

Electronic Supplementary Information

Analytical validation of an ATR-FTIR based method for quantifying the amount of polysorbate 80 adsorbed on the PLGA nanoparticles

Nabodita Sinha,^a Abhayraj Shrikrishna Joshi^b and Ashwani Kumar Thakur^{*c}

^a Biological Sciences and Bioengineering, IIT Kanpur, India

^b DTU Biosustain, Denmark, Europe.

^c Biological Sciences and Bioengineering, IIT Kanpur, India

Contents:

- 1. Table S1: Coating of nanoparticles with different concentrations of polysorbate 80**
- 2. Table S2: Dynamic light scattering analysis of the coated nanoparticles after 240 minutes of coating**
- 3. Table S3: Intraday precision of extracted amount of polysorbate 80 after 240 minutes of coating**
- 4. Table S4: Extraction amount of polysorbate 80 after 240 minutes of coating on different days**
- 5. Table S5: Mean amount of polysorbate 80 extracted after 240 minutes of coating and inter-day RSD %**

Table S1: Coating of nanoparticles with different concentrations of polysorbate 80

Nanoparticle Volume (μ l) (Mass ~2.24 mg)	Volume of stock polysorbate 80 (50 mg/ml) or (*200 mg/ml) added (μ l)	Water Added (μ l)	Final Concentration of coating (mg/ml)
1000	2.5	247.5	0.1
1000	7.5	242.5	0.3
1000	12.5	237.5	0.5
1000	25	225	1
1000	75	175	3
1000	125	125	5
1000	187.5	62.5	7.5
1000	62.5*	187.5	10
1000	125*	125	20
1000	187.5*	62.5	30

Table S2: Dynamic light scattering analysis of the coated nanoparticles after 240 minutes of coating

Coating Concentration (mg/ml)	Hydrodynamic Diameter (nm)	Polydispersity Index	Zeta Potential (millivolts)
Uncoated	158.33 $\pm$ 0.65	0.048 $\pm$ 0.0011	-20.73 $\pm$ 0.49
0.1	160.16 $\pm$ 1.16	0.041 $\pm$ 0.0020	-20.83 $\pm$ 1.47
0.3	162.60 $\pm$ 1.47	0.041 $\pm$ 0.0015	-20.93 $\pm$ 1.26
0.5	160.43 $\pm$ 0.40	0.042 $\pm$ 0.0026	-20.81 $\pm$ 1.34
1.0	159.63 $\pm$ 1.40	0.042 $\pm$ 0.0032	-21.83 $\pm$ 1.70
3.0	160.66 $\pm$ 0.72	0.042 $\pm$ 0.0011	-20.97 $\pm$ 0.87
5.0	160.73 $\pm$ 1.65	0.047 $\pm$ 0.0038	-22.26 $\pm$ 2.45
7.5	160.03 $\pm$ 2.08	0.042 $\pm$ 0.0028	-20.46 $\pm$ 0.75
10.0	160.20 $\pm$ 2.45	0.046 $\pm$ 0.0011	-23.36 $\pm$ 2.41
20.0	161.06 $\pm$ 0.75	0.043 $\pm$ 0.0023	-21.26 $\pm$ 1.90
30.0	159.13 $\pm$ 1.73	0.042 $\pm$ 0.0031	-23.43 $\pm$ 1.62

Table S3: Intraday precision of extracted amount of polysorbate 80 after 240 minutes of coating

Concentration of polysorbate 80 added for coating (mg/ml)	Day 1 Extracted polysorbate 80 (µg/mg)	Intraday RSD% Day 1	Day 2 Extracted polysorbate 80 (µg/mg)	Intraday RSD% Day 2	Day 3 Extracted polysorbate 80 (µg/mg)	Intraday RSD% Day 3	Mean intraday RSD%
0.1	4.0±0.2342	5.85	4.54±0.2905	6.40	3.17±0.1562	4.93	5.72667
0.3	2.80±0.1439	5.14	3.58±0.1564	4.37	4.15±0.2174	5.24	4.91667
0.5	11.56±0.4115	3.56	10.35±0.4233	4.09	10.43±0.5162	4.95	4.20000
1	7.75±0.2906	3.75	5.25±0.2992	5.70	6.45±0.3031	4.70	4.71667
3	20.84±1.2337	5.92	21.87±1.5046	6.88	19.56±0.9858	5.04	5.94667
5	11.07±0.1184	1.07	12.0±0.2976	2.48	10.87±0.1500	1.38	1.64333
7.5	11.25±0.2823	2.51	10.93±0.1628	1.49	12.56±0.2084	1.66	1.88667
10	5.78±0.1745	3.02	7.825±0.3591	4.59	7.21±0.3677	5.10	4.23667
20	5.78±0.2479	4.29	6.25±0.2343	3.75	7.38±0.3453	4.68	4.24000
30	7.14±0.3662	5.13	8.75±0.4480	5.12	8.06±0.5239	6.50	5.58333

Table S4: Extraction amount of polysorbate 80 after 240 minutes of coating on different days

Concentration of polysorbate 80 added for coating (mg/ml)	Day 1 Extracted polysorbate 80 (µg/mg)	Day 2 Extracted polysorbate 80 (µg/mg)	Day 3 Extracted polysorbate 80 (µg/mg)	Mean Amount of extracted polysorbate 80 (µg/mg)	Standard error of mean
0.1	4.0	4.54	3.17	3.903	0.6901
0.3	2.80	3.58	4.15	3.510	0.6777
0.5	11.56	10.35	10.43	10.780	0.6766
1	7.75	5.25	6.45	6.483	1.2503
3	20.84	21.87	19.56	20.756	1.1572
5	11.07	12.0	10.87	11.313	0.6030
7.5	11.25	10.93	12.56	11.580	0.8636
10	5.78	7.825	7.21	6.938	1.0492
20	5.78	6.25	7.38	6.470	0.8223
30	7.14	8.75	8.06	7.983	0.8077

Table S5: Mean amount of polysorbate 80 extracted after 240 minutes of coating and inter-day RSD%

Concentration of polysorbate 80 used for coating (mg/ml)	Mean amount of PS 80 obtained from standard curve (µg)	Extraction limit of PS 80 after 240 minutes (µg/mg)	% Relative Standard Deviation
0.1	0.0087	3.903±0.6901	17.68
0.3	0.0078	3.51±0.6777	19.30
0.5	0.0241	10.78±0.6776	6.28
1.0	0.0145	6.483±1.2503	19.28
3.0	0.0464	20.756±1.1572	5.57
5.0	0.0253	11.313±0.6030	5.33
7.5	0.0259	11.58±0.8636	7.45
10.0	0.0155	6.939±1.0496	15.12
20.0	0.0144	6.47±0.8223	12.70
30.0	0.0178	7.983±0.8077	10.11