Supplementary Material

Revisiting the Lipid Ratio in Lipid Nanoparticles: A Comparison of OFAT and Design of Experiments Approaches

Jade Forrester[1], Eleanor Vaughan[2], Benjamin R. Knappett[2] and Yvonne Perrie[1]

[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, 161 Cathedral St, University of Strathclyde, Glasgow, G4 0RE, UK.

[2]Unchained Labs Royston Ltd, Unit 1 Anglian Business Park, Royston, SG8 5TW, UK.

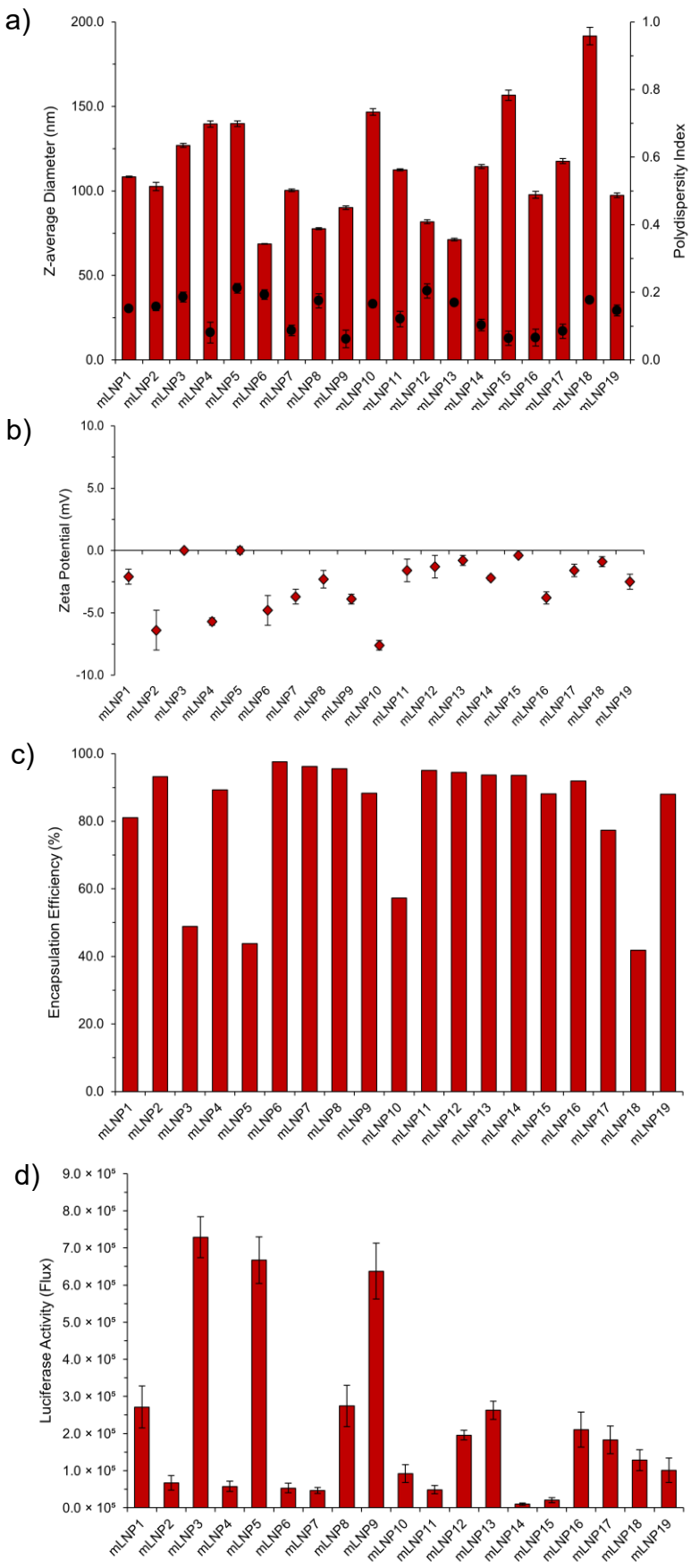

Figure S1. CQAs for mDoE LNP Formulations. (a) represents the z-average diameter (nm) (bars) and polydispersity index (circles), while (b) and (c) represent the zeta potential (mV) and encapsulation efficiency (%) respectively. (d) shows the protein expression (Flux) at a treatment concentration of 2 µg/mL mRNA for mDoE formulations. Data (excluding encapsulation efficiency) are presented as mean ± SD of three technical replicate measurements (n = 1).

Table S1. Predicted and Actual CQAs from OFAT LNPs and Predictive mLNP Model.

