Appendix A. TEM Micrographs of Ch-AO-NPs

A1. Additional TEM images of optimised Ch-AO-NPs

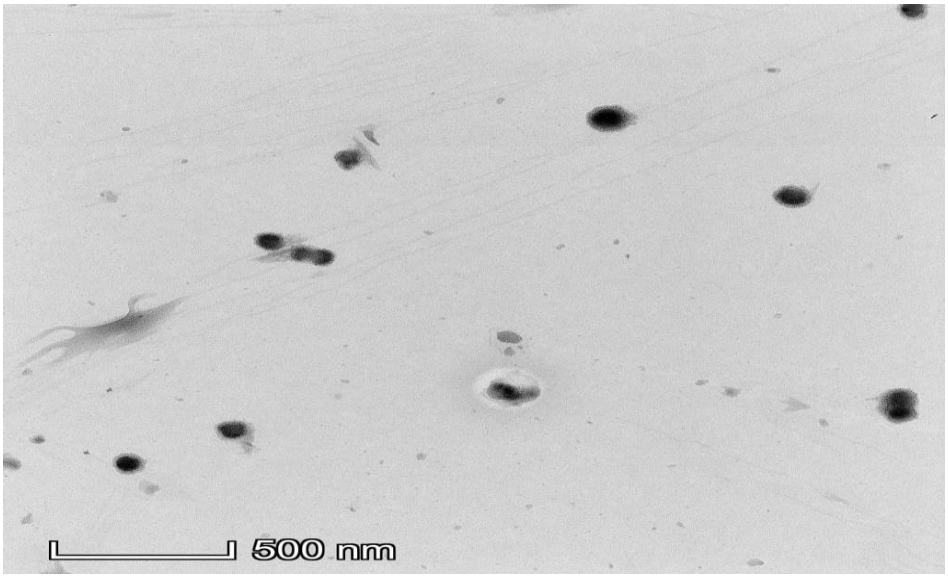

Figure A1. Additional TEM micrograph of Ch-AO-NPs at lower magnification. Scale bar = 500 nm.

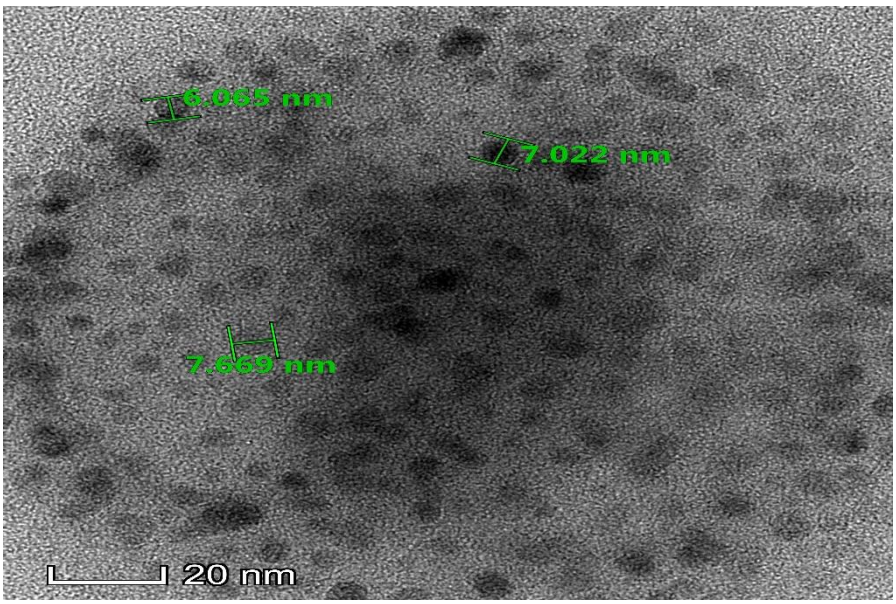

Figure A2. Additional TEM micrograph of Ch-AO-NPs at higher magnification showing internal nanostructural features. Scale bar = 20 nm.

