



Supporting Information

for

Development of *Protium heptaphyllum* essential oil-loaded nanocapsules: experimental design and biological activity

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Additional experimental data

Table S1: Model summary statistics for the fitted response surface model evaluating particle size. S: standard deviation of the regression (residual standard error); R²: coefficient of determination; R² (adj): adjusted coefficient of determination; R² (pred): predicted coefficient of determination and adequate precision.

Model Summary					
	S	R ²	R ² (adj)	R ² (pred)	Adeq. Precision
Size	30.7290	90.78%	87.71%	82.44%	18.91
PDI	0.0445652	47.91%	30.54%	1.18%	7.46
Zeta	2.05155	78.82%	71.76%	58.32%	12.39

Table S2: Analysis of variance (ANOVA) for the fitted response surface model evaluating the effects of PCL, essential oil, and polysorbate concentration on the size. DF: degrees of freedom; Adj. SS: adjusted sum of squares; Adj. MS: adjusted mean square. Significant effects were determined at $p < 0.05$. The model was statistically significant, with no significant lack of fit.

Analysis of Variance (ANOVA) for Size						
Source	DF	Adj. SS	Adj. MS	F-Value	P-Value	
Model	11	306780	27889	29.54	0.000	
Blocks	2	147	74	0.08	0.925	
Linear	3	258509	86170	91.26	0.000	
PCL	1	243050	243050	257.39	0.000	
EO	1	14391	14391	15.24	0.000	
Tween 80	1	1068	1068	1.13	0.295	
Quadratic	3	24212	8071	8.55	0.000	
PCL * PCL	1	6557	6557	6.94	0.013	
EO * EO	1	17816	17816	18.87	0.000	
Tween 80 * Tween 80	1	460	460	0.49	0.490	
Two-Factor Interaction	3	23912	7971	8.44	0.000	
PCL * EO	1	408	408	0.43	0.515	
PCL * Tween 80	1	15480	15480	16.39	0.000	
EO * Tween 80	1	8024	8024	8.50	0.006	
Error	33	31161	944			
Lack of Fit	27	25958	961	1.11	0.492	

Pure Error	6	5203	867	*	*
Total	44	337941	—	—	—

Table S3: Analysis of variance (ANOVA) for the fitted response surface model evaluating the effects of PCL, essential oil, and polysorbate concentration on the PDI. DF: degrees of freedom; Adj. SS: adjusted sum of squares; Adj. MS: adjusted mean square. Significant effects were determined at $p < 0.05$. The model was statistically significant, with no significant lack of fit.

Analysis of Variance (ANOVA) for PDI					
Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Model	11	0.060272	0.005479	2.76	0.012
Blocks	2	0.006010	0.003005	1.51	0.235
Linear	3	0.020818	0.006939	3.49	0.026
PCL	1	0.005441	0.005441	2.74	0.107
EO	1	0.014955	0.014955	7.53	0.010
Tween 80	1	0.000422	0.000422	0.21	0.648
Quadratic	3	0.027610	0.009203	4.63	0.008
PCL * PCL	1	0.012843	0.012843	6.47	0.016
EO * EO	1	0.000200	0.000200	0.10	0.753
Tween 80 * Tween 80	1	0.012454	0.012454	6.27	0.017
Two-Factor Interaction	3	0.005834	0.001945	0.98	0.414
PCL * EO	1	0.001076	0.001076	0.54	0.467
PCL * Tween 80	1	0.003827	0.003827	1.93	0.174
EO * Tween 80	1	0.000931	0.000931	0.47	0.498
Error	33	0.065540	0.001986		
Lack of Fit	27	0.059975	0.002221	2.40	0.139
Pure Error	6	0.005564	0.000927	*	*
Total	44	0.125812	—	—	—