	Z-average Diameter (nm)			Polydispersity Index			Zeta Potential (mV)			Encapsulation Efficiency (%)			Cell Expression (Flux)		
	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy
LNP1	123.2	110.0	112.0	0.081	0.105	77.1	-3.6	1.8	-200.0	83.2	94.4	88.1	242848	83087484.2	0.3
LNP3	149.0	111.6	133.5	0.092	0.121	76.0	-1.0	1.0	-100.0	59.4	74.6	79.6	448939	5957067.5	7.5
LNP5	172.9	99.8	173.2	0.102	0.318	32.1	1.4	3.2	43.8	37.4	69.3	54.0	639573	35406150.8	1.8
LNP6	110.2	111.2	99.1	0.075	0.057	131.6	-4.9	0.7	-700.0	95.1	97.3	97.7	139803	74111959.2	0.2
LNP8	109.9	91.2	120.5	0.174	0.113	154.0	-1.3	0.4	-325.0	73.5	77.2	95.2	298430	4331792.5	6.9
LNP9	103.2	92.7	111.3	0.221	0.121	182.6	-0.1	-1.7	68.6	68.6	41.8	164.1	326221	20285874.4	1.6

Table S2. Predicted and Actual CQAs from OFAT LNPs and Predictive aLNP Model

	Z-average Diameter (nm)			Polydispersity Index			Zeta Potential (mV)			Encapsulation Efficiency (%)			Cell Expression (Flux)			In Vivo Expression (Flux)		
	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy	Predicted	Actual	Accuracy
LNP1	105.1	110	95.5	0.081	0.105	77.1	13.1	1.8	727.8	89.9	94.4	95.2	2411289	83087484.2	2.9	393766639	427752520	92.1
LNP3	76.8	111.6	68.8	0.043	0.121	35.5	13.4	1	1340.0	82.5	74.6	110.6	2251148	5957067.5	37.8	312387166	328152520	95.2
LNP5	50.5	99.8	50.6	0.008	0.318	2.5	13.7	3.2	428.1	75.7	69.3	109.2	2103019	35406150.8	5.9	237111153	25254186.67	938.9
LNP6	119.3	111.2	107.3	0.099	0.057	173.7	13	0.7	1857.1	93.5	97.3	96.1	2491359	74111959.2	3.4	434456376	278877520	155.8
LNP8	106.4	91.2	116.7	0.115	0.113	101.8	11	0.4	2750.0	88.7	77.2	114.9	478310	4331792.5	11.0	252570419	281874186.7	89.6
LNP9	107.1	92.7	115.5	0.132	0.121	109.1	9.9	-1.7	-582.4	88.1	41.8	210.8	-488180	20285874.4	-2.4			

Table S3. Model Fit Analysing of mDoE and aDoE Regression Model used for Predictions in Table S1 and S2.

	mDoE Model Fit		aDoE Model Fit	
	R^2	p-value	R^2	p-value
Size	51.62	0.011	81.74	<0.001
Polydispersity Index	51.27	0.011	47.61	0.019
Zeta Potential	21.72	0.284	4.66	0.864
Encapsulation Efficiency	16.27	0.432	44.27	0.029
In Vitro	35.69	0.078	54.25	0.007
In Vivo	N/A	N/A	18.05	0.379

