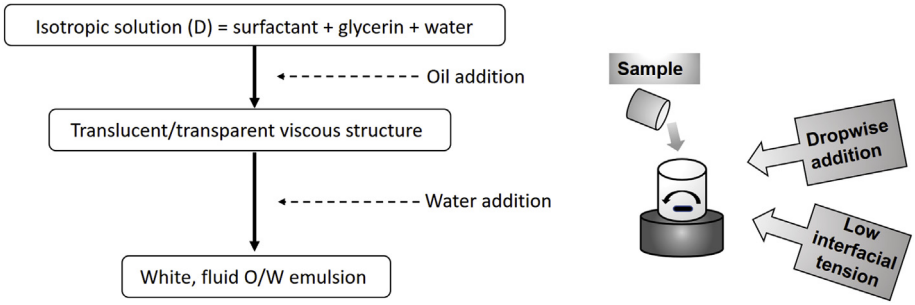


Fig. 1. Nanoemulsion preparation by DPE process.

Table 3
Box-Behnken experiment for nanoemulsion preparations by HPH process.

Order	Pressure (bar)	Cycle	Surfactant (% w/w)	Oil (% w/w)	Glycerin (% w/w)	Mean Particle Size (MPS)	Polydispersity Index (PdI)
1	450	5	2.5	7.5	2.0	287.0 ± 3.4	0.29 ± 0.07
2	600	4	2.5	10.0	1.0	265.1 ± 6.1	0.25 ± 0.08
3	300	4	2.5	5.0	1.0	236.5 ± 3.3	0.27 ± 0.06
4	450	4	1.0	7.5	0.0	386.1 ± 7.7	0.33 ± 0.12
5	600	3	2.5	7.5	1.0	239.8 ± 3.5	0.25 ± 0.03
6	450	4	4.0	7.5	2.0	283.00 ± 5.7	0.40 ± 0.02
7	450	3	2.5	5.0	1.0	238.1 ± 5.7	0.26 ± 0.02
8	450	4	4.0	5.0	1.0	202.2 ± 3.4	0.30 ± 0.04
9	450	4	4.0	7.5	0.0	248.5 ± 0.8	0.31 ± 0.07
10	600	4	2.5	7.5	2.0	241.6 ± 4.9	0.24 ± 0.04
11	450	4	1.0	7.5	2.0	376.8 ± 21.0	0.16 ± 0.10
12 (CP)	**450**	**4**	**2.5**	**7.5**	**1.0**	**297.4 ± 0.4**	**0.34 ± 0.03**
13	300	4	4.0	7.5	1.0	262.7 ± 7.5	0.36 ± 0.01
14	450	4	1.0	5.0	1.0	340.8 ± 16.7	0.22 ± 0.06
15	450	4	2.5	10.0	0.0	310.7 ± 18.7	0.23 ± 0.08
16	450	5	4.0	7.5	1.0	249.4 ± 2.5	0.30 ± 0.05
17 (CP)	**450**	**4**	**2.5**	**7.5**	**1.0**	**270.0 ± 2.5**	**0.40 ± 0.01**
18	450	3	2.5	7.5	0.0	300.3 ± 2.2	0.40 ± 0.03
19	450	4	1.0	10.0	1.0	367.7 ± 138.0	0.40 ± 0.04
20	600	4	1.0	7.5	1.0	374.4 ± 11.1	0.35 ± 0.10
21	300	5	2.5	7.5	1.0	324.0 ± 4.2	0.45 ± 0.01
22	450	5	2.5	5.0	1.0	216.9 ± 4.0	0.27 ± 0.02
23	600	4	4.0	7.5	1.0	200.9 ± 1.7	0.24 ± 0.01
24 (CP)	**450**	**4**	**2.5**	**7.5**	**1.0**	**282.3 ± 2.4**	**0.40 ± 0.01**
25	450	5	2.5	10.0	1.0	297.0 ± 4.3	0.25 ± 0.03
26	450	4	2.5	7.5	1.0	289.2 ± 8.5	0.36 ± 0.05
27	300	4	2.5	7.5	2.0	314.5 ± 3.6	0.34 ± 0.0
28 (CP)	**450**	**4**	**2.5**	**7.5**	**1.0**	**287.1 ± 8.6**	**0.20 ± 0.09**
29	300	4	2.5	7.5	0.0	315.9 ± 4.3	0.33 ± 0.11
30	300	3	2.5	7.5	1.0	321.1 ± 8.6	0.36 ± 0.07
31	450	5	2.5	7.5	0.0	278.6 ± 5.7	0.24 ± 0.11
32	450	4	4.0	10.0	1.0	300.3 ± 11.67	0.20 ± 0.19
33	600	5	2.5	7.5	1.0	235.6 ± 2.5	0.26 ± 0.08
34	300	4	1.0	7.5	1.0	454.6 ± 12.9	0.45 ± 0.02
35	450	3	2.5	7.5	2.0	299.3 ± 0.8	0.34 ± 0.06
36 (CP)	**450**	**4**	**2.5**	**7.5**	**1.0**	**295.8 ± 7.3**	**0.29 ± 0.08**
37	450	3	4.0	7.5	1.0	241.5 ± 2.3	0.31 ± 0.04
38	450	3	2.5	10.0	1.0	308.4 ± 15.7	0.16 ± 0.12
39	600	4	2.5	5.0	1.0	204.2 ± 0.3	0.25 ± 0.02
40	300	4	2.5	10.0	1.0	313.8 ± 17.8	0.16 ± 0.10
41	450	3	1.0	7.5	1.0	400.8 ± 18.4	0.28 ± 0.17
42	450	4	2.5	5.0	0.0	236.1 ± 1.8	0.27 ± 0.01
43	450	4	2.5	5.0	2.0	227.8 ± 0.9	0.25 ± 0.02
44	450	4	2.5	10	2.0	298.4 ± 15.1	0.20 ± 0.11
45	450	5	1.0	7.5	1.0	376.6 ± 24.8	0.18 ± 0.08
46	600	4	2.5	7.5	0.0	239.7 ± 1.2	0.22 ± 0.04

CP = central point.

glycerol were previously dissolved, at a specific temperature (Table 2) under stirring. The pre-heated olive oil at the same temperature was added dropwise into this surfactant solution, under magnetic stirring, at 250 rpm. After completion of the addition of the oil, the system was kept under stirring at 250 rpm for 20 min, at a constant temperature. The process was followed by the dropwise addition of pre-heated remaining water to obtain the final O/W emulsion. After complete addition of the remaining water, the emulsion was cooled down to 25 °C (Fig. 1).

