

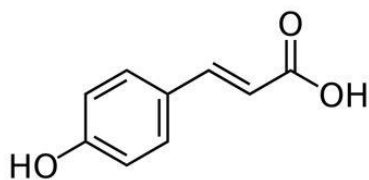
Novel microemulsion systems to enhance p-Coumaric acid oral bioavailability: *in-vitro* and *in-vivo* studies

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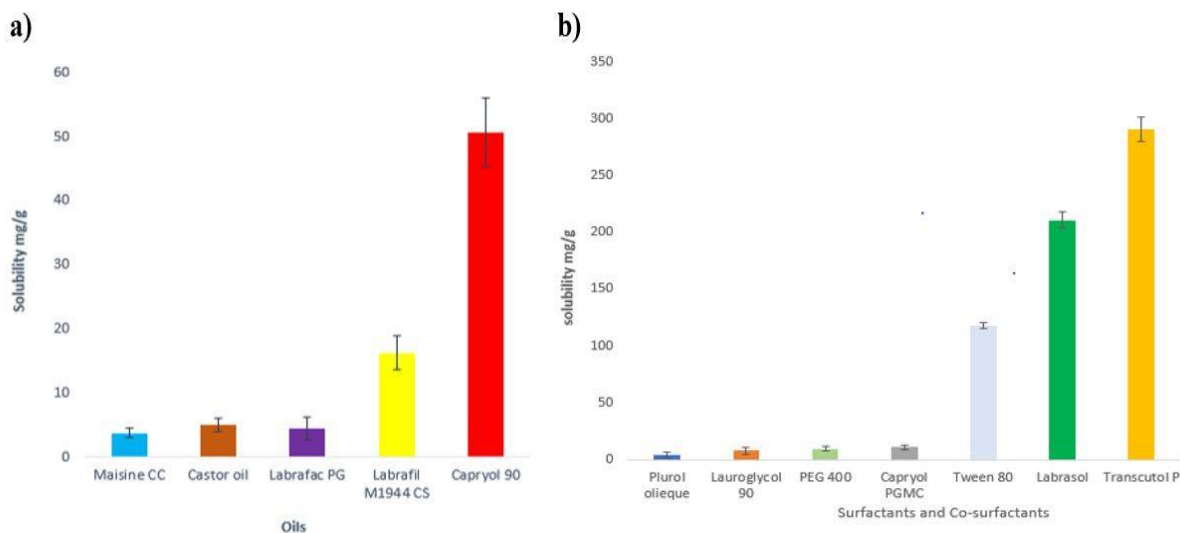
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Supplementary Material, Fig.1. The chemical structure of p-Coumaric acid.

Supplementary Material, Table 1: The upper and lower limits of the independent variables used of the two selected triangles in the pseudo-ternary phase diagrams.

Variable	Lower Limit (%)	Upper Limit (%)
Oil	0.50	16.00
S/ CoS	64.50	80.00
Water	4.00	19.50



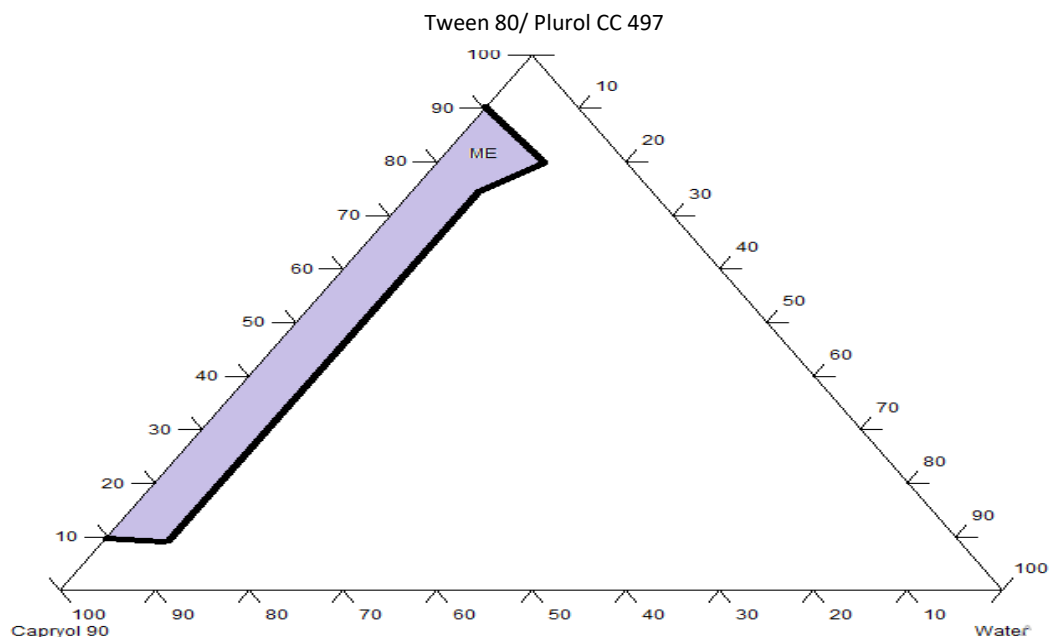
Supplementary Material, Fig. 2. The Solubility of p-Coumaric acid in different components; a) Oils, and b) Surfactants and co-surfactants.

Statistical Analysis of the droplet size model:

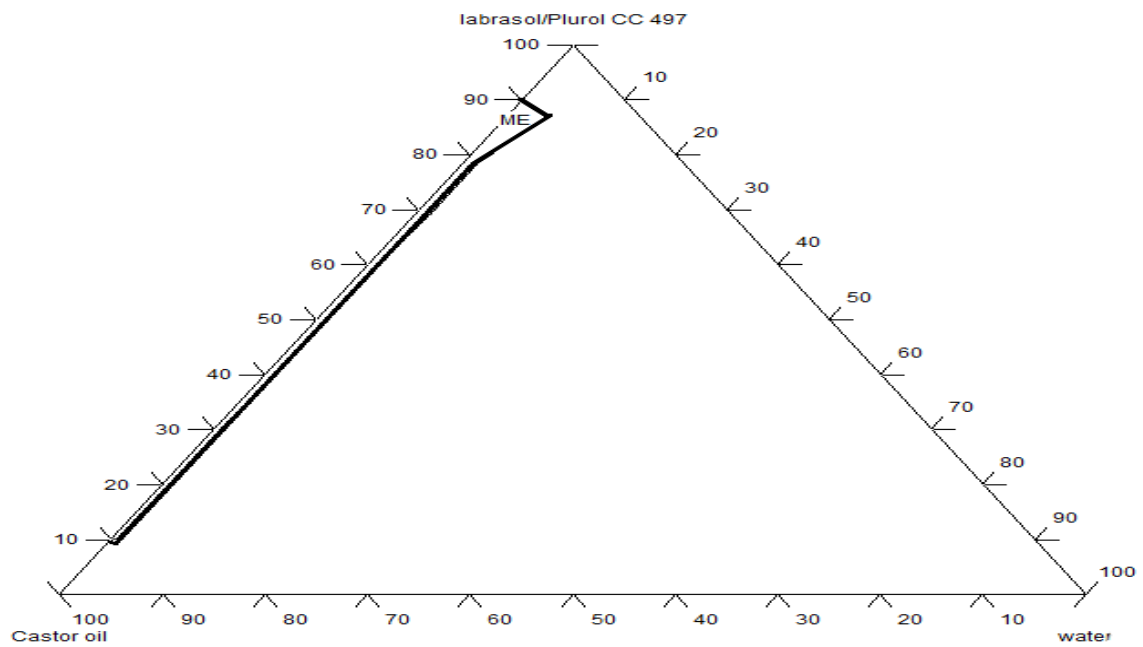
Supplementary Material, Table 3 displays the analysis results of droplet size using ANOVA showing that different models were suggested in each case for both systems using the predicted residual sum of square (PRESS) statistics for identification of the most appropriate fitting model²⁰. All suggested models were extremely significant as the p-value was very low. Moreover, the terms “ R^2 and the adjusted R^2 ” measure the variation of the results around the mean explained by the model and their fitting to the reduced model (elimination of insignificant term), respectively. Based on this, all models of both systems highly fitted the data with R^2 and adjusted R^2 greater than 0.7 and reached more than 0.9 in many of them. The predicted R^2 measures the efficiency of a model to predict the results of an unperformed run, its value must be within 0.2 of adjusted R^2 to be reasonably accepted, which was the case in all models of both systems. Finally, the Adequate precision measures the signal-to-noise ratio, where it is desired to be greater than 4 to navigate any design space and this was also applied to all suggested models^{52–54}.

The graphical presentation of the predicted versus actual data, showing the correlation between predicted and experimental data, coincided with the obtained R^2 in the provided models where all points closely fitted to the 45° line. Also, the Box-Cox power transformation graphs that suggests the best lambda (power) to obtain highly fitting models suggested; lambda 1 for all CTTL ME system models, while for LTL ME system, a lambda equivalent to -1 was suggested for the 2 h formulations before and after dilution models, -0.5 for the 24 h formulations before dilution model and 0.5 for the 24 h formulations after dilution model, where they all lied between the suggested low and high confidence intervals making these systems’ models suitable to be utilized in optimization and prediction (**Supplementary Material, figure 4**).

