Supporting Information

Microfluidic manufacturing of liposomes: Development and optimization by design of experiment and machine learning.

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1. Cross-validation process of ANN model

A cross-validation on time series was applied in our study the data was split during the steps of cross validation as it is illustrated in **figure S1**.

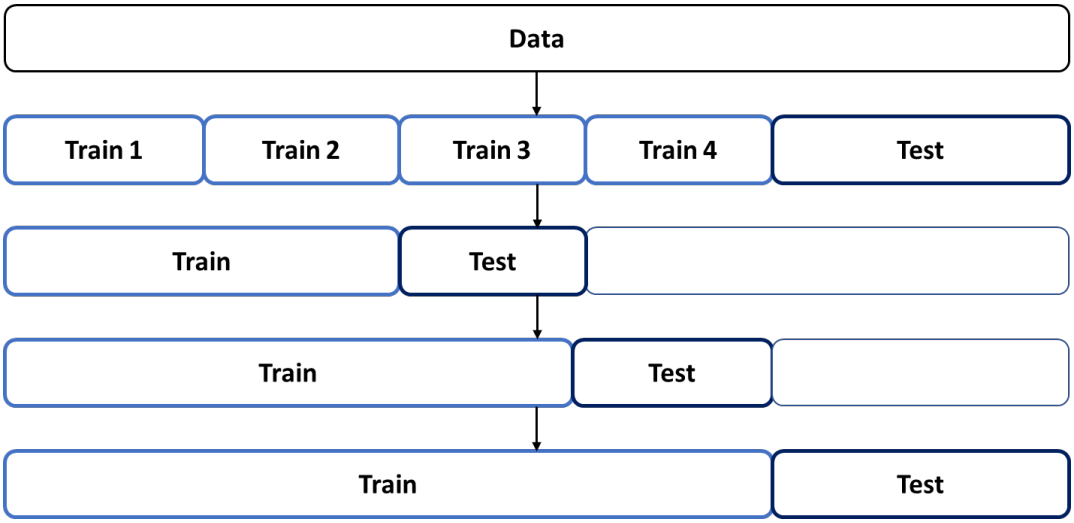

Figure S1. Schematic representation of the dataset split for the training and time series cross-validation of the ANN.

2. Verification of the microfluidic system set-up

The system set-up was checked by measuring the volume of 3 repetitions infused through the syringes at different flow rates (0.1, 1, and 3 mL/min) and flow rate ratios (1, 20, and 50). Using MilliQ water as the only liquid, 2 mL samples were collected at the exit of the syringe, at the end of the plastic tube, and at the output line of the chip. The final weights of water collected at the three different sites showed no significant difference (**Figure S2**).

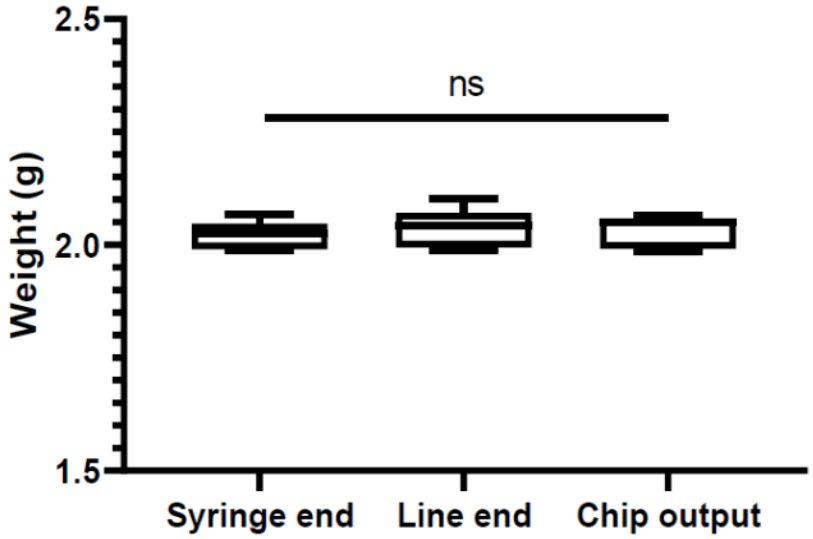

Figure S2. Registered weights of infused water at different stages of the microfluidics system; data presented as mean ± SD.