The process and formulation parameter were the temperature and the concentrations of olive oil, glycerin, surfactant and initial water (input factors), respectively. The independent variables or input factors are shown in Table 2. A total of 46 formulas with 5 central points were prepared in random order using the statistical software Minitab 17

Table 4
Analysis of variance for the different models fitted-response for mean particle size of nanoemulsion by HPH process.

Source	DF	SS	MS	F-Value	P-Value
Model	8	127493	15936.7	88.30	0.001
Linear	5	106307	21261.4	117.80	0.001
Pressure (bar)	**1**	**16318**	**16318.1**	**90.41**	**0.001**
Number of cycle	1	440	440.0	2.4	0.127
Surfactant (% w/w)	**1**	**70022**	**70022.4**	**387.98**	**0.001**
Oil (% w/w)	**1**	**19516**	**19516.1**	**108.13**	**0.001**
Glycerin (%w/w)	1	10	10.2	0.06	0.813
Square	2	19919	9959.5	55.18	0.001
Surfactant (% w/w)* Surfactant (% w/w)	**1**	**13196**	**13196.3**	**73.12**	**0.001**
Oil (% w/w)* Oil (% w/w)	**1**	**4075**	**4074.7**	**22.58**	**0.001**
Interaction	1	1267	1267.4	7.02	0.012
Surfactant (% w/w)* Oil (% w/w)	**1**	**1267**	**1267.4**	**7.02**	**0.012**
Error	37	6678	180.5		
Lack of fit	**32**	**6176**	**193.0**	**1.92**	**0.241**
Pure error	5	502	100.3	*	*
Total	45	134171			
S = 13.43 R^2 = 95.02%	R^2 adj = 93.95%			R^2 pred = 90.93%	

DF = degrees of freedom, SS = sequential sums of squares, MS = sequential mean squares, F-Value = value on the F distribution, P-Value = lack-of-fit adjustment, S = standard error of the regression, R^2 = multiple correlation coefficient, R^2_{adj} = adjusted multiple correlation coefficient, R^2_{pred} = predicted correlation coefficient.

(State College PA, USA).

3.2. Optimization procedure

Statistical software Minitab 17 (State College PA, USA) response optimizer tool was used to identify process and formulation parameters that provide nanoemulsion containing the same composition and presenting similar MPS, regardless of the use of the HPH or DPE process. The composite desirability ranges from zero to one. One represents the target MPS; zero indicates that one or more responses are outside acceptable limits.

3.3. Model validation

Based on the optimization procedure, a new preparation was carried out, one for each process (HPH and DPE). The observed and predicted MPS values for the obtained nanoemulsions were compared to evaluate the adequacy of the final models.

3.4. Mean particle size (MPS) and polydispersity index (PdI) analysis

The measurements of MPS and PdI were carried out using Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK), by photon correlation spectroscopy. Samples were diluted in purified water prior to analysis to avoid multiple scattering effects. This measurement is based on the principle of dynamic light scattering.

3.5. Preparation of nanoemulsion gel

The nanoemulsion gels were prepared using the optimized formulations to achieve a final carbopol concentration of 0.2% (w/w) with pH adjusted to 5.0–5.5. These nanoemulsion gel preparations were kept in closed borosilicate glass vessels and a stability test was carried out for three months at 4 °C and 25 °C, by determination of MPS and by visual inspection of the formulations.

4. Results and discussion

4.1. Box-Behnken statistical design

4.1.1. HPH process

The design of experiment (DoE) allows evaluating multiple interactions between independent variables in experiments, differing from the conventional one-factor-at-a-time method [23]. By analyzing these interactions between the input factors (process and formulation parameters) and associated output response (CQA) [28], it allows for increasing understanding the product and process, once integrated with mechanistic-based studies [29]. This DS approach has gained attention in recent years, considered a powerful tool for implementing QbD [30]. In this study, a Box-Behnken design revealed the main effects and interactions of the evaluated factors, with the advantage of requiring a lower number of runs compared to other Response Surface Methods (RSM) (e.g. Central Composite Method). The results are shown in Table 3, with the MPS (output factor) ranging from 202.2 to 454.6 nm (PdI from 0.16 to 0.45).

The analysis of variance (ANOVA) of the resultant quadratic polynomial models for the MPS of olive oil nanoemulsion is shown in Table 4. This study was carried out to identify the significant terms (input factors) and conduct a statistical analysis of the regression model [28]. The effects corresponding to the investigated input factors (pressure [X_1], number of cycles of homogenization [X_2], concentrations of surfactant [X_3], olive oil [X_4], and glycerin [X_5]) for the MPS was evaluated. The p-value represents the significance of the regression coefficients for a polynomial equation, where a p-value lower than 0.05

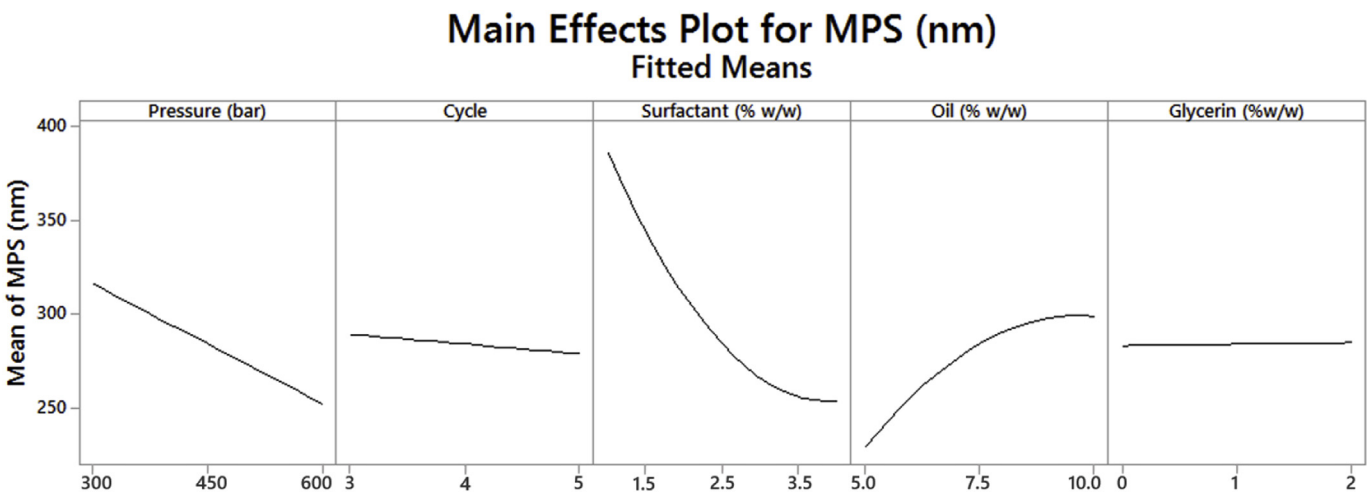


Fig. 2. Main effect plots for mean particle size as a function of components and preparation variables by HPH process.

