



Supporting Information

for

Quality by design optimization of microemulsions for topical delivery of *Passiflora setacea* seed oil

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Additional figures and tables

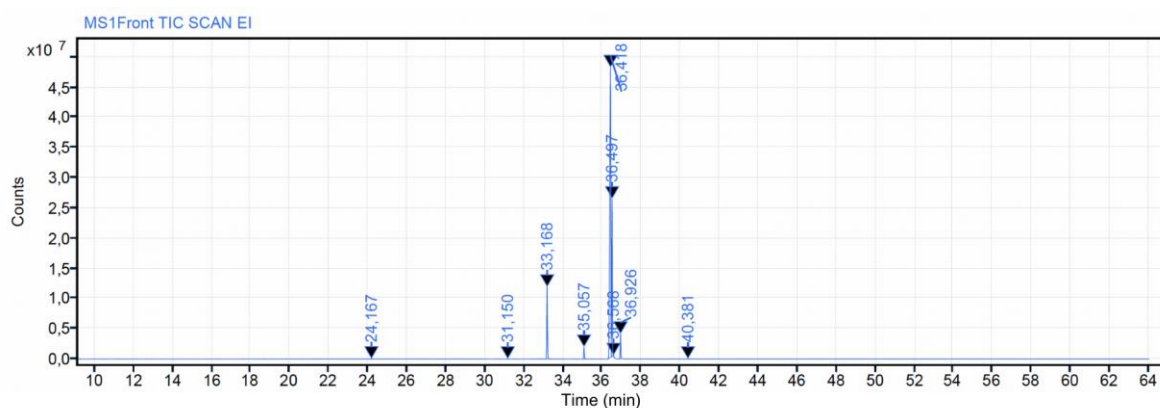


Figure S1: Gas chromatography coupled with mass spectroscopy (GC-MS) chromatogram of the constituents of transesterified *P. setacea* oil. Arrows indicate the retention time of each compound identified in Table 1.

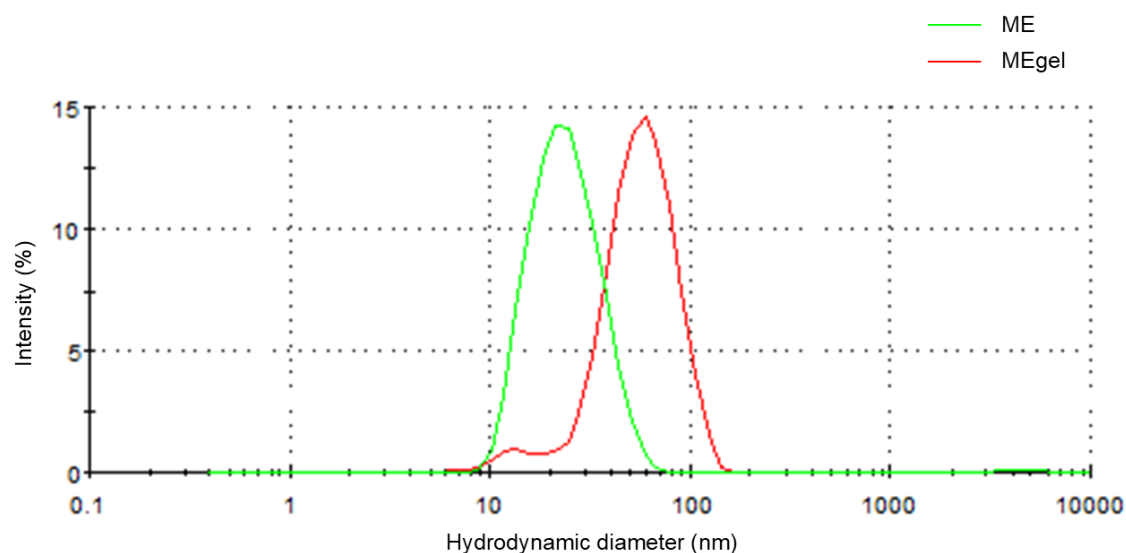


Figure S2: Hydrodynamic diameter distribution by intensity of the microemulsion (ME, green line) and its gelled form (MEgel, red line) 24 h after production.

