

Supplementary data

**Development of Minoxidil-Loaded Calcium Citrate  
Nanoparticles**

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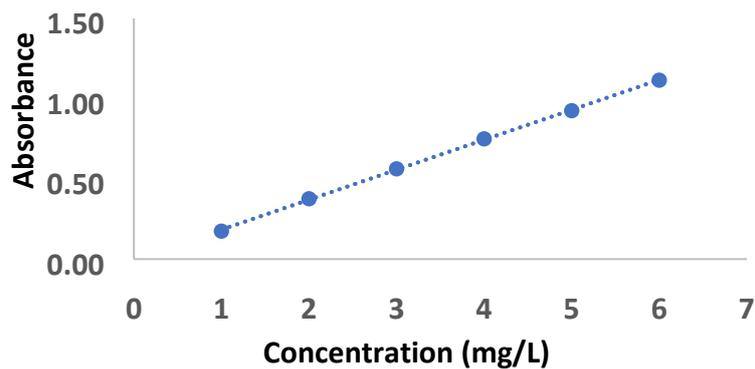


Figure S1. Calibration curve of Minoxidil in H<sub>2</sub>O/EtOH

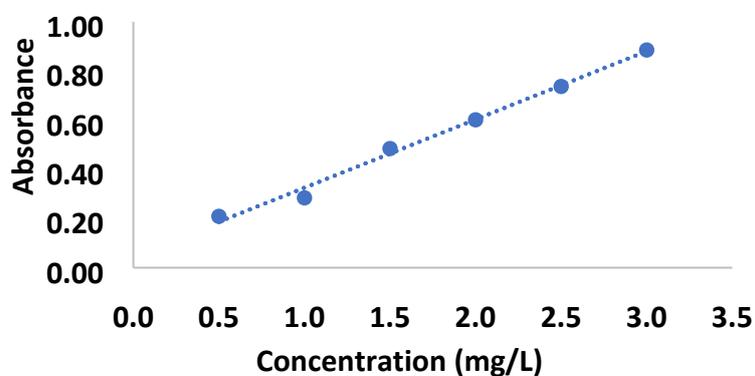


Figure S2. Calibration curve of Minoxidil in PBS pH 5.6/EtOH

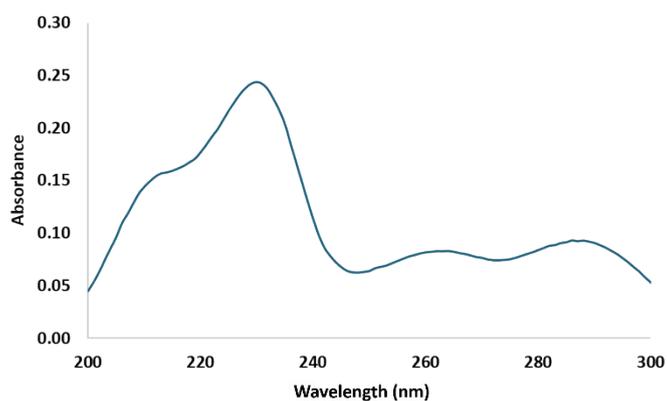


Figure S3. The UV-Vis spectrum graphs of Minoxidil for the drug loading and encapsulation efficiency assessment.

(Blank: Unloaded CaCit NPs)

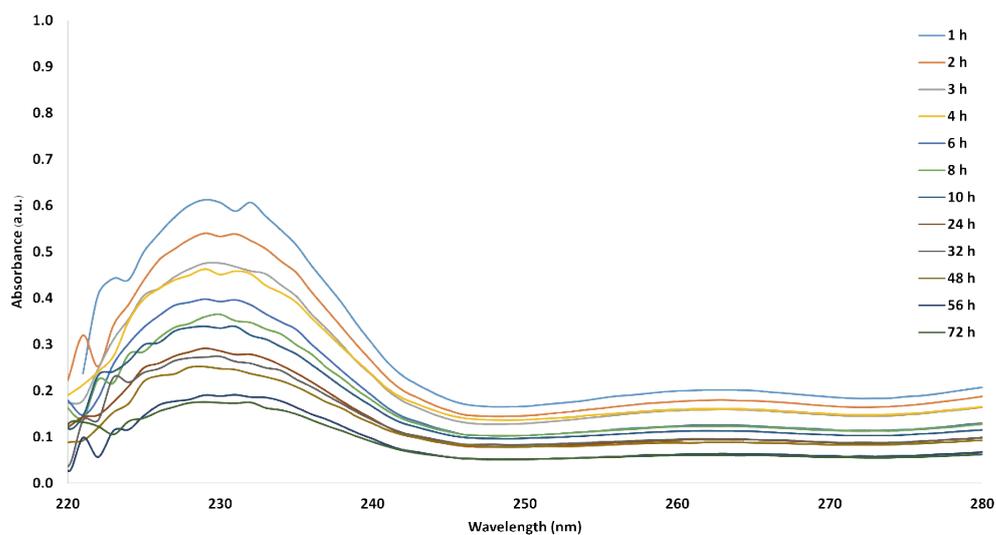


Figure S4. The UV-Vis spectrum graphs of Minoxidil  
for drug release study of MNX-CaCit NPs  
(Blank: Unloaded CaCit NPs)

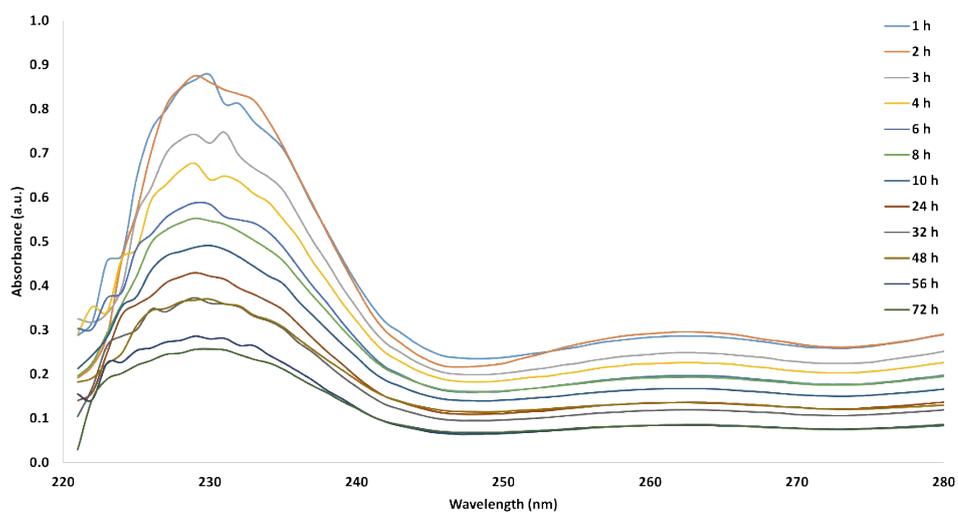


Figure S5. The UV-Vis spectrum graphs of Minoxidil  
for drug release study of MNX-CaCit NPs with SEPIMAX™ ZEN  
(Blank: Unloaded CaCit NPs with SEPIMAX™ ZEN)

