

Multivariate Chemometric Design of Nitric Oxide-Releasing Chitosan Nanoparticles for Skin-Related Biomedical Applications

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Table S 1 - Experiments performed in the Plackett-Burman Design.

Experiment	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
1	2.1	1	650	6	1.5	25	1.5	50	1	-1	1
2	2.1	5	350	9	1.5	25	0.5	50	1	1	-1
3	0.5	5	650	6	5	25	0.5	0	1	1	1
4	2.1	1	650	9	1.5	100	0.5	0	-1	1	1
5	2.1	5	350	9	5	25	1.5	0	-1	-1	1
6	2.1	5	650	6	5	100	0.5	50	-1	-1	-1
7	0.5	5	650	9	1.5	100	1.5	0	1	-1	-1
8	0.5	1	650	9	5	25	1.5	50	-1	1	-1
9	0.5	1	350	9	5	100	0.5	50	1	-1	1
10	2.1	1	350	6	5	100	1.5	0	1	1	-1
11	0.5	5	350	6	1.5	100	1.5	50	-1	1	1
12	0.5	1	350	6	1.5	25	0.5	0	-1	-1	-1
13	1.3	3	500	7.5	3.25	62.5	1	25	0	0	0
14	1.3	3	500	7.5	3.25	62.5	1	25	0	0	0
15	1.3	3	500	7.5	3.25	62.5	1	25	0	0	0

Factor	Unit
X ₁ Initial chitosan concentration	mg/mL
X ₂ TPP solution concentration	mg/mL
X ₃ TPP solution addition rate	mL/min
X ₄ pH of TPP solution	-
X ₅ CS:TPP mass ratio	g _{Ch} /g _{TPP}
X ₆ Final GSH concentration	mmol/L
X ₇ Initial acetic acid concentration	% m/V
X ₈ Additional ionic strength	mM
X ₉ , X ₁₀ , and X ₁₁ are inert factors (dummy variables)	

Table S 2 - Response matrix of the Plackett-Burman Design.

Experiment	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇	Y ₈	Y ₉
1	179.3	0.244	19.1	1.1	1.1	1.0	1.0	1.1	1.0
2	287.1	0.459	12.5	2.4	1.5	1.0	3.5	1.5	1.1
3	189.2	0.929	38.9	0.9	1.1	1.0	1.4	1.0	0.9
4	167.9	0.243	14.8	1.2	1.2	1.1	1.3	1.3	1.0
5	240.8	0.547	39.3	1.0	1.7	1.0	1.2	1.8	0.9
6	334.6	0.465	15.6	1.0	1.0	1.4	1.0	0.9	1.6
7	99.95	0.225	16.8	1.3	1.2	1.1	1.1	0.9	1.1
8	146	0.512	29.4	1.0	0.8	1.1	1.1	0.8	0.9
9	145.1	0.821	26.8	0.9	0.4	0.8	1.3	0.8	0.9
10	250.9	0.982	31.1	0.7	0.6	1.0	0.8	0.5	1.1
11	146.6	0.167	18.3	1.1	1.3	0.9	1.1	1.1	0.8
12	116.8	0.449	18.2	1.4	1.0	1.0	1.5	0.8	0.7
13	169.2	0.403	25.4	1.0	1.0	1.1	1.0	1.0	1.2
14	175.1	0.359	26.7	1.0	0.9	1.0	1.0	1.0	1.1
15	172.7	0.342	27.5	1.0	1.0	0.8	1.0	1.0	1.1

Y₁: average diameter immediately post-synthesis; Y₂: PDI immediately post-synthesis, Y₃: ZP immediately post-synthesis, Y₄: normalized average diameter 7 days post-synthesis at room temperature; Y₅: normalized PDI 7 days post-synthesis at room temperature; Y₆: normalized ZP 7 days post-synthesis at room temperature; Y₇: normalized average diameter 7 days post-synthesis at 4 °C; Y₈: normalized PDI 7 days post-synthesis at 4 °C; Y₉: normalized ZP 7 days post-synthesis at 4 °C.

Table S 3 - Experiments performed in the Face-Centered Design.

Experiment	Final GSH concentration (mmol/L)	CS:TPP mass ratio (g _{CH} /g _{TPP})
1	20	1.5
2	40	1.5
3	20	3
4	40	3
5	20	2.25
6	40	2.25
7	30	1.5
8	30	3
9	30	2.25
10	30	2.25
11	30	2.25

Table S 4 - Response matrix of the Face-Centered Design.

Experiment	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇	Y ₈	Y ₉
1	125.4	0.321	9.63	1.2	0.9	1.1	1.3	1.0	1.0
2	157.1	0.843	29.9	0.9	0.9	1.0	1.1	0.9	1.0
3	107.7	0.233	11.3	1.1	0.9	1.1	1.1	1.0	1.0
4	125.3	0.713	27.4	1.0	0.9	1.0	1.1	0.9	0.9
5	112.7	0.324	11.9	1.2	0.9	1.0	1.3	1.0	0.9
6	111.9	0.537	29.3	1.0	0.8	1.0	1.1	0.8	0.9
7	131.5	0.718	19.4	1.0	0.8	1.0	1.0	0.9	1.0
8	105.7	0.36	23.5	1.0	0.8	1.0	1.0	0.9	0.9
9	95.64	0.309	19.2	1.0	1.0	1.0	1.1	1.0	1.1
10	105.2	0.389	16.5	0.9	0.9	0.9	1.0	0.9	1.0
11	96.3	0.298	17.7	1.0	0.9	1.1	1.0	0.9	1.2

Y₁: average diameter immediately post-synthesis; Y₂: PDI immediately post-synthesis, Y₃: ZP immediately post-synthesis, Y₄: normalized average diameter 7 days post-synthesis at room temperature; Y₅: normalized PDI 7 days post-synthesis at room temperature; Y₆: normalized ZP 7 days post-synthesis at room temperature; Y₇: normalized average diameter 7 days post-synthesis at 4 °C; Y₈: normalized PDI 7 days post-synthesis at 4 °C; Y₉: normalized ZP 7 days post-synthesis at 4 °C.

