SUPPLEMENTARY MATERIAL

Design and formulation of eudragit coated zein/pectin nanoparticles for the colon delivery of resveratrol

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Content

S.1 Centrifugal FFF basic theory

The separation in CF3 is based on the mass of the sample components. The retention time t_r of an analyte is related to the retention ratio R according to

$$\frac{t_0}{t_r} = R = \frac{v_{zone}}{\langle v \rangle} \quad \text{S1}$$

where R is the ratio of the average velocity of the analyte zone v_{zone} and the average velocity of the carrier liquid $\langle v \rangle$. In FFF [24], R is related to an adimensional retention parameter named λ, as:

$$R = 6\lambda \left[coth\left(\frac{1}{2\lambda} - 2\lambda\right)\right] \quad \text{S2}$$

which becomes under high retention conditions (i.e. when λ < 0.01)

$$R \cong 6\lambda \quad \text{S3}$$

λ is, in turn, related to the applied field and some analyte physical chemical properties

$$\lambda = \frac{kT}{m_{eff}\left(1-\frac{\rho_l}{\rho_p}\right)Gw} \quad \text{S4}$$

where k is the Boltzmann's constant, T the absolute temperature during elution, m_{eff} the particle effective mass, ρ_l the carrier density, ρ_p the particle density, G the centrifugal field strength and w the channel thickness.

When a sample contains particles of a very broad mass range, it is convenient to decrease the centrifugal field strength G as the run proceeds so that eluting species at different times are subjected to different average field strengths. The program for the field decay applied to this study is:

$$G = G_0 \left(\frac{t_1 - t_a}{t - t_a}\right)^p \quad \text{S5}$$

which guarantees a uniform fractionation power throughout a broad diameter range.

Table S1: Centrifugal field flow fractionation analysis conditions. The flow rate was set to 1.0 mL min^{-1} and the UV-vis detection was recorded at 254 nm. The particle density was taken from the literature: 1.226 g mL^{-1} [25].

Initial RPM	Final RPM	Eq time (min)	t_1 (min)	t_a (min)
1000	20	10	5	-40
2000	20	5	5	-80

S.2 In vitro release studies.

Table S2: Accurately weighted amount of raw RSV or ZP-RSV-US and ZP-RSV-US-ES 100 particles for the in vitro release studies. Particles were dispersed in three aqueous media at three different pH conditions to simulate the pH conditions of the physiological fluids in the mouth (pH 7), stomach (pH 3), and colon (pH 8).

		pH neutral	**pH** ≈3	**pH** ≈8
		H_2O:CH_3CH_2OH (70:30 % v/v)	PB*:CH_3CH_2OH (70:30 %v/v)	Tris/HCl**:CH_3CH_2 OH (70:30 % v/v)
Samples	**Vol (mL)**	**weight(mg)**	**weight (mg)**	**weight (mg)**
ZP-RSV	20	2.70	3.14	2.97
ZP-RSV-ES100	4	4.37	4.47	4.36
Pure RSV	20	0.13	0.09	0.09
Blank ZP	20	3.24	3.14	3.56
Blank ZP-ES100	4	4.35	4.01	4.00

*Phosphate buffer 10 mM, pH=3. 100 mL of 0.20 M H_3PO_4 were prepared from the concentrated reagent (85% w/w, d=1.68 g/mL). The pH was adjusted with NaOH 2 M to get a final pH = 3 (PB 0.1 M). The buffer was diluted to get a final 10 mM concentration.

** Tris-HCl buffer 50 mM, pH=8. The buffer was prepared by dissolving 30.29 g of Trizma base in 350 mL of UP water. The pH was adjusted with HCl conc. to 8. This solution was diluted with UP water to get a final 50 mM Tris-HCl buffer.

S.3. Yield of production, RSV loading content and entrapment efficiency

Table S3: UV-VIS spectrophotometric measurements at λ = 306 nm: calibration curves used to determine the yield of production (Yp(%)), the drug (RSV) loading content (DLC(%)) and entrapment efficiency (EE(%)); ref. §3.2.3. and **Table 1**.

Tested samples	**Conc. range (μM) and (ppm)**	**Equation based on ppm**	**r^2**
	Prot-1 - DMSO		
ZP-RSV-US	0.438 – 17.5 0.1 – 4.0	y= 0.1009x – 0.0101	0.997
ZP-RSV-MS	2.19 – 17.5 0.5 – 4.0	y= 0.1293x – 0.0062	1.000
ZP-RSV-MS	2.19 – 17.5 0.5 – 4.0	y= 0.1169x + 0.0111	0.997
	Prot-2 – EtOH:H_2O 50:50		
ZP-RSV-US	0.438 – 21.9 0.1 – 5.0	y= 0.1199x+ 0.017	0.999
ZP-RSV-UT	0.438 – 21.9 0.1 – 5.0	y= 0.1159x+ 0.030	0.998

S.4 Additional figures

Figure captions

Fig. S1: HPLC-MS chromatograms for the (**a**) gastric and (**b**) duodenal phases for the RSV (100 ppm) analyzed in duplicate. Full mass 227 and ms/ms for the ion 227 → 185. Injection volume was 2 µL, retention time 4.10 min; flow programmed conditions (50% - 5% of CH_3COONH_4 in 8 min, then back to 50% of CH_3COONH_4 and 50% solvent acetonitrile for 8 min), flow rate 100 µL min^{-1} .