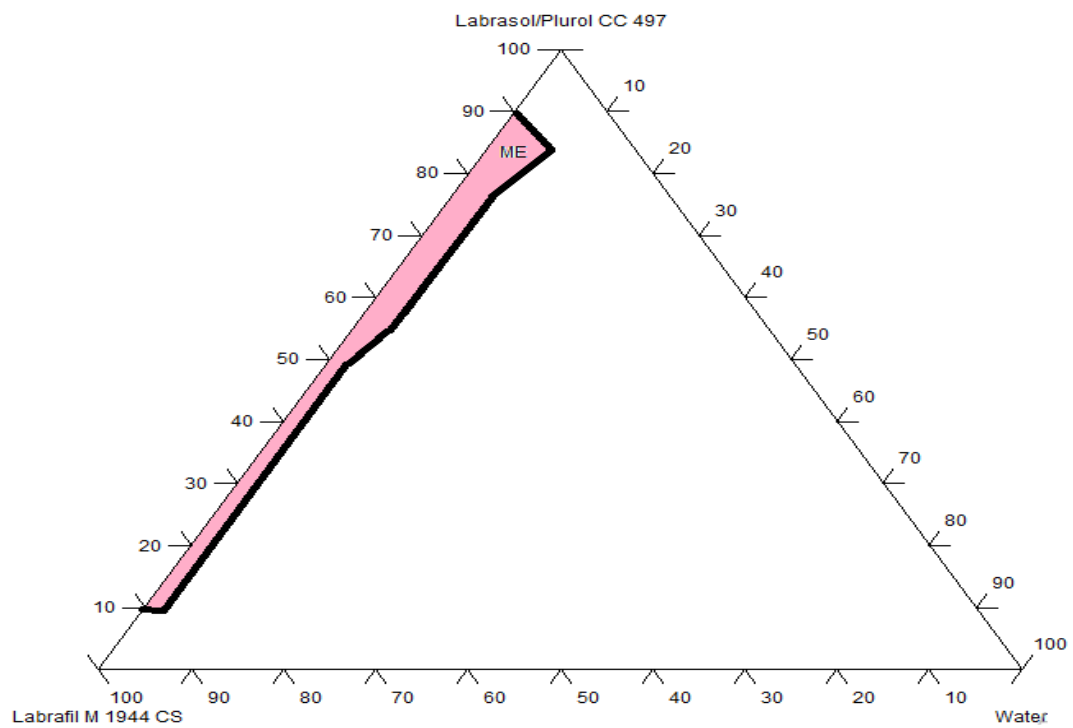
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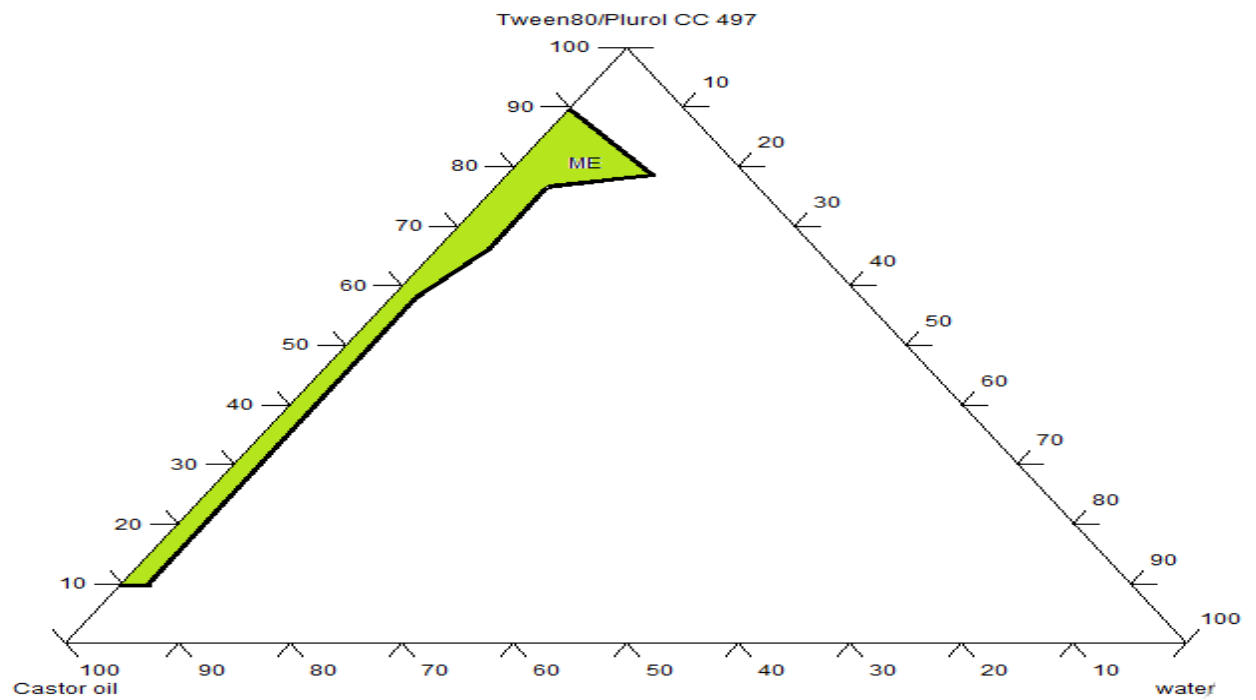
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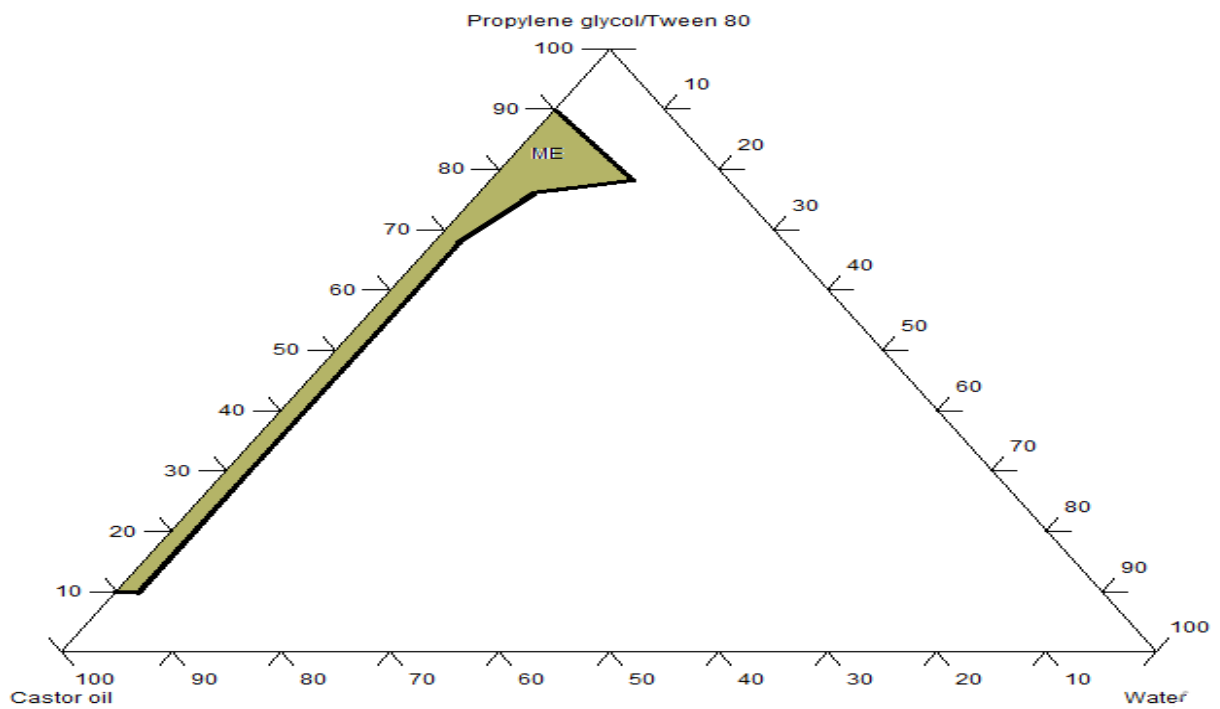
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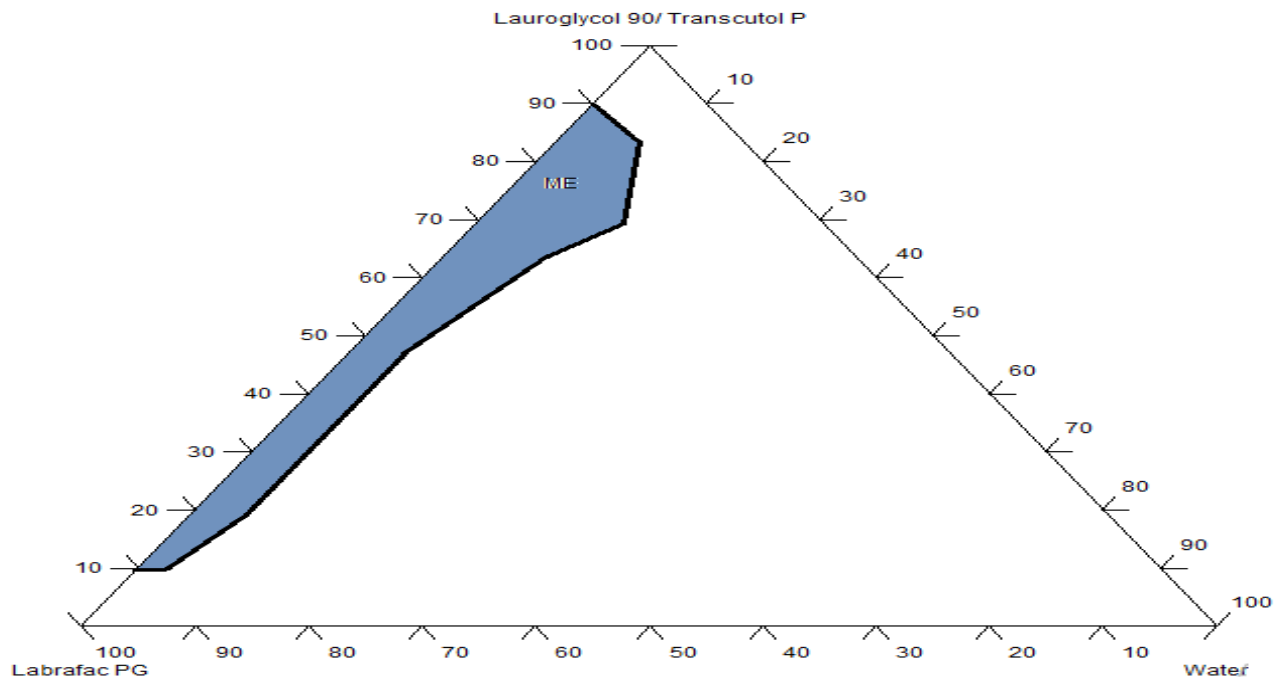
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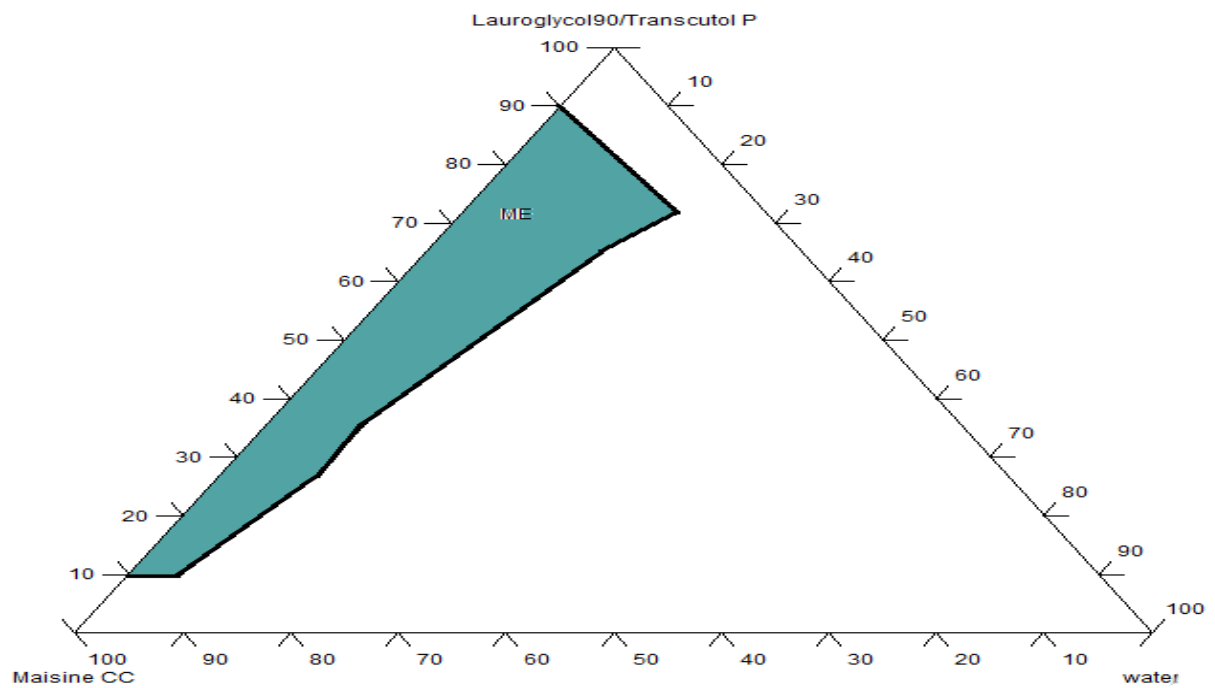
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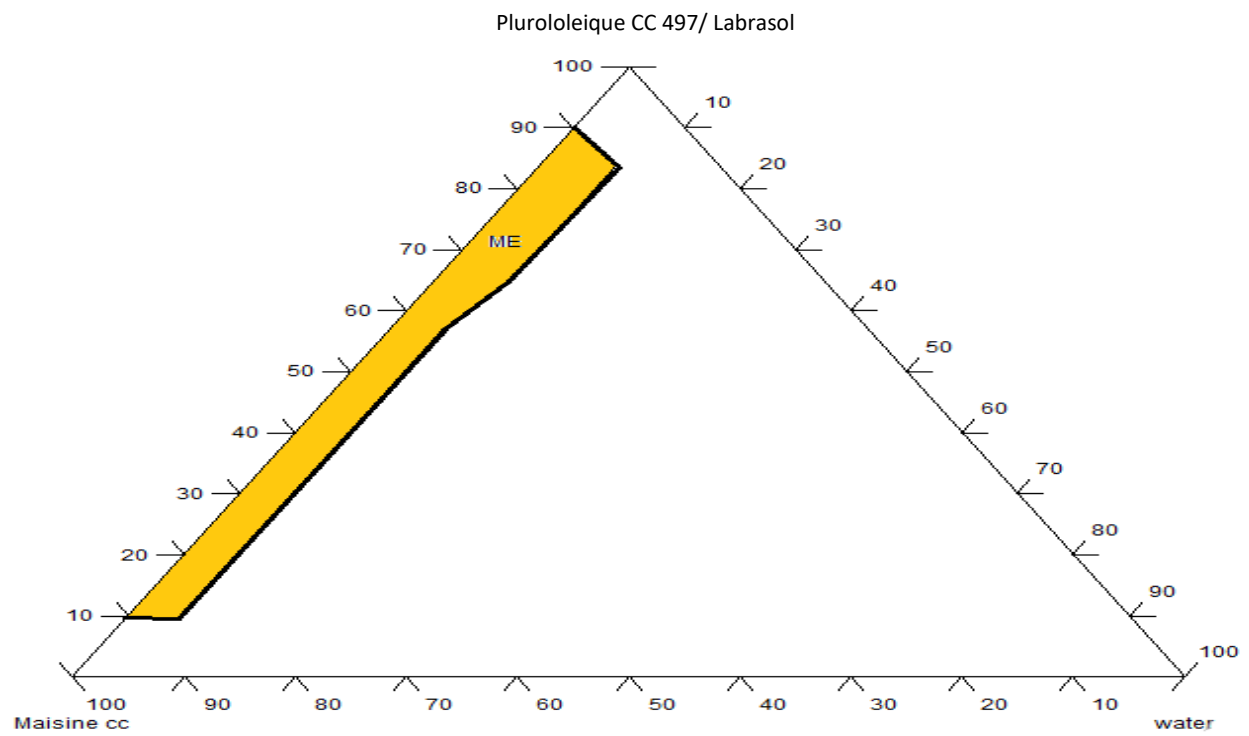
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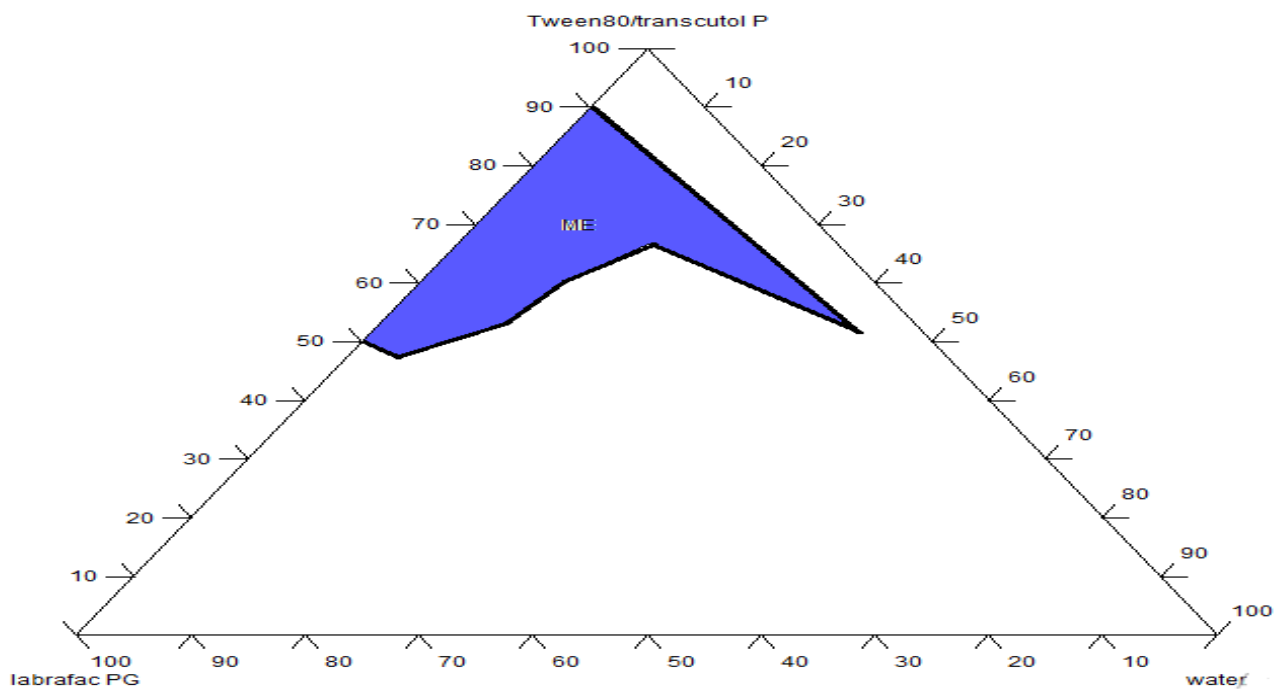
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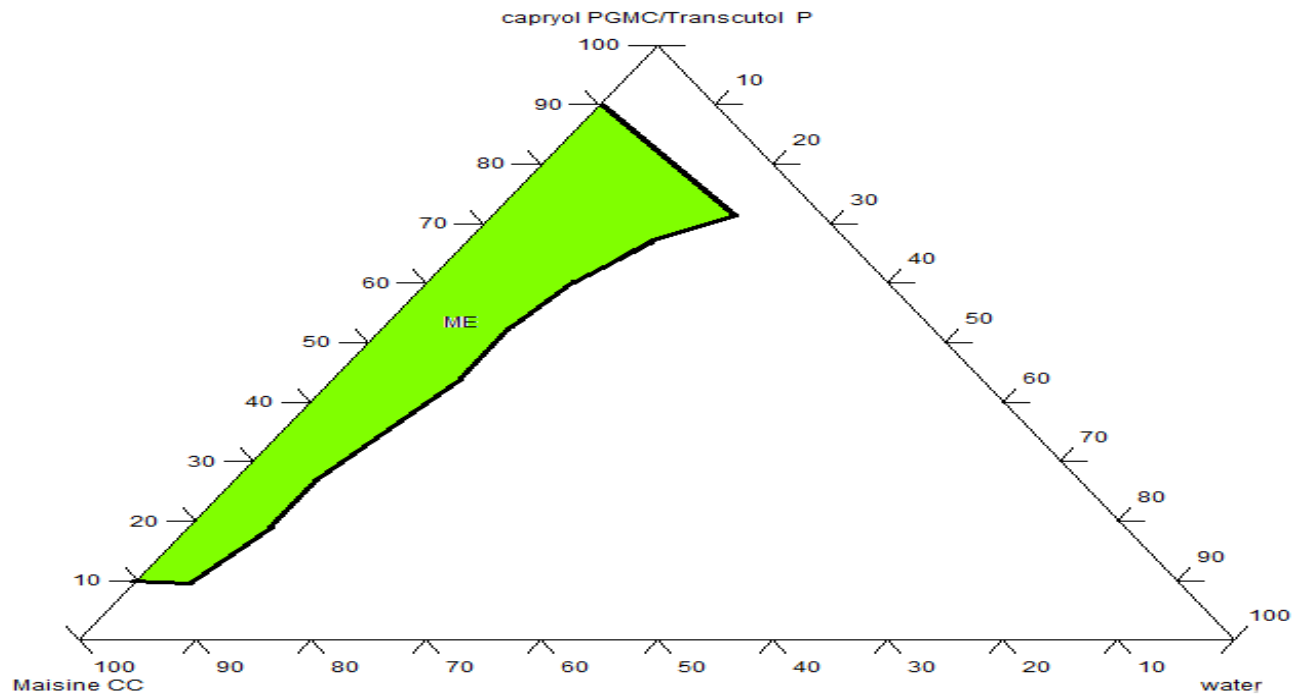
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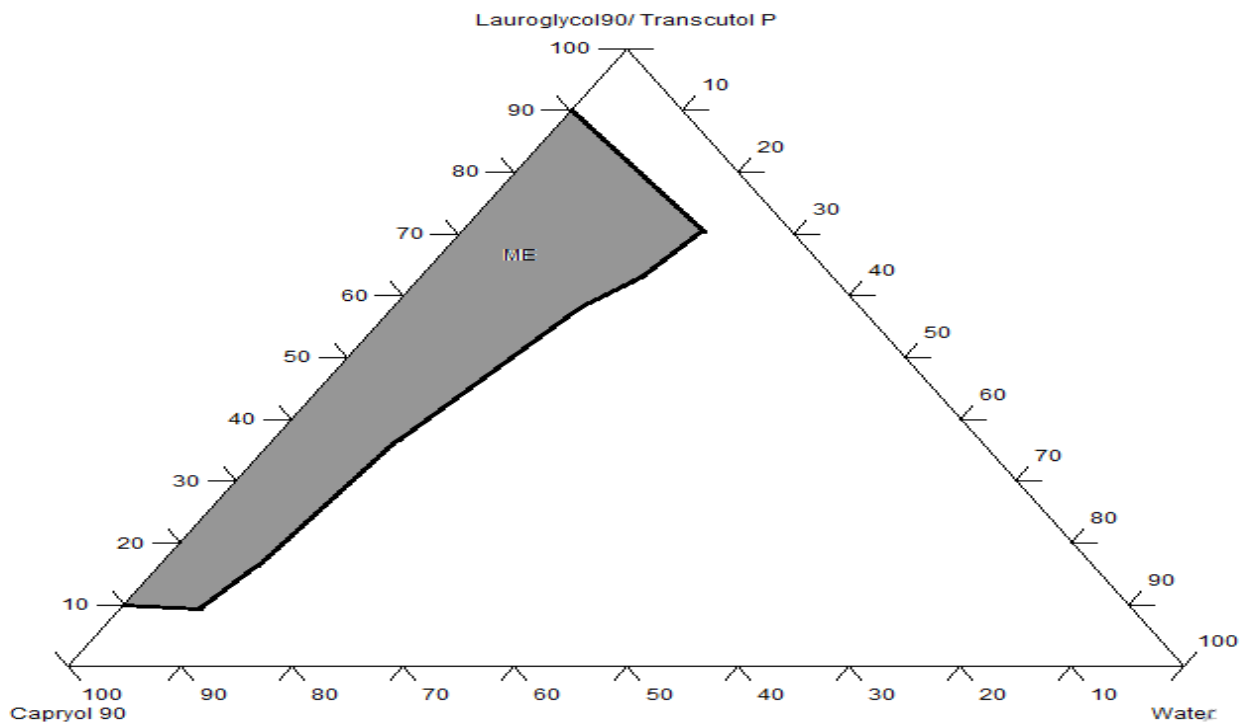
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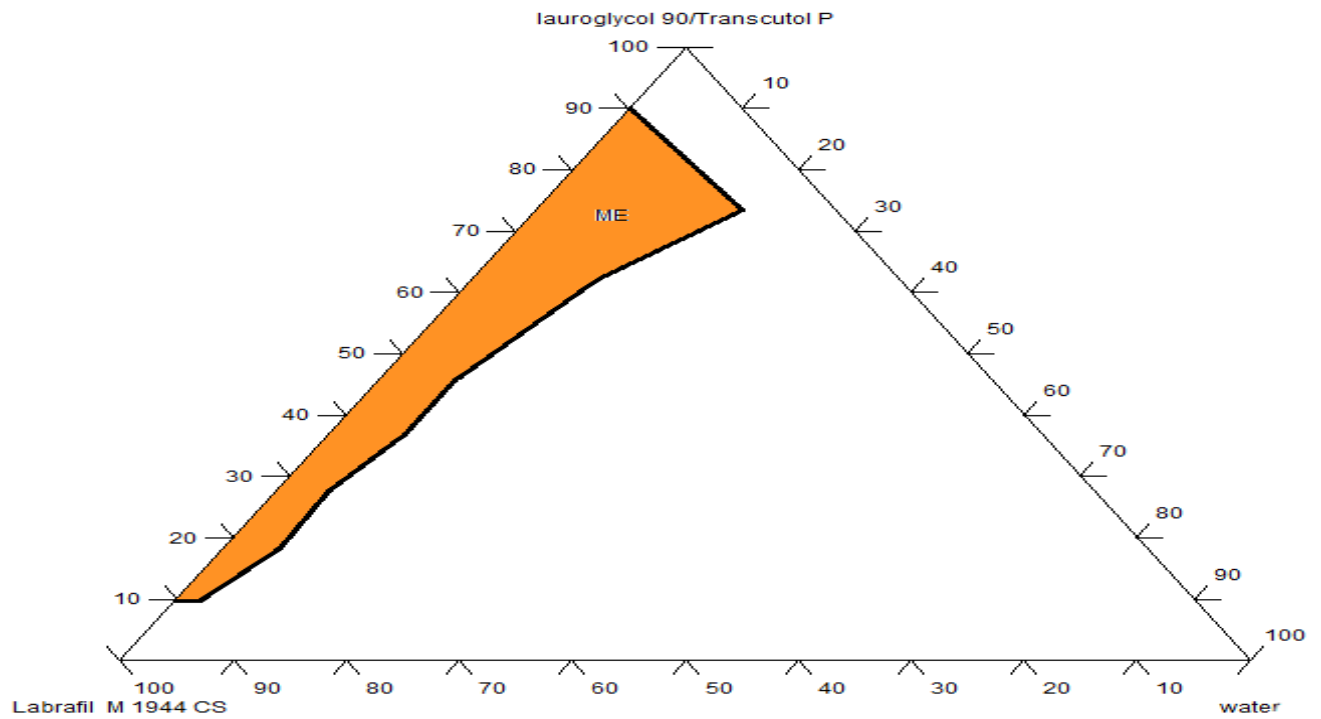
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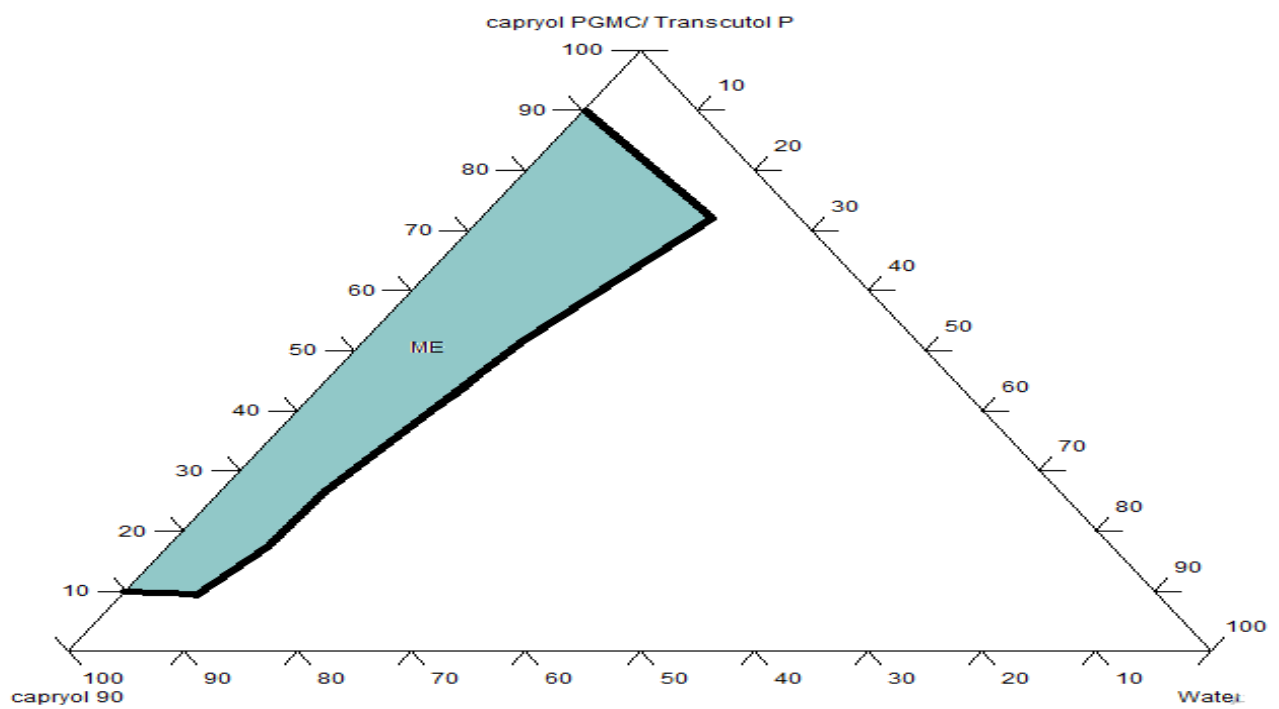
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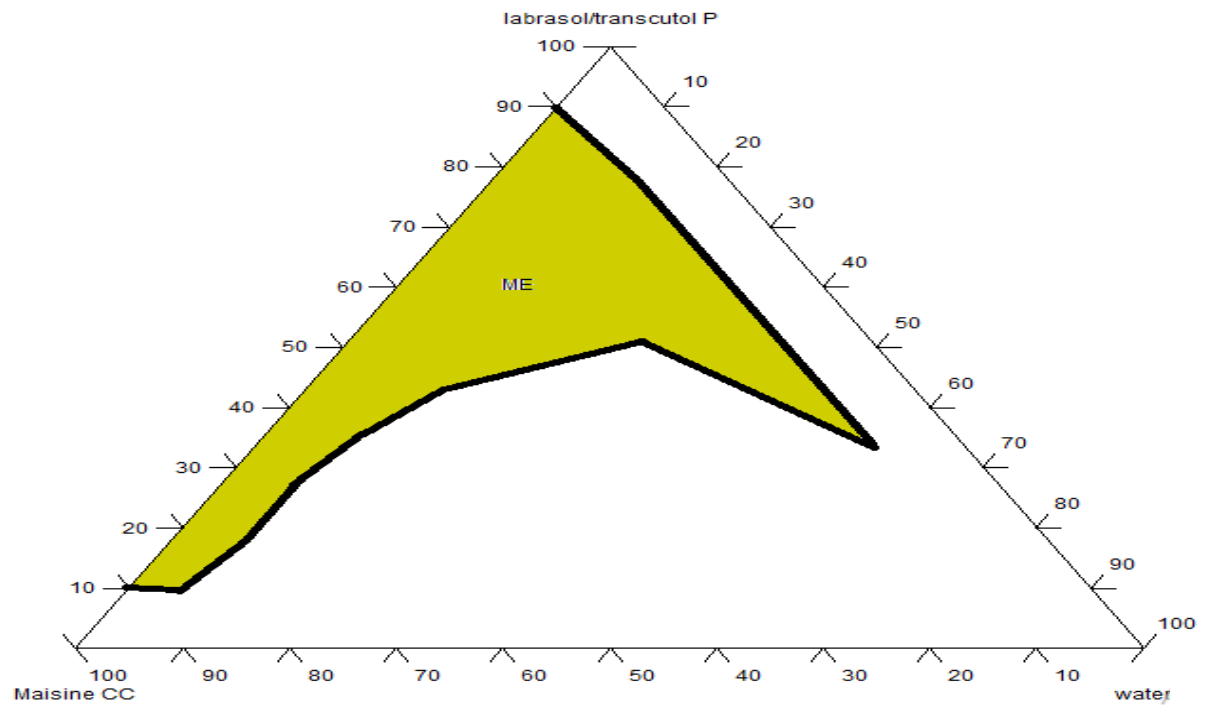
l)



