DoE studies for obtaining a self-assembling nanostructured system based on citronellol homopolymers: a brand-new application for a green lipophilic material

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Abstract

Background: Self-assembling systems are those which get their supramolecular structure spontaneously. The aim of this work was to study the ability of citronellol homopolymers in forming self-assembling nanostructured lipid carriers using regular emulsification processes with non-ionic surfactants.

Method: Four 2^3 DoE were used, where percentages of surfactants, propylene glycol, and content of citronellol homopolymer in the oily phase were factors studied. All formulations were produced by emulsification and visually inspected, pictured, and analysed by laser diffraction. The data were assessed in statistical software for Response Surface Regression applying ANOVA and principal effects presented as Pareto charts. The results were used to run optimization studies and estimate the desirability index for a target formulation.

Results: The visual inspection suggested that different emulsified systems were formed. Most of the tested formulations were unable to form a monodisperse system of nanoparticles, although nanostructures were present in several of them. Principal effects in a Pareto chart revealed that the proportion of citronellol homopolymer was the principal factor of variation. The ANOVA applied for RSR showed most of the p-values < 0.05 for the models in DoE, however the R^2 for models were poor setting below than 0.90. Two optimization studies were done for each DoE, where desirability was higher than 0.75 for all.

Conclusion: It was possible to obtain self-assembling formulations using citronellol homopolymer as part of the oily phase in lipid nanoparticles. The homopolymer has shown an interesting feature as solvent for lipophilic actives and might be used to carrier them in formulations.

Key words: DoE, citronellol homopolymer; self-assembling nanostructured system, lipid nanoparticles.

Introduction

Self-assembling drug delivery systems represent a great interest in the field of nanocarriers development since the supramolecular devices obtained are capable to load actives molecules for different uses such as cosmetics, drugs and foods [1]. These systems constituted by one or more materials have as feature the ability to get spontaneously a level of organization, structuring isolated and stable particles, fibers, gels, among others, able to convey and delivery interest molecules [1], [2].

There are different types of compounds being used as self-assembly materials originating those kinds of supramolecular structures. Polymers, surfactants, lipids, proteins, and peptides are some examples [2]–[4]. For these different classes of compounds, a property is present at all, the amphiphilicity. The balance between hydrophilic and hydrophobic parts in molecules allow them interacting one with others and also with the medium through weak forces such as ionic, dipole-dipole, Van-der-Walls, and hydrogen-bond, that summed become sufficient to maintain the supramolecular aggregates stable [2], [5]. This is the case of emulsions, which are constituted by three fundamental classes of compounds, surfactants, lipophilic compounds and water, as placed together under specific conditions [6].

Emulsified systems are present at nature playing several roles and might be obtained with adequate proportions of each compound, and some energy, which will vary depending on the self-assembly capacity presented by the gather of compounds and the medium they are placed. Thus, some systems like microemulsions are formed in a complete spontaneous way [7], whereas for polymeric micelles some heating and cooling are necessary [5]. The same might be considered for different types of liposomes, where multilamellar vesicles are obtained spontaneously by hydration of phospholipids, whereas small unilamellar vesicles require a more energetic process [8].

In this work we seek developing a nanocarrier based on a self-assembling emulsified system focusing on the use of a new lipophilic compound, citronellol homopolymer, a terpene present in several kinds of plants worldwide. Depending as the synthesis reaction is conducted the homopolymer can be obtained with different lengths of chain and then assume specific features. Here, we use two marketed types of citronellol homopolymers, one with low viscosity (CL) and another with high viscosity (CH), to obtain lipid nanoparticles using citronellol homopolymer as constituent of lipid matrix, in a simple process of emulsification. DoE (design of experiments) was used to evaluate proportions of CL ou CH, blend of surfactants and amount of co-surfactant (glycol propylene) able to provide the lowest size and more homogeneous system after emulsification.

Citronellol homopolymers are derived from citronellol, a natural acyclic monoterpenoid found in citronella oils. The citronellol is derived from terpenes that are extracted from the sap of sustainably grown pine trees. These raw material terpenes are Forest Stewardship Council® (FSC) certified. The citronellol can be converted in its homopolymer by way of a green, clean, mild, and high yielding conversion process called Process Intensified Continuous Etherification (PICE™) [9]. In our days, the seek for materials from renewable resources must be constant to maintain the balance between the necessity of new and more efficient product and its composition and manufacturing more environmental responsible.

Material and Methods

Material

Citronellol homopolymer (Citropol® 1A – low viscosity (CL); and Citropol F – high viscosity (CH)) were kindly donated by P2 (P2 Science, Inc., Woodbridge, USA), poloxamer 188 (Kolliphor® 188 P188)) and polyvinyl pyrrolidone k30 (Kollidon® K30 (PVPk30)) were donated by Basf (BASF S.A., São Paulo, Brazil), ethoxylated oleic alcohol (EOA), capric/caprylic triglycerides (Polymol® 812 (CCT) and glyceryl monostearate (GMS)) were donated by Aqia (AQIA Ltda., Guarulhos, Brazil), polysorbate 80 (Tw80) and propylene glycol (PPG) were purchased with Labsynth (Labsynth Ltda., Diadema, Brazil). Water used in experiments was ultrapurified in a Reverse Osmose equipment.