Fig. S2. Percentage of RSV amounts detected in simulated gastrointestinal fluids at the end of gastric and duodenal phases. RSV was introduced in simulated fluids as a water solution. Data are reported as the mean ± S.D. of three independent experiments.

Fig. S3: CF3 comparison between the ZP-RSV-US particles dispersed in water (green lines) and in a mixture ethanol:water 30:70 (pink lines). Particles were stored at room temperature (~ 25°C) for 1 day. **a**) Elugrams detected with the UV on-line detector set at 254 nm, carrier Fl70 0.1% v/v, 1.0 flow rate mL min^{-1}. **b**) Particle size distribution computed from the elugrams by using 1.226 g mL^{-1} as value for the particle density.

Fig. S4 (**a**) CF3 comparison between the particle size distributions of the ZP-RSV-US NPs (green line) and the ZP-RSV-US-ES100 obtained with a core: ES100 shell ratio 1:1 (red line). Particle size distribution computed from the elugrams by using 1.226 g mL^{-1} as value for the particle density. The UV on-line detector was set at 254 nm, carrier Fl70 0.1% v/v, 1.0 flow rate mL min^{-1}, initial rpm were 2000 and 1000 rpm respectively. (**b**) Elugrams of the ZP-RSV-US-ES100 obtained with a core : ES100 ratio of 1:1 and 1:2. Initial rpm 1000.

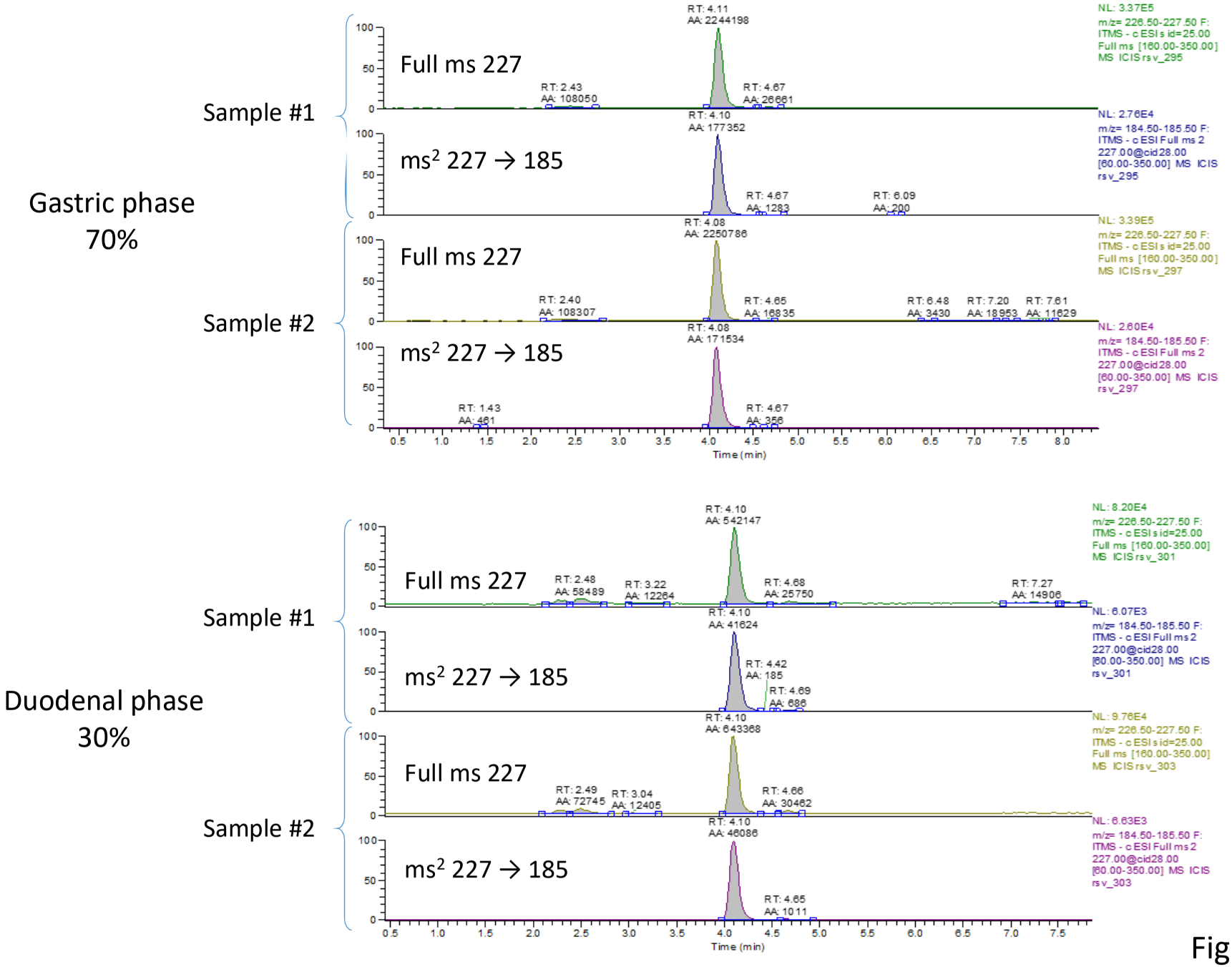
Gastric phase
70%
Sample #1
Full ms 227
RT: 4.11
AA: 2244198
RT: 2.43
AA: 108050
RT: 4.67
AA: 26661
NL: 3.37E5
m/z= 226.50-227.50 F:
ITMS - c ESI s id=25.00
Full ms [160.00-350.00]
MS ICIS rs v_295
ms2 227 → 185
RT: 4.10
AA: 177352
RT: 4.67
AA: 1283
RT: 6.09
AA: 200
NL: 2.76E4
m/z= 184.50-185.50 F:
ITMS - c ESI Full ms 2
227.00@cid28.00
[60.00-350.00] MS ICIS
rs v_295
Sample #2
Full ms 227
RT: 4.08
AA: 2250786
RT: 2.40
AA: 108307
RT: 4.65
AA: 16835
RT: 6.48
AA: 3430
RT: 7.20
AA: 18953
RT: 7.61
AA: 11629
NL: 3.39E5
m/z= 226.50-227.50 F:
ITMS - c ESI s id=25.00
Full ms [160.00-350.00]
MS ICIS rs v_297
ms2 227 → 185
RT: 4.08
AA: 171534
RT: 1.43
AA: 461
RT: 4.67
AA: 356
NL: 2.60E4
m/z= 184.50-185.50 F:
ITMS - c ESI Full ms 2
227.00@cid28.00
[60.00-350.00] MS ICIS
rs v_297
Time (min)
Duodenal phase
30%
Sample #1
Full ms 227
RT: 4.10
AA: 542147
RT: 2.48
AA: 58489
RT: 3.22
AA: 12264
RT: 4.68
AA: 25750
RT: 7.27
AA: 14906
NL: 8.20E4
m/z= 226.50-227.50 F:
ITMS - c ESI s id=25.00
Full ms [160.00-350.00]
MS ICIS rs v_301
ms2 227 → 185
RT: 4.10
AA: 41624
RT: 4.42
AA: 185
RT: 4.69
AA: 686
NL: 6.07E3
m/z= 184.50-185.50 F:
ITMS - c ESI Full ms 2
227.00@cid28.00
[60.00-350.00] MS ICIS
rs v_301
Sample #2
Full ms 227
RT: 4.10
AA: 643368
RT: 2.49
AA: 72745
RT: 3.04
AA: 12405
RT: 4.66
AA: 30462
NL: 9.76E4
m/z= 226.50-227.50 F:
ITMS - c ESI s id=25.00
Full ms [160.00-350.00]
MS ICIS rs v_303
ms2 227 → 185
RT: 4.10
AA: 46086
RT: 4.65
AA: 1011
NL: 6.63E3
m/z= 184.50-185.50 F:
ITMS - c ESI Full ms 2
227.00@cid28.00
[60.00-350.00] MS ICIS
rs v_303
Time (min)

Figure S1

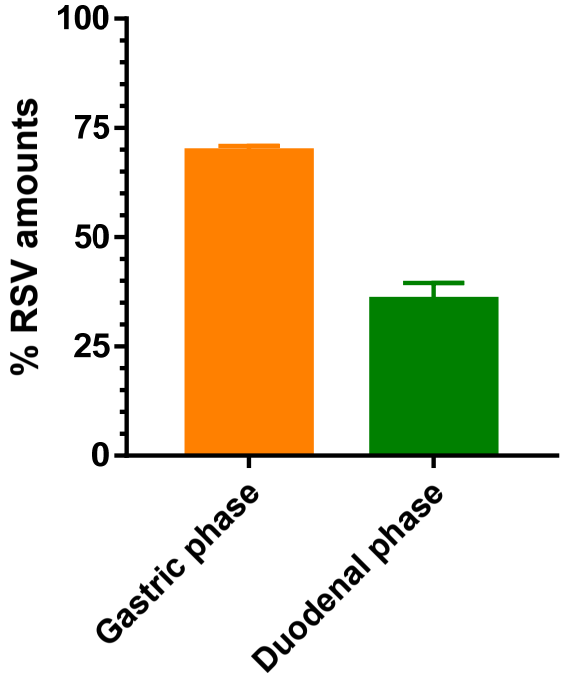

Figure S2