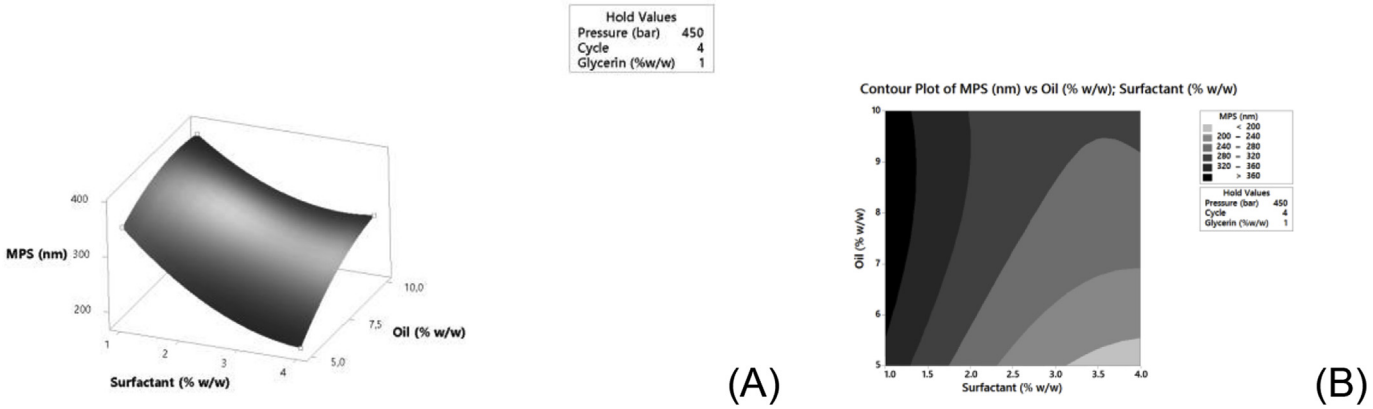


Fig. 3. Response surface (A) and contour plot (B) of means particle size as a function of component variables by HPH process.

Table 5
Box-Behnken experiment for nanoemulsion preparations by DPE process.

Order	Surfactant (% w/w)	Oil (% w/w)	Glycerin (% w/w)	Initial Water (% w/w)	Tempera ture (°C)	Mean Particle Size (MPS)	Polydispersity Index (PdI)
1 (CP)	**2.5**	**7.5**	**2.0**	**2.0**	**60**	**295.8 ± 8.5**	**0.22 ± 0.03**
2	2.5	10.0	3.0	2.0	60	S	–
3	2.5	5.0	2.0	1.0	60	S	–
4	2.5	7.5	1.0	3.0	60	S	–
5	2.5	7.5	1.0	2.0	70	S	–
6	1.0	10.0	2.0	2.0	60	323.3 ± 21.5	0.23 ± 0.19
7 (CP)	**2.5**	**7.5**	**2.0**	**2.0**	**60**	**279.5 ± 8.3**	**0.17 ± 0.04**
8	2.5	7.5	2.0	3.0	70	274.7 ± 2.9	0.19 ± 0.04
9	1.0	7.5	2.0	2.0	70	S	–
10	1.0	7.5	2.0	1.0	60	S	–
11 (CP)	**2.5**	**7.5**	**2.0**	**2.0**	**60**	**334.2 ± 14.2**	**0.11 ± 0.08**
12	1.0	7.5	2.0	2.0	50	337.1 ± 13.5	0.11 ± 0.08
13	2.5	5.0	3.0	2.0	60	S	–
14	2.5	10.0	2.0	2.0	70	S	–
15	2.5	5.0	2.0	2.0	50	298.0 ± 5.2	0.16 ± 0.07
16	4.0	10.0	2.0	2.0	60	276.8 ± 23,6	0.18 ± 0.09
17	2.5	7.5	3.0	2.0	70	S	–
18	2.5	7.5	2.0.	1.0	50	S	–
19	2.5	7.5	1.0	1.0	60	S	–
20	2.5	10.0	2.0	2.0	50	371.1 ± 9.1	0.25 ± 0.08
21	4.0	5.0	2.0	2.0	60	307.2 ± 5.9	0.11 ± 0.08
22	2.5	7.5	2.0	3.0	50	327.5 ± 18.2	0.18 ± 0.12
23	1.0	7.5	2.0	3.0	60	1064.0 ± 104.0	1.00
24 (CP)	**2.5**	**7.5**	**2.0**	**2.0**	**60**	**338.8 ± 7.2**	**0.25 ± 0.04**
25	4.0	7.5	2.0	2.0	50	214.5 ± 1.4	0.17 ± 0.02
26	4.0	7.5	2.0	3.0	60	324.0 ± 10.4	0.15 ± 0.08
27 (CP)	**2.5**	**7.5**	**2.0**	**2.0**	**60**	**358.9 ± 20.6**	**0.17 ± 0.07**
28	2.5	7.5	3.0	3.0	60	356.5 ± 12.0	0.14 ± 0.10
29	2.5	7.5	2.0	2.0	60	297.8 ± 4.7	0.12 ± 0.11
30	2.5	10.0	1.0	2.0	60	S	–
31	2.5	5.0	2.0	3.0	60	242.7 ± 3.6	0.10 ± 0.04
32	1.0	7.5	1.0	2.0	60	S	–
33	2.5	7.5	2.0	1.0	70	S	–
34	1.0	7.5	3.0	2.0	60	606.0 ± 35.8	0.68 ± 0.44
35	4.0	7.5	2.0	2.0	70	S	–
36	2.5	10.0	2.0	1.0	60	S	–
37	4.0	7.5	2.0	1.0	60	S	–
38	4.0	7.5	1.0	2.0	60	S	–
39	4.0	7.5	3.0	2.0	60	S	–
40	2.5	7.5	3.0	1.0	60	S	–
41	2.5	7.5	1.0	2.0	50	S	–
42	2.5	7.5	3.0	2.0	50	263.1 ± 2.5	0.15 ± 0.01
43	2.5	5.0	1.0	2.0	60	237.4 ± 5.0	0.16 ± 0.03
44	2.5	10.0	2.0	3.0	60	283.1 ± 11.7	0.17 ± 0.13
45	2.5	5.0	2.0	2.0	70	S	–
46	1.0	5.0	2.0	2.0	60	751.3 ± 61.7	0.55 ± 0.43

S = Separation in one day, CP = central point.