Methods

Four 2^3 DoE (Design of Experiments) were used, where percentages of surfactants 1:1 (polysorbate 80 (Tw80) added of ethoxylated oleic alcohol (EOA) or poloxamer 188 (P188) - 4 and 8% w/w), propylene glycol (PPG) (5 and 15% w/w), and content of citronellol homopolymer (low or high viscosity, CL or CH, respectively) in the oily phase (13 and 87% w/w) were factors and levels studied. Besides, two intermediate levels were included with regard to the content of citronellol homopolymer in the oily phase (25 and 75% w/w), resulting in 16 formulations for each DoE (DoE1: EOA-CL; DoE2: EOA-CH; DoE3: EOA-CL; DoE4: EOA-CH) as presenting in Table 01. All the formulations were produced by emulsification.

For that, oily phase (MEG, CL or CH, EOA or P188) and aqueous phase (water, Tw80, PVPk30 and PPG) were heated up to 70 – 80 °C and homogenized in a magnetic stirrer (Magnetic Stirrer, VELP, Inc., Italy). As both phases have gotten the temperature, the aqueous phase was poured over the oily phase maintaining the stirring for 10 minutes at 80 °C. Afterward, the formulations were transferred for other magnetic stirrer without heating where were kept until room temperature.

Table 01. Sequence of four DoEs executed with factors and levels used. Oil Phase and Aqueous Phase show amounts of materials used to prepare 10 g formulation. Each formulation was analysed in triplicate by laser diffraction.

DoEs (DoE1: EOA-CL; DoE2: EOA-CH; DoE3: EOA-CL; DoE4: EOA-CH)					Compounds grouping						
					Oil Phase				Aqueous Phase		
Formu-lation	Tested order	[%sur-factants]	[%CL or %CH] in oil phase	[%Propy-lene glycol]	CL or CH (g)	GMS (g)	Propy-lene glycol (g)	EOA or P188 (g)	Tw80 (g)	PVPK30 2% dispersion (g)	Purified Water (g)
13	1	8	25	5	0.063	0.188	0.500	0.400	0.400	5.000	3.450
5	2	4	25	5	0.063	0.188	0.500	0.200	0.200	5.000	3.850
2	3	4	87	15	0.218	0.033	1.500	0.200	0.200	5.000	2.850
15	4	8	13	5	0.033	0.218	0.500	0.400	0.400	5.000	3.450
8	5	4	13	15	0.033	0.218	1.500	0.200	0.200	5.000	2.850
4	6	4	75	15	0.188	0.063	1.500	0.200	0.200	5.000	2.850
7	7	4	13	5	0.033	0.218	0.500	0.200	0.200	5.000	3.850
10	8	8	87	15	0.218	0.033	1.500	0.400	0.400	5.000	2.450
6	9	4	25	15	0.063	0.188	1.500	0.200	0.200	5.000	2.850
9	10	8	87	5	0.218	0.033	0.500	0.400	0.400	5.000	3.450
11	11	8	75	5	0.188	0.063	0.500	0.400	0.400	5.000	3.450
1	12	4	87	5	0.218	0.033	0.500	0.200	0.200	5.000	3.850
3	13	4	75	5	0.188	0.063	0.500	0.200	0.200	5.000	3.850
12	14	8	75	15	0.188	0.063	1.500	0.400	0.400	5.000	2.450
14	15	8	25	15	0.063	0.188	1.500	0.400	0.400	5.000	2.450
16	16	8	13	15	0.033	0.218	1.500	0.400	0.400	5.000	2.450

The formulations were visually inspected, pictured, and analysed by laser diffraction (range of particle size analysis 40 nm – 2.5 mm) (Cilas 1190, XXXXX, xxxxx) using ultrapurified water as the diluent. The Mie method was used by software for calculation of sizes and size distribution, allowing the recording of d10, d50, d90, mean size (for volume and number passing), and the percentage of particles for a specific size range. These data made possible the calculation of span (size dispersion) and uniformity ratio, as parameters of uniformity of size distribution. The data were assessed in statistical software for Response Surface Regression (RSR) applying ANOVA with significance ≤ 0.05 and Principal Component Analysis (PCA)

presented as Pareto charts for each parameter analyzed. The results were used to run optimization studies and estimate the desirability index for a target formulation.