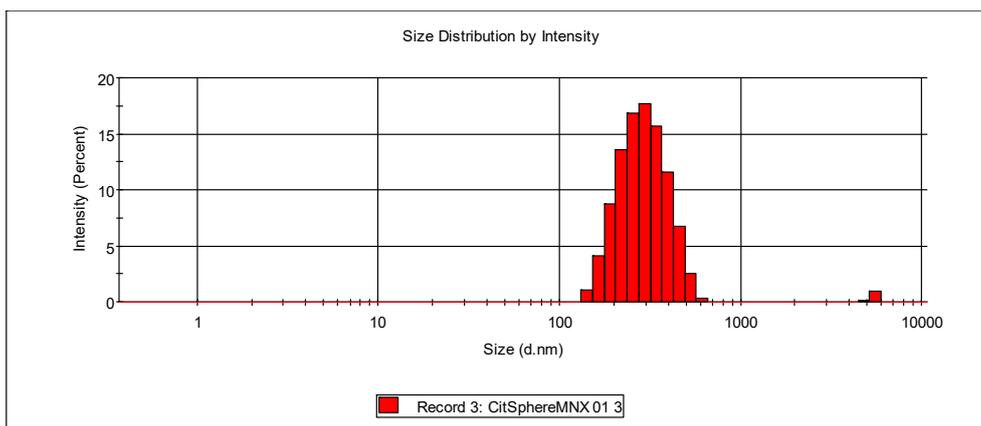


Figure S6. Particle size distribution graph of MNX-CaCit NPs at 0 hours

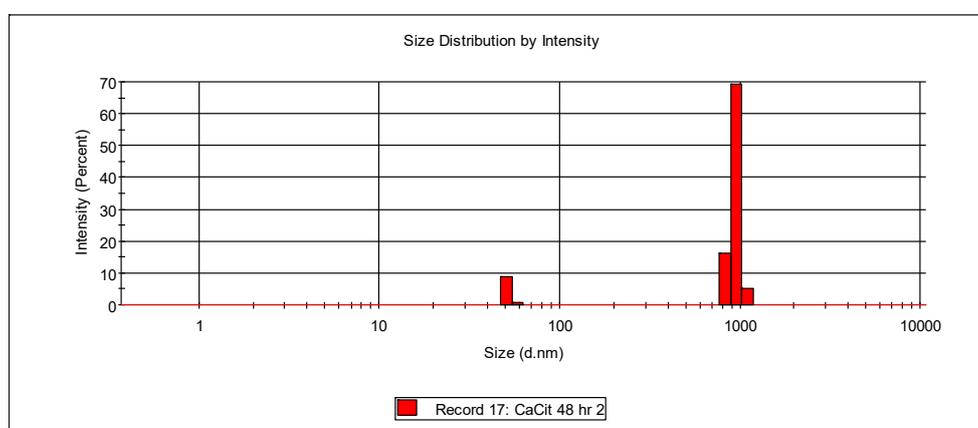


Figure S7. Particle size distribution graph of MNX-CaCit NPs at 48 hours

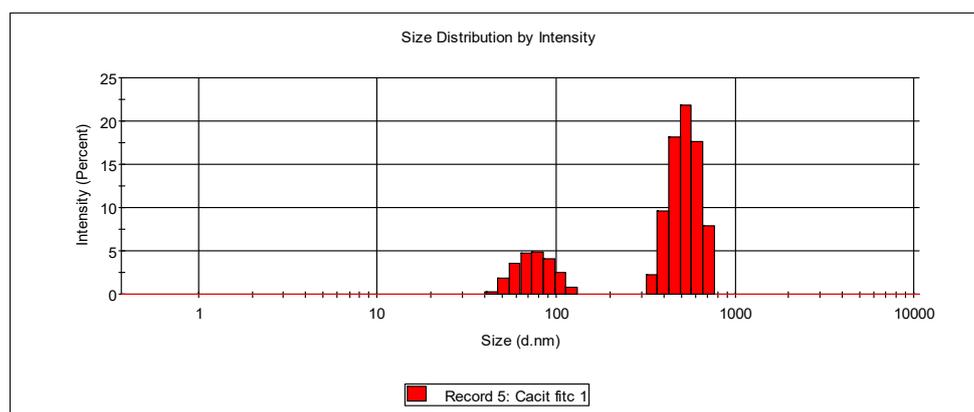


Figure S8. Particle size distribution graph of FITC-MNX-CaCit NPs