3. Reliability of size and PDI data

Particle size and PDI of the liposomes were measured using two different DLS apparatus to ensure the reliability of the data, which was confirmed by the similar values obtained (**Figure S3**). Due to its ease of use, all the subsequent measurements employed for the analysis were performed utilizing the Zetasizer Nanoseries Nano-ZS.

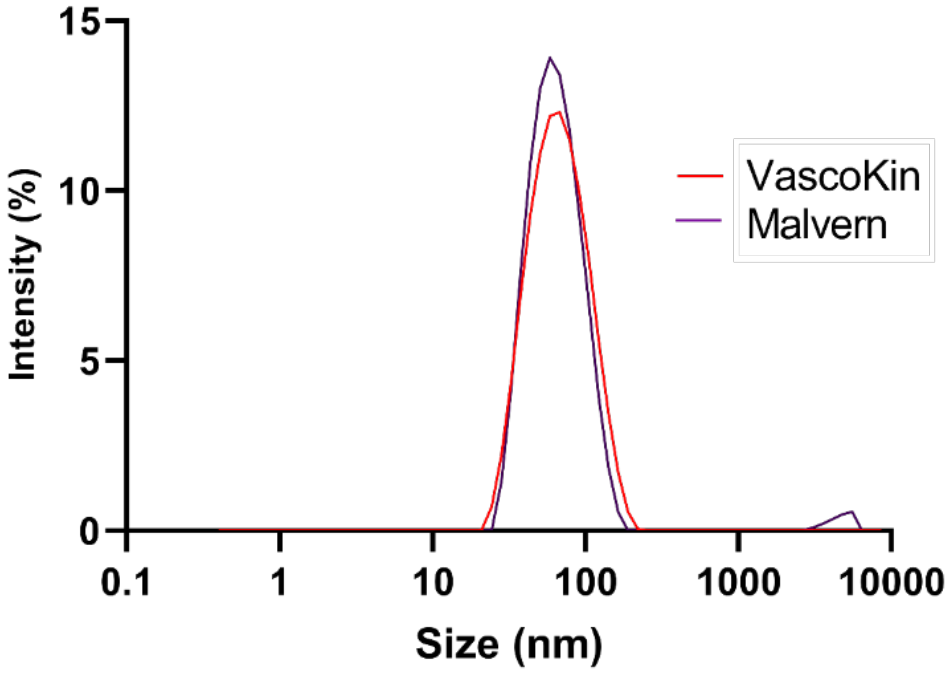

Figure S3. Particle size distribution results obtained by Zetasizer and VascoKin. The results correspond to the run 4 of the screening experiment (see **Table S1**).

4. DLS experimental results of all performed runs

Table S1. Experimental conditions and DLS results of all performed runs.

Experiment	[PEGylated lipid] (%)	[Cholesterol] (%)	TFR (mL/min)	FRR	[Salt] (mg/mL)	Temperature (°C)	Particle size (nm)	PDI
Screening 1	1	0	0.2	3	0	15	87.31	0.148
Screening 2	1	0	0.5	9	9	25	87.85	0.261
Screening 3	1	0	1.0	15	18	35	94.43	0.304
Screening 4	1	0	1.5	19	9	25	68.60	0.203
Screening 5	1	0	2.0	39	0	15	109.80	0.253
Screening 6	1	0	2.0	39	0	15	103.70	0.245
Screening 7	1	19	0.2	9	18	25	77.19	0.206
Screening 8	1	19	0.5	15	9	15	71.44	0.232
Screening 9	1	19	0.5	15	9	15	60.04	0.204
Screening 10	1	19	1.0	19	0	15	80.48	0.236
Screening 11	1	19	1.5	39	0	25	105.20	0.246
Screening 12	1	19	2.0	3	9	35	62.12	0.230
Screening 13	1	41	0.2	15	0	25	75.25	0.403
Screening 14	1	41	0.5	19	0	35	101.10	0.308
Screening 15	1	41	1.0	39	9	25	77.80	0.367
Screening 16	1	41	1.5	3	18	15	48.46	0.220
Screening 17	1	41	1.5	3	18	15	48.43	0.224
Screening 18	1	41	2.0	9	9	15	71.32	0.462
Screening 19	1	19	0.2	19	9	15	NA	NA
Screening 20	1	19	0.5	39	18	15	81.71	0.355
Screening 21	1	19	1.0	3	9	25	99.66	0.287
Screening 22	1	19	1.5	9	0	35	55.70	0.161
Screening 23	1	19	2.0	15	0	25	71.89	0.339
Screening 24	1	0	0.2	39	9	35	99.25	0.293
Screening 25	1	0	0.5	3	0	25	74.89	0.147
Screening 26	1	0	1.0	9	0	15	65.56	0.210
Screening 27	1	0	1.5	15	9	15	70.04	0.236
Screening 28	1	0	2.0	19	18	25	86.49	0.336
Surface-response 1	5	10	1.3	4	11	20	55.89	0.130
Surface-response 2	5	30	1.3	4	11	20	48.25	0.123
Surface-response 3	5	10	1.7	4	11	20	52.47	0.141
Surface-response 4	5	10	1.7	4	11	20	53.37	0.147
Surface-response 5	5	30	1.7	4	11	20	45.23	0.119
Surface-response 6	5	10	1.3	12	11	20	51.45	0.137
Surface-response 7	5	30	1.3	12	11	20	45.52	0.140
Surface-response 8	5	30	1.3	12	11	20	47.11	0.139
Surface-response 9	5	10	1.7	12	11	20	51.10	0.125
Surface-response 10	5	30	1.7	12	11	20	45.23	0.161
Surface-response 11	5	10	1.5	8	11	20	50.80	0.123