Results

The visual inspection of the produced formulations suggested that different emulsified systems were formed, since microemulsions (transparent formulations with nanometric dimensions - DoE1-F14) until nanoemulsions (translucid and nanometric - DoE3-F15), and ordinary emulsions, opalescent formulations containing particles with tens of micrometers, with (DoE3-F4) or without (DoE3 – F8) phase separation (Figure 1). Overall, the best results were observed for DoE3, where only three formulations showed spontaneous and easily to observe phase separation (F02, F04, and F12). For these three formulations CH proportion was in 75 and 87% w/w in oil phase. Additionally, for those three formulations glycol propylene was used in the highest concentration (15% w/w in formulation). It might suggest that the high proportion of CH in oil phase could contribute to destabilization of the emulsified system and the association with highest proportion of glycol propylene could become favorable that destabilization.

These observations could be in part confirmed in DoE 1, 2 and 4 since all seven formulations in DoE-1 (F 01, 02, 03, 04, 09, 10, and 12) and DoE-2 (F 01, 02, 04, 09, 10, 11, and 12), and five from six in DoE4 (F 01, 02, 04, 09, and 10), presented phase separation as citronellol homopolymer was used in the two higher proportion in oil phase, and without dependence of glycol propylene proportion since both used showed destabilization.

The visual inspection also suggests that high-viscosity citronellol homopolymer was that with the best potential for emulsification in the formulation-base and process used in this work. Furthermore, the association of EOA and Tw80 allowed to obtain formulations lightly opalescence until almost transparent indicating a more effective size reduction allowing to obtain formulations with high proportion of nanoparticles below to 500 nm. Despite that, most of the tested formulations were unable to form a nanostructured system with good uniformity, especially those using P188 as surfactant (Figure 1 and Table 2-5), although nanostructures were present in several of them to different DoE.