Appendix B. In-vitro drug release profile of Ch-AO-NPs

B1. Full release curves at pH 4.8 and pH 8.5

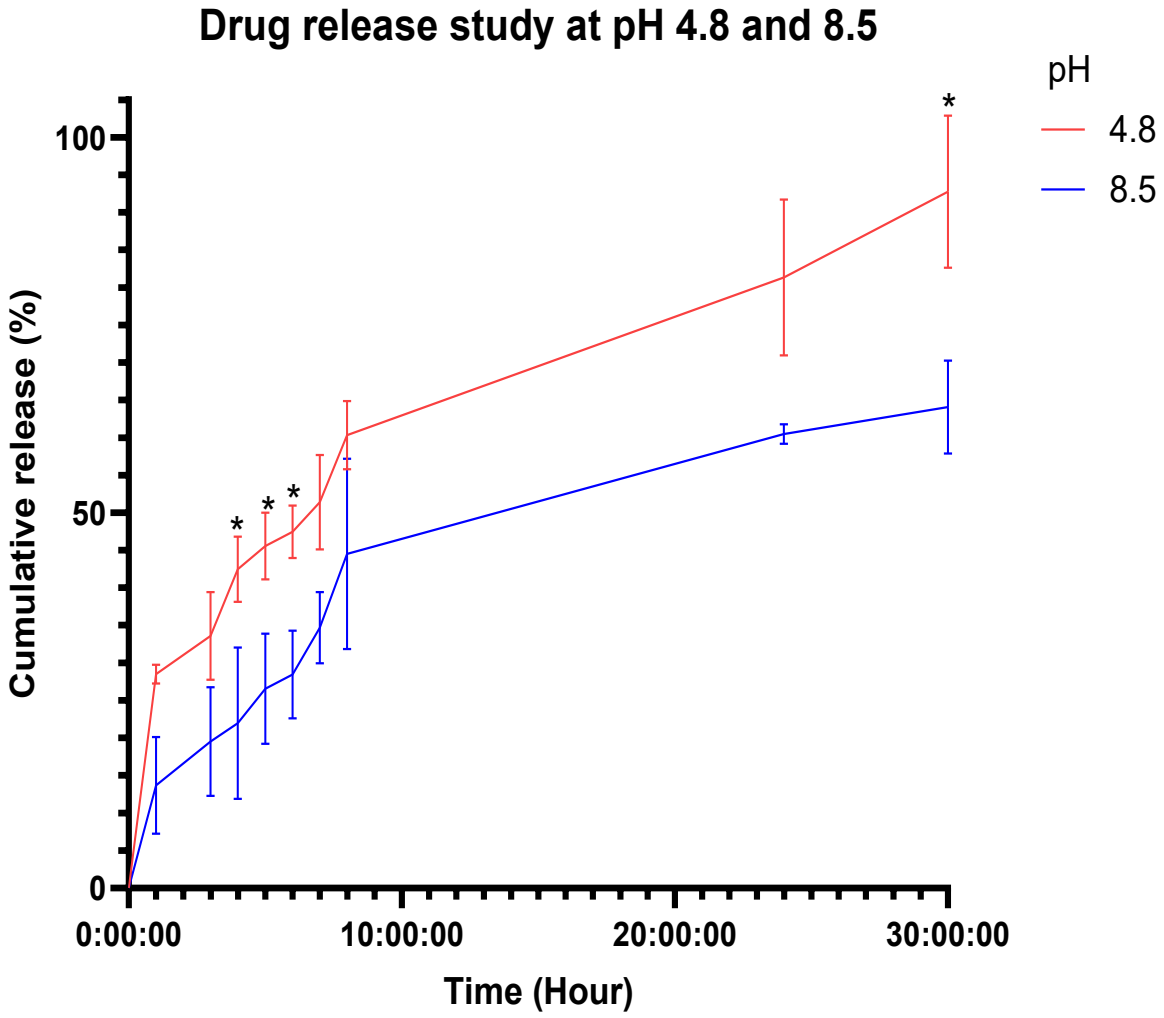

Figure B1. Drug release study of Ch-AO-NPs at pH 4.8 and 8.5. Data are mean ± SD (n = 3). Asterisks indicate significant differences between pH conditions at the corresponding time points ($p < 0.05$ at 4 h, 5 h, 6 h, and 30 h).

Statistical analysis was performed using a paired t-test comparing pH 4.8 vs pH 8.5 at each time point ($p < 0.05$).

Appendix C. Short-term stability data of Ch-AO-NPs

All stability data reflect mean ± SD of triplicate measurements. No statistical comparisons were performed, as stability assessments were descriptive in nature