($\alpha = 0.05$) indicates that the corresponding coefficient was significant [12,22,23]. The significant input factors were the pressure (p-value equal to 0.001), the surfactant and olive oil concentration (p-value equal to 0.001) and the interaction between the surfactant and olive oil (p-value equal to 0.012). As observed in the main effect graph, due to the quadratic effect of surfactant, the lowest MPS was achieved in the vertex region of the parabola, corresponding to its highest concentration (3.5–4.0% w/w). In contrast, the influence of olive oil

Table 6
Second phase of Box-Behnken experiment for nanoemulsion preparations by DPE process.

Order	Surfactant (% w/w)	Oil (% w/w)	Initial water (% w/w)	Mean Particle Size (MPS)	Polydispersity Index (PdI)
1 (CP)	**3.25**	**7.5**	**2.5**	**295.0 ± 2.6**	**0.29 ± 0.01**
2	2.50	7.5	3.0	726.3 ± 4.8	0.31 ± 0.01
3	3.25	10.0	2.0	669.5 ± 5.6	0.29 ± 0.01
4	2.50	7.5	2.0	625.2 ± 4.4	0.25 ± 0.01
5	4.00	10.0	2.5	477.3 ± 9.1	0.29 ± 0.07
6	3.25	5.0	2.0	790.3 ± 20.6	0.36 ± 0.20
7	4.00	7.5	2.0	1399.0 ± 48.2	0.87 ± 0.22
8	4.00	5.0	2.5	648.8 ± 17.7	0.14 ± 0.12
9	2.50	5.0	2.5	485.0 ± 4.9	0.21 ± 0.01
10	4.00	7.5	3.0	289.0 ± 16.6	0.20 ± 0.06
11	3.25	10.0	3.0	288.6 ± 18.6	0.17 ± 0.07
12	3.25	5.0	3.0	384.7 ± 15.0	0.11 ± 0.03
13 (CP)	**3.25**	**7.5**	**2.5**	**371.5 ± 11.3**	**0.21 ± 0.01**
14	2.50	10.0	2.5	401.4 ± 25.8	0.35 ± 0.29
15 (CP)	**3.25**	**7.5**	**2.5**	**320.0 ± 21.02**	**0.19 ± 0.11**

CP = central point.

Table 7
Analysis of variance for the different models fitted-response for mean particle size of nanoemulsion by DPE process.

Source	DF	SS	MS	F-value	P-value
Model	6	1168879	194813	102.60	0.001
Linear	3	472281	157427	82.91	0.001
Surfactant (%w/w)	**1**	**41501**	**41501**	**21.86**	**0.002**
Oil (%w/w)	**1**	**27848**	**27848**	**14.67**	**0.005**
Initial water (%w/w)	**1**	**402933**	**402933**	**212.22**	**0.001**
Square	2	329907	164953	86.88	0.001
Surfactant (%w/w)* Surfactant (%w/w)	**1**	**152245**	**152245**	**80.19**	**0.001**
Initial water (%w/w)* Initial water (%w/w)	**1**	**200966**	**200966**	**105.85**	**0.001**
Interaction	1	366691	366691	193.13	0.001
Surfactant (%w/w)* Initial water (%w/w)	**1**	**366691**	**366691**	**193.13**	**0.001**
Error	8	15189	1899		
Lack of fit	6	12146	2024	1.33	**0.489**
Pure error	2	3043	1522	*	*
Total	14	1184068			
S = 43.57 R^2 = 98.72%	R^2_{adj} = 97.76%			R^2_{pred} = 93.72%	

DF = degrees of freedom, SS = sequential sums of squares, MS = sequential mean squares, F-Value = value on the F distribution, P-Value = lack-of-fit adjustment, S = standard error of the regression, R^2 = multiple correlation coefficient, R^2_{adj} = adjusted multiple correlation coefficient, R^2_{pred} = predicted correlation coefficient.

concentration in MPS achieved the lowest value in its lowest concentration, at 5.0% (w/w) (Fig. 2). The lack-of-fit was non-significant (p-value equal to 0.241, higher than 0.05), indicating minimum pure errors (e.g. experimental errors) [12,31]. This fact indicates the well fitness of the proposed quadratic polynomial model.

The quadratic regression model demonstrated the coefficient of determination (R^2) for the MPS of 95.02%, indicating that this response value could be attributed to the identified input factors. The R^2_{adj}, which reflects the correlation between the experimental and predicted values, was 93.95%. This is a closer value to R^2, indicating a good statistical model. The predicted coefficient of determination (R^2_{pred}) was 90.93% indicating how well the model predicts responses for new observations (Table 4).

The number of cycles and the concentration of glycerin in the evaluated range did not show a significant influence on the output response. Hence, both input factors were not critical to the MPS. Thus, these factors were excluded (except for those required to support hierarchy) [12,23] and the final reduced quadratic model for MPS of olive oil nanoemulsion by HPH process was generated, as demonstrated in the following regression equation, in terms of the uncoded factor.

$$MPS(nm) = 414.2 - 0.2129\,X_1 - 5.24\,X_2 - 159.6\,X_3 + 50.1\,X_4 + 0.80\,X_5 + 15.99\,X_3^2 - 3.198\,X_4^2 + 4.75\,X_3X_4$$

The effect of the input factor that synergistically influences the MPS reduction is demonstrated by a negative value in the regression equation, and the inverse effect of the input factor that influences the increase in MPS is demonstrated by a positive value [12].

The interaction between the input factors and the output response was demonstrated under construction of the three-dimensional (3D) surface response and the contour plots. The effects of surfactant and olive oil concentration on MPS by the HPH process are clearly demonstrated in Fig. 3.

In brief, this approach allowed identifying pressure as a critical process parameter for MPS, a critical quality attribute of nanoemulsion. The DS also revealed that the lowest MPS was achieved using the highest surfactant and the lowest olive oil concentrations. Thus, it was possible to identify the input factors and their ranges within which consistent quality can be achieved.

As a complementally information, Dordević et al. [23] applied a general factorial design approach, to evaluating the effect of preparation and process factors that affect the CQA of the nanoemulsion prepared by HPH method. The HPH process (hot temperature) was identified as the main factor of MPS reduction (one of CQA), followed by aqueous phase type and co-emulsifier type. This indicated that both, preparation and process factors, influenced the MPS of this nanoemulsion prepared by HPH, which is consistent with our results.