Surface-response 12	5	30	1.5	8	11	20	47.96	0.156
Surface-response 13	5	20	1.3	8	11	20	50.42	0.125
Surface-response 14	5	20	1.7	8	11	20	48.09	0.143
Surface-response 15	5	20	1.7	8	11	20	50.15	0.166
Surface-response 16	5	20	1.5	4	11	20	48.60	0.102
Surface-response 17	5	20	1.5	12	11	20	45.74	0.147
Surface-response 18	5	20	1.5	8	11	20	48.24	0.112
Surface-response 19	5	20	1.5	8	11	20	48.96	0.118
Surface-response 20	5	20	1.5	8	11	20	48.58	0.131
Surface-response 21	5	16	1.45	7.3	11	20	48.58	0.135
Surface-response 22	5	16	1.45	7.3	11	20	50.28	0.130
Surface-response 23	5	24	1.45	7.3	11	20	45.37	0.104
Surface-response 24	5	20	1.6	7.3	11	20	47.14	0.146
Surface-response 25	5	20	1.5	10	11	20	49.54	0.160
[PEGylated lipid] 1	4	35	1.8	3	11	20	41.51	0.078
[PEGylated lipid] 2	4	15	1.8	5	11	20	50.36	0.165
[PEGylated lipid] 3	4	15	1.8	5	11	20	50.76	0.132
[PEGylated lipid] 4	2	15	1.8	5	11	20	53.27	0.132
[PEGylated lipid] 5	2	35	1.0	5	11	20	82.65	0.322
[PEGylated lipid] 6	4	15	1.0	3	11	20	58.08	0.165
[PEGylated lipid] 7	2	35	1.8	3	11	20	52.93	0.149
[PEGylated lipid] 8	2	35	1.8	3	11	20	50.23	0.154
[PEGylated lipid] 9	4	35	1.0	5	11	20	46.90	0.182
[PEGylated lipid] 10	2	15	1.0	3	11	20	59.54	0.122
[PEGylated lipid] 11	3	25	1.4	4	11	20	58.13	0.153
[PEGylated lipid] 12	3	25	1.4	4	11	20	57.74	0.132
Complementary 1	5	35	1.5	10	11	20	49.47	0.146
Complementary 2	1	30	1.4	4	11	20	61.39	0.156
Complementary 3	1	30	1.4	4	11	20	61.63	0.157
Complementary 4	5	30	1.4	4	11	20	50.75	0.121
Complementary 5	5	30	1.4	4	11	20	50.99	0.127
Model prediction 1.1	1.5	10	2	6	11	20	51,82	0.144
Model prediction 1.2	1.5	10	2	6	11	20	53.50	0.147
Model prediction 1.3	1.5	10	2	6	11	20	52.08	0.150
Model prediction 2.1	1	5	1.51	4	11	20	62.29	0.171
Model prediction 2.2	1	5	1.51	4	11	20	62.36	0.167
Model prediction 2.3	1	5	1.51	4	11	20	58.32	0.153
Model prediction 3.1	3.5	22	1.7	8	11	20	52.20	0.146
Model prediction 3.2	3.5	22	1.7	8	11	20	49.31	0.140
Model prediction 3.3	3.5	22	1.7	8	11	20	47.56	0.148