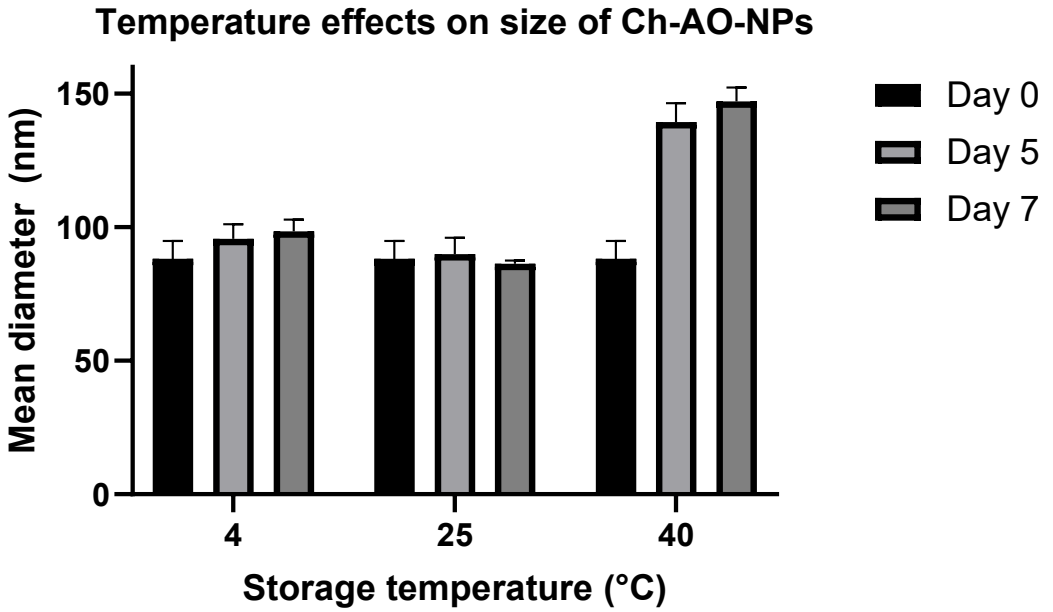

Figure C1. The effect of temperature on the mean diameter of Ch-AO-NPs over 7 days.

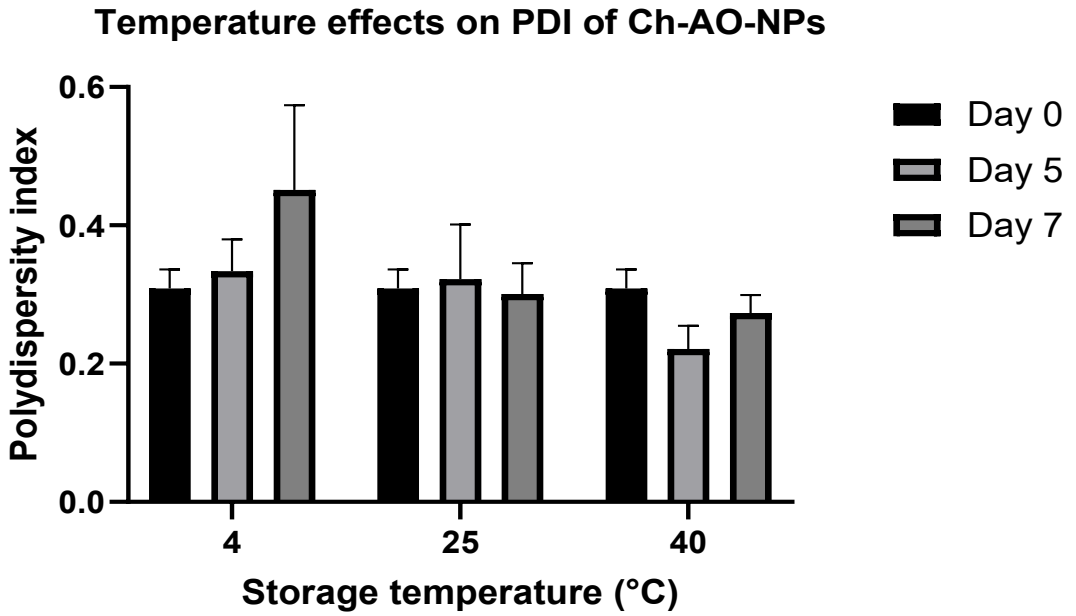

Figure C2. The effect of temperature on the PDI of Ch-AO-NPs over 7 days.