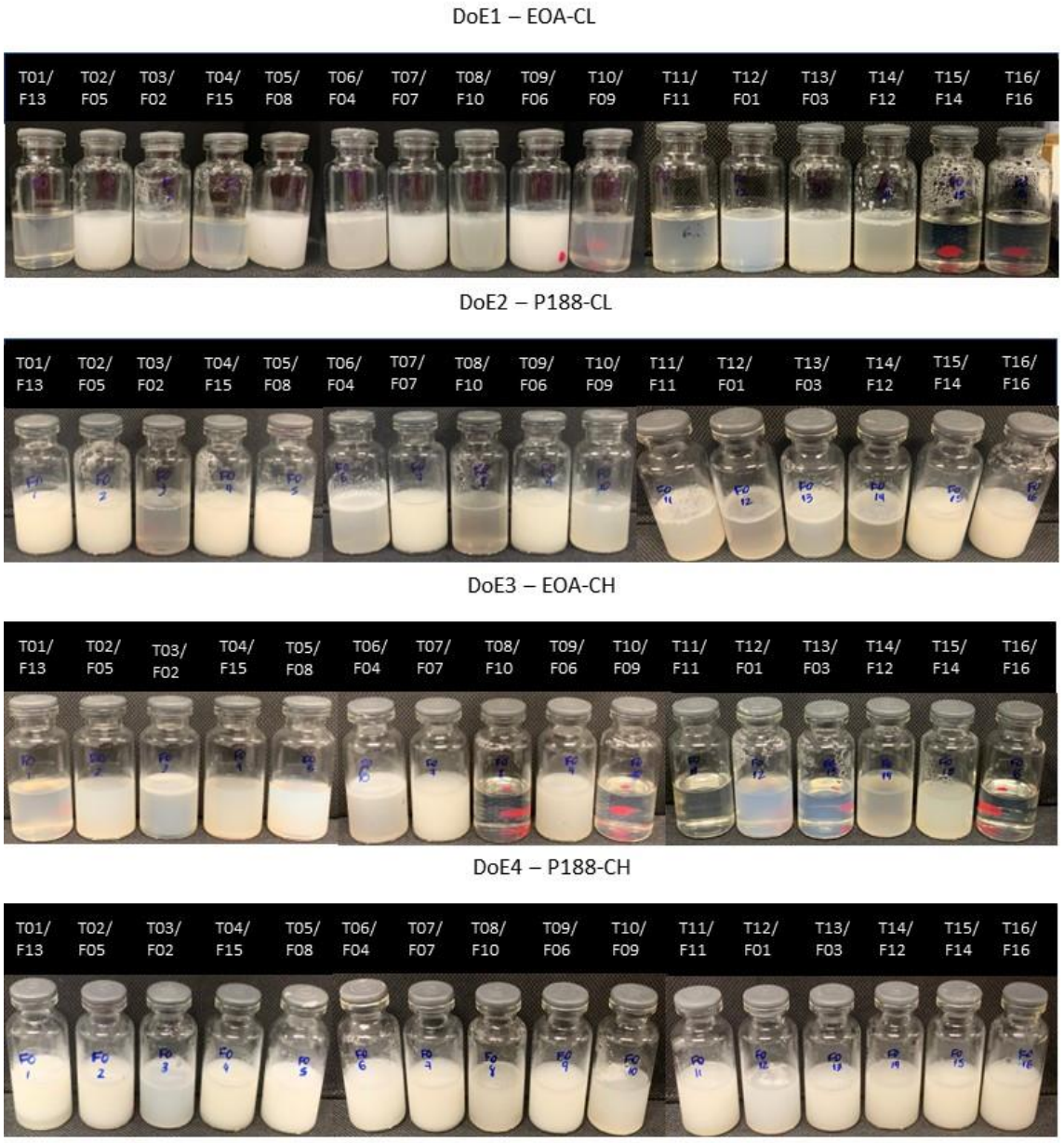

Figure 1. Pictures of sixteen formulation from DoE 1 – 4 obtained by emulsification process. T01-T16 refer to the tested order which the formulations were executed and F01-F16 refers to formulations tested with different factors and levels.

Nanostructures could be observed in all four DoE evaluated since the most formulations showed a percentage of particles higher than 90% for particle size equal or lower than 300 nm (%300n) as shown in Tables 2-4. For example, in the DoE1 (Table 2), only the formulations F08, 13, 15 e 16 showed percentage of particles lower than 90% for particle size ≤ 300 nm. In fact, for these formulations laser diffraction analysis did not detect any nanoparticles below 300 nm. The same behavior was observed in seven formulations for DoE2 (Table 3), two formulations for DoE3 (Table 4) and five formulations for DoE4 (Table 5).

Table 2. Data of size analysis from DoE1 formulations by laser diffraction.

DOE1	d10v (µm)	d50v (µm)	d90v (µm)	SPAMv	dmv (µm)	dmn (µm)	UR	%500v (%)	%300v (%)	%300n (%)	%100n (%)
F01	0,86	5,87	13,24	2,11	6,58	0,06	109,67	5,81	3,38	98,29	96,07
F02	0,64	3,89	10,38	2,51	4,88	0,06	81,28	7,86	4,30	98,10	95,59
F03	0,50	3,40	9,33	2,60	4,27	0,09	95,19	9,90	5,57	94,60	86,60
F04	2,38	54,16	272,69	5,01	98,28	0,11	893,42	2,16	1,28	94,40	85,85
F05	1,26	7,93	21,42	2,54	9,82	0,13	75,51	4,61	2,48	92,27	81,25
F06	1,74	18,61	49,25	2,55	22,52	0,11	204,76	4,57	2,44	93,66	83,11
F07	1,09	6,75	17,48	2,43	8,18	0,06	136,28	4,90	2,84	98,35	96,19
F08	3,88	23,08	49,80	1,99	25,87	1,58	16,34	0,00	0,00	0,00	0,00
F09	0,51	4,72	12,82	2,61	5,94	0,11	53,95	9,86	5,19	93,54	82,83
F10	0,77	2,18	10,07	4,27	3,91	0,12	32,61	4,30	2,08	91,67	87,05
F11	0,46	3,61	11,64	3,09	5,07	0,11	46,06	11,03	6,14	94,31	84,33
F12	0,60	4,13	12,42	2,86	5,56	0,12	46,33	8,32	4,49	93,03	82,34
F13	3,59	16,04	34,49	1,93	17,98	1,63	11,22	0,00	0,00	0,00	0,00
F14	**0,06**	**0,22**	**0,47**	**1,91**	**0,26**	**0,05**	**5,10**	**91,75**	**70,33**	**99,47**	**96,36**
F15	3,83	17,51	40,77	2,11	20,55	1,41	14,61	0,00	0,00	0,00	0,00
F16	4,77	23,84	58,87	2,27	28,58	2,01	14,47	0,00	0,00	0,00	0,00

d10v, d50v, and d90v depict the particle size assumed by 10, 50, and 90% of particles, respectively, express in passing volume; SPAMv is the dispersity determined through equation [(d90v-d10v)/d50v] considering data in passing volume; dmv and dmn are mean diameters in passing volume and passing number, respectively; UR (uniformity ratio) is the ratio between dmv/dmn; %500v, %300v, %300n, and %100n represent the percentage of particles equal or lower than 500 or 300 nm in passing volume, or 300 or 100 nm in passing number, respectively.

Despite most of formulations in all DoE showing more than 90% of particles with a size ≤ 300 nm (%300n), these results are not corroborated by %300v data. Actually, if considering data depicted in passing volume the percentages come down below 10% for the majority. It shows that the process and formulations are able to provide nanostructures, however the yield of the process is low since the volume of material in particles with size bigger than 300 nm is higher than 95% for most of formulations. As an example, in formulation F03 (Table 3), it was registered %300n of 96.3% of particles ≤ 300 nm, but 98,9% (%300v = 1,1%) of volume of particles above 300 nm. Therefore, for the major fraction of the material constitutive of particles 98,9% represent particles above 300 nm.

Table 3. Data of size analysis from DoE2 formulations by laser diffraction.