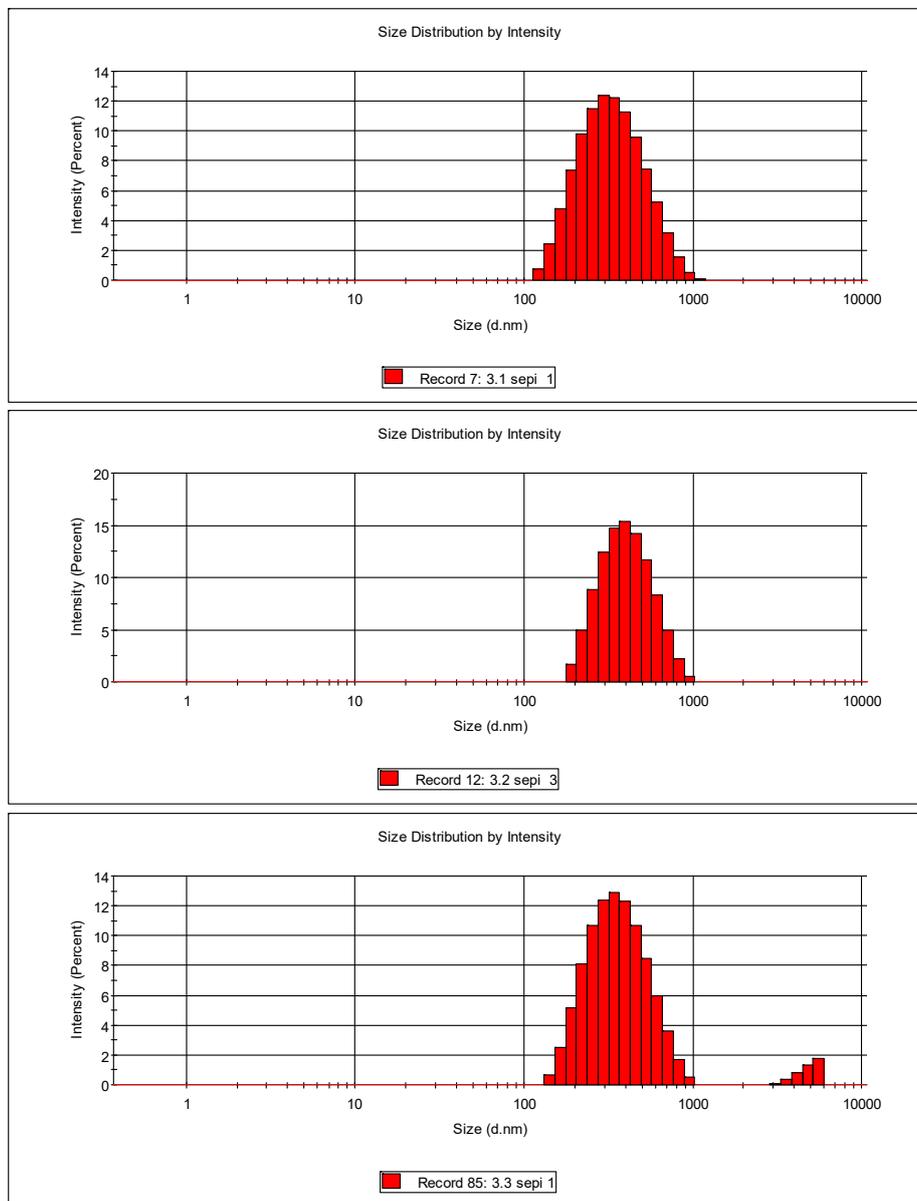


Figure S9. Particle size distribution graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 0 hours: 0.5, 1, and 1.5 % (w/w), respectively.

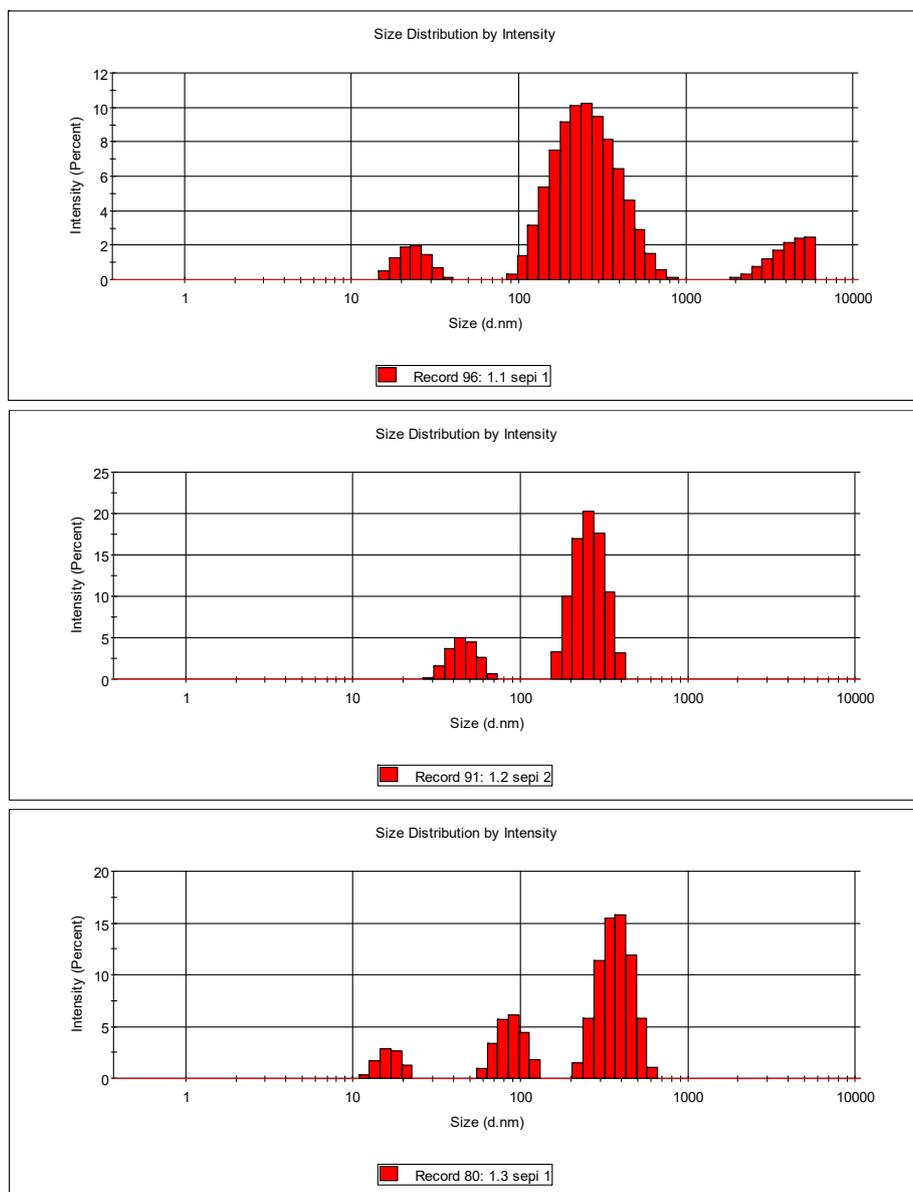


Figure S10. Particle size distribution graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 48 hours: 0.5, 1, and 1.5 % (w/w), respectively.