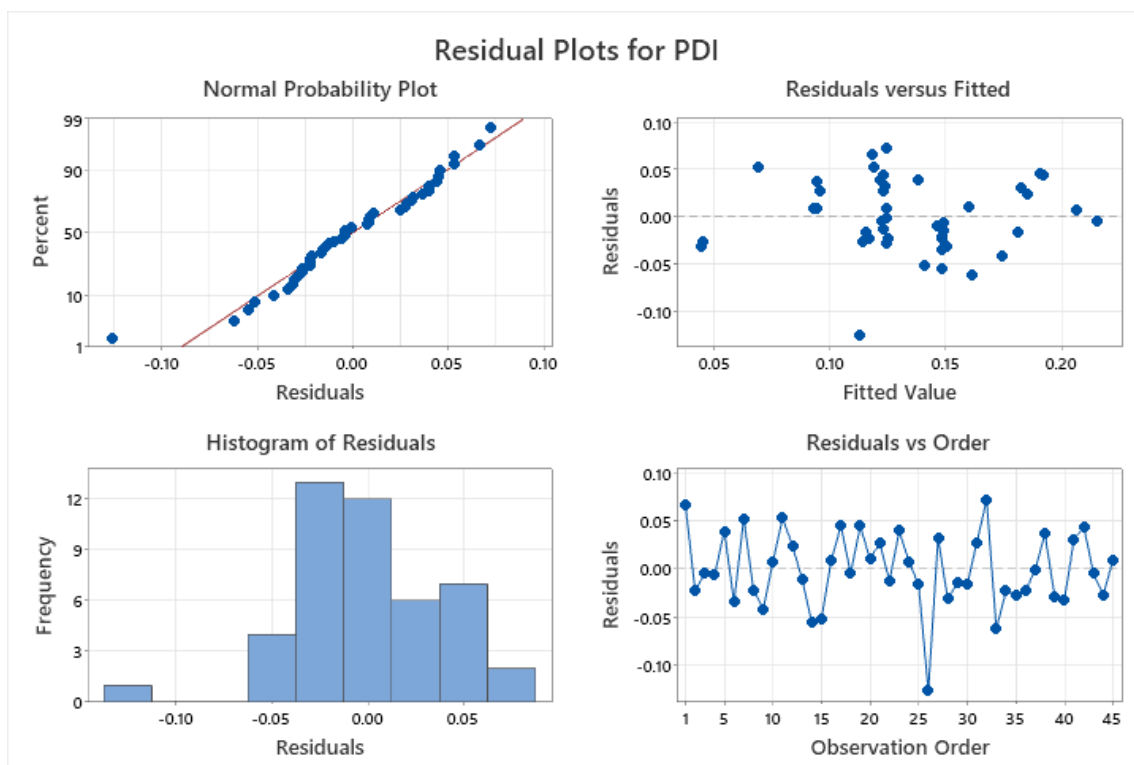


Figure S1: Residual PDI diagnostic plots for the fitted response surface models.

Table S4: Analysis of variance (ANOVA) for the fitted response surface model evaluating the effects of PCL, essential oil, and polysorbate concentration on the zeta potential. DF: degrees of freedom; Adj. SS: adjusted sum of squares; Adj. MS: adjusted mean square. Significant effects were determined at $p < 0.05$. The model was statistically significant, with no significant lack of fit.

Analysis of Variance (ANOVA) for Zeta Potential						
Source	DF	Adj. SS	Adj. MS	F-Value	P-Value	
Model	11	516.804	46.982	11.16	0.000	
Blocks	2	5.140	2.570	0.61	0.549	
Linear	3	153.717	51.239	12.17	0.000	
PCL	1	8.809	8.809	2.09	0.157	
EO	1	0.014	0.014	0.00	0.955	
Tween 80	1	144.894	144.894	34.43	0.000	
Quadratic	3	180.315	60.105	14.28	0.000	
PCL * PCL	1	173.533	173.533	41.23	0.000	
EO * EO	1	0.361	0.361	0.09	0.771	
Tween 80 * Tween 80	1	12.538	12.538	2.98	0.094	
Two - Factor Interaction	3	177.632	59.211	14.07	0.000	

PCL * EO	1	77.368	77.368	18.38	0.000
PCL * Tween 80	1	0.385	0.385	0.09	0.764
EO * Tween 80	1	99.879	99.879	23.73	0.000
Error	33	138.893	4.209		
Lack of Fit	27	129.730	4.805	3.15	0.078
Pure Error	6	9.162	1.527	*	*
Total	44	655.696	—	—	—