DOE2	d10v (µm)	d50v (µm)	d90v (µm)	SPAMv	dmv (µm)	dmn (µm)	RU	%500v (%)	%300v (%)	%300n (%)	%100n (%)
F01	0,58	3,52	12,94	3,51	5,42	0,12	46,5	8,5	4,9	93,3	83,4
F02	0,62	5,06	20,12	3,85	8,13	0,12	69,8	8,2	4,8	93,9	83,9
F03	3,74	41,58	85,61	1,97	44,22	0,09	491,3	1,8	1,1	96,3	90,0
F04	1,12	10,43	40,14	3,74	16,23	0,11	147,5	5,7	3,3	94,4	84,7
F05	4,40	27,48	62,71	2,12	31,31	1,53	20,5	0,0	0,0	0,0	0,0
F06	5,06	22,45	50,57	2,03	25,81	1,66	15,5	0,0	0,0	0,0	0,0
F07	4,66	25,30	58,59	2,13	29,11	1,80	16,2	0,0	0,0	0,0	0,0
F08	3,71	21,99	50,37	2,12	25,18	1,55	16,2	0,0	0,0	0,0	0,0
F09	0,82	4,36	13,15	2,83	5,88	0,06	98,1	6,1	3,5	98,0	95,6
F10	0,93	6,32	19,86	3,00	8,57	0,06	136,0	4,9	2,8	97,6	94,9
F11	0,52	4,61	17,37	3,65	7,11	0,09	79,0	9,7	6,0	96,0	89,0
F12	1,34	8,86	35,57	3,86	14,41	0,12	120,1	5,0	2,8	93,7	83,3
F13	4,22	25,35	62,54	2,30	30,24	0,14	216,0	1,3	0,7	91,7	80,5
F14	6,61	22,37	48,31	1,86	25,42	1,86	13,7	0,0	0,0	0,0	0,0
F15	4,85	25,08	59,82	2,19	29,44	1,57	18,8	0,0	0,0	0,0	0,0
F16	4,38	20,06	46,02	2,08	23,19	1,59	14,6	0,0	0,0	0,0	0,0

d10v, d50v, and d90v depict the particle size assumed by 10, 50, and 90% of particles, respectively, express in passing volume; SPAMv is the dispersity determined through equation [(d90v-d10v)/d50v] considering data in passing volume; dmv and dmn are mean diameters in passing volume and passing number, respectively; UR (uniformity ratio) is the ratio between dmv/dmn; %500v, %300v, %300n, and %100n represent the percentage of particles equal or lower than 500 or 300 nm in passing volume, or 300 or 100 nm in passing number, respectively.

Table 4. Data of size analysis from DoE3 formulations by laser diffraction.

DOE3	d10v (µm)	d50v (µm)	d90v (µm)	SPAMv	dmv (µm)	dmn (µm)	RU	%500v (%)	%300v (%)	%300n (%)	%100n (%)
F01	1,61	13,59	34,12	2,39	16,12	0,09	179,1	4,2	2,5	96,1	88,9
F02	2,02	13,88	37,22	2,54	17,19	0,12	143,3	3,6	1,9	93,3	82,5
F03	1,19	12,03	31,79	2,56	14,63	0,48	96,7	7,5	4,2	63,7	57,4
F04	2,60	20,33	63,18	2,98	27,85	0,13	214,3	2,1	1,2	92,8	82,4
F05	0,17	3,33	20,49	6,10	7,27	0,05	145,3	23,7	18,5	99,6	97,8
F06	1,56	13,43	32,87	2,33	15,58	0,11	137,7	4,4	2,5	93,9	83,8
F07	0,29	1,34	4,57	3,19	2,01	0,06	33,6	18,1	10,4	97,8	94,9
F08	4,09	14,76	35,22	2,11	17,91	1,47	12,2	0,0	0,0	0,0	0,0
F09	0,27	8,15	26,43	3,21	11,23	0,06	204,5	17,3	12,5	98,2	93,9
F10	0,07	0,30	3,59	11,41	1,05	0,05	24,8	69,3	51,8	99,2	96,1
F11	0,31	10,38	36,43	3,46	14,81	0,07	237,7	14,7	10,4	98,3	93,9
F12	0,56	5,16	27,86	5,28	10,28	0,11	96,7	9,0	5,2	94,8	85,6
F13	1,34	12,98	31,69	2,34	15,11	0,11	137,3	5,9	3,2	94,4	84,2
F14	3,39	14,94	33,93	2,04	17,21	1,87	9,2	0,0	0,0	0,0	0,0
F15	**0,05**	**0,18**	**0,33**	**1,56**	**0,19**	**0,04**	**4,8**	**100,0**	**85,1**	**99,8**	**97,9**
F16	**0,05**	**0,19**	**0,35**	**1,58**	**0,20**	**0,04**	**5,0**	**99,8**	**82,1**	**99,8**	**98,0**

d10v, d50v, and d90v depict the particle size assumed by 10, 50, and 90% of particles, respectively, express in passing volume; SPAMv is the dispersity determined through equation [(d90v-d10v)/d50v] considering data in passing volume; dmv and dmn are mean diameters in passing volume and passing number, respectively; UR (uniformity ratio) is the ratio between dmv/dmn; %500v, %300v, %300n, and %100n represent the percentage of particles equal or lower than 500 or 300 nm in passing volume, or 300 or 100 nm in passing number, respectively.