Table S1: Risk estimation matrix by three-level interdependence scale (1: low, 3: medium, 9: high) between QTPP and CQAs.

	Droplet size	Polydispersity index (PDI)	pH	Viscosity	Visual clarity	Surface tension	Stability
Dosage form	9	9	1	9	1	9	1
Route of administration	1	1	9	9	1	1	1
Delivery type	3	3	1	9	1	1	1
Appearance	9	9	1	1	9	1	1
Active pharmaceutical ingredient (API)	3	3	1	1	1	1	1
Stability	9	9	3	1	3	1	9
Safety	1	1	3	1	1	1	3
Efficacy	9	9	1	1	1	1	1

Table S2: Risk estimation matrix by three-level interdependence scale (1: low, 3: medium, 9: high) between CQAs and CMA/CPP.

	OPS concentration	Smix ratio	Smix concentration	Vitamin E concentration	NaCMC concentration	Preservative concentration
Droplet size	9	9	9	3	1	1
Polydispersity index	9	9	9	1	1	1
pH	1	1	1	1	1	1
Viscosity	1	1	1	1	9	1
Visual clarity	3	3	9	1	9	1
Surface tension	3	9	9	1	1	1
Stability	9	9	9	9	3	9

Table S3: Risk estimation matrix by three-level interdependence scale (1: low, 3: medium, 9: high) between CQAs and CMA/CPP.

	Stirring speed	Stirring time	Temperature during mixing	Water addition rate	Homogenization sequence	Cooling rate after formation
Droplet size	9	9	9	9	3	1
Polydispersity index	9	3	9	9	3	1
pH	1	1	1	1	1	1
Viscosity	1	1	1	1	1	1
Visual clarity	3	3	3	9	3	1
Surface tension	1	1	1	1	1	1
Stability	1	1	1	9	3	1

Table S4: 2³ Full factorial design (FFD) and their responses.

Execution order	Factor A (%)	Factor B (mass ratio)	Factor C (%)	Hydrodynamic diameter(nm)	Polydispersity index
1	15	7:3	20	35.2	0.23
2	5	9:1	10	24.5	0.29
3	5	9:1	20	14.9	0.12
4	10	8:2	15	25.6	0.29
5	10	8:2	15	26.0	0.24
6	5	7:3	20	14.1	0.21
7	15	9:1	20	37.6	0.23
8	15	7:3	10	91.4	0.20
9	5	7:3	10	23.2	0.23
10	15	9:1	10	152.0	0.24
11	10	8:2	15	25.7	0.26

Factor A: Oil concentration; Factor B: Ratio of PEG 30 castor oil and Span® 80; Factor C: Concentration of the surfactant mixture.

Table S5: ANOVA for the hydrodynamic diameter (nm) of the ME FFD.

Factor	Sum of squares	df	Medium square	F-value	p-value	Comments
Model	15952.46	6	2658.74	19.13	0.0172	Significant
A-Oil concentration	7170.63	1	7170.63	51.59	0.0056	Significant
B-Surfactants ratio	530.89	1	530.89	3.82	0.1457	Not significant
C-S _{mix} concentration	4475.05	1	4475.05	32.20	0.0108	Significant
AB	464.36	1	464.36	3.34	0.1650	Not significant
BC	2881.54	1	2881.54	20.73	0.0199	Significant
BC	429.98	1	429.98	3.09	0.1768	Not significant
Curvature	1189.19	1	1189.19	8.56	0.0612	Not significant
Residue	416.98	3	138.99			
Lack of fit	416.88	1	416.88	8888.76	0.0001	Significant
Pure error	0.0938	2	0.0469			
Total correlation	17558.63	10				

df = degrees of freedom.