4.1.2. DPE process

The Box-Behnken design used in the HPH process was also applied to evaluate the influence of input factors on the DPE process. These input factors, components and their concentration, were similar to the components used in the HPH process (Table 2). The results of MPS and PdI for 46 formulas are shown in Table 5. However, it resulted in a wide range of outside specification nanoemulsions (separation in one day), which did not allow subsequent statistical analysis of data. Nevertheless, these results informed the decisions for further improvements in the process. Furthermore, they allowed us to identify and solve the unpredictability of specific process and formulation parameters in this exploration stage of DS, in the DPE process. This DS step identified the optimized ranges of the input parameter and improved understanding the process [30], as described below, bringing the output response into the specification range (CQA).

The concentration of glycerin at 1.0% (w/w) demonstrated that 7 out of 8 preparations presented separation in one day, and at 3.0% (w/w) of glycerin, 5 out of 8 preparations showed the same behavior. At 2.0% (w/w), 15 out of 26 nanoemulsions were successfully obtained. The central points, containing 2.0% (w/w) glycerin, resulted in nanoemulsion with MPS around 300 nm. This result is in agreement with the previous study [27], confirming that the concentration of glycerin strongly impacts the MPS of nanoemulsion in the DPE process.

Temperatures of 50 °C and 60 °C showed 6 out of 7 and 12 out of 26 successful nanoemulsions, respectively. Although at 50 °C the ratio was higher, we decided to use the temperature at 60 °C since it generated an intermediate phase with appropriate viscosity, which allowed for easier manipulation than at 50 °C.

Thus, using the fixed amount of glycerin (2.0% w/w) and setting the temperature to 60 °C, tighten the concentration of surfactant (2.50; 3.25; 4.00%) and initial water (2.0; 2.5; 3.0%) (all w/w), the subsequent and more accurate study was performed for DS building and optimization. The input factor, olive oil, was kept at the previous concentration (5.0; 7.5; 10.0%). The output responses, MPS and PdI, for designed preparations (15 formulations with 3 central points), are

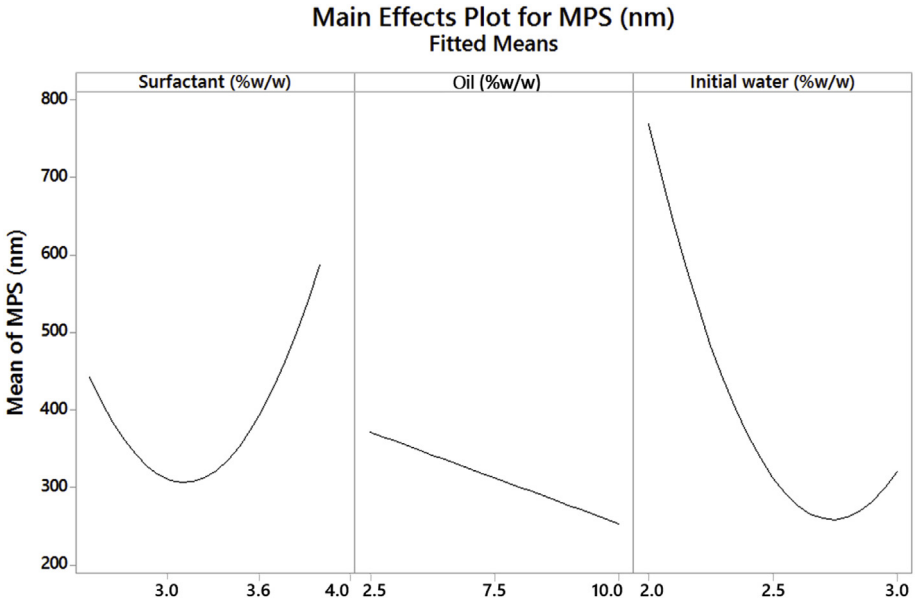


Fig. 4. Main effect plots for mean particle size as a function of components and preparation variables by DPE process.

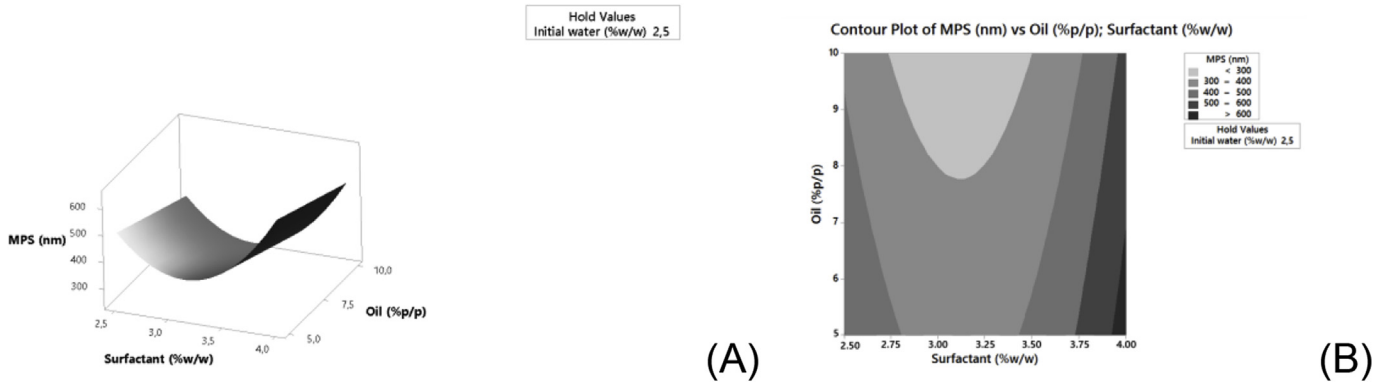


Fig. 5. Response surface (A) and contour plot (B) of means particle size as a function of component variables by DPE process.

shown in Table 6. The results showed the MPS ranging from 288.6 nm to 1399.0 nm (PdI from 0.11 to 0.87).

The analysis of variance (ANOVA) of the resultant quadratic polynomial models for the MPS of olive oil nanoemulsion is shown in Table 7. The effects corresponding to the investigated input factors (surfactant [Z_1], olive oil [Z_2], glycerin [Z_3], initial water [Z_4], and temperature [Z_5]) for MPS was evaluated. The significant input factors were surfactant, olive oil and initial water concentrations (p-value equal to 0.005, 0.002 and 0.001, respectively) and the interaction between the surfactant and initial water (p-value equal to 0.001). In the graph of the main effect, we observed that the quadratic effect of the surfactant concentration in MPS provided the lowest value around 3.2% (w/w). For the initial water concentration, the quadratic effect revealed the highest MPS value around 2.8% (w/w) (Fig. 4). The lack-of-fit was non-significant (p-value equal to 0.489, higher than 0.05), indicating the well fitness of the proposed quadratic polynomial model (Table 7). Due to the quadratic effect of the surfactant, the lowest MPS was achieved in the narrow vertex region of the parabola. A similar result was obtained for the initial water concentration (Fig. 4). Thus, the DPE process showed a greater influence of the compositions, as input factor, on the MPS (output response), compared to HPH.