Table 5. Data of size analysis from DoE4 formulations by laser diffraction.

DOE4	d10v (µm)	d50v (µm)	d90v (µm)	SPAMv	dmv (µm)	dmn (µm)	RU	%500v (%)	%300v (%)	%300n (%)	%100n (%)
F01	0,16	1,94	10,17	5,17	3,72	0,05	74,33	25,78	19,48	99,47	97,53
F02	0,23	14,11	173,19	12,83	56,35	0,05	1127,00	18,03	13,97	99,60	97,76
F03	2,10	21,89	55,21	2,43	25,82	0,11	234,73	2,92	1,71	94,47	85,41
F04	1,25	8,03	22,05	2,59	10,11	0,13	77,77	4,39	2,43	92,30	81,22
F05	2,59	19,63	49,10	2,37	23,26	0,59	144,39	2,52	1,42	63,02	56,42
F06	1,73	17,39	42,66	2,35	20,20	0,12	173,53	4,58	2,43	93,49	82,81
F07	4,54	18,77	44,65	2,14	22,21	1,87	11,88	0,00	0,00	0,00	0,00
F08	2,10	18,99	47,39	2,38	22,33	0,12	186,11	2,82	1,62	93,41	83,07
F09	0,50	3,40	12,04	3,39	5,06	0,12	42,14	9,94	5,54	93,29	82,76
F10	1,48	12,19	44,93	3,56	18,34	0,08	255,75	3,32	1,96	96,77	92,32
F11	0,51	11,26	46,25	4,06	18,06	0,10	180,57	9,76	5,53	95,01	85,30
F12	1,67	13,73	41,72	2,92	18,14	0,12	151,19	3,92	2,10	92,65	81,66
F13	4,35	23,62	58,61	2,30	28,35	1,50	18,86	0,00	0,00	0,00	0,00
F14	3,34	12,45	28,02	1,98	14,38	2,00	7,19	0,00	0,00	0,00	0,00
F15	5,35	26,35	62,00	2,15	30,90	1,54	20,11	0,00	0,00	0,00	0,00
F16	3,21	13,59	32,30	2,14	16,06	1,88	8,53	0,00	0,00	0,00	0,00

d10v, d50v, and d90v depict the particle size assumed by 10, 50, and 90% of particles, respectively, express in passing volume; SPAMv is the dispersity determined through equation [(d90v-d10v)/d50v] considering data in passing volume; dmv and dmn are mean diameters in passing volume and passing number, respectively; UR (uniformity ratio) is the ratio between dmv/dmn; %500v, %300v, %300n, and %100n represent the percentage of particles equal or lower than 500 or 300 nm in passing volume, or 300 or 100 nm in passing number, respectively.

Differently, DoE1 F14, and DoE3 F15 and F16, presented high percentages for both values %300n and %300v, indicating a high yield for formulations and process in providing nanoparticles below 300 nm. This obviously becomes closer the results of dmv and dmn, favoring to obtain UR close to 1,0. Actually, UR = 1,0 would be the gold result for this parameter since mean diameters depict in volume and number passing should be equal. Our experience with laser diffraction analysis for nanostructured systems suggests that values between 1,0 and 5,0 unveil a great uniformity in particle size distribution and a good yield in the process for a specific range of size, whereas values between 5,1 and 10,0 suggest a just acceptable uniformity and yield. On the other hand, values of UR higher than 20,0 suggest a wide and undesirable size distribution, considering at the same time, the number of particles and the amount of material used in those particles.

This behavior can be observed in Figure 2, where the histograms of size distribution from formulations illustrate the profiles with great (DoE3-F15), acceptable (DoE3-F14) and undesirable (DoE2-F01) size distribution.