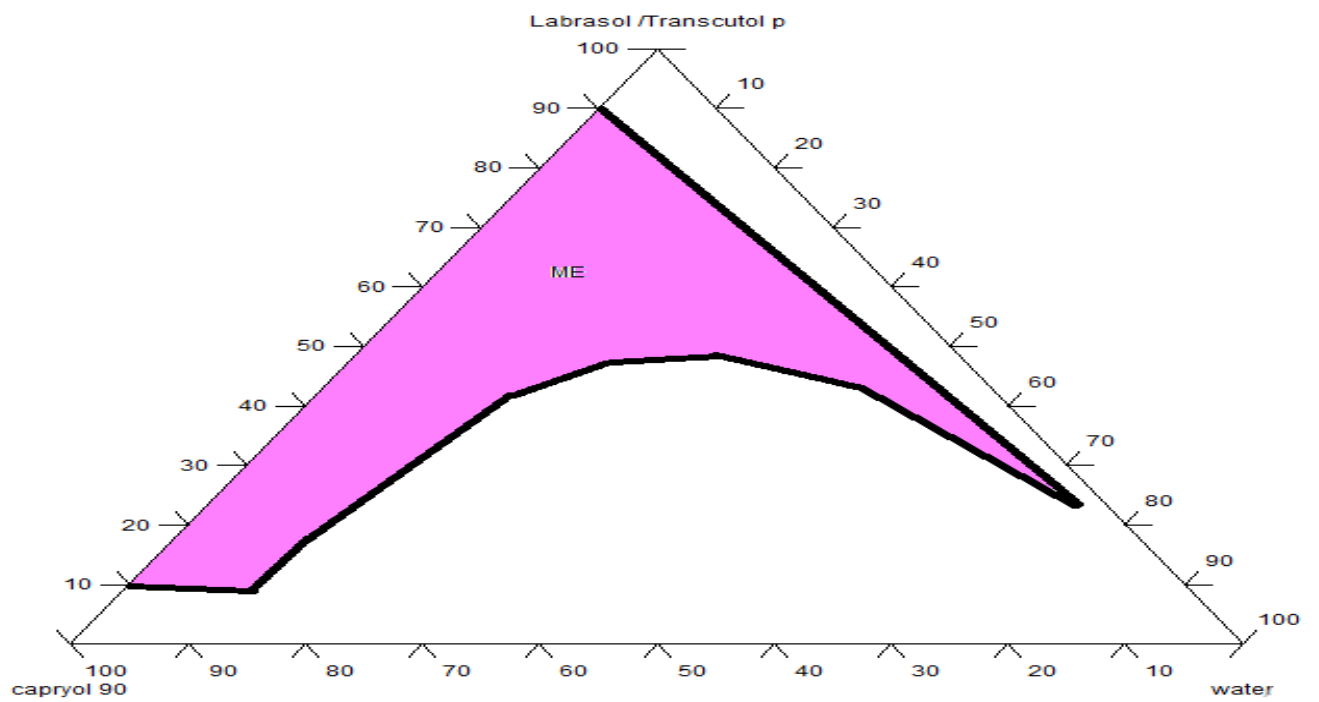
m)