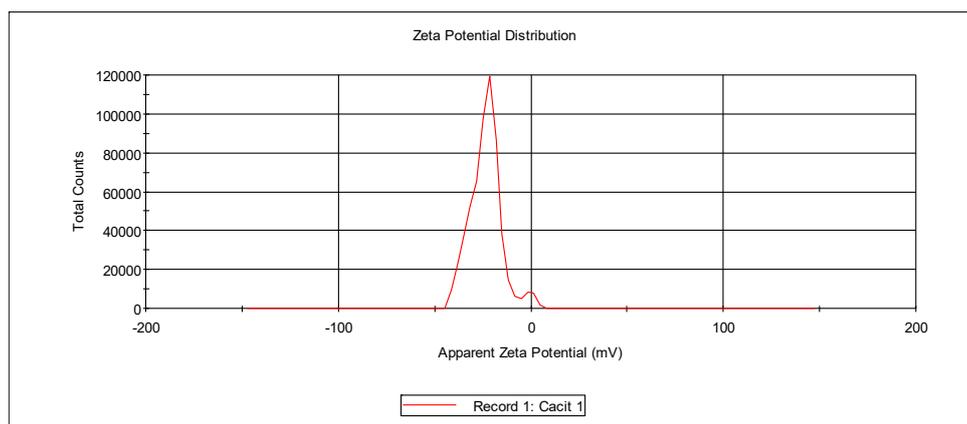


Figure S11. Zeta potential graph of MNX-CaCit NPs at 0 hours.

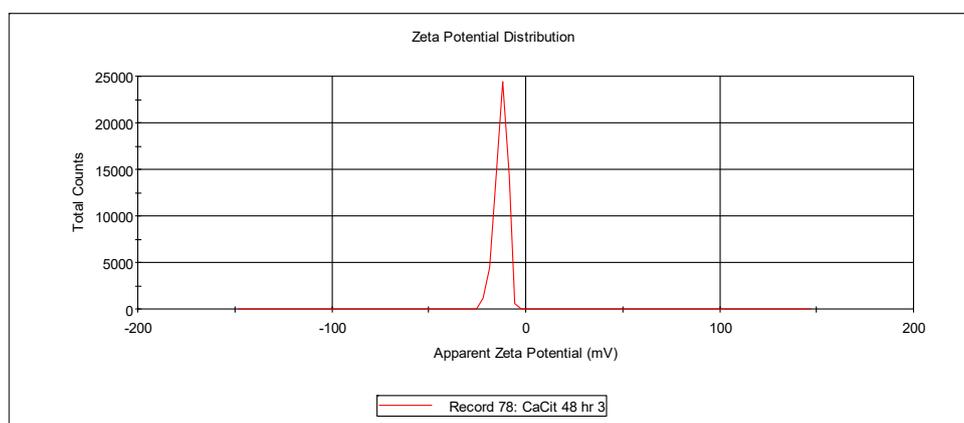


Figure S12. Zeta potential graph of MNX-CaCit NPs at 48 hours.

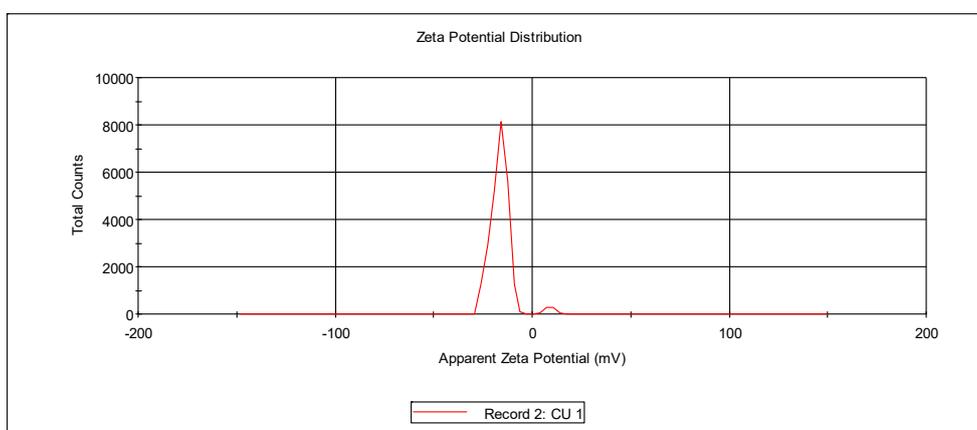


Figure S13. Zeta potential graph of FTIC-MNX-CaCit NPs.