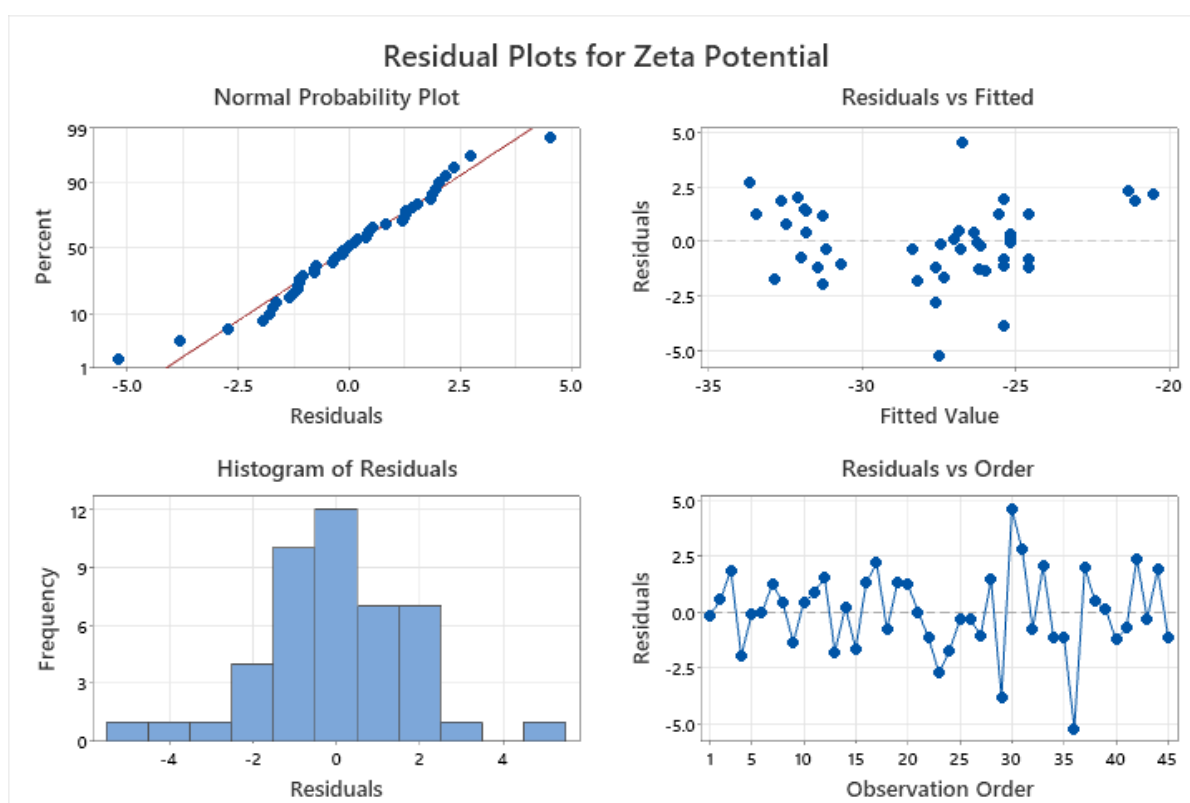


Figure S2: Residual zeta potential diagnostic plots for the fitted response surface models.

Morphology of nanocapsules

Experimental

Transmission electron microscopy (TEM) was performed to evaluate morphology and structural integrity of the nanocapsules prior to optimized formulation. Analyses were carried out using a ZEISS EM900 transmission electron microscope (Belém, PA, Brazil), equipped with a high-resolution MegaView 3G digital camera, operated at an acceleration voltage of 80 kV. Samples, both in their concentrated form and diluted at a 1:100 ratio in Milli-Q® water, were deposited onto carbon-coated copper grids and allowed to stand for 30 s. Non-adhered nanocapsules were removed using filter paper, and a drop of 2% phosphotungstic acid (pH 7.4) was applied as a negative stain for 30 s. Excess stain was removed with filter paper, and the grids were air-dried at room temperature before analysis, as described by Hedayati et al. [1].

This procedure was conducted in collaboration with the Evandro Chagas Institute (Belém, PA, Brazil).

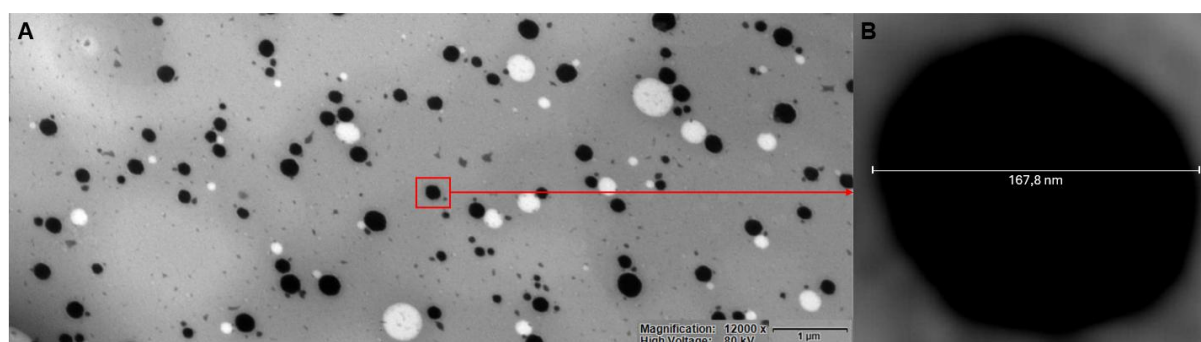


Figure S3: TEM images of NPNC obtained by nanoprecipitation. The image shows the overall particle distribution (magnification 12000 $\times$ and 80 kV) (A), with a higher magnification highlighting a single nanocapsule (B).

References

1. Hedayati, S.; Niakousari, M.; Mohsenpour, Z. *Int. J. Biol. Macromol.* **2020**, *143*, 136–142. doi:10.1016/j.ijbiomac.2019.12.003