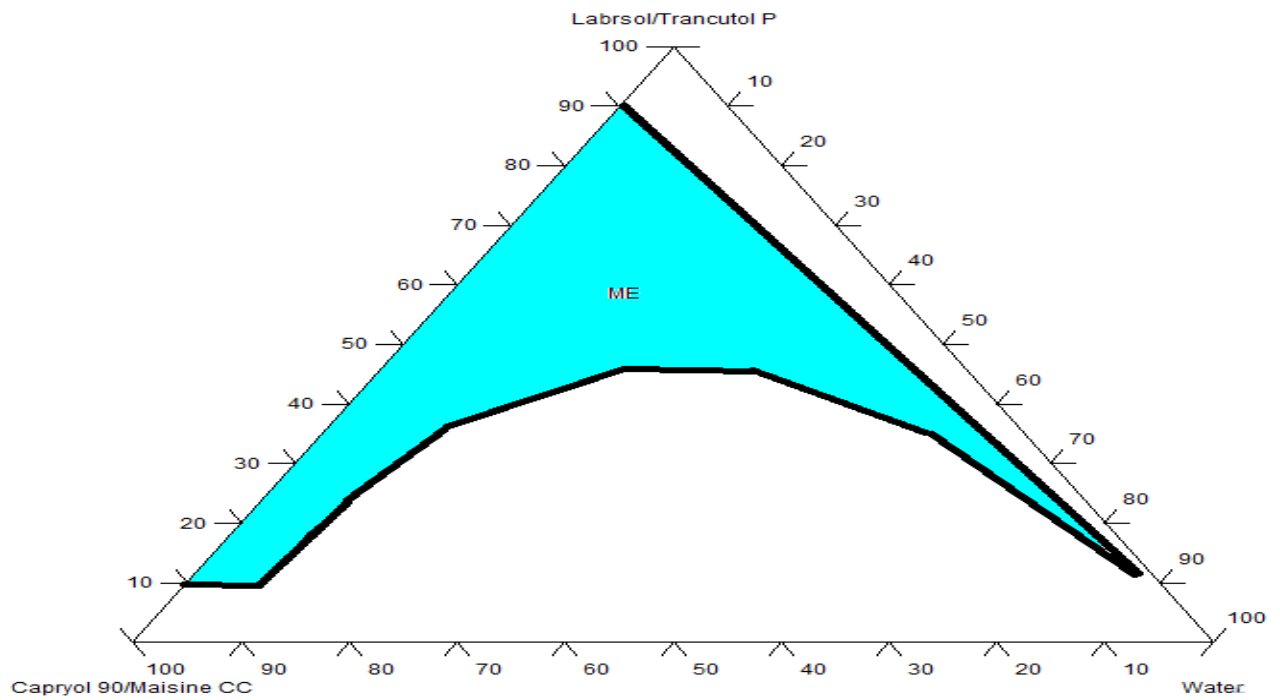
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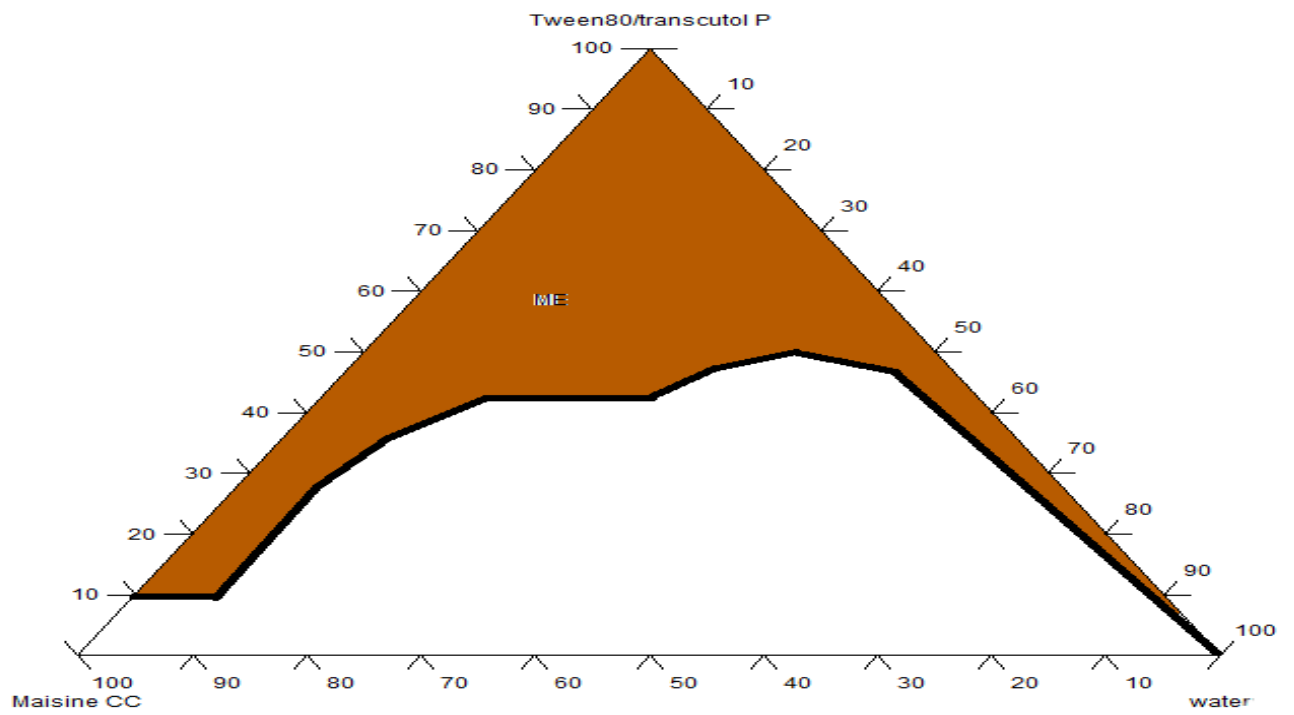
o)



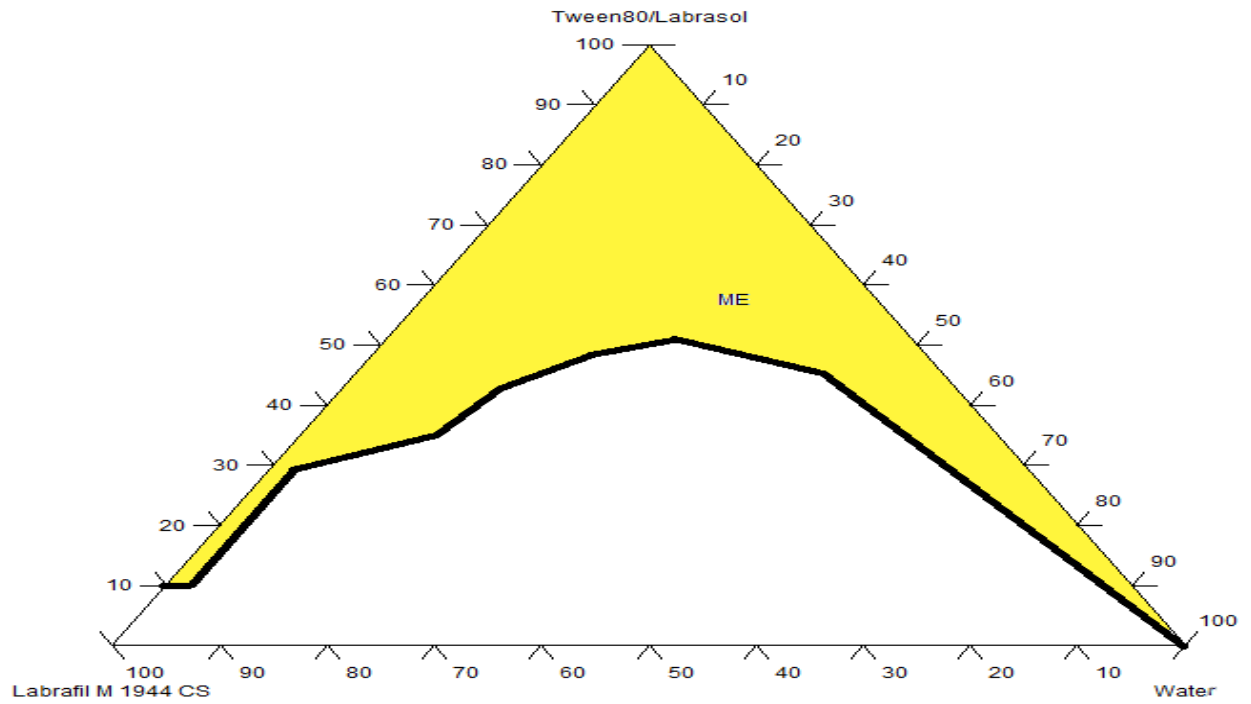
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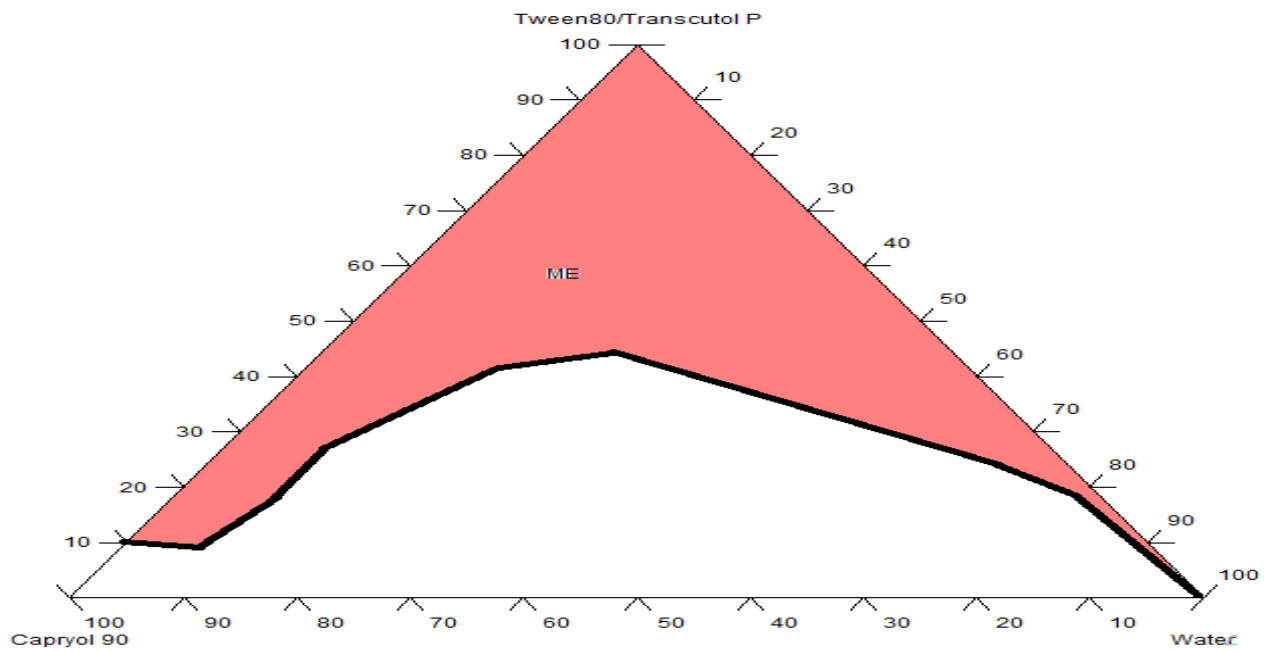
q)