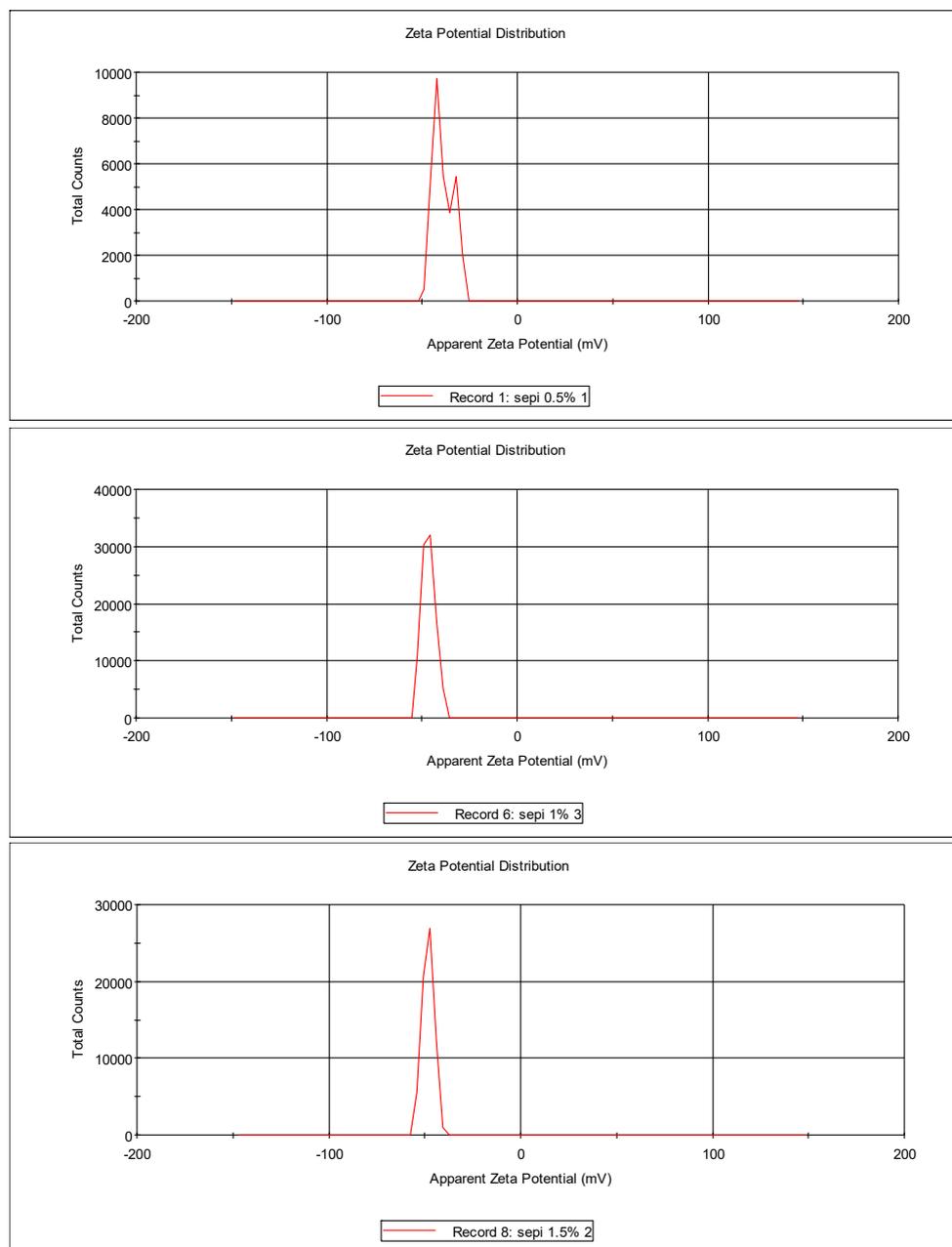


Figure S14. Zeta potential graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 0 hours: 0.5, 1, and 1.5 % (w/w), respectively.

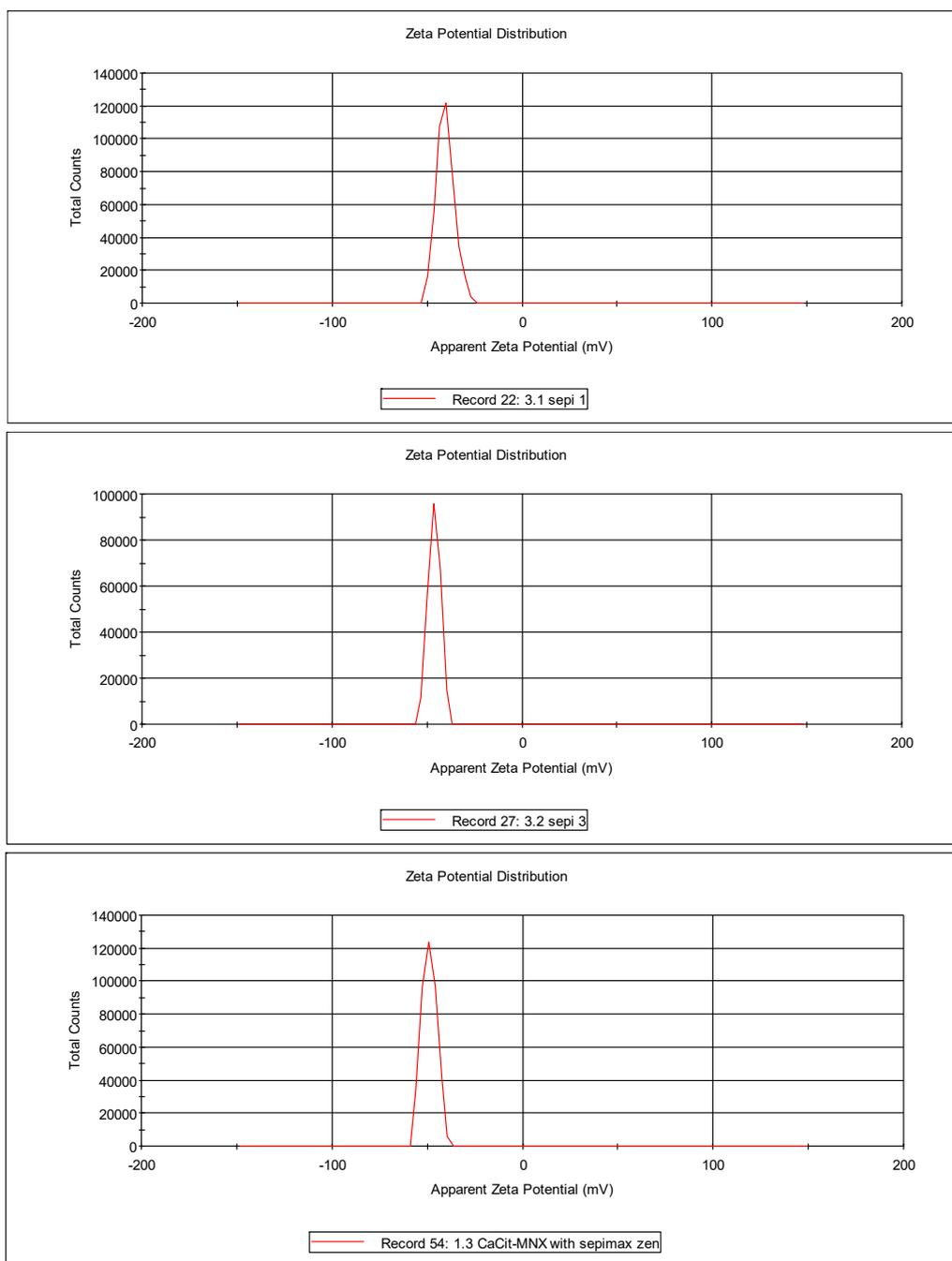


Figure S15. Zeta potential graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 48 hours: 0.5, 1, and 1.5 % (w/w), respectively.

Table S1. Particle size and zeta potential of nanoparticles.

	0 h			48 h		
	Particle size (nm)	PDI	Zeta potential (mV)	Particle size (nm)	PDI	Zeta potential (mV)
FITC-MNX-CaCit NPs	525.33±57.62	0.791±0.198	-13.4±2.442	-	-	-
MNX-CaCit NPs	311.17±13.44	0.278±0.059	-22.5±2.1	911.73±25.46	0.846±0.142	-12.2±1.7
MNX-CaCit NPs with 0.5% Tween80	314.83±67.09	0.372±0.067	-21.4±1.2	681.67±32.22	0.588±0.067	-15.3±1.2
MNX-CaCit NPs with 1% Tween80	308.87±31.38	0.403±0.116	-20.0±4.0	475.30±36.85	0.640±0.153	-15.4±2.8
MNX-CaCit NPs with 1.5% Tween80	312.73±102.53	0.262±0.189	-21.8±0.2	446.23±51.50	0.741±0.186	-16.3±2.7
MNX-CaCit NPs with 0.5% CMC	300.70±21.36	0.798±0.350	-30.7±1.2	510.17±57.02	0.305±0.219	-18.6±2.0
MNX-CaCit NPs with % 1% CMC	287.70±66.38	0.777±0.386	-36.7±0.8	669.00±55.75	0.479±0.333	-22.7±1.9
MNX-CaCit NPs with 1.5% CMC	372.80±107.86	0.777±0.386	-36.4±3.9	863.90±39.60	0.549±0.288	-25.0±2.0
MNX-CaCit NPs with 0.5% SEPI	325.47±102.91	0.513±0.169	-40.9±0.5	296.10±158.7 5	0.341±0.295	-39.4±3.0
MNX-CaCit NPs with 1% SEPI	325.00±21.18	0.573±0.365	-46.4±0.7	315.13±67.97	0.611±0.213	-44.9±2.9
MNX-CaCit NPs with 1.5% SEPI	350.50±17.95	0.571±0.172	-48.2±0.8	330.67±40.84	0.734±0.105	-47.2±2.6