5. Determination of liposomes stability

The stability of liposomes prepared by the microfluidic technique was evaluated by measuring the size and PDI of the liposomes of surface-response formulations after five months of storage. The variance percentage was also calculated and presented in **Table S2**. The results showed a slight increase in the size of liposomes (**Figure S4**) with small variations regarding the PDI (**Figure S5**). All performed runs presented a size less than 75 nm and a PDI under 0.2.

Table S2. DLS results of surface-response formulations after five months of storage.

Experiment	Size (nm) Day 1	Size (nm) Month 5	Size Variance percentage (%)	PDI Day 1	PDI Month 5	PDI Variance percentage (%)
Surface-response 1	55.89	71.8	28.5	0.13	0.129	0.77
Surface-response 2	48.25	62.63	29.8	0.123	0.131	6.50
Surface-response 3	52.47	61.99	18.1	0.141	0.147	4.26
Surface-response 4	53.37	62.97	18.0	0.147	0.129	12.24
Surface-response 5	45.23	49.84	10.2	0.119	0.126	5.88
Surface-response 6	51.45	58.61	13.9	0.137	0.137	0.00
Surface-response 7	45.52	49.21	8.1	0.14	0.101	27.86
Surface-response 8	47.11	54.1	14.8	0.139	0.156	12.23
Surface-response 9	51.1	56.14	9.9	0.125	0.125	0.00
Surface-response 10	45.23	47.14	4.2	0.161	0.187	16.15
Surface-response 11	50.8	56.38	11.0	0.123	0.129	4.88
Surface-response 12	47.96	51.05	6.4	0.156	0.161	3.21
Surface-response 13	50.42	62.9	24.8	0.125	0.143	14.40
Surface-response 14	48.09	50.11	4.2	0.143	0.138	3.50
Surface-response 15	50.15	57.16	14.0	0.166	0.193	16.27
Surface-response 16	48.6	54.11	11.3	0.102	0.117	14.71
Surface-response 17	45.74	48.63	6.3	0.147	0.126	14.29
Surface-response 18	48.24	51.24	6.2	0.112	0.121	8.04
Surface-response 19	48.96	54.82	12.0	0.118	0.122	3.39
Surface-response 20	48.58	54.56	12.3	0.131	0.126	3.82
Surface-response 21	48.58	51.5	6.0	0.135	0.152	12.59
Surface-response 22	50.28	51.99	3.4	0.13	0.129	0.77
Surface-response 23	45.37	46.96	3.5	0.104	0.127	22.12
Surface-response 24	47.14	47.87	1.5	0.146	0.123	15.75
Surface-response 25	49.54	51.77	4.5	0.16	0.163	1.88