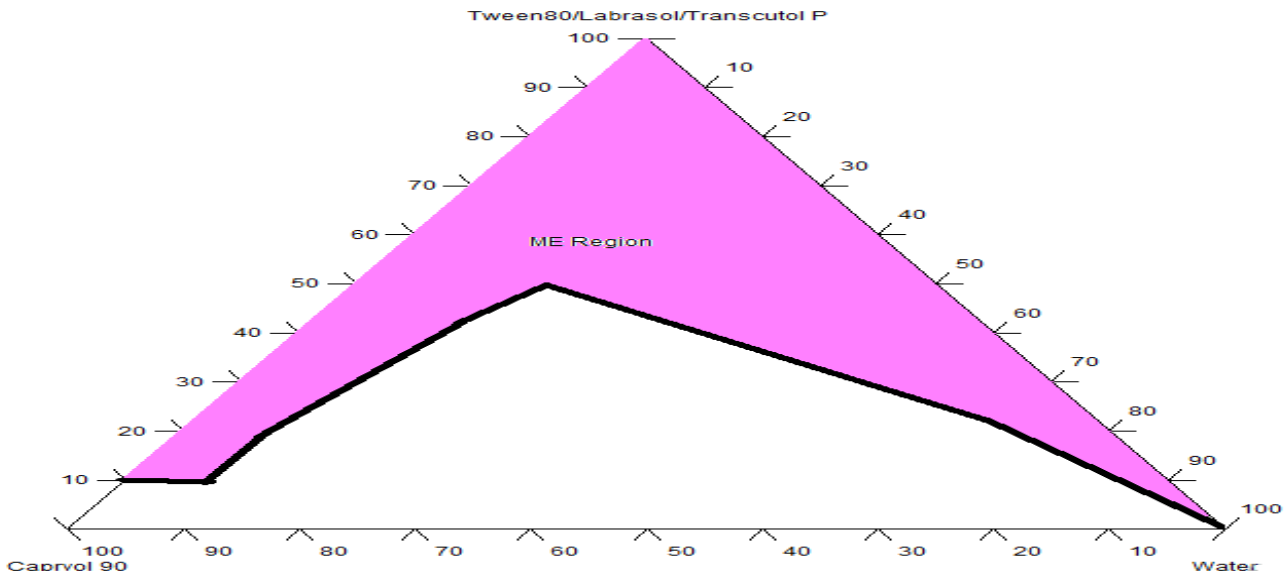
r)



s)



t)



Supplementary Material, figure 3. The pseudo-ternary phase diagrams (a-t) of the 20 different ME combinations.

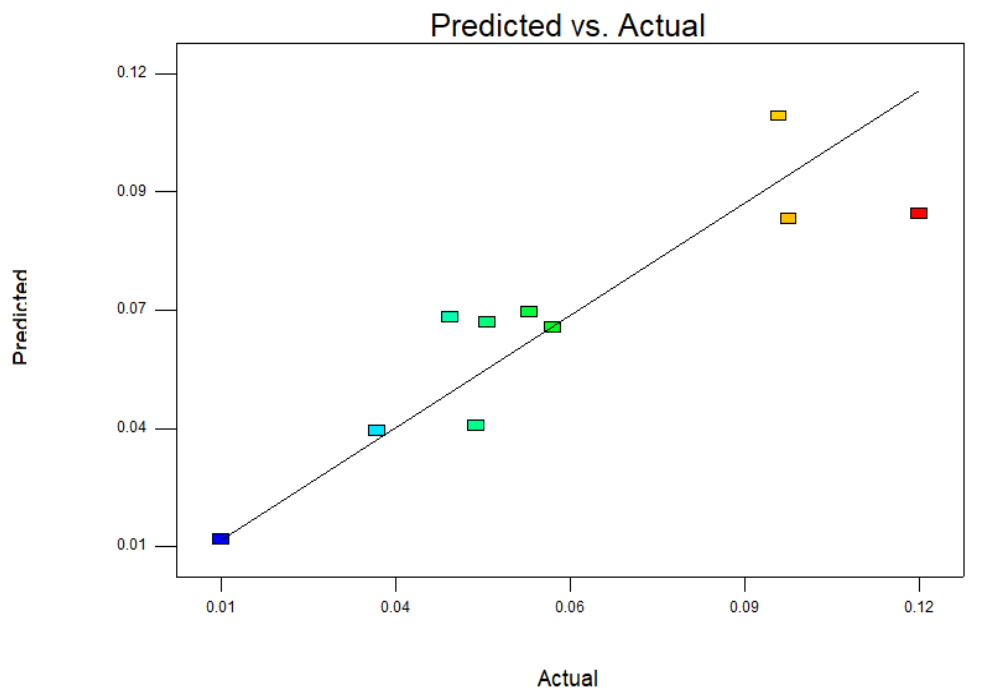
Supplementary Material, Table 2. Droplet size and PDI of the prepared formulations of the two selected microemulsion systems after 2 h and 24 h from preparation and after 100 folds dilution, where the significant changes colored in red.

	Oil %	S/CoS %	Water %	Diameter (nm)		PDI		Diameter (nm) after dilution		PDI after dilution	
				2 h	24 h	2 h	24 h	2 h	24 h	2 h	24 h
System 1. Labrafil M 1944 CS/ Tween 80/ Labrasol/ Water ME system (LTL)											
F1	5.67	77.42	16.92	21.75±1.195	24.74±0.376	1 ±0	0.942±0.058	9.681±0.19869	10.02±0.1724	0.047±0.009	0.064±0.014
F2	16.00	72.25	11.75	17.32±1.462	20.92±2.967	0.687±0.008	0.774±0.083	74.35±0.6576	104.7±0.9899	0.209±0.013	0.098±0.004
F3	16.00	80.00	4.00	86.02±4.859	93.1±3.02	1 ±0	1±0	32.26±0.3818	60.51±1.181	0.247±0.001	0.162±0.015
F4	8.25	72.25	19.50	8.615±1.005	13.33±1.027	0.556±0.072	0.659±0.022	13.84±2.016	10.69±0.02121	0.179±0.114	0.141±0.074
F5	0.50	80.00	19.50	17.30±4.818	19.98±6.456	0.911 ±0.096	0.887±0.117	10.82±0.885	12.39±0.1061	0.145±0.051	0.284±0.027
F6	13.42	69.67	16.92	10.34±0.163	14.53±2.009	0.608±0.007	0.699±0.142	27.19±0.1697	53.37±0.3536	0.224±0.004	0.248±0.001
F7	16.00	64.50	19.50	12.29±1.63	12.9±1.247	0.598±0.0853	0.614±0.046	98.14 ± 2.54	113±1.908	0.168±0.005	0.090±0.014
F8	10.83	74.83	14.33	19.40±2.494	31.03±4.885	0.859±0.082	0.982±0.036	12.34±1.415	16.36±0.6133	0.092±0.033	0.388±0.008
F9	13.42	77.42	9.17	28.53±2.037	51.83±3.753	0.961±0.046	1±0	27.14±0.03536	49.35±0.5728	0.228±0.002	0.196±0.01
F10	8.25	80.00	11.75	20.06±4.545	31.72±2.517	0.879±0.102	1±0	12.95±0.2475	10.61±0.1414	0.238±0.031	0.064±0.006
System 2. Capryol 90/ Tween 80/ Transcutol p/ Labrasol/ Water ME system (CTTL)											
F1	5.67	77.42	16.92	23.69±6.217	39.66±16.21	1±0	1±0	64.07±2.082	121.7±1.044	0.168±0.008	0.515±0.011
F2	16.00	72.25	11.75	85.30±3.661	133±8.576	0.439±0.053	0.358±0.031	163.9±6.580	186.1±6.757	0.552±0.031	0.537±0.008
F3	16.00	80.00	4.00	88.33±5.515	164.6±22.64	0.262±0.027	0.495±0.443	172.4±1.701	207.3±5.250	0.508±0.006	0.476±0.007
F4	8.25	72.25	19.50	17.83±5.669	23.85±13.07	0.992±0.014	1±0	87.12±0.3844	141.8±2.669	0.170±0.024	0.387±0.061
F5	0.50	80.00	19.50	3.105±0.9332	4.934±1.278	0.493±0.044	0.628±0.033	10.79±0.1833	11.04±0.1206	0.142±0.032	0.167±0.009
F6	13.42	69.67	16.92	46.27±1.705	93.12±9.514	0.590±0.076	0.649±0.155	179.3±1.701	184.3±2.515	0.526±0.003	0.516±0.006
F7	16.00	64.50	19.50	24.65±9.914	78.08±1.421	1±0	0.561±0.016	133±3.951	214.4±0.9644	0.408±0.028	0.304±0.009
F8	10.83	74.83	14.33	57.22±6.725	138.1±11.88	0.641±0.042	0.859±0.199	142.2±3.214	180.2±1.343	0.314±0.003	0.447±0.07
F9	13.42	77.42	9.17	79.51±5.665	145±15.22	0.276±0.064	0.333±0.144	173±1.904	188.5±7.067	0.481±0.015	0.510±0.017
F10	8.25	80.00	11.75	66.36±6.067	138.6±7	0.732±0.265	0.765±0.173	87.36±1.06	147.5±1.732	0.146±0.003	0.514±0.005

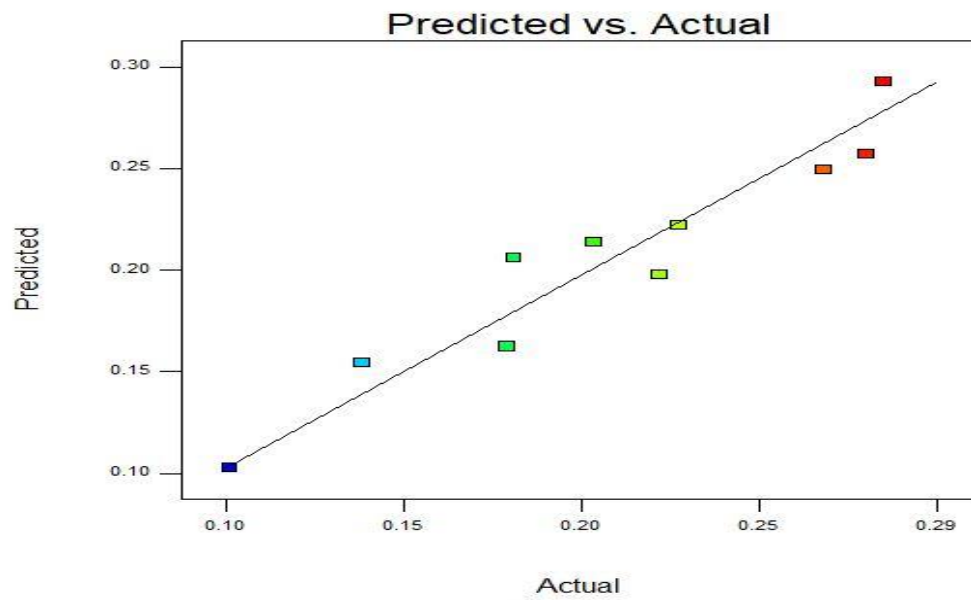
Supplementary Material, Table 3. Statistical analysis results of the experimental response (droplet size) for both systems.