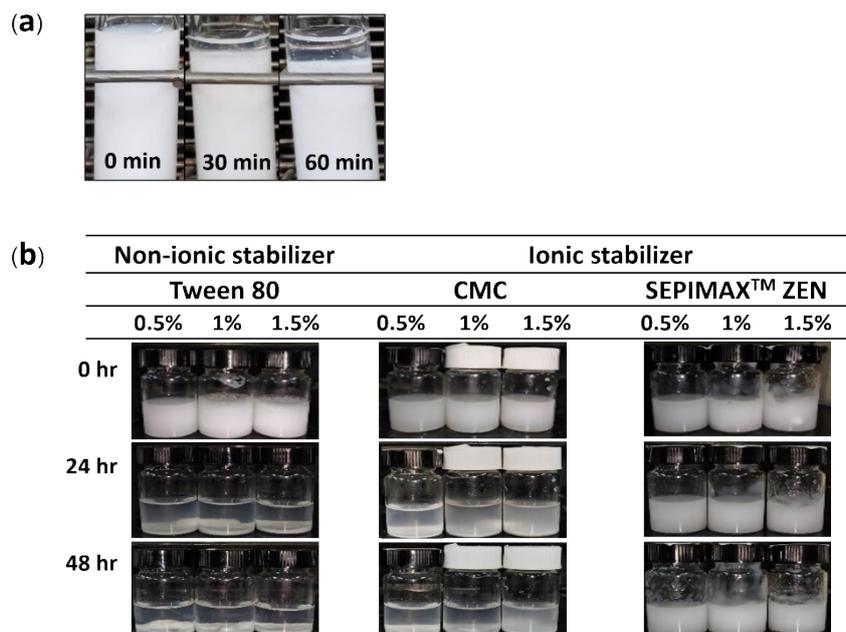


Figure S16. Visual appearance of colloidal solutions of (a) MNX-CaCit NPs and (b) MNX-CaCit NPs with non-ionic and ionic stabilisers, demonstrating their stability.

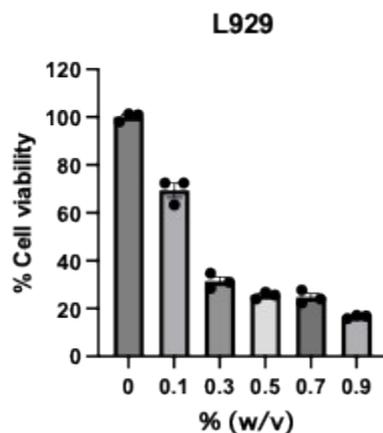


Figure S17. Percentage of L929 cell viability after treatment with various concentrations of MNX-CaCit NPs (% w/v). Each data point represents the mean  $\pm$  SD of triplicate measurements.