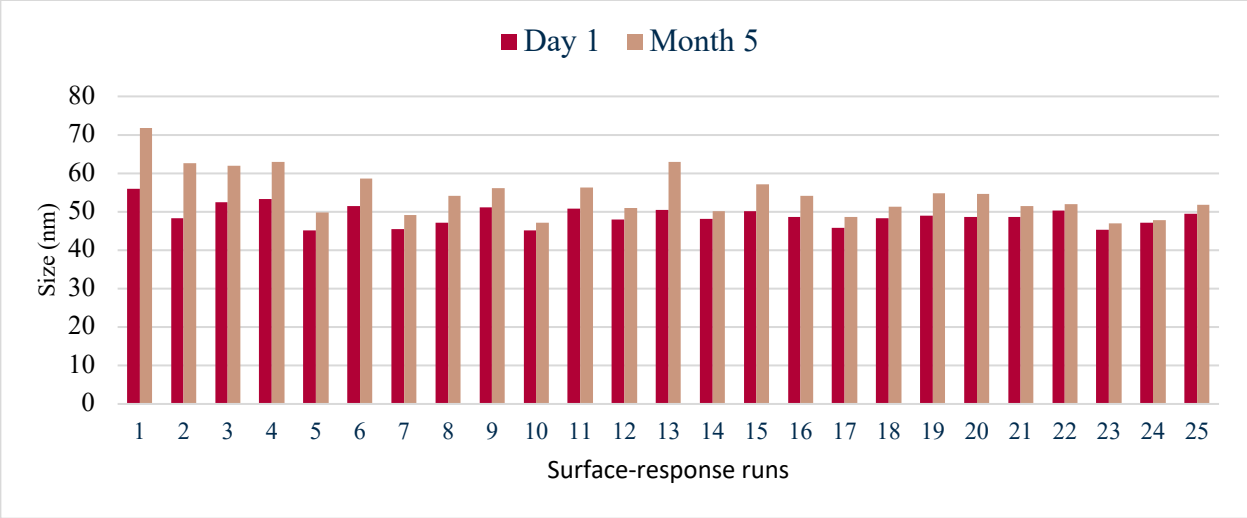

Figure S4. The average size of liposomes of surface-response formulations after five months of storage.

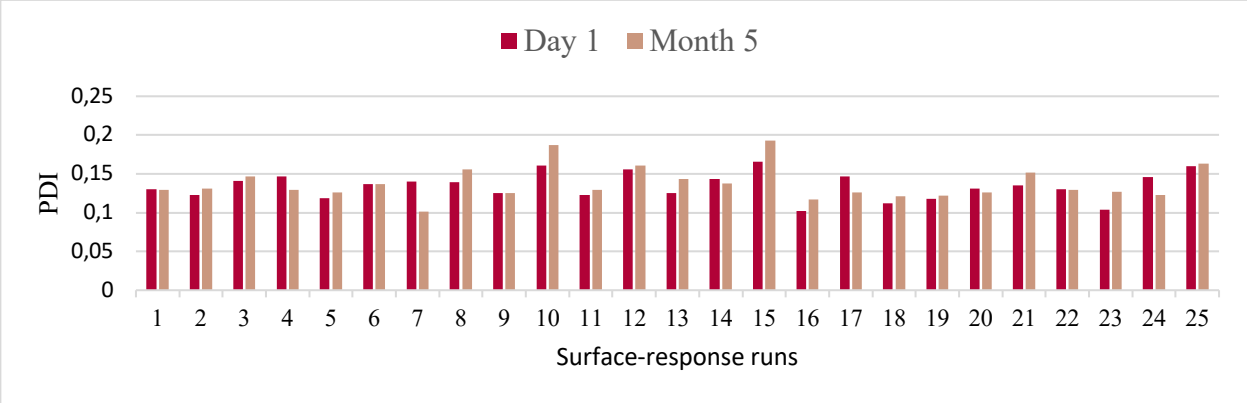

Figure S5. PDI of liposomes of surface-response formulations after five months of storage.

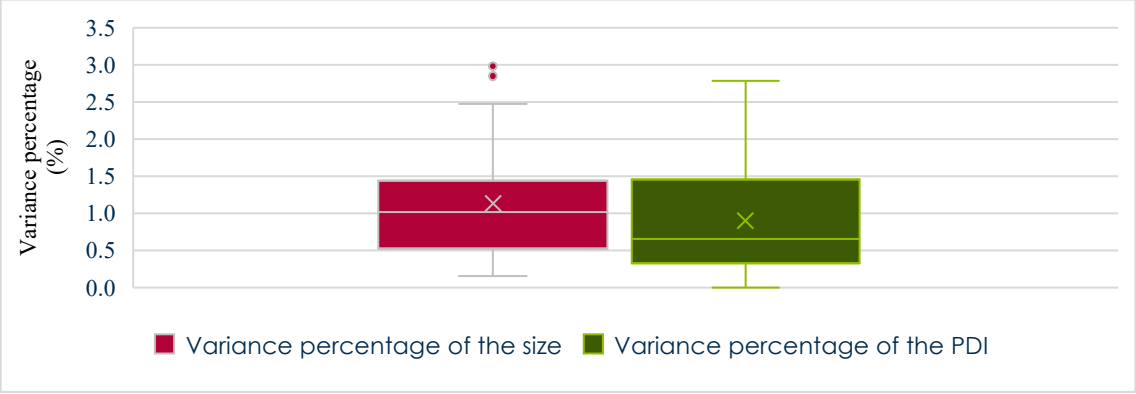

Figure S6. Percentage variance of size and PDI of surface response formulations between the two measurements.