Optimization, Thermo-Oxidative Stability, and Biological Activities of Vitamin E-Chitosan-TPP Nanoformulations

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Supplementary Materials

Table S1. Preliminary screening for nanoemulsion preparation factors

F	Oil (mL)	Chitosan (w/v%)	Aq Phase (mL)	TPP: Chitosan Ratio	Size (nm)	PDI	Charge (mV)
1	2	0.5	8	0	475	0.251	24.8
2	2.5	0.5	7.5	0	753	0.252	28.7
3	3	0.5	7	0	772	0.198	22.2
4	2	1	8	0	254	0.128	26
5	2.5	1	7.5	0	691	0.175	26.7
6	3	1	7	0	820	0.181	29.9
7	2	0.5	8	0.1	398	0.232	26.1
8	2.5	0.5	7.5	0.1	644	0.225	30.5
9	3	0.5	7	0.1	671	0.173	31.1
10	2	1	8	0.1	204	0.119	28.2
11	2.5	1	7.5	0.1	353	0.154	28.7
12	3	1	7	0.1	381	0.177	27.7

Table S2. Assessment of the Box-Behnken design results demonstrating the influence of the examined formulation variables

Responses	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	F-Value	P-Value	Significant Factors
Size (Y1)	0.9174	0.8211	0.5355	11.8423	8.00	0.0022	X_1, X_2, X_3
PDI (Y2)	0.6428	0.2260	-1.0273	4.9077	1.70	0.2150	X_1, X_2
Charge (Y3)	0.9840	0.9654	0.9081	20.8161	53.40	<0.0001	X_1, X_4

* All models are quadratic

Table S3. Coefficients table demonstrating the p-values for the estimated responses among various nanoformulations

	Intercept	X_1	X_2	X_3	X_4	X_1X_2	X_1X_3	X_1X_4	X_2X_3	X_2X_4	X_3X_4	X_1^2	X_2^2	X_3^2	X_4^2
size	796.68	**-1207.5**	**350.15**	-103.52	42.725	**-543.48**	130.48	-339.41	**689.71**	36.208	85.183	**1094.3**	75.327	154.68	101.11
p-values		**< 0.0001**	**0.0251**	0.4639	0.7602	**0.0407**	0.5921	0.1777	**0.0131**	0.8811	0.7255	**0.0002**	0.7200	0.4656	0.6312
PD	0.1855	-0.0009	0.0006	0.0419	0.0031	**0.2269**	-0.0323	0.0160	-0.0165	-0.0725	0.1284	0.0747	0.1164	-0.0379	0.0852
p-values		0.9826	0.9877	0.3426	0.9423	**0.0095**	0.6681	0.8306	0.8263	0.3434	0.1065	0.2635	0.0927	0.5624	0.2055
Charge	30.188	**25.650**	-0.125	0.775	0.3775	-1.2583	-1.3	**-4.7575**	-2.9083	0.975	2.7166	**-21.486**	0.2431	1.2515	1.6802
p-values		**< 0.0001**	0.9139	0.5064	0.7444	0.5329	0.5197	**0.0319**	0.1636	0.6278	0.1909	**< 0.0001**	0.8884	0.4751	0.3417

*p-value: (Bold) **p < 0.05,** (Normal) $0.05 \leq p < 0.1$

Table S4. Scavenging DPPH radical by various tocopherol formulations (results are the mean of triplicate ±SD)

Conc. (µM)	% Inhibition (± SD)			
	α-TQ	α-TQA	α-TQ/CS-TPP/NPs	α-TQA/CS-TPP/NPs
3.68	25.78 (± 0.265)	0.234 (± 0.116)		
14.71	40.22 (± 0.347)	0.234 (± 0.116)	77.55 (± 0.174)	0.455 (± 0.228)
22.06	69.21 (± 0.265)	0.334 (± 0.352)	83.43 (± 0.237)	0.531 (± 0.348)
36.77	81.95 (± 1.906)	0.502 (± 0.301)	86.04 (± 0.431)	0.607 (± 0.525)
73.63	87.56 (± 0.174)	0.334 (±0.153)	88.21 (± 0.174)	0.683 (± 0.521)
110.3	87.06 (± 0.174)	0.535 (± 0.058)	90.82 (± 0.066)	0.872 (± 0.066)
515.43		0.502 (± 0.201)		0.872 (± 0.174)

Table S5. Scavenging hydroxyl radical by various tocopherol formulations (results are the mean of triplicate ±SD)