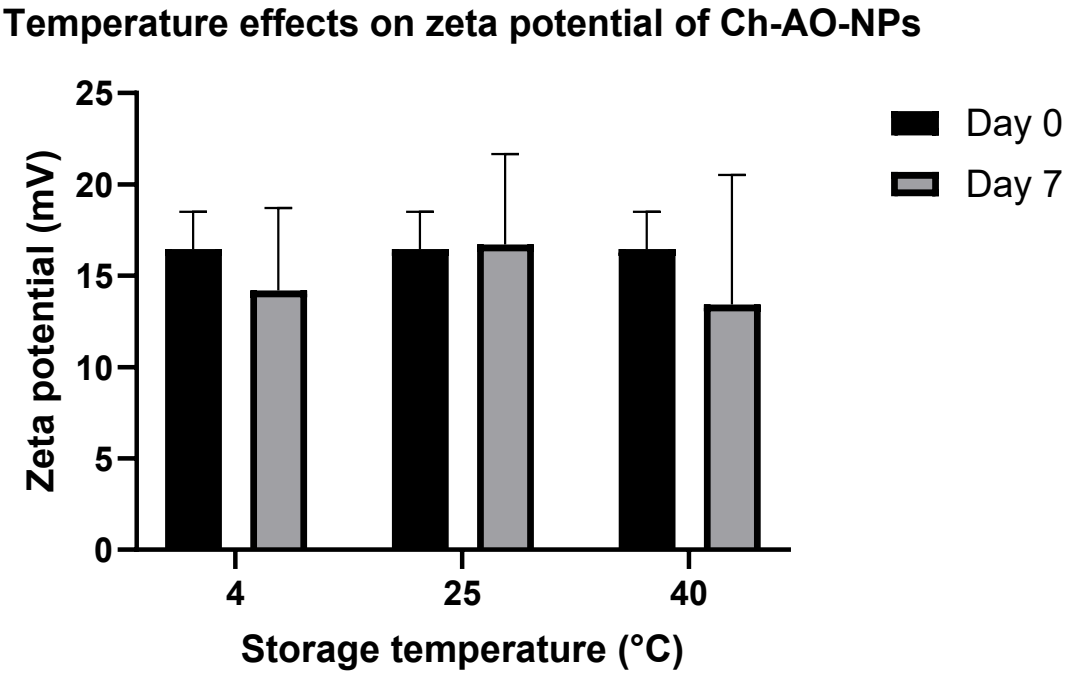

Figure C3. The effect of temperature on the zeta potential of Ch-AO-NPs between day 0 and day 7.

Appendix D. Formulation screening and optimisation data

Table D1. Formulation parameters of Ch-AO-NPs (n = 39).

Formula	Mass ratio of CS: TPP	Wt Tween 80 (% w/v)	Volume ratio of CS: AO (mL)	Stirring rate (rpm)	Stirring time (hour)
F1	2:1	0.67	1:0.01	500	1
F2	4:1	0.67	1:0.01	500	1
F3	5:1	0.67	1:0.01	500	1
F4	6:1	0.67	1:0.01	500	1
F5	5:1	0.56	1:0.01	500	1
F6	5:1	0.78	1:0.01	500	1
F7	5:1	0.67	1:0.03	500	1
F8	5:1	0.67	1:0.05	500	1
F9	5:1	0.67	1:0.01	300	1
F10/Z3	5:1	0.67	1:0.01	700	1
F11	5:1	0.67	1:0.01	700	2
Z1	5:1	-	-	700	1
Z2	5:1	0.67	-	700	1

Table D2. Particle size and PDI of Ch-AO-NP formulations, n=33.

Formula	Mean diameter (nm) ± SD	PDI ± SD
F1	287.2 ± 75.55	0.28 ± 0.01
F2	181.04 ± 21.35	0.26 ± 0.01
F3	154.86 ± 6.76[A]	0.28 ± 0.02
F4	210.16 ± 24.16	0.28 ± 0.01
F5	226.17 ± 33.75	0.24 ± 0.02
F6	184.51 ± 8.41	0.26
F7	414.13 ± 99.36	0.27 ± 0.03
F8	1135.37 ± 236.69	0.29 ± 0.04
F9	2425 ± 402.3[B]	0.32 ± 0.02[C]
*F10	90.75 ± 0.85	0.27 ± 0.01
F11	78.6 ± 5.19	0.26 ± 0.01

Footnotes: *Represents the optimised formulation.
A: p <0.05, significant against F1, F5, and F8.
B and C: p <0.05, significant against F3 and F10.

Table D3. Zeta potential of Ch-AO-NPs and Blank Nanoparticles, n=9.

Formula	AO	Tween 80	Mean diameter (nm) ± SD	PDI ± SD	Zeta potential (mV) ± SD	EE (%) ± SD	LC (%) ± SD	Yield (%) ± SD
Z1	x	x	564.33 ± 90.99	0.26 ± 0.03	+27.37 ± 0.45	-	-	-
Z2	x	✓	554.5 ± 32.24	0.26 ± 0.01	+24.87 ± 1.97	-	-	-
*Z3/ F10	✓	✓	90.75 ± 0.85[A]	0.27± 0.01	+17.59 ± 1.33[B]	80.23 ± 0.87	24.84 ± 1.85	15.49 ± 0.66

Footnotes: *Represents the optimised formulation.
A and B: p <0.05 is significant against Z1 and Z2 formulations.