	2 h from preparation	24 h from preparation	2 h after dilution	24 h after dilution
System 1. Labrafil M 1944 CS/ Tween 80/ Labrasol/ Water ME system (LTL)				
Suggested model	Linear	Linear	linear	Quadratic
Equation	$1/(GS)=+2.34018e^{0.03} \cdot \text{Oil}$ $-6.08275e^{0.04} \cdot \text{S/CoS}$ $+5.74259e^{0.03} \cdot \text{water}$	$1/\text{sqrt}(GS)=+4.3637e^{0.03} \cdot \text{Oil}$ $-1.90697e^{0.04} \cdot \text{S/CoS}$ $+0.012053 \cdot \text{water}$	$1/(GS)=-4.94361e^{0.03} \cdot \text{Oil}$ $+1.40617e^{0.03} \cdot \text{S/CoS}$ $+1.99119e^{0.04} \cdot \text{water}$	$\text{sqrt}(GS)=+5.63832 \cdot \text{Oil}$ $+0.083747 \cdot \text{S/CoS}$ $-0.14516 \cdot \text{water}$ $-0.066709 \cdot \text{Oil} \cdot \text{S/CoS}$ $-0.041646 \cdot \text{Oil} \cdot \text{water}$
P-value	0.0033	0.0001	0.0024	0.0003
R ²	0.8043	0.9196	0.8223	0.9764
Adjusted R ²	0.7484	0.8966	0.7716	0.9576
Predicted R ²	0.5950	0.8575	0.5788	0.8761
Adequate precision	11.298	18.66	11.298	19.157
C.V. %	25.6	9.02	29.17	10.08
PRESS	$3.666e^{0.03}$	$4.276e^{0.03}$	$4.198e^{0.03}$	9.69
System 2. Capryol 90/ Tween 80/ Transcutol p/ Labrasol/ Water ME system (CTTL)				
Suggested model	Quadratic	Linear	Linear	Quadratic
Equation	$(GS)=+4.57597 \cdot \text{Oil}$ $-0.027771 \cdot \text{S/CoS}$ $-34.55603 \cdot \text{water}$ $+0.27489 \cdot \text{Oil} \cdot \text{water}$ $+0.43150 \cdot \text{S/CoS} \cdot \text{water}$	$(GS)=+5.72894 \cdot \text{Oil}$ $+1.55363 \cdot \text{S/CoS}$ $-5.75110 \cdot \text{water}$	$(GS)=+9.80157 \cdot \text{Oil}$ $+0.42114 \cdot \text{S/CoS}$ $-1.14316 \cdot \text{water}$	$(GS)=-40.50705 \cdot \text{Oil}$ $+0.46882 \cdot \text{S/CoS}$ $-1.78884 \cdot \text{water}$ $+0.59885 \cdot \text{Oil} \cdot \text{S/CoS}$ $+0.76345 \cdot \text{Oil/Water}$
P-value	<0.0001	0.0017	0.0007	0.0002
R ²	0.9881	0.8373	0.8755	0.9797
Adjusted R ²	0.9785	0.7908	0.8399	0.9635
Predicted R ²	0.9455	0.6610	0.6901	0.8265
Adequate precision	27.158	12.498	13.675	23.634
C.V. %	9.14	27.11	18.67	7.17
PRESS	462.21	9852.86	8939.41	5516.75