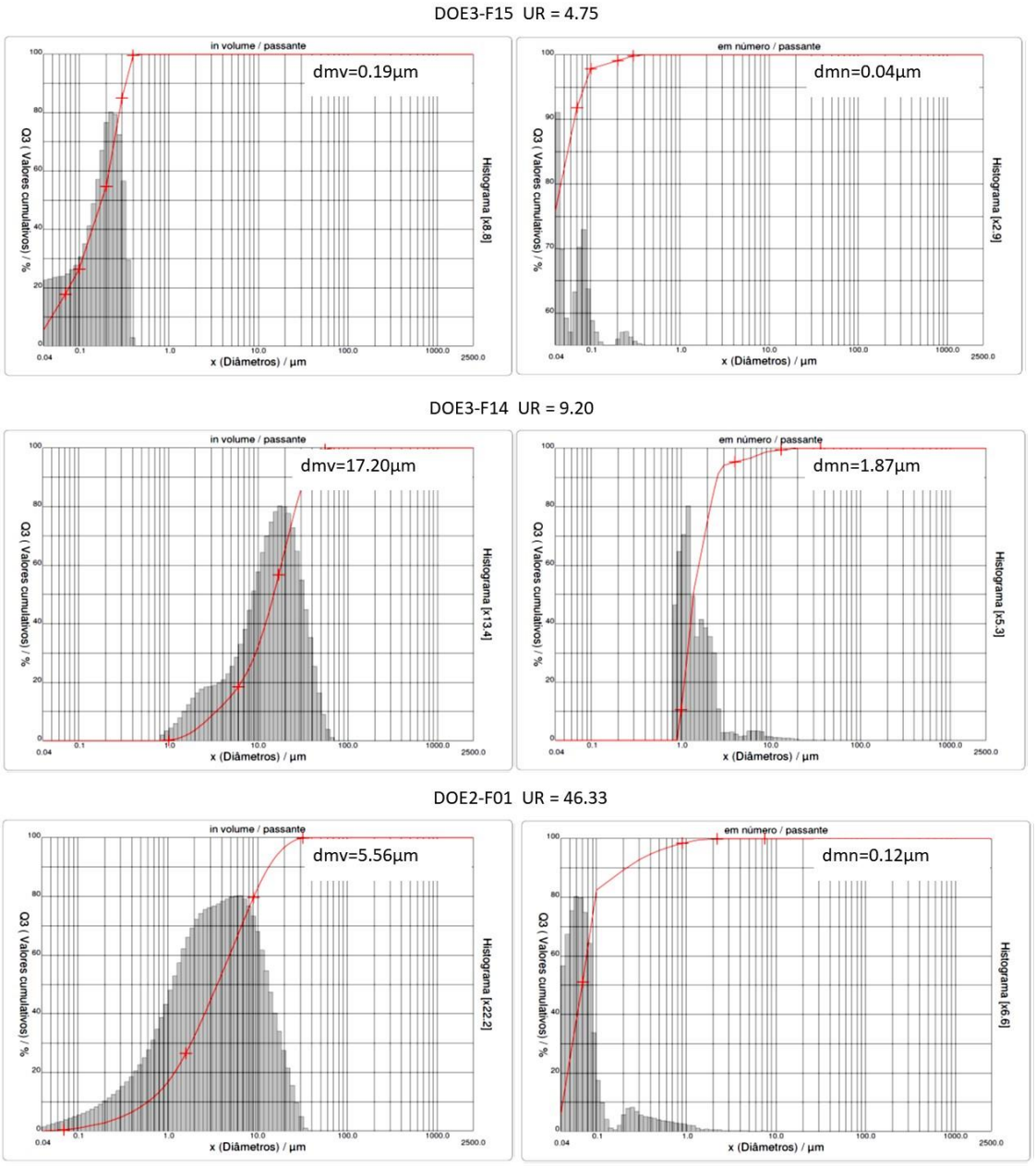

Figure 2. Histograms obtained by laser diffraction analysis from formulations with different UR depicting the uniformity of size distribution.

The ANOVA applied for Response Surface Regression (RSR) considering the studied parameters showed most of the p-values lower than 0.05 for the models (Table 6). The same was observed for p-values for each factor, either isolated or in association, showing that factors studied are important for the size properties in tested formulations (data not shown). Despite p-values were good for all DoE and parameters, the determination coefficients (R^2) were all below 0,9, and some lower than 0.6 (Table 6), which was consider the limit to include the parameter in optimization study.

Table 6. ANOVA applied for Response Surface Regression (RSR) for all four DoE tested.

Parameter	DoE1		DoE2		DoE3		DoE4	
	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2
d50v	0,002	0,430	0,000	0,670	0,000	0,780	0,000	0,616
SPAMv	0,000	0,543	0,000	0,723	0,000	0,716	0,000	0,591
dmv	0,005	0,388	0,000	0,696	0,000	0,794	0,001	0,424
dmn	0,000	0,676	0,000	0,871	0,003	0,419	0,000	0,812
UR	0,001	0463	0,002	0,415	0,000	0,716	0,000	0,512
%500v	0,010	0,361	0,000	0,826	0,000	0,567	0,000	0,721
%300v	0,008	0,369	0,000	0,812	0,000	0,565	0,000	0,703
%300n	0,000	0,697	0,000	0,881	0,005	0,394	0,000	0,847
%100n	0,000	0,670	0,000	0,850	0,004	0,400	0,000	0,847

Principal components presented in a Pareto chart revealed that the proportion of citronellol homopolymer in the oily phase was the principal factor of variation for most of the parameter analyzed - be it alone, associated with another factor, or quadratic - including those related to uniformity (span and uniformity ratio). An example of this is presented Figure 3, where is possible to note the great influence of citronellol homopolymer concentration in the several parameters related to size and uniformity.