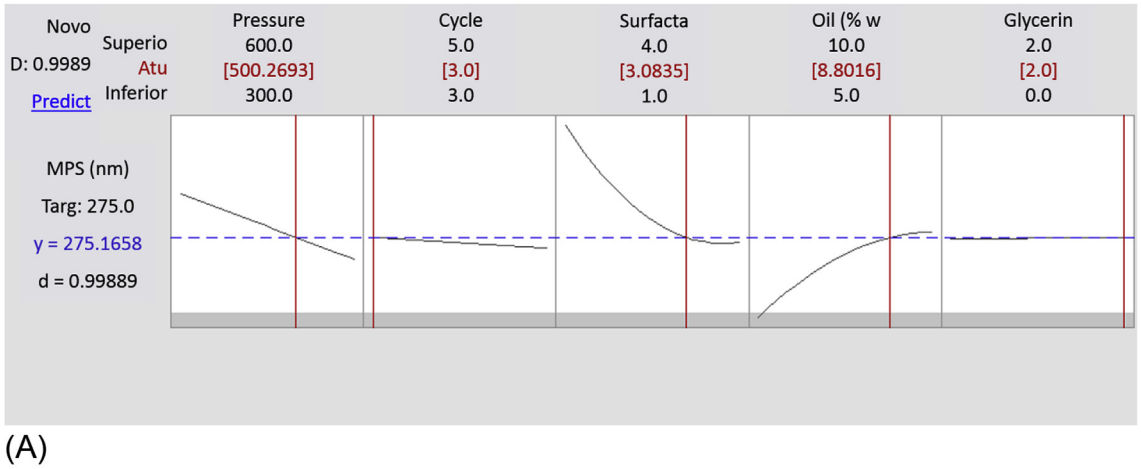
The quadratic regression model demonstrated the coefficient of determination (R^2) for the MPS of 98.72%, indicating that this response value could be attributed to the identified input factors. The R^2_{adj}, which reflects the correlation between the experimental and predicted values, was 97.76%. This is a closer value to R^2, indicating a good statistical model. The predicted coefficient of determination (R^2_{pred}) was 93.72%, indicating how well the model predicts responses for new observations (Table 7).

The final reduced quadratic model for MPS of olive oil nanoemulsion by DPE process was generated, as demonstrated in the following regression equation, in terms of the uncoded factor.

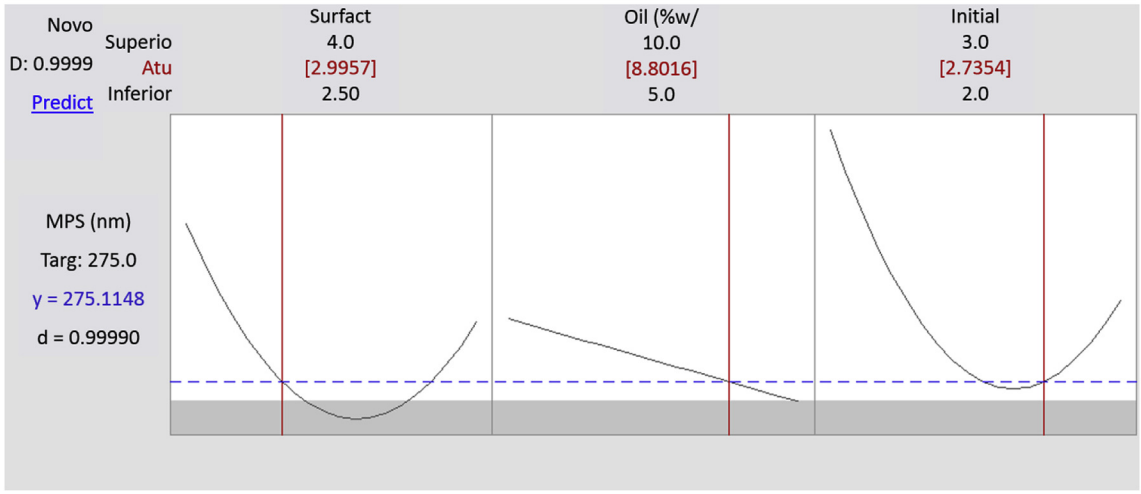
$$\text{MPS (nm)} = 4357 - 225\,Z_1 - 23.60\,Z_2 - 2477\,Z_4 + 359.9\,Z_1^2 + 930.4\,Z_4^2 - 807.4\,Z_1Z_4$$

The interaction between the input factors and the responses was demonstrated by the three-dimensional (3D) surface response and the contour plots. The effects of the surfactant and the olive oil concentration on the MPS, by the DPE process, are shown in Fig. 5. It is notable that the MPS was reduced as the concentration of olive oil was increased, in a narrow region of the surfactant concentration, which is the opposite phenomenon observed in the HPH process. This behavior of olive oil in this process was also observed in our previous study [27].

As an additional comment, unlike conventional low-energy methods such as PIT and PIC, DPE allowed to obtain nanoemulsions using a single highly hydrophilic surfactant: oleth-20 (HLB = 15.3). There was no need to combine lipophilic surfactant with oleth-20 to adjust the final HLB of O/W nanoemulsion containing olive oil (HLB = 7.0). This proves that the strict adjustment of HLB when DPE method is applied, is not necessary.



(A)



(B)

Fig. 6. Optimization plot for HPH process (A) and DPE process (B).

Table 8
Theoretical and experimental value of MPS of optimized formulas by HPH and DPE processes.

Formula	Theoretical MPS	Experimental MPS	Experimental PdI
HPH Opt	275.1	285.9 ± 12.8	0.32 ± 0.15
DPE Opt	275.1	278.2 ± 10.3	0.28 ± 0.07

MPS = mean particle size, PdI = polydispersity index.

4.2. Optimization procedure

Figs. 1 and 3 revealed the opposite effect of the olive oil concentration on the MPS for the DPE and HPH processes. Therefore, identifying optimized nanoemulsions by the HPH and DPE processes with the same composition to obtain a similar MPS was quite a challenge. This challenge was successfully overcome using the desirability function (target 275 nm d, 0.99), which revealed a MPS of 275.1 nm for the HPH process, as well for the DPE process (Fig. 6).