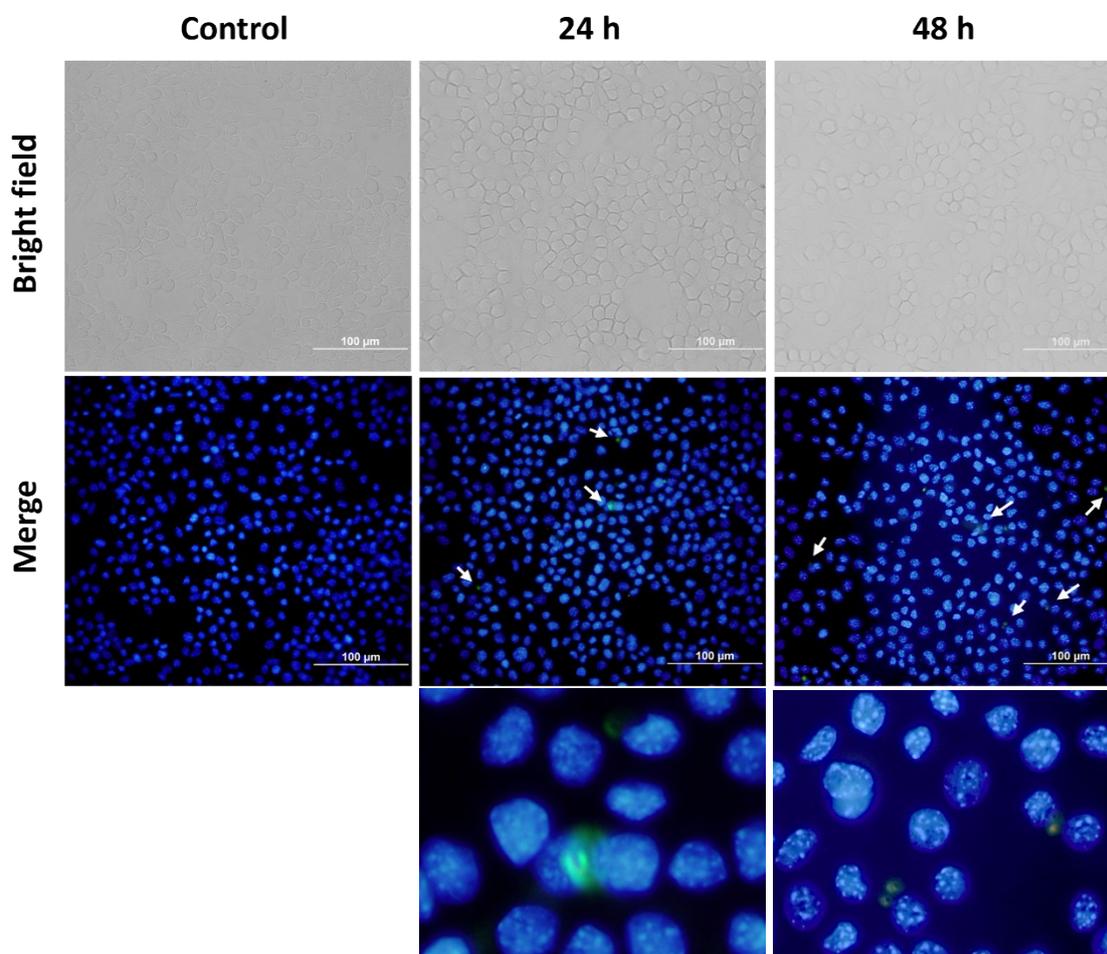


Figure S18. Cellular uptake of FITC-labelled MNX-CaCit NPs in L929 cells. Representative inverted fluorescence microscopy images of cells incubated with unlabelled MNX-CaCit NPs (Control), or with FITC-labelled NPs for 24 h and 48 h (0.1% w/v). Blue: cell nuclei (stained with Hoechst). Green: FITC-labelled NPs. Merge: Overlay of blue and green channels. Bright-field (BF) images are included to show cell morphology. Scale bar = 100  $\mu$ m.

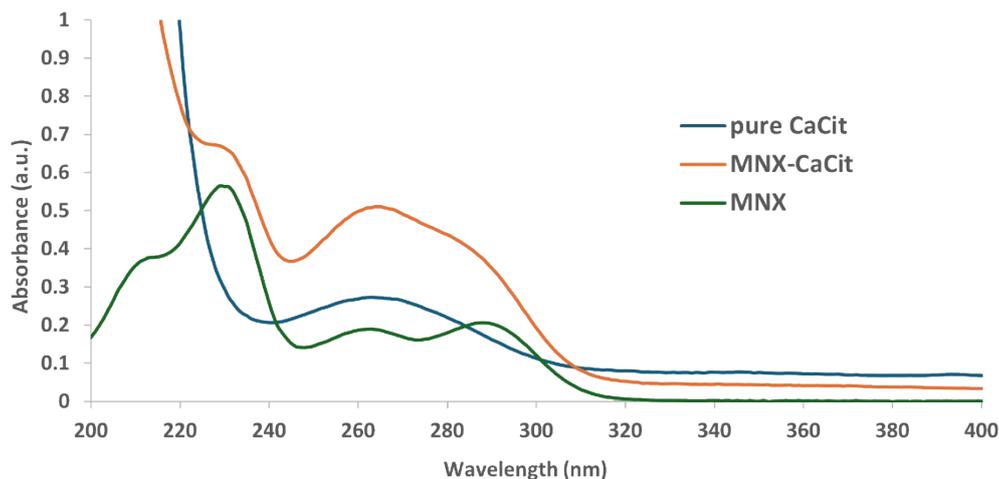


Figure S19. The UV-Vis spectrum graphs of pure CaCit, MNX, and MNX-CaCit NPs. This graph compares the characteristic peaks of each component to confirm successful drug encapsulation.

Table S2. Percentage of cumulative drug release at pH 5.6 and standard deviation (SD) of MNX-CaCit NPs.

MNX-CaCit NPs	% cumulative release (mean)	SD
1 hr	20.88	1.317
2 hr	21.38	1.634
3 hr	22.61	1.634
4 hr	24.00	2.091
6 hr	24.82	2.418
8 hr	25.38	2.844
10 hr	26.34	3.021
24 hr	26.57	2.788
32 hr	27.58	2.966
48 hr	27.65	3.486

Table S3. Percentage of cumulative drug release at pH 5.6 and standard deviation (SD) of MNX-CaCit NPs with 1.5% SEPIMAX™ ZEN

MNX-CaCit NPs with 1.5% SEPI	% cumulative release (mean)	SD
1 hr	13.10	0.540
2 hr	14.52	0.349
3 hr	15.30	0.438
4 hr	15.70	0.425
6 hr	16.15	0.266
8 hr	17.03	0.618
10 hr	17.33	0.456
24 hr	17.57	0.724
32 hr	17.45	0.436
48 hr	18.48	0.361

Table S4. Percentage of cumulative drug release at pH 7.4 and standard deviation (SD) of MNX-CaCit NPs.

MNX-CaCit NPs	% cumulative release (mean)	SD
1 hr	21.44	0.159
2 hr	22.99	0.446
3 hr	24.31	0.034
4 hr	24.75	0.684
6 hr	25.19	0.249
8 hr	26.72	0.413
10 hr	26.61	0.412
24 hr	27.33	1.233
32 hr	27.39	0.319
48 hr	27.92	0.194

Table S5. Percentage of cumulative drug release at pH 7.4 and standard deviation (SD) of MNX-CaCit NPs with 1.5% SEPIMAX™ ZEN

MNX-CaCit NPs with 1.5% SEPI	% cumulative release (mean)	SD
1 hr	12.55	0.087
2 hr	12.39	0.067
3 hr	13.52	0.102
4 hr	13.84	0.026
6 hr	14.81	0.125
8 hr	14.64	0.135
10 hr	14.90	0.244
24 hr	15.46	0.073
32 hr	15.52	0.115
48 hr	15.20	0.058

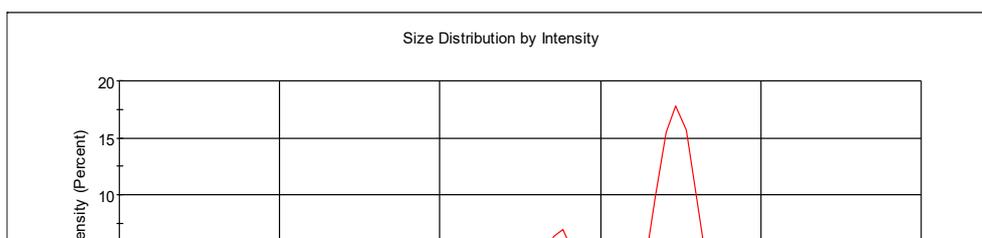


Figure S20. Particle size graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 4°C.