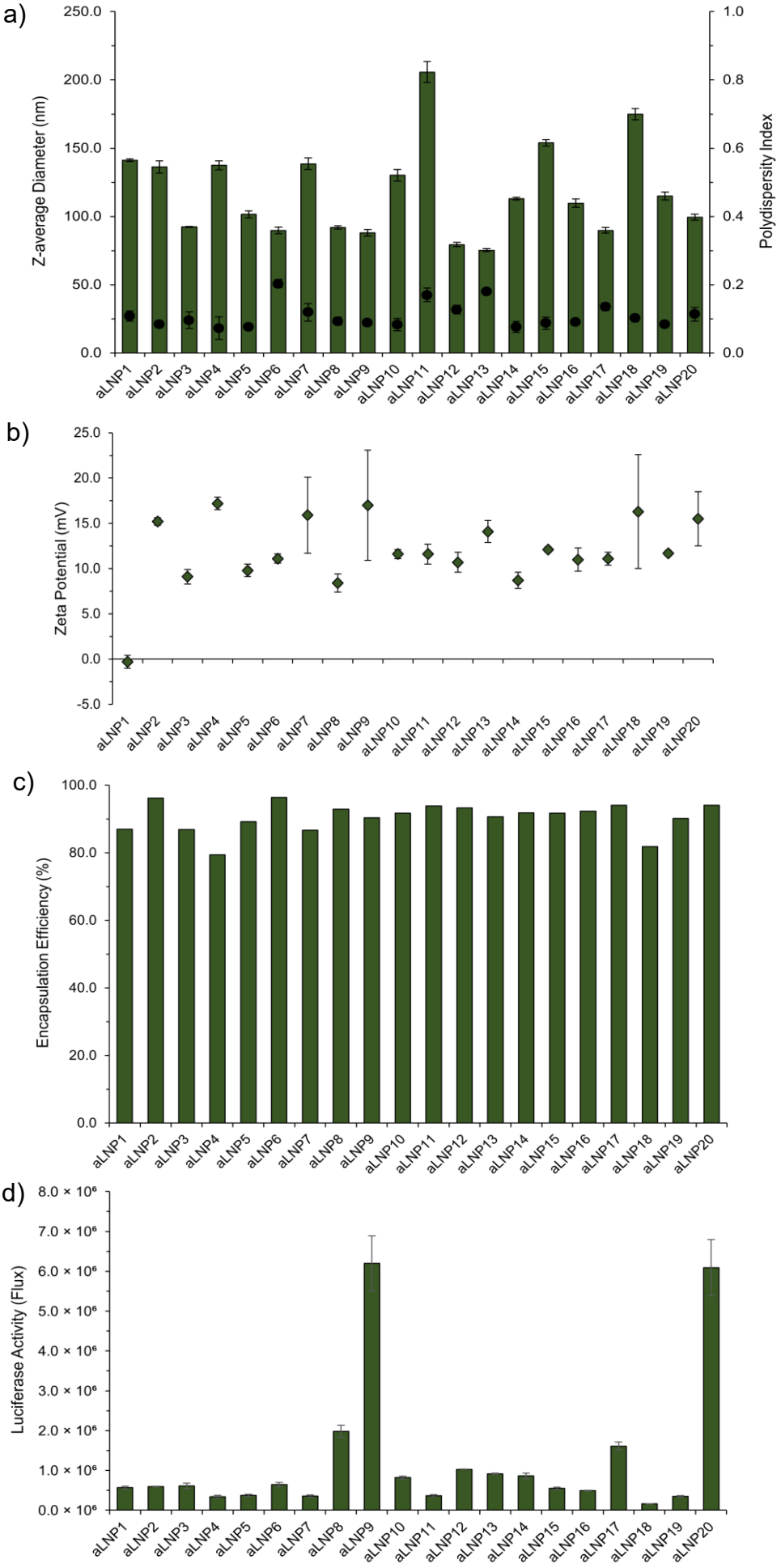

Figure S2 (continued).

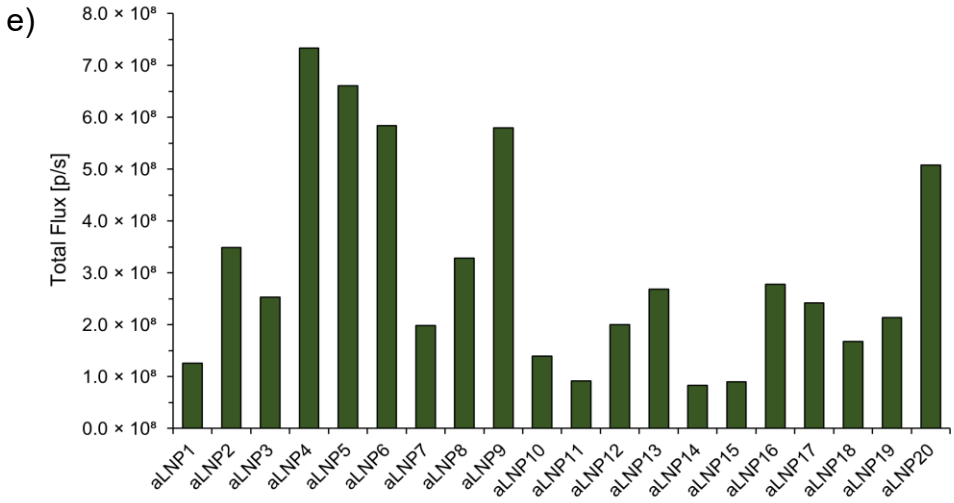

Figure S2 (continued). CQAs for aDoE LNP Formulations. (a) represents the z-average diameter (nm) (bars) and polydispersity index (circles), while (b) and (c) represent the zeta potential (mV) and encapsulation efficiency (%) respectively. (d) shows the protein expression (Flux) in HEK-293 cells at a treatment concentration of 2 µg/mL mRNA for aDoE formulations while (e) represents the protein expression (total flux [p/s]) at the injection site of BALB/c mice 6 hours post intramuscular injection with aDoE formulations. aLNP20 represents the standard molar ratio. Data (excluding encapsulation efficiency and in vivo expression) are presented as mean ± SD of three technical replicate measurements (n = 1).