The optimization composition of the nanoemulsion for both processes was 2.0% glycerin, 3.0% surfactant and 8.8% olive oil at (all w/w). The pressure at 500 bars and 3 cycles were fixed for HPH process. The 2.7% (w/w) initial water and temperature at 60 °C were set for the DPE process as complementary preparation parameters to achieve this MPS.

4.3. Fitting model verification at the selected range

For both HPH and DPE processes, the similar predicted and observed MPS obtained from the optimized formulas, as shown in Table 8, validated the proposed models.

4.4. Nanoemulsion gel stability test

A viscosity agent was incorporated in the nanoemulsion to yield a nanoemulsion gel. Carbomer is a polymeric thickener applied in emulsion preparations. This is well known for its safety and the effectiveness to improve viscosity and texture of nanoemulsions for oral or topical applications. Moreover, it provides stability enhancement since nanoemulsion is driven by Ostwald ripening phenomenon – the main cause of instability of this system [32]. Electrostatic or steric stabilization are generally applied in order to overcome this problem [3]. Carbomer offered the stearic stabilization for this system, as shown in the following results:

The 3-month stability test for the optimized gel formulations showed no change in MPS. For nanoemulsion gel prepared by HPH process, the initial MPS was 305.9 ± 12.6 nm (PdI = 0.32 ± 0.09). After 3 months of stability, the results were 284.3 ± 6.3 nm (PdI = 0.23 ± 0.07) and 294.1 ± 4.3 nm (PdI = 0.23 ± 0.11) at 25 °C and 4 °C, respectively. For the nanoemulsion gel prepared by the

DPE process, the initial MPS was 328.8 ± 11.9 nm (PdI = 0.24 ± 0.05), and after 3 months of stability the results were 327.8 ± 17.7 nm (PdI = 0.10 ± 0.09) and 342.2 ± 10.4 nm (PdI = 0.18 ± 0.06) at 25 °C and 4 °C, respectively. No phase separation was observed for both processes during this time interval by visual evaluation.

As an outcome of this study, we observed that in the HPH process, the identification of CPP was fairly simple using the one-step Box-Behnken design. For the DPE process, an additional step was necessary to identify the input factors affecting the CQA, which were represented by the narrower acceptable ranges of component and process parameters (tight concentration range of surfactant and initial water, glycerin set at 2% w/w and temperature at 60 °C), when compared to the HPH process. The process and formulation parameters of the HPH method, which affect the CQA of the nanoemulsion, were the pressure, concentration of surfactant and concentration of olive oil. For DPE, these input factors were the surfactant, olive oil and initial water concentrations (glycerin concentration and temperature were fixed). As a result, a deeper understanding of the HPH and DPE processes was achieved revealing the DS for the required CQA. Additionally, a similar MPS for the same nanoemulsion composition, independent of the process used, was obtained according to the purpose of this study. This opens opportunities for regulatory flexibility in the selection of these processes for the manufacture of olive oil nanoemulsion.

5. Conclusion

In the present study, a systematic design of experiments approach was successfully applied to identify and understand the relationship between input factors and their associated output response in the development of olive oil nanoemulsion, by HPH and DPE processes. This approach allowed us to identify the optimized ranges of the input parameter and to improve the process understanding, bringing the output response into the specification range (CQA). The implementation applying RSM optimization provided a unique range of CPP within the design space, where nanoemulsions with similar mean particle sizes of 275 nm could be achieved with equal composition, for both HPH and DPE processes.

These nanoemulsions containing olive oil can provide therapeutic effects such as antioxidant and anti-inflammatory properties, for topical applications. In addition, there is the possibility of its applications as carriers of poorly water-soluble drugs in nanoemulsions.

CRediT author statement

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Conflicts of interest

The authors declare no conflict of interest in this article.

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Appendix A. Supplementary data