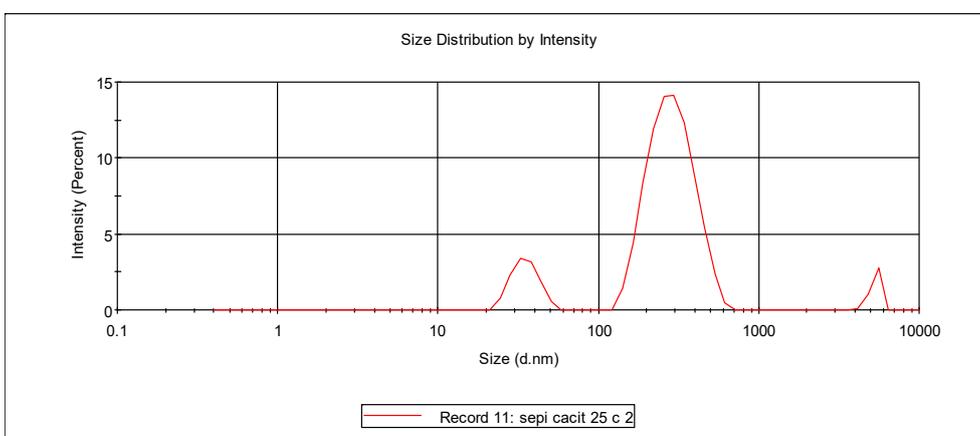


Figure S21. Particle size graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 25°C.

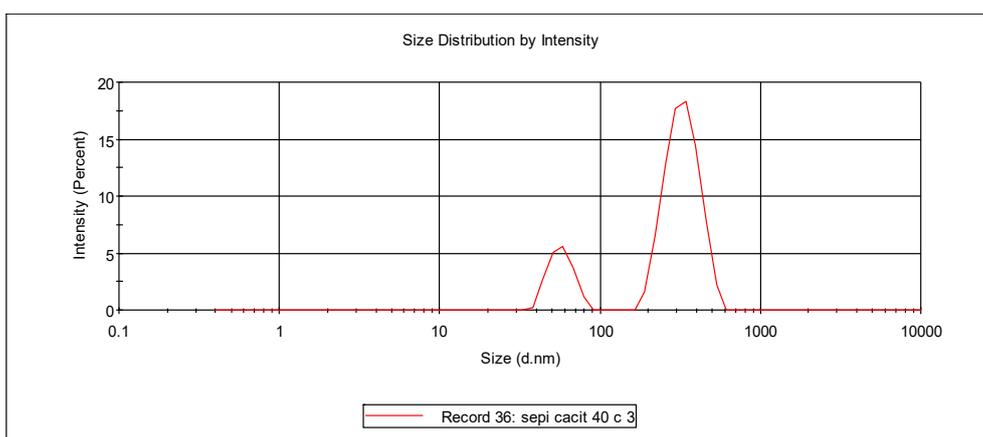


Figure S22. Particle size graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 40°C.

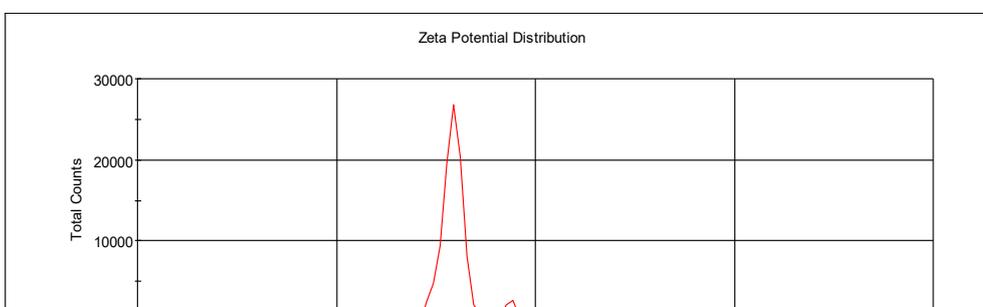


Figure S23. Zeta potential graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 4°C.

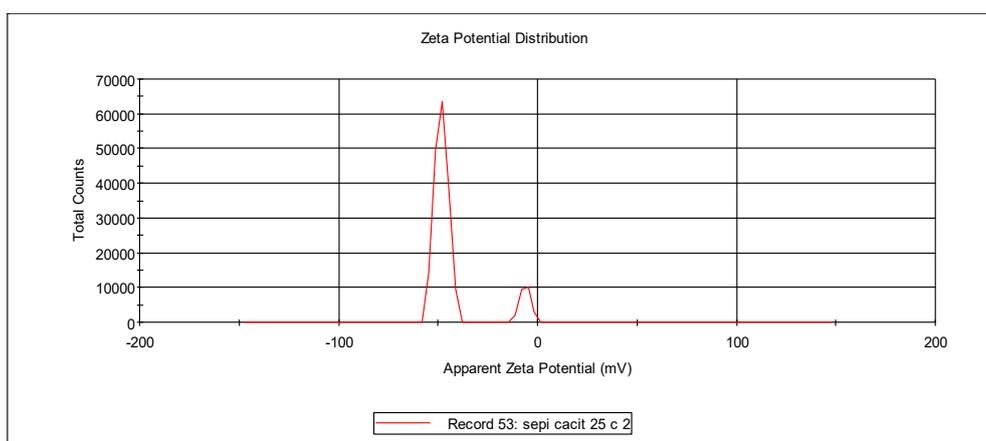


Figure S24. Zeta potential graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 25°C.

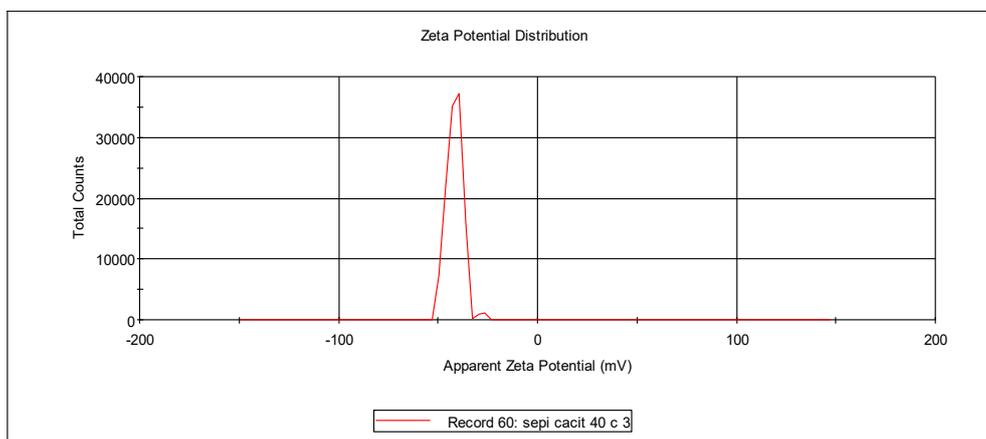


Figure S25. Zeta potential graph of MNX-CaCit NPs with SEPIMAX™ ZEN at 40°C.