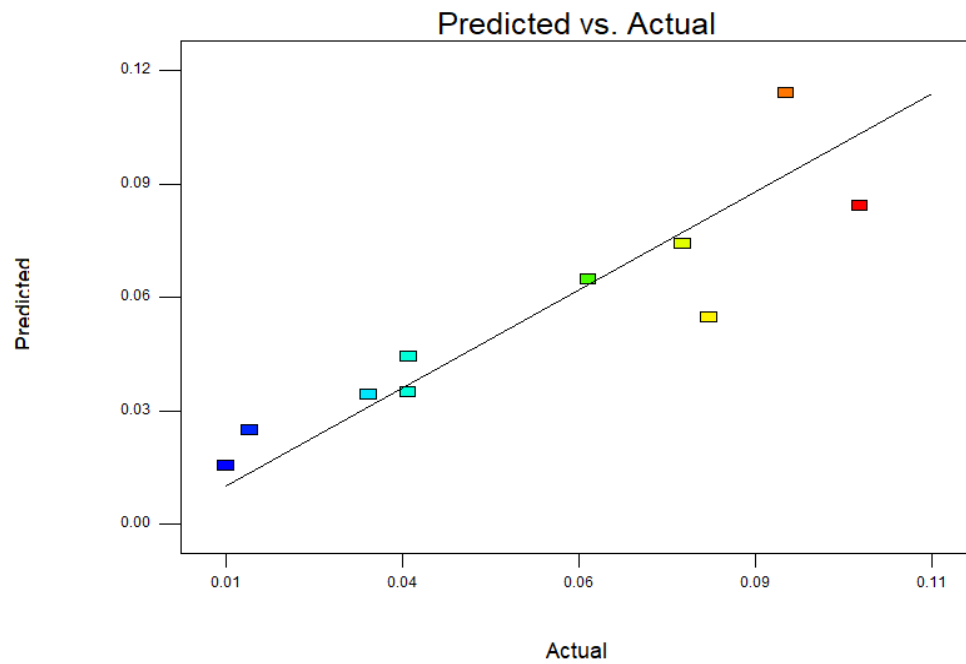
a) After 2h



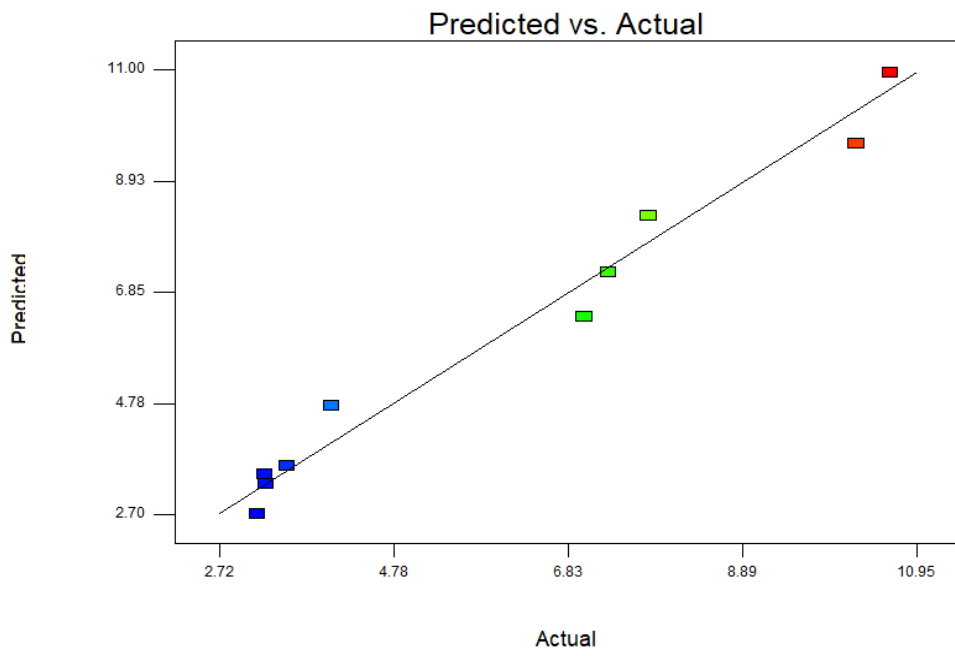
b) After 24h



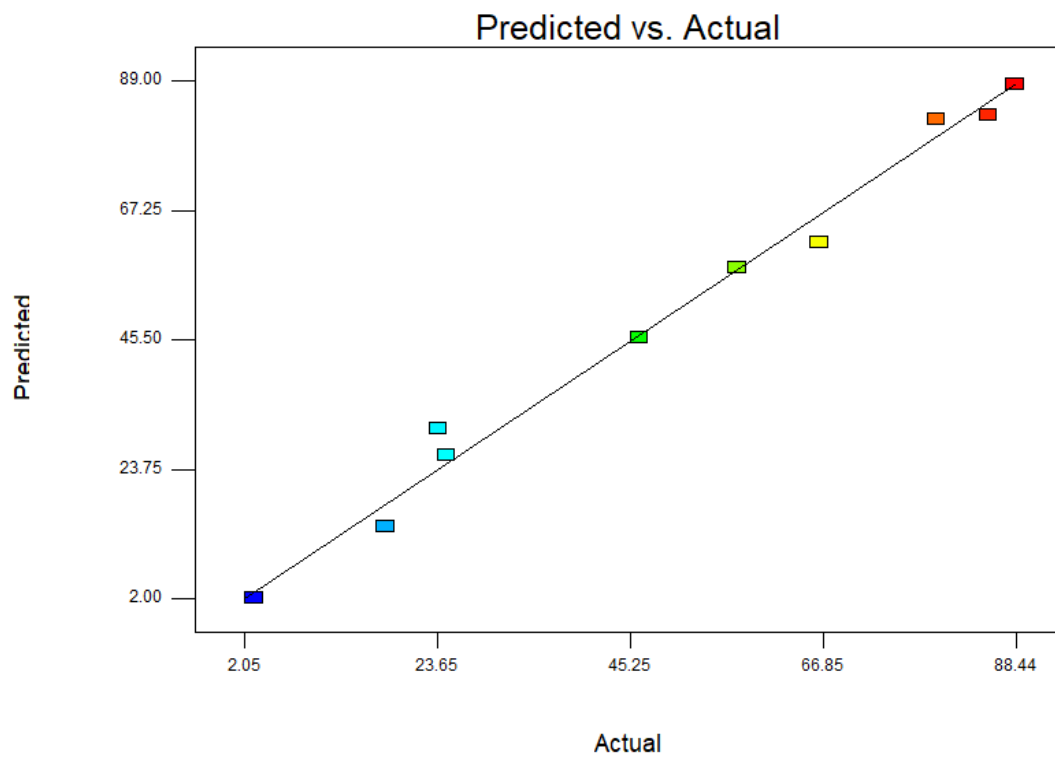
c) 2h after dilution



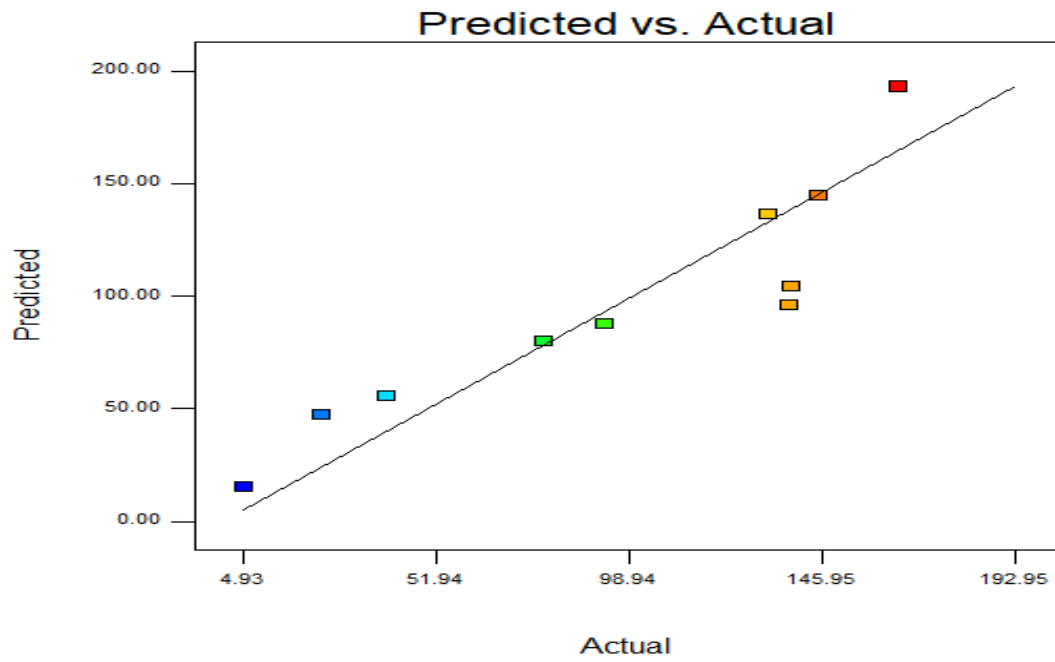
d) 24h after dilution



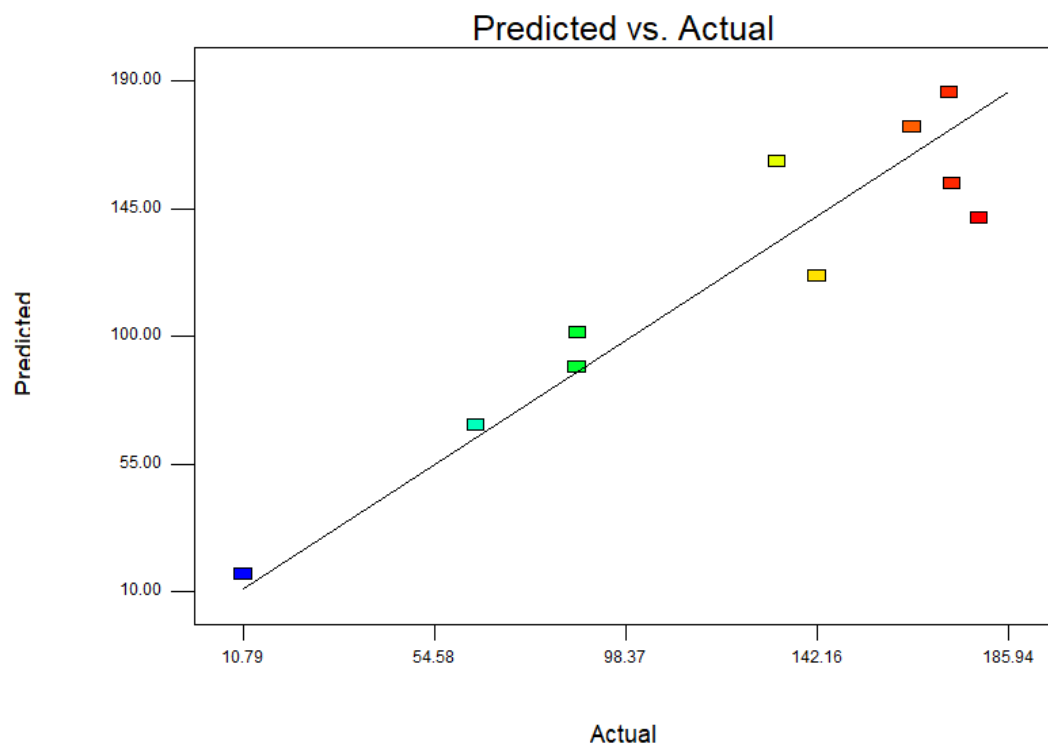
e) After 2h



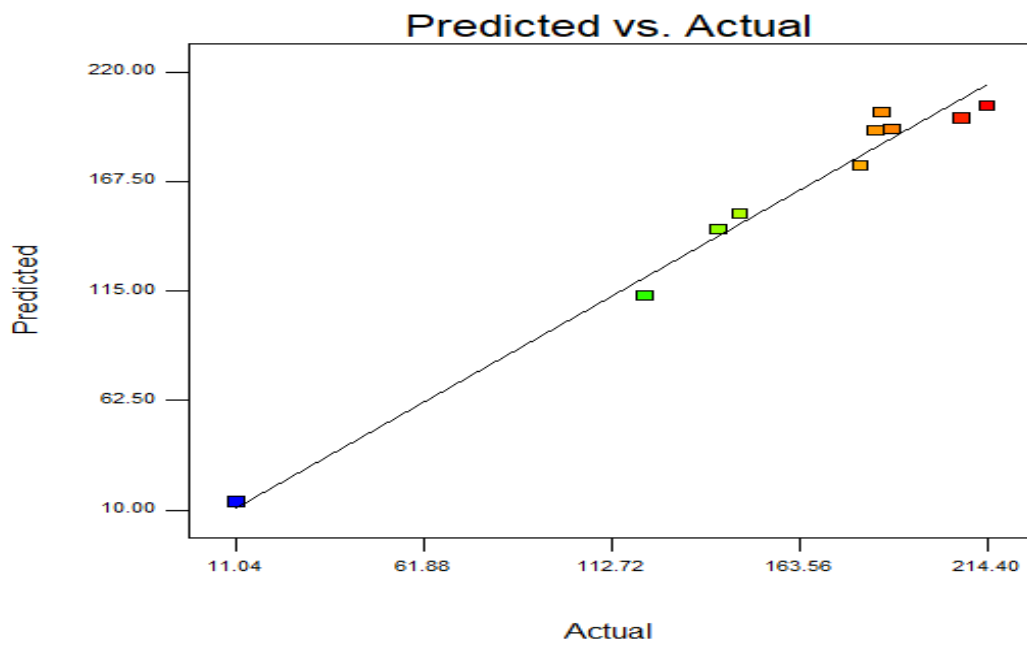
f) After 24h



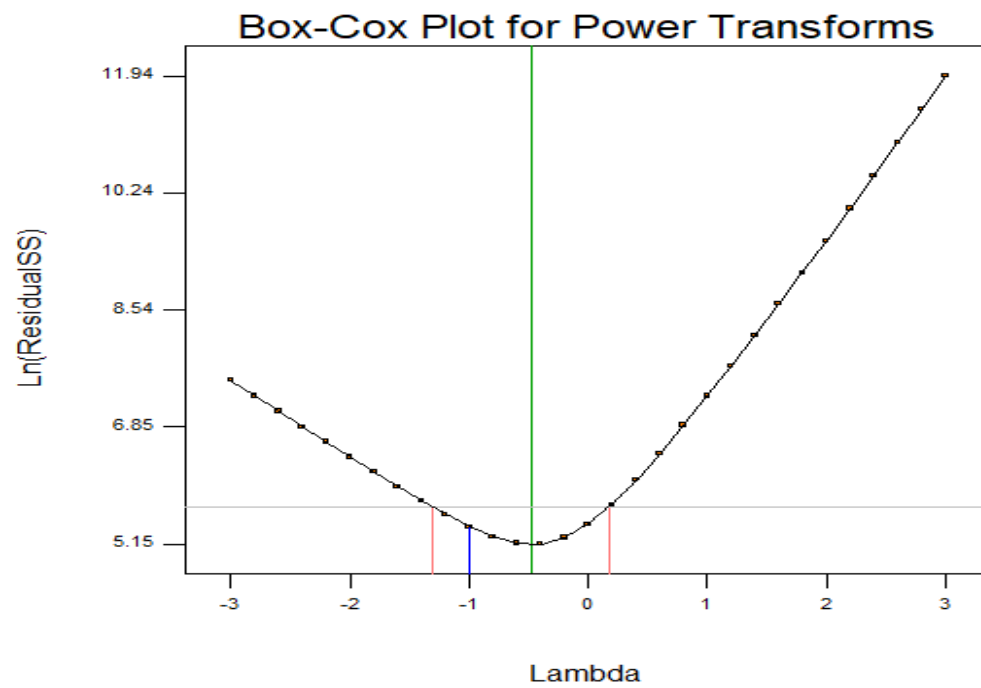
g) 2h after dilution



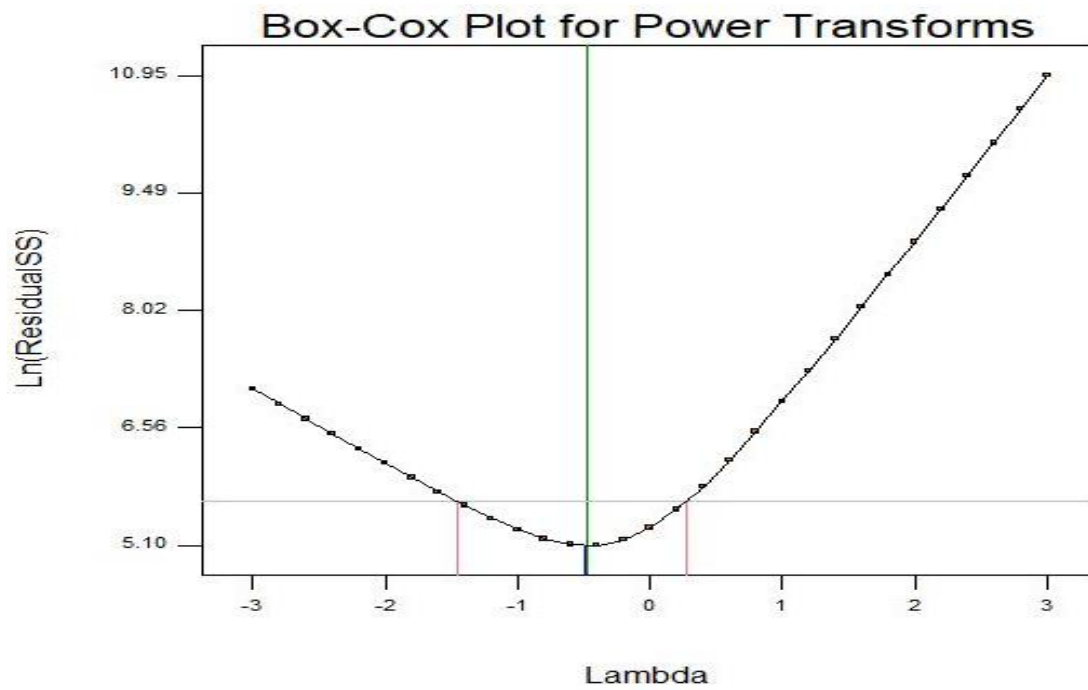
h) 24h after dilution



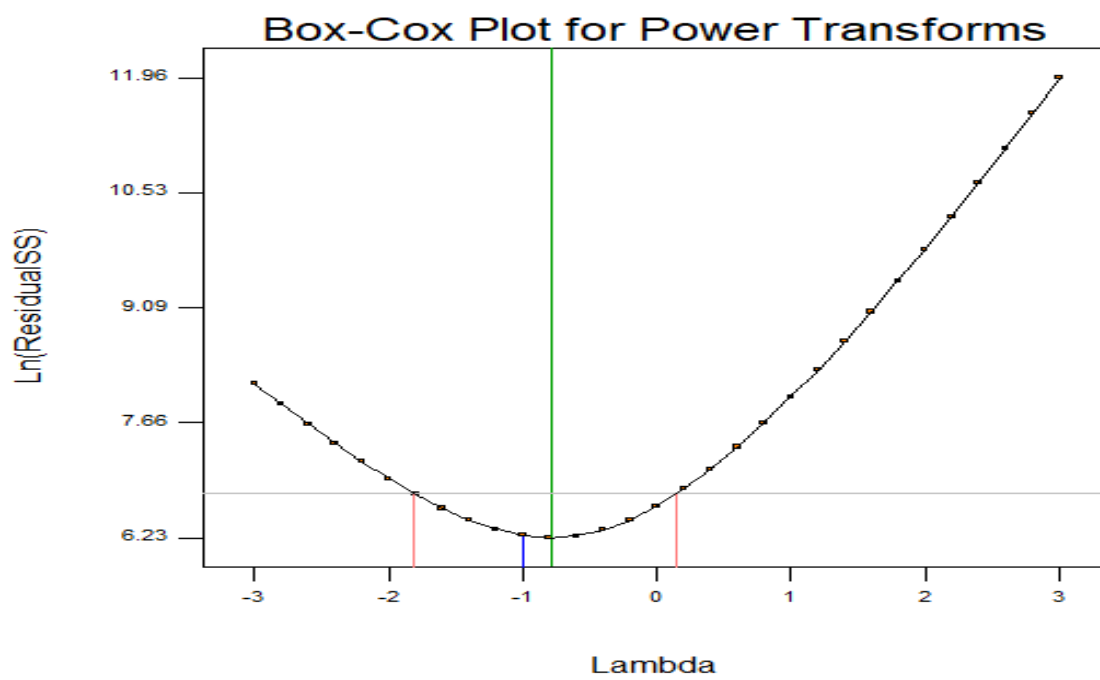
i) After 2h



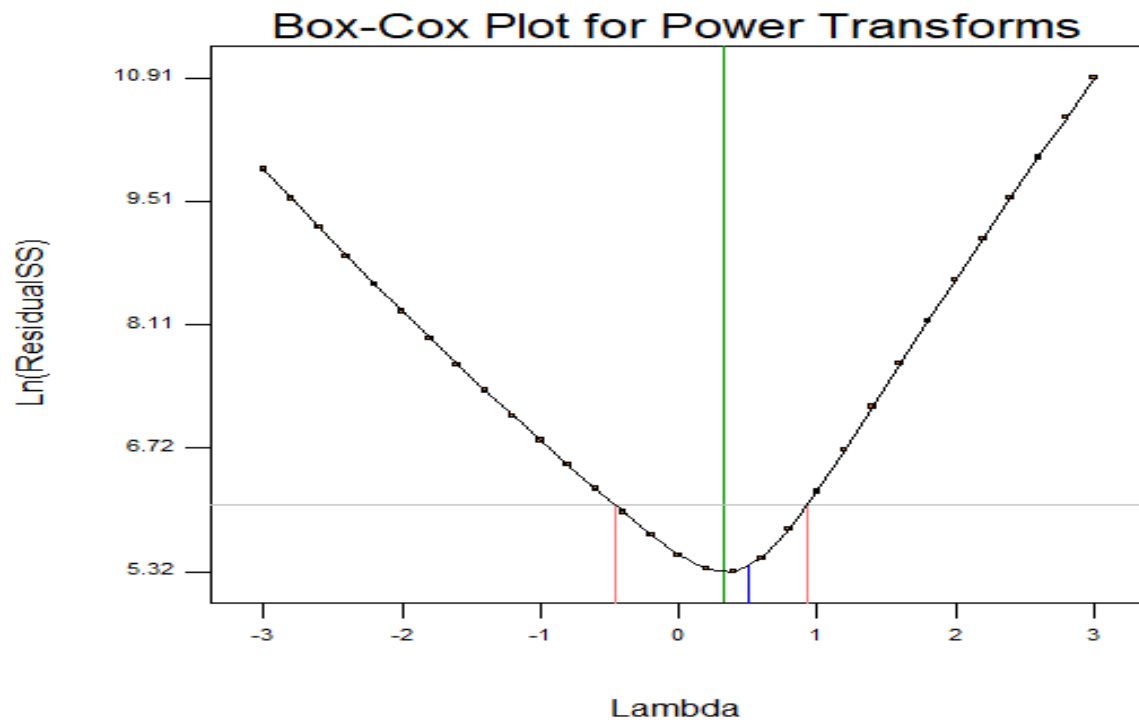
j) After 24h



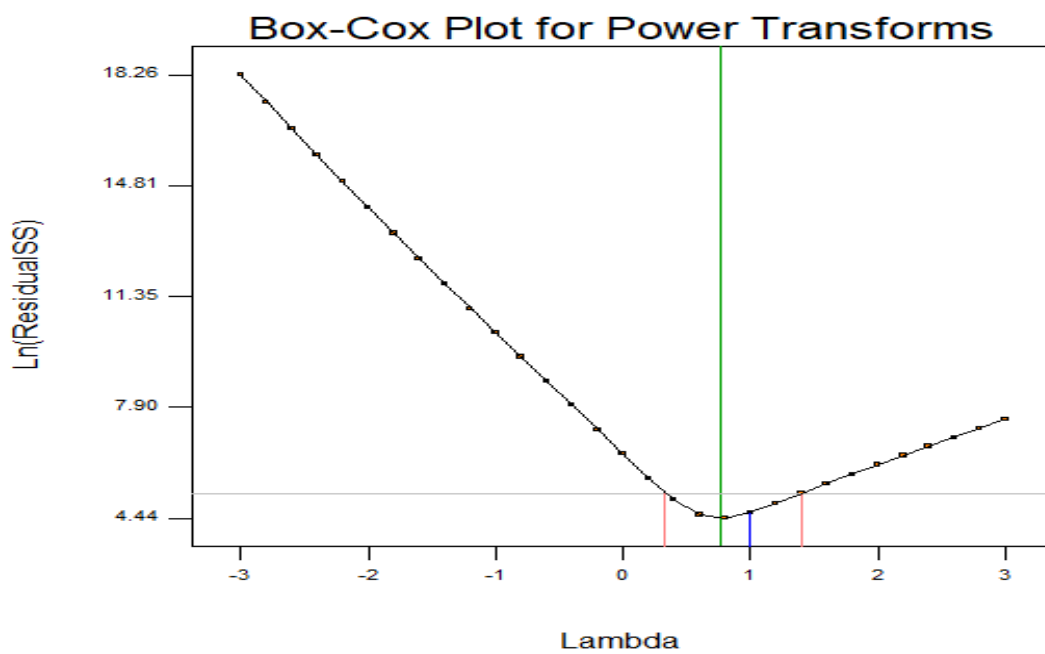
k) 2h after dilution



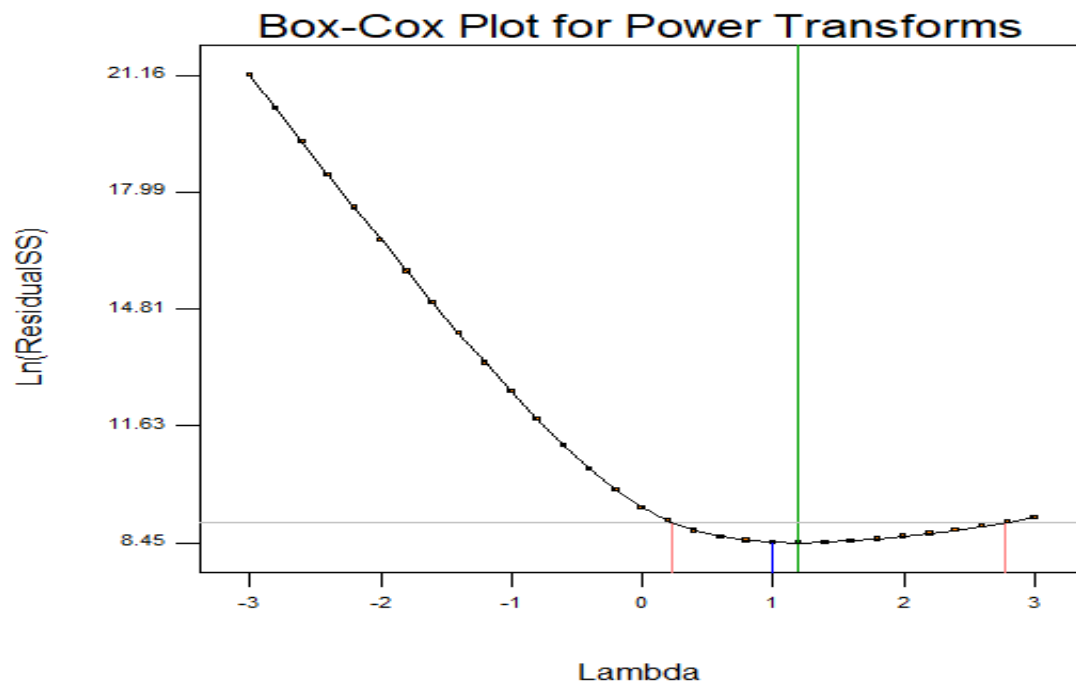
l)24h after dilution



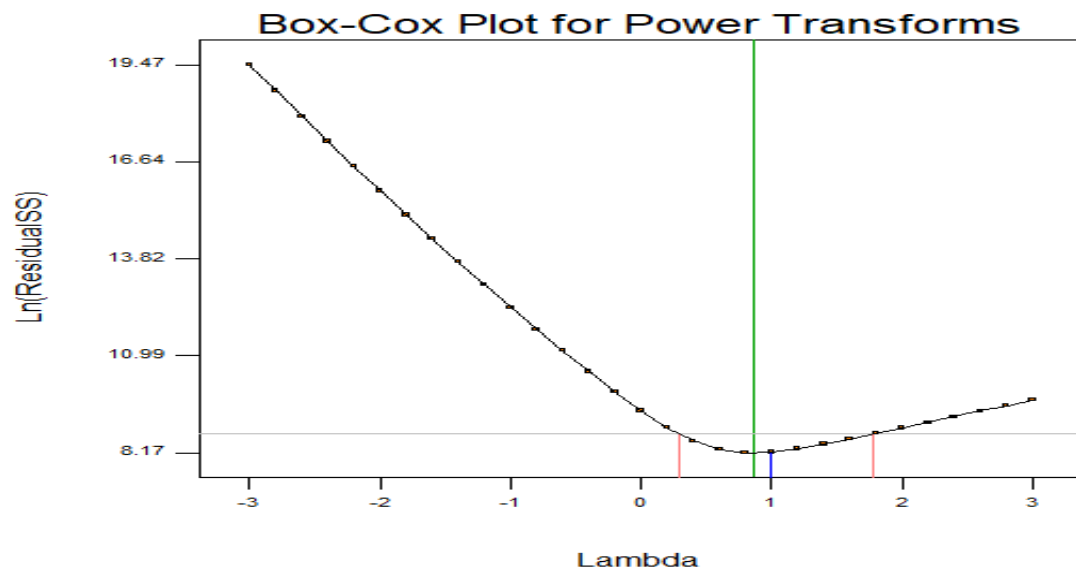
m)After 2h



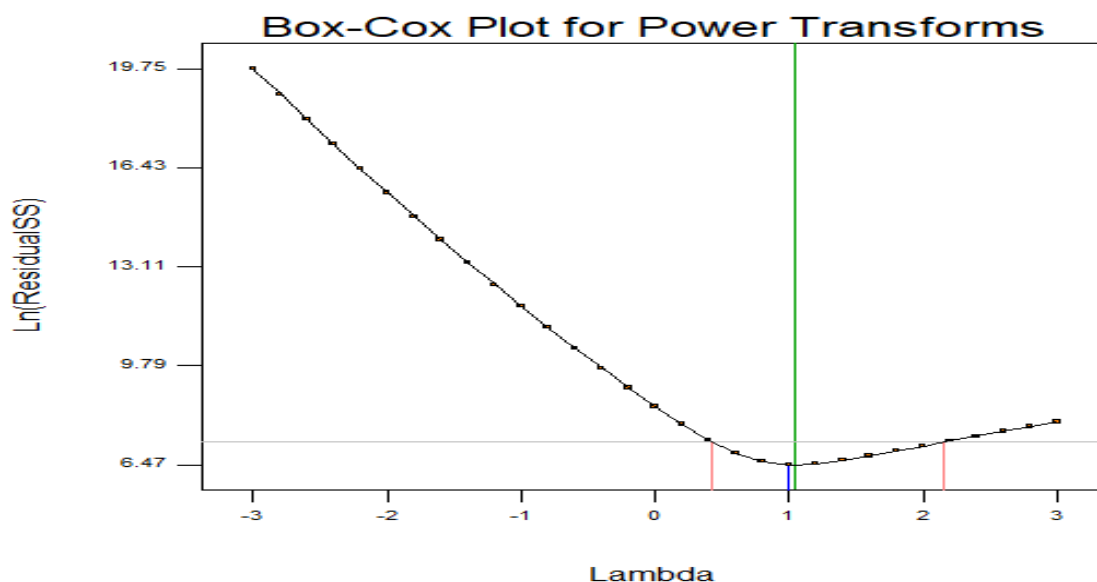
n) After 24h



o) 2h after dilution



p) 24h after dilution



Supplementary Material, figure 4. Actual versus predicted plots for globule size of LTL (a-d) and CTTL (e-h) ME systems before and after dilution. Where, a&e, b&f, c&g, d&h correspond to 2 h after preparation, 24 h after preparation, 2 h after dilution, and 24 h after dilution, respectively. While i-p show the graphical presentation of Box-Cox power transformation of globule size for LTL (i-l) and CTTL (m-p) ME system. Where, i&m, j&n, k&o, l&p correspond to 2 h after preparation, 24 h after preparation, 2 h after dilution, and 24 h after dilution, respectively.

Physical stability study

The loaded optimized formulae were maintained in sealed glass vials and stored at room ($25 \pm 0.2^\circ\text{C}$) and refrigerator ($4 \pm 0.2^\circ\text{C}$) temperatures to determine the physical stability of the formulae in terms of droplet size, PDI, and physical appearance by visualization of drug precipitation or turbidity formation. Samples were withdrawn over 6 months i.e., after 15, 30, 60, 90, 120, 180 days, and compared with the baseline (0 days) to check the effect of storage and temperature on the stability of formulae.

The results presented in **Supplementary Material, Table 4** revealed that both formulations showed no significant change in the physical appearance in terms of color and drug precipitation. Moreover, both formulae stored at different conditions showed negligible changes in the droplet size and PDI measured at different time intervals, this result demonstrated that the chances of aggregation of droplets were minimum after such a long storage period. Thus, the stability study confirmed the resilience of the ME formulae by proving an adequate results at the tested stability conditions.

Supplementary Material, Table 4. Physical Stability of the optimized formulations.

	Time (days)	Physical appearance		Room temperature (25°C ± 0.2)		Refrigerator (4°C ± 0.2)	
		precipitation	Color	Droplet size (nm) ± SD	PDI ± SD	Droplet size (nm) ± SD	PDI ± SD
F1 LTL ME formulation	0	NO	Transparent	19.64 ± 2.76	1 ± 0	20.32 ± 1.78	1 ± 0
	30	NO	Transparent	20.01 ± 5.43	1 ± 0	20.98 ± 3.12	1 ± 0
	60	NO	Transparent	20.42 ± 3.98	0.965 ± 0.030	22.13 ± 3.98	1 ± 0
	90	NO	Transparent	22.23 ± 1.18	0.911 ± 0.064	24.22 ± 1.73	1 ± 0