Figure S 1 - Normalized variations in the average diameter, PDI, and ZP of GSH-CSNPs produced in the PB Design over time at room temperature and under refrigeration.

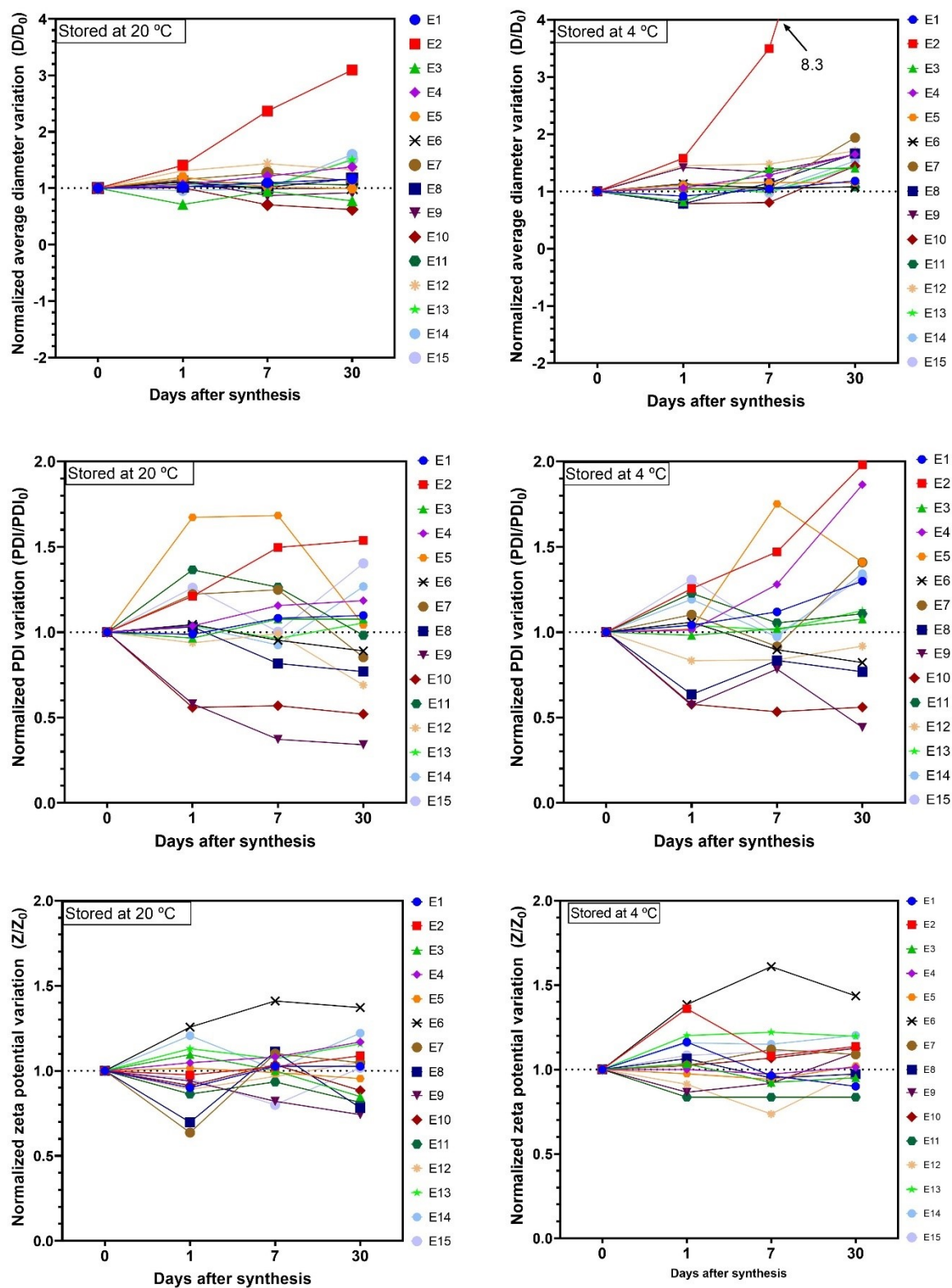


Table S 5 - Model terms and statistical indicators for the FCD design, adjusted for initial parameters of GSH-CSNPs measured immediately post-synthesis.

Model parameter*	Initial Average Diameter		Initial PDI		Initial ZP	
	<i>Coefficient</i>	<i>p-value</i>	<i>Coefficient</i>	<i>p-value</i>	<i>Coefficient</i>	<i>p-value</i>
Intercept	99.7	<0.01	0.371	<0.01	19.6	<0.01
x₁	8.1	0.0368	0.202	0.0014	9.0	<0.01
x₂	-12.5	0.0060	-0.096	0.0454	-	-
x₁ · x₂	-	-	-	-	-	-
x₁²	11.7	0.0457	-	-	-	-
x₂²	18.0	0.0083	0.160	0.0293	-	-
R²	0.90	-	0.85	-	0.93	-
R²_{adj}	0.83	-	0.78	-	0.92	-
Std. Dev.	7.4	-	0.0967	-	2.0	-

* **x₁** = CS:TPP mass ratio; **x₂** = Final GSH concentration.