Table S6: ANOVA for the ME FFD polydispersity index.

Factor	Sum of squares	df	Medium square	F-value	p-value	Comments
Model	0.0144	6	0.0024	2.70	0.2223	Not significant
A-Oil concentration	0.0002	1	0.0002	0.18	0.6983	Not significant
B-Surfactant ratio	0.0000	1	0.0000	0.02	0.8959	Not significant
C-S _{mix} concentration	0.0039	1	0.0039	4.35	0.1282	Not significant
AB	0.0008	1	0.0008	0.85	0.4234	Not significant
BC	0.0049	1	0.0049	5.51	0.1006	Not significant
BC	0.0047	1	0.0047	5.29	0.1050	Not significant
Curvature	0.0038	1	0.0038	4.24	0.1316	Not significant
Residue	0.0027	3	0.0009			
Lack of fit	0.0016	1	0.0016	2.85	0.2335	Not significant
Pure error	0.0011	2	0.0006			
Total correlation	0.0209	10				

df = degrees of freedom.

Table S7: ANOVA for the hydrodynamic diameter (nm) of the BBD of the ME.

Source	Sum of squares	df	Mean square	F-value	p-value	Comments
Model	199.52	11	18.14	401.93	< 0.0001	Significant
A-Oil conc.	49.38	1	49.38	1094.34	< 0.0001	
B-Surfac. ratio	27.48	1	27.48	608.97	< 0.0001	
C- S _{mix} conc.	24.97	1	24.97	553.32	< 0.0001	
AB	17.32	1	17.32	383.89	< 0.0001	
AC	10.91	1	10.91	241.69	< 0.0001	
BC	9.22	1	9.22	204.26	< 0.0001	
A ²	4.49	1	4.49	99.49	0.0002	
B ²	4.77	1	4.77	105.74	0.0001	
C ²	1.14	1	1.14	25.32	0.0040	
A ² C	0.3941	1	0.3941	8.73	0.0317	
AC ²	0.9575	1	0.9575	21.22	0.0058	
Residual	0.2256	5	0.0451			
Lack of fit	0.0141	1	0.0141	0.2676	0.6322	not significant
Pure error	0.2115	4	0.0529			
Total correlation	199.74	16				

df = degrees of freedom.

Table S8: ANOVA for Pdl of ME BBD.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Comments
Model	6.81	7	0.9732	19.41	< 0.0001	Significant
A-Oil conc.	4.16	1	4.16	83.05	< 0.0001	
B-Surfac. ratio	0.0195	1	0.0195	0.3886	0.5485	
C-S _{mix} conc.	0.5370	1	0.5370	10.71	0.0096	
AB	0.2176	1	0.2176	4.34	0.0669	
AC	0.6001	1	0.6001	11.97	0.0072	
A ²	0.9673	1	0.9673	19.29	0.0017	
B ²	0.3681	1	0.3681	7.34	0.0240	
Residual	0.4513	9	0.0501			
Lack of fit	0.2068	5	0.0414	0.6766	0.6654	Not significant
Pure Error	0.2445	4	0.0611			
Total correlation	7.26	16				

df = degrees of freedom.

Table S9: ANOVA for visual classification of ME BBD.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Comments
Model	13.14	6	2.19	56.32	< 0.0001	significant
A-Oil conc.	8.00	1	8.00	205.71	< 0.0001	
B-Surfac. ratio	0.1250	1	0.1250	3.21	0.1032	
C- S _{mix} conc.	3.13	1	3.13	80.36	< 0.0001	
AB	1.0000	1	1.0000	25.71	0.0005	
BC	0.2500	1	0.2500	6.43	0.0296	
A ²	0.6405	1	0.6405	16.47	0.0023	
Residual	0.3889	10	0.0389			
Lack of Fit	0.3889	6	0.0648			
Pure Error	0.0000	4	0.0000			
Total correlation	13.53	16				

df = degrees of freedom.