Conc. (mM)	% Inhibition (± SD)			
	α-TQ	α-TQA	α-TQ/CS-TPP/NPs	α-TQA/CS-TPP/NPs
0.529	11.29 (± 0.894)	0.00 (± 0.256)	24.52 (± 0.054)	0.38 (± 0.094)
0.882	14.30 (± 0.148)	0.52 (± 0.148)	38.02 (± 0.143)	1.09 (± 0.610)
1.235	36.01 (± 0.194)	0.58 (± 0.194)	49.98 (± 0.286)	1.19 (± 0.443)
1.941	50.50 (± 0.244)	0.61 (± 0.279)	68.67 (± 0.379)	3.28 (± 1.543)
2.647	60.76 (± 0.979)	1.00 (± 0.868)	83.97 (± 0.162)	4.47 (± 0.603)

Table S6. Reducing power of various tocopherol formulations (results are the mean of triplicate ±SD)

Conc. (µM)	Absorbance (± SD)			
	α-TQ	**α-TQA**	**α-TQ/CS-TPP/NPs**	**α-TQA/CS-TPP/NPs**
3.836	0.099 (± 0.001)	0.004 (± 0.002)	0.144 (± 0.006)	0.009 (± 0.003)
9.59	0.168 (± 0.001)	0.003 (± 0.002)	0.542 (± 0.001)	0.0137 (± 0.002)
38.359	0.623 (± 0.021)	0.005 (± 0.002)	0.705 (± 0.008)	0.022 (± 0.001)
76.719	0.771 (± 0.005)	0.011 (± 0.005)	0.836 (± 0.008)	0.037 (± 0.003)
172.617	0.905 (± 0.021)	0.027 (± 0.002)	1.679 (± 0.106)	0.051 (± 0.002)

Table S7. Oxidative stability of various tocopherol formulations against H_2O_2 (results are the mean of triplicate ±SD)

Time(h)	Remaining Percentage (± SD)			
	α-TQ	**α-TQA**	**α-TQ/CS-TPP/NPs**	**α-TQA/CS-TPP/NPs**
Zero	100 (± 0.400)	100 (± 0.724)	100 (± 0.423)	100 (± 3.673)
24	90.73 (± 0.646)	98.65 (± 1.016)		
48	82.72 (± 1.042)	92.94 (± 0.982)		
72	69.53 (± 0.704)	86.86 (± 0.450)		
96	68.62 (± 0.500)	86.86 (± 0.450)	99.96 (± 0.573)	99.84 (± 2.301)

Table S8. Oxidative stability of various tocopherol formulations against HOCl (results are the mean of triplicate ±SD)

Time (min)	Remaining Percentage (± SD)			
	α-TQ	**α-TQA**	**α-TQ/CS-TPP/NPs**	**α-TQA/CS-TPP/NPs**
Zero	100 (± 0.075)	100 (± 0.598)	100 (± 0.00)	100 (± 3.017)
5	37.55 (± 1.091)	89.91 (± 0.470)		
15	22.29 (± 0.418)	77.79 (± 0.470)		
5760	22.22 (± 0.437)	26.44 (± 0.690)	99.79 (± 0.376)	100.91 (± 0.346)

Table S9. % of MDA formation of heated oils (5 h), relative to the unheated sample, with different tocopherol formulations (results are the mean of triplicate ±SD)

Time (h)	Formation Percentage of MDA (± SD)			
	Oil+α-TQ	**Oil+α-TQA**	**Oil+α-TQ/CS-TPP/NE**	**Oil+α-TQA/CS-TPP/NE**
1	10.06 (± 1.040)	71.21 (± 0.631)	6.61 (± 0.826)	51.24 (± 0.826)
2	18.32 (± 0.509)	78.8 (± 0.530)	8.65 (± 1.018)	68.45 (± 0.881)
3	27.6 (± 0.107)	83.36 (± 1.029)	9.12 (± 0.565)	71.975 (± 0.770)
4	37.47 (± 0.644)	84.1 (± 0.921)	12.08 (± 0.670)	72.02 (± 0.322)
5	47.89 (± 0.294)	91.58 (± 0.504)	14.67 (± 0.922)	73.25 (± 0.922)

