

Supplementary Material

Polysaccharide-Based Multilayer Nano-Emulsions Loaded with Oregano Oil: Production, Characterization, and In Vitro Digestion Assessment

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Table S1. Process parameters and their corresponding levels for the preparation of NE I, II and III.

Nanoemulsion	Process Parameters and Formulation	Levels
NE I	OSA-MS concentration	7.5 mg/mL
	LMW chitosan concentration	0.12, 0.16, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mg/mL
NE II	MMW chitosan concentration	0.12, 0.16, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mg/mL
	Ultra turrax-ultrasound	With and without
NE III	Na-CMC concentration	0.2, 0.4, 0.8, 1.2, 1.6, 2.0 mg/mL

Table S2. Particle size (PS) of NE II for increasing concentration of low and medium molecular weight chitosan (LMW and MMW, respectively).

Chitosan concentration (mg/mL)	PS (nm)*		
	LMW Chitosan	MMW Chitosan	<i>p</i> -value
0.12	205.86 ± 0.48	226.90 ± 50.57	4.05 × 10 ⁻¹
0.16	205.68 ± 11.28	229.02 ± 60.73	5.90 × 10 ⁻¹
0.20	201.01 ± 37.64	230.00 ± 5.58	1.41 × 10 ⁻³
0.40	251.35 ± 47.77	232.24 ± 52.05	3.35 × 10 ⁻²
0.80	262.74 ± 33.58	218.28 ± 44.68	2.03 × 10 ⁻¹
1.20	206.75 ± 69.62	347.82 ± 91.65	3.52 × 10 ⁻¹
1.60	373.80 ± 24.21	189.56 ± 26.88	8.79 × 10 ⁻⁴
2.00	227.98 ± 11.39	392.30 ± 125.33	1.52 × 10 ⁻¹
3.00	292.70 ± 59.58	226.42 ± 64.93	5.19 × 10 ⁻³
4.00	218.43 ± 180.99	399.12 ± 134.76	6.82 × 10 ⁻¹
5.00	379.90 ± 57.57	195.36 ± 107.33	6.50 × 10 ⁻³
6.00	335.20 ± 57.25	224.16 ± 162.17	7.26 × 10 ⁻²
7.00	335.20 ± 34.76	187.42 ± 73.15	3.79 × 10 ⁻³
<i>p</i> -value	3.51 × 10 ⁻¹¹	1.01 × 10 ⁻⁹	

* The values are presented as the average of five true replicates ± standard deviation.

Table S3. Effect of Na-CMC concentration on the particle size (PS), polydispersity index (PDI) and zeta potential (ZP) of NE III.

Na-CMC Concentration (mg/mL)	PS (nm)*	PDI*	ZP (mV)*
0.2	246.68 ± 81.20	0.495 ± 0.068	21.64 ± 2.72
0.4	221.68 ± 13.50	0.294 ± 0.063	18.12 ± 2.84
0.8	268.80 ± 63.17	0.405 ± 0.052	13.10 ± 1.65
1.2	258.52 ± 40.45	0.640 ± 0.037	9.90 ± 0.38
1.6	345.02 ± 72.43	0.627 ± 0.097	2.50 ± 2.08
2.0	306.08 ± 92.66	0.769 ± 0.033	-6.40 ± 2.42
<i>p</i> -value	4.89 × 10 ⁻²	7.86 × 10 ⁻¹⁰	2.36 × 10 ⁻¹³

* The values are presented as the average of five true replicates ± standard deviation.

Table S4. Particle size (PS), polydispersity index (PDI) and zeta potential (ZP) of loaded NE III during time at 4 and 20 °C.

Time (days)	PS (nm)*		PDI*		ZP (mV)*	
	20 °C	4 °C	20 °C	4 °C	20 °C	4 °C
1	324.21 ± 0.97	324.23 ± 0.86	0.64 ± 0.09	0.64 ± 0.14	−0.95 ± 2.68	−0.96 ± 3.14
3	529.78 ± 5.52	513.94 ± 3.56	0.73 ± 0.14	0.71 ± 0.10	3.88 ± 3.01	3.61 ± 2.21
5	735.64 ± 26.88	703.77 ± 10.24	0.87 ± 0.16	0.80 ± 0.13	8.21 ± 5.22	8.14 ± 2.73
7	870.16 ± 44.67	754.22 ± 22.30	>1	>1	10.74 ± 1.07	12.27 ± 4.31
14	>1000	>1000	>1	>1	1.23 ± 2.51	5.09 ± 4.76
21	>1000	>1000	>1	>1	−11.61 ± 1.33	−15.31 ± 1.15

* The values are presented as the average of five samples ± standard deviation. .