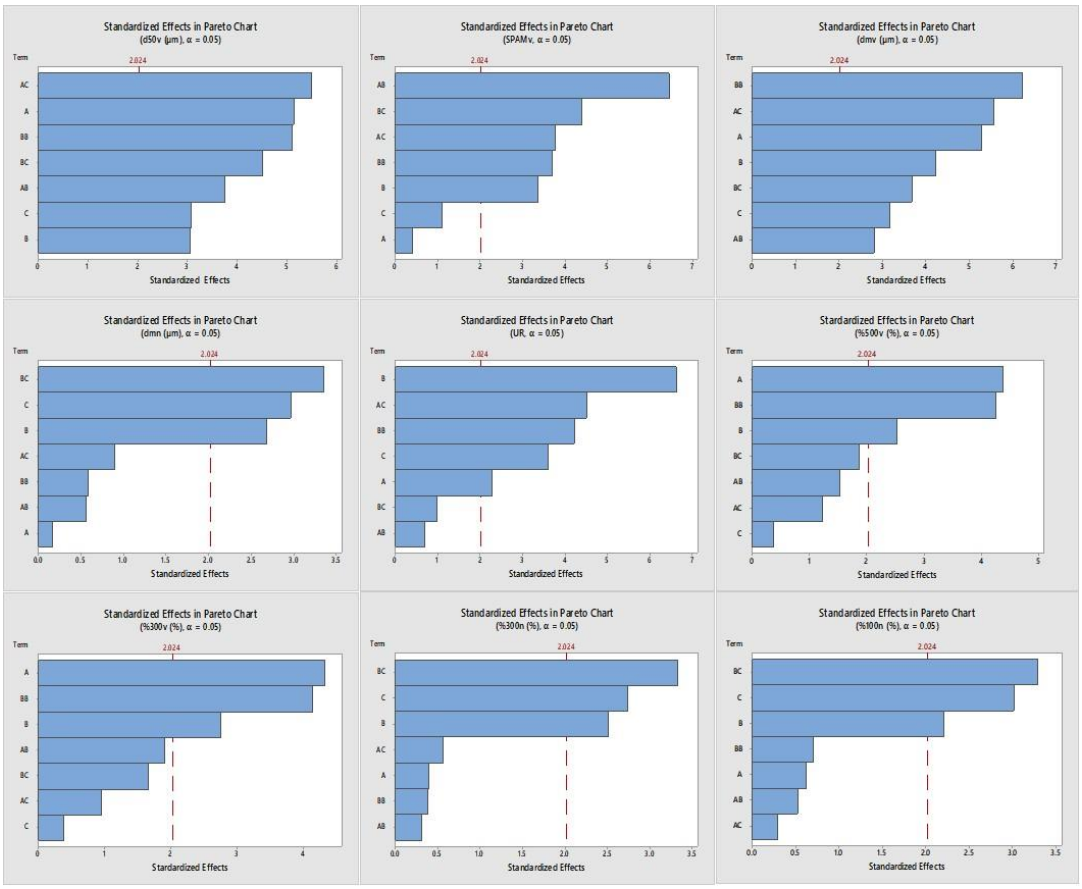

Figure 3. Standardized effects in Pareto Chart from DoE1 analysis using Response Surface Regression model. The bars crossing the dashed line are statically significant with p-value < 0.05. Factors A: [EOA+Tw80]; B: [CH in oil phase]; C: [Propylene glycol].

Two optimization studies were done for each DoE, considering parameters that had presented significance for the models in RSR and R^2 > 0,6. The desirability obtained was higher for those studies where size parameters (dmn, dmn, d50v) and uniformity parameter (SPAMv and UR) were minimized and parameter related to percentage of particles (%500v, %300v, %300n, and %100n) were maximized, D > 0,83 for all DoEs, whereas the indication of targets for size resulted in a lower desirability index, with D = 0.75 for DoE2, as an example. Concerning the optimization study, the formulation suggested for reaching the parameters in DoE3, which allowed to obtain two formulations with features of a monodispersed nanoparticles, was 8% of surfactants (EOA+Tw80), 13% of CH in oil phase, and 9% of propylene glycol.

Conclusion

It was possible to obtain self-assembling formulations using citronellol homopolymer as part of the oily phase in lipid nanoparticles and ordinary surfactants as polysorbate 80 and EOA. The citronellol homopolymer has shown an interesting feature as solvent for lipophilic actives what might be interesting to carrier them in cosmetic and pharmaceutical formulations in a sustainable and ecological way.

Parameters used in DoE study unveiled that it is important monitoring the size of particles depict in different ways since measures of size just in passing number is not able to demonstrate the real yield of process to provide in a specific range. Thus, the uniformity ratio might be used to point the process and formulation more capable to provide size range and yield specified.

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Conflict of Interest Statement

NONE

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