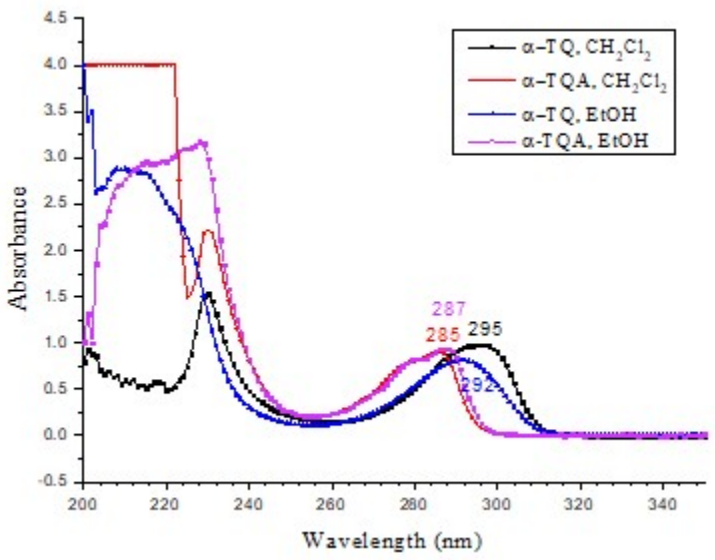

Fig. S1. UV-Vis spectra of α-TQ, and α-TQA in CH_2Cl_2, and EtOH

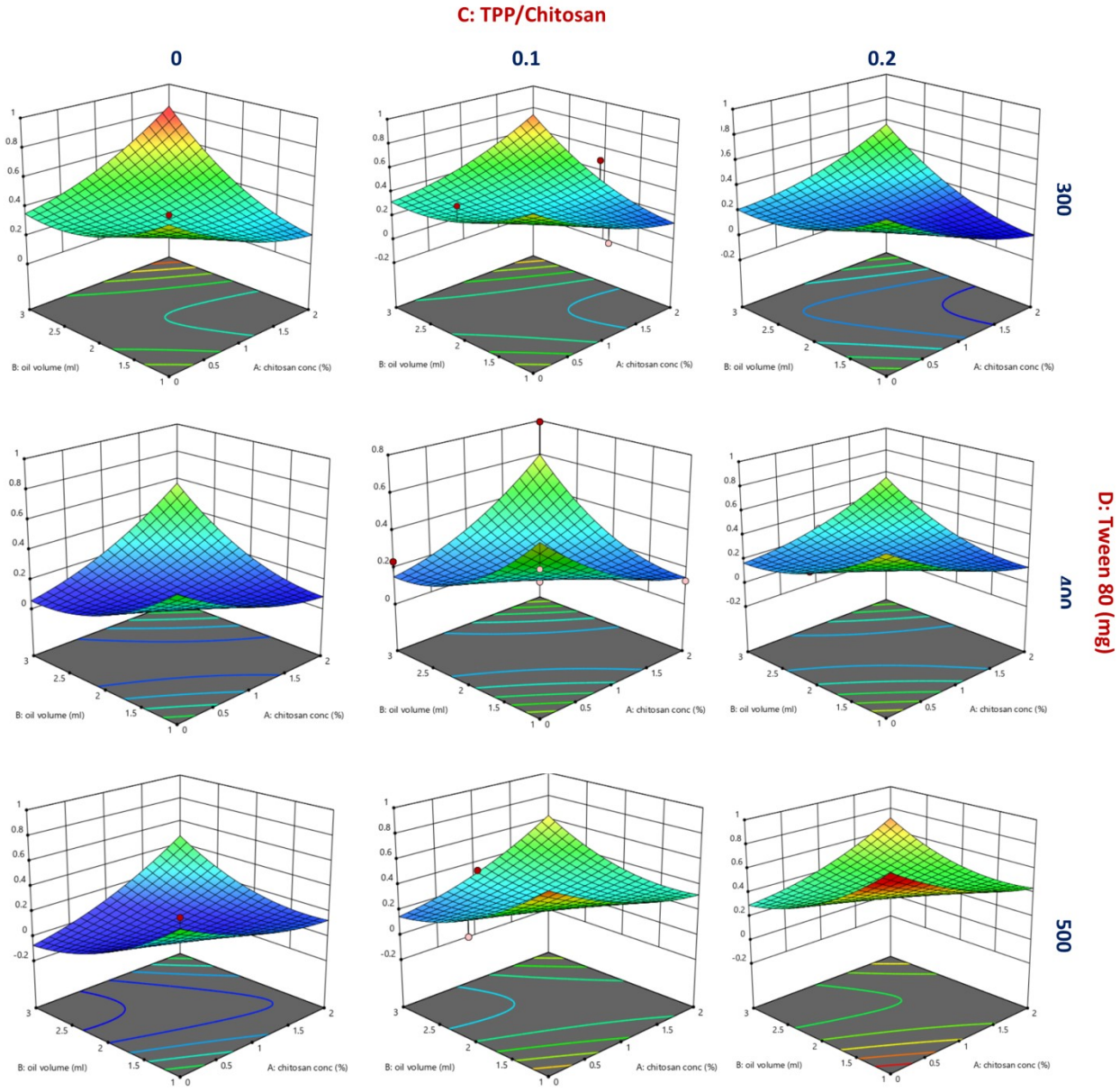

Fig. S2. RSM of the effect of CS concentration (A) and oil volume (B) on PDI (Y2) under different conditions of TPP:Chitosan ratio (C) and Tween 80 (D)

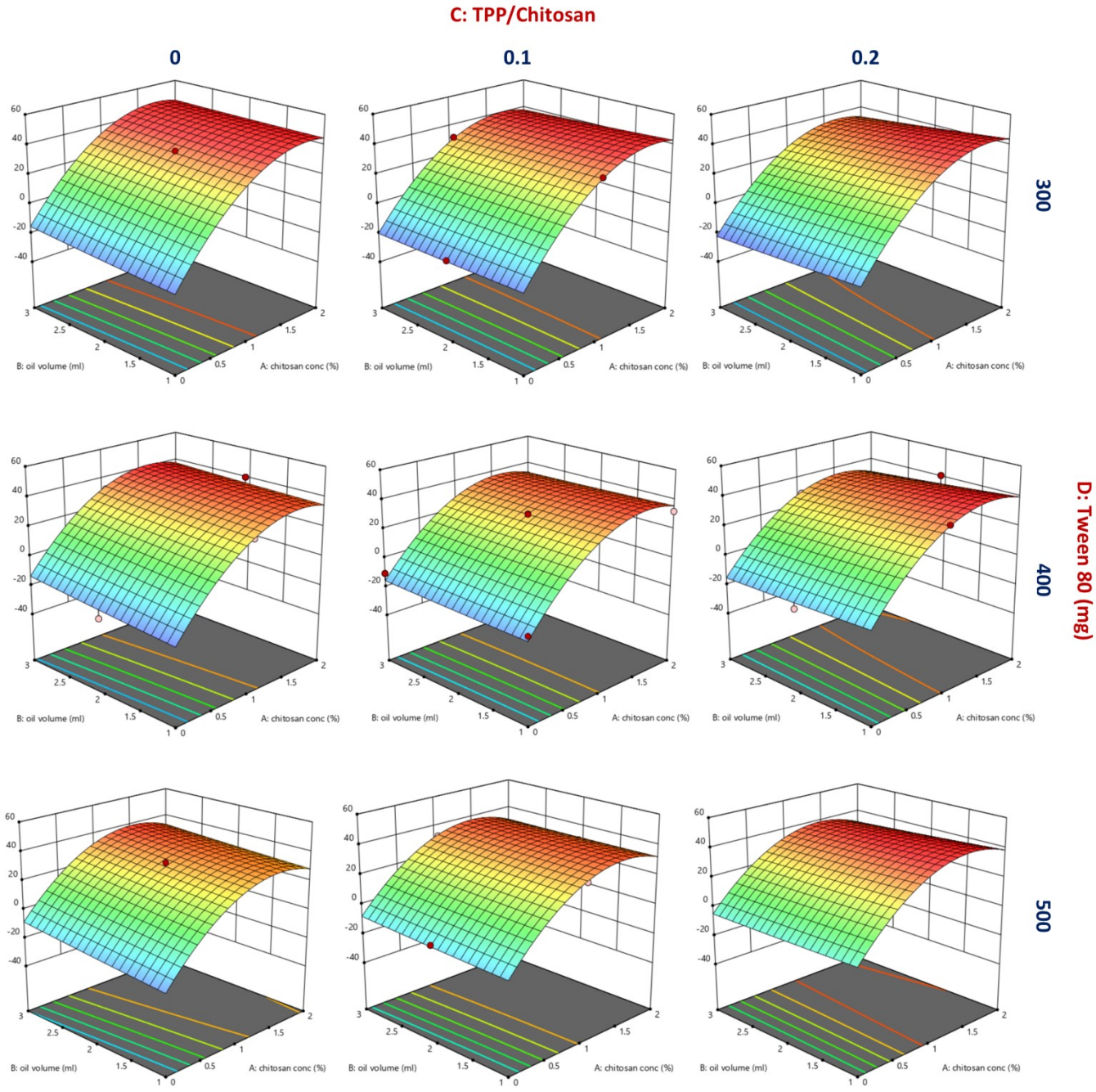

Fig. S3. RSM of the effect of Chitosan concentration (A) and Oil volume (B) on Surface Charge (Y3) under different conditions of TPP:Chitosan ratio (C) and Tween 80 (D)

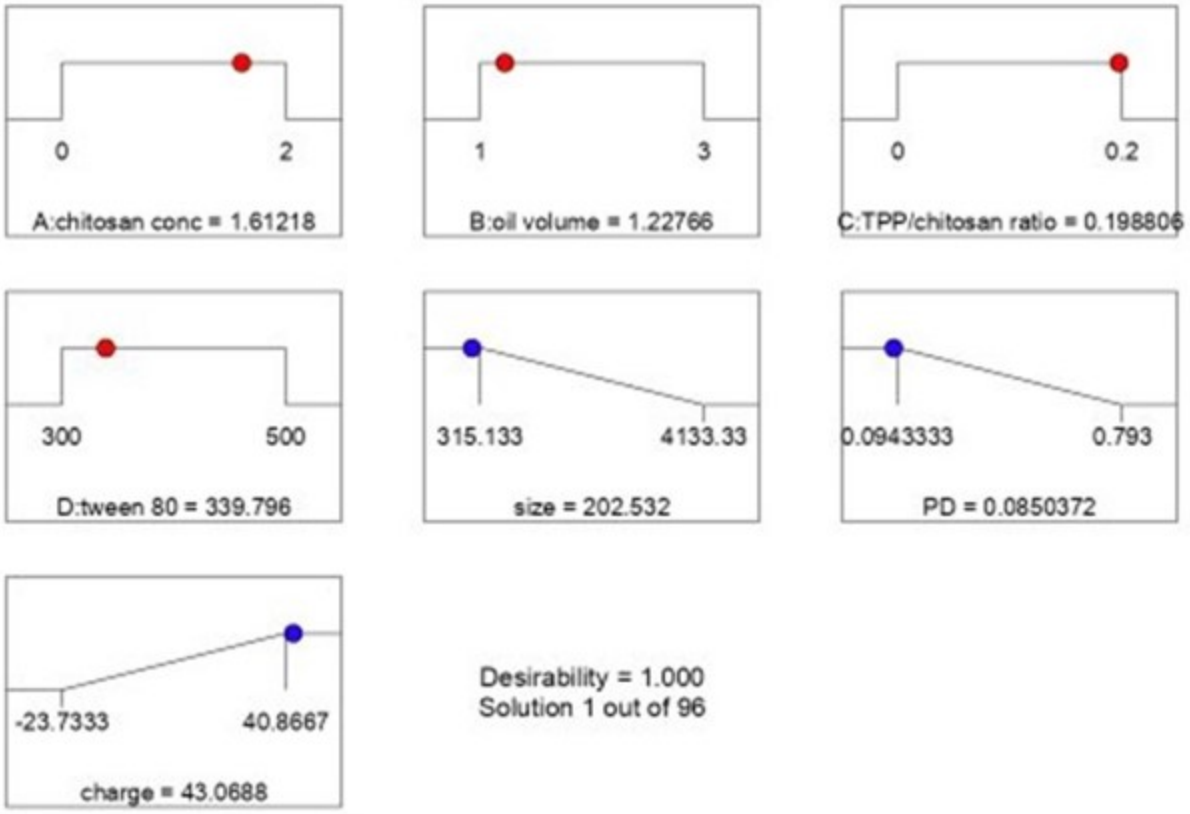

Fig. S4. Design-Expert optimization showing target constraints, optimized factors, and predicted responses for α-TQ/CS-TPP/NE**.** The desirability was adjusted to minimize both particle size (Y1) and PDI (Y2), while maximizing the zeta potential (Y3)**.** The optimized factors were: (A) **c**hitosan solution concentration = 1.612% w/v**,** (B) Oil volume = 1.227 mL, (C) TPP: Chitosan mass ratio = 0.198, and (D) Tween-80 amount = 339.76 mg**.** The model outcome predicts the optimized formulation with an overall desirability = 1.000, where the predicted size = 202.532 nm**,** PDI = 0.085**,** and Zeta potential = +43.068 mV**.** Red and blue circles indicate the chosen factors and predicted responses, respectively, within the allowable ranges.