	120	NO	Transparent	24.77 ± 2.30	1 ± 0	26.10 ± 4.12	1 ± 0
	180	NO	Transparent	26.11 ± 4.10	1 ± 0	26.35 ± 2.31	1 ± 0
F5 CTTL ME formulation	0	NO	Transparent	2.98 ± 1.30	0.417 ± 0.054	3.15 ± 0.56	0.534 ± 0.022
	30	NO	Transparent	2.86 ± 1.56	0.399 ± 0.077	3.33 ± 1.14	0.579 ± 0.099
	60	NO	Transparent	3.51 ± 2.63	0.578 ± 0.033	4.10 ± 0.91	0.737 ± 0.051
	90	NO	Transparent	4.58 ± 2.31	0.512 ± 0.011	5.21 ± 1.11	0.681 ± 0.012
	120	NO	Transparent	5.13 ± 1.98	0.721 ± 0.098	6.05 ± 2.33	0.842 ± 0.079
	180	NO	Transparent	5.32 ± 3.34	0.632 ± 0.035	6.46 ± 1.98	0.883 ± 0.034

Development and validation of high-performance liquid chromatography (HPLC) method for accurate determination of p-Coumaric acid in rat's plasma.

Development and validation of new HPLC method for pCA quantification in rat's plasma

A new sensitive, selective, and accurate HPLC method was developed and validated for pCA quantification in rat's plasma using Diclofenac sodium as an internal standard (IS). The assay was performed on the HPLC PerkinElmer system (PerkinElmer, California, USA) equipped with a variable wavelength UV detector and autosampler. The Liquid chromatographic separation was carried out at room temperature, and achieved on a Phenomenex column (C18, 250mm × 4.6mm, 5µm) using water: acetonitrile: glacial acetic acid (62: 37: 1 v/v) as mobile phase at a flow rate 1.5 ml/min and detected at 310 nm. The injection volume was 30 µl.

The stock solutions of both pCA and IS were prepared by dissolving in acetonitrile to obtain 100 µg/ ml. pCA stock solution was diluted to obtain working solutions ranging from 25-400 ng/ml. while Diclofenac sodium stock solution was diluted to obtain a working solution of 100 ng/ml. Moreover, Plasma samples were spiked with pCA and IS with the same prepared concentrations. Then for extraction, 0.5 ml of a mixture of acetonitrile-methanol (1:1) was added to 0.5 ml of plasma sample, vortexed for 1 minute, and then centrifuged for 10 minutes at 5000 rpm to separate denatured proteins. The organic phase was then transferred to another vial and evaporated to dryness in a thermostatic controller at 30°C. The residue was dissolved in the mobile phase and 30

µl were injected into the HPLC. Calibration curves were obtained by dividing the peak area of each pCA concentration by its corresponding IS' peak area. This method was validated according to specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision.

Validation of the newly developed HPLC method to determine pCA concentration in rat's plasma showed good specificity with no interference of chromatogram peaks of pCA and the internal standard (IS) either spiked or unspiked (data shown in supplementary data S3). Also, the obtained linearity R^2 was 0.998, LOD at 0.07 ng/ml, LOQ at 0.212 ng/ml, with recovery percentage of 98.21% and the coefficient of variation percentage (CV %) of precision was less than 15% which is the maximum accepted limit. The intra-day precision had a % CV ranged from 0.62 to 1.4%, while the inter-day precision had % CV in the range from 0.606 to 2.798 %⁷². All results were acceptable and confirmed that this new developed method is suitable for its intended use.