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References

[1] R. Parveen, S. Baboota, J. Ali, A. Ahuja, S.S. Vasudev, S. Ahmad, Oil based nanocarrier for improved oral delivery of silymarin: in vitro and in vivo studies, Int. J. Pharm. 413 (2011) 245–253, https://doi.org/10.1016/j.ijpharm.2011.04.041.
[2] S. Ganta, M. Talekar, A. Singh, T.P. Coleman, M.M. Amiji, Nanoemulsions in translational research—opportunities and challenges in targeted cancer therapy, AAPS PharmSciTech 15 (2014) 694–708, https://doi.org/10.1208/s12249-014-0088-9.
[3] M.N. Yukuyama, E.T.M. Kato, R. Löbenberg, N.A. Bou-Chacra, Challenges and future prospects of nanoemulsion as a drug delivery system, Curr. Pharmaceut. Des. 23 (2017), https://doi.org/10.2174/1381612822666161027111957.
[4] S. Akhavan, E. Assadpour, I. Katouzian, S.M. Jafari, Lipid nano scale cargos for the protection and delivery of food bioactive ingredients and nutraceuticals, Trends Food Sci. Technol. 74 (2018) 132–146, https://doi.org/10.1016/j.tifs.2018.02.001.
[5] C. Qian, D.J. McClements, Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size, Food Hydrocolloids 25 (2011) 1000–1008, https://doi.org/10.1016/j.foodhyd.2010.09.017.
[6] C. Solans, P. Izquierdo, J. Nolla, N. Azemar, M.J. Garcia-Celma, Nano-emulsions, Curr. Opin. Colloid Interface Sci. 10 (2005) 102–110, https://doi.org/10.1016/j.cocis.2005.06.004.
[7] L.A. Trujillo-Cayado, J. Santos, P. Ramírez, M.C. Alfaro, J. Muñoz, Strategy for the development and characterization of environmental friendly emulsions by microfluidization technique, J. Clean. Prod. 178 (2018) 723–730, https://doi.org/10.1016/j.jclepro.2018.01.028.
[8] P. Izquierdo, J. Esquena, T.F. Tadros, J.C. Dederen, J. Feng, M.J. Garcia-Celma, N. Azemar, C. Solans, Phase behavior and nano-emulsion formation by the phase inversion temperature method, Langmuir 20 (2004) 6594–6598, https://doi.org/10.1021/la049566h.
[9] M.N. Yukuyama, D.D.M. Ghisleni, T.J.A. Pinto, N.A. Bou-Chacra, Nanoemulsion: process selection and application in cosmetics - a review, Int. J. Cosmet. Sci. 38 (2016) 13–24, https://doi.org/10.1111/ics.12260.
[10] H. Sagitani, formation of fine emuisions by surface chemical methods focusing on the mechanism of the inversion emulsification method and the surfactant (D) phase emulsification method, J. Oleo Sci. 35 (1986), https://doi.org/10.5650/jos1956.35.198 198–2.
[11] Y. Yang, C. Marshall-Breton, M.E. Leser, A.A. Sher, D.J. McClements, Fabrication of ultrafine edible emulsions: comparison of high-energy and low-energy homogenization methods, Food Hydrocolloids 29 (2012) 398–406, https://doi.org/10.1016/j.foodhyd.2012.04.009.
[12] S. Kotta, A.W. Khan, S.H. Ansari, R.K. Sharma, J. Ali, Formulation of nanoemulsion: a comparison between phase inversion composition method and high-pressure homogenization method, Drug Deliv. 22 (2015) 455–466, https://doi.org/10.3109/10717544.2013.866992.
[13] A. Bajoub, T. Pacchiarotta, E. Hurtado-Fernández, L. Olmo-García, R. García-Villalba, A. Fernández-Gutiérrez, O.A. Mayboroda, A. Carrasco-Pancorbo, Comparing two metabolic profiling approaches (liquid chromatography and gas chromatography coupled to mass spectrometry) for extra-virgin olive oil phenolic compounds analysis: a botanical classification perspective, J. Chromatogr. A 1428 (2016) 267–279, https://doi.org/10.1016/j.chroma.2015.10.059.
[14] T. Keceli, M.H. Gordon, The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil, J. Sci. Food Agric. 81 (2001) 1391–1396, https://doi.org/10.1002/jsfa.959.
[15] E. Tripoli, M. Giammanco, G. Tabacchi, D. Di Majo, S. Giammanco, M. La Guardia, The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health, Nutr. Res. Rev. 18 (2005) 98, https://doi.org/10.1079/NRR200495.
[16] S. Cicerale, L.J. Lucas, R.S.J. Keast, Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil, Curr. Opin. Biotechnol. 23 (2012) 129–135, https://doi.org/10.1016/j.copbio.2011.09.006.
[17] R.W. Owen, A. Giacosa, W.E. Hull, R. Haubner, G. Würtele, B. Spiegelhalder, H. Bartsch, Olive-oil consumption and health: the possible role of antioxidants, Lancet Oncol. 1 (2000) 107–112, https://doi.org/10.1016/S1470-2045(00)00015-2.
[18] M.A. Carluccio, L. Siculella, M.A. Ancora, M. Massaro, E. Scoditti, C. Storelli, F. Visioli, A. Distante, R. De Caterina, Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 622–629, https://doi.org/10.1161/01.ATV.0000062884.69432.A0.
[19] A. Budiyanto, Protective effect of topically applied olive oil against photocarcinogenesis following UVB exposure of mice, Carcinogenesis 21 (2000) 2085–2090, https://doi.org/10.1093/carcin/21.11.2085.
[20] J. Rodríguez-Morató, L. Xicota, M. Fitó, M. Farré, M. Dierssen, R. De La Torre, Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases, Molecules 20 (2015) 4655–4680, https://doi.org/10.3390/molecules20034655.
[21] Y. Sun, Z. Xia, J. Zheng, P. Qiu, L. Zhang, D.J. McClements, H. Xiao, Nanoemulsion-based delivery systems for nutraceuticals: influence of carrier oil type on

bioavailability of pterostilbene, J. Funct. Foods. 13 (2015) 61–70, https://doi.org/10.1016/j.jff.2014.12.030.
[22] Ich, Pharmaceutical development Q8, ICH harmon, Tripart. Guidel. 8 (2009) 1–28.
[23] S.M. Dordević, N.D. Cekić, M.M. Savić, T.M. Isailović, D.V. Randelović, B.D. Marković, S.R. Savić, T.T. Stamenić, R. Daniels, S.D. Savić, Parenteral nanoemulsions as promising carriers for brain delivery of risperidone: design, characterization and in vivo pharmacokinetic evaluation, Int. J. Pharm. 493 (2015) 40–54, https://doi.org/10.1016/j.ijpharm.2015.07.007.
[24] G. Amasya, U. Badilli, B. Aksu, N. Tarimci, Quality by design case study 1: design of 5-fluorouracil loaded lipid nanoparticles by the W/O/W double emulsion - solvent evaporation method, Eur. J. Pharm. Sci. 84 (2016) 92–102, https://doi.org/10.1016/j.ejps.2016.01.003.
[25] F.R. Lourenço, F.L. Francisco, M.R.S. Ferreira, T.D.J.A. Pinto, R. Löbenberg, N. Bou-Chacra, Design space approach for preservative system optimization of an anti-aging eye fluid emulsion, J. Pharm. Pharmaceut. Sci. 18 (2015) 551–561.
[26] H. Endoo, M. Sagitani, Preparation of triglyceride O/W emulsions by D phase emulsification, J. Oil Sci. 40 (1991) 133–139, https://doi.org/10.5650/jos1956.40.133.
[27] M.N. Yukuyama, P.L.F. Oseliero, E.T.M. Kato, R. Lobënberg, C.L.P. de Oliveira, G.L.B. de Araujo, N.A. Bou-Chacra, High internal vegetable oil nanoemulsion: D-phase emulsification as a unique low energy process, Colloids Surfaces A Physicochem. Eng. Asp. 554 (2018) 296–305, https://doi.org/10.1016/j.colsurfa.2018.06.023.
[28] G. Choi, T.H. Le, S. Shin, A new multidimensional design space identification method for a quality-oriented drug development process, Total Qual. Manag. Bus. Excel. 27 (2016) 804–817, https://doi.org/10.1080/14783363.2016.1187999.
[29] L.X. Yu, G. Amidon, M.A. Khan, S.W. Hoag, J. Polli, G.K. Raju, J. Woodcock, Understanding pharmaceutical quality by design, AAPS J. 16 (2014) 771–783, https://doi.org/10.1208/s12248-014-9598-3.
[30] H. Bhatia, E. Read, C. Agarabi, K. Brorson, S. Lute, S. Yoon, A design space exploration for control of Critical Quality Attributes of mAb, Int. J. Pharm. 512 (2016) 242–252, https://doi.org/10.1016/j.ijpharm.2016.08.046.
[31] C.L. Ngan, M. Basri, F.F. Lye, H.R. Fard Masoumi, M. Tripathy, R.A. Karjiban, E. Abdul-Malek, Comparison of process parameter optimization using different designs in nanoemulsion-based formulation for transdermal delivery of fullerene, Int. J. Nanomed. 9 (2014) 4375–4386, https://doi.org/10.2147/IJN.S65689.
[32] O. Sonneville-Aubrun, N.M. Yukuyama, A. Pizzino, Chapter 14 - application of nanoemulsions in cosmetics, Nanoemulsion (2018) 435–475, https://doi.org/10.1016/B978-0-12-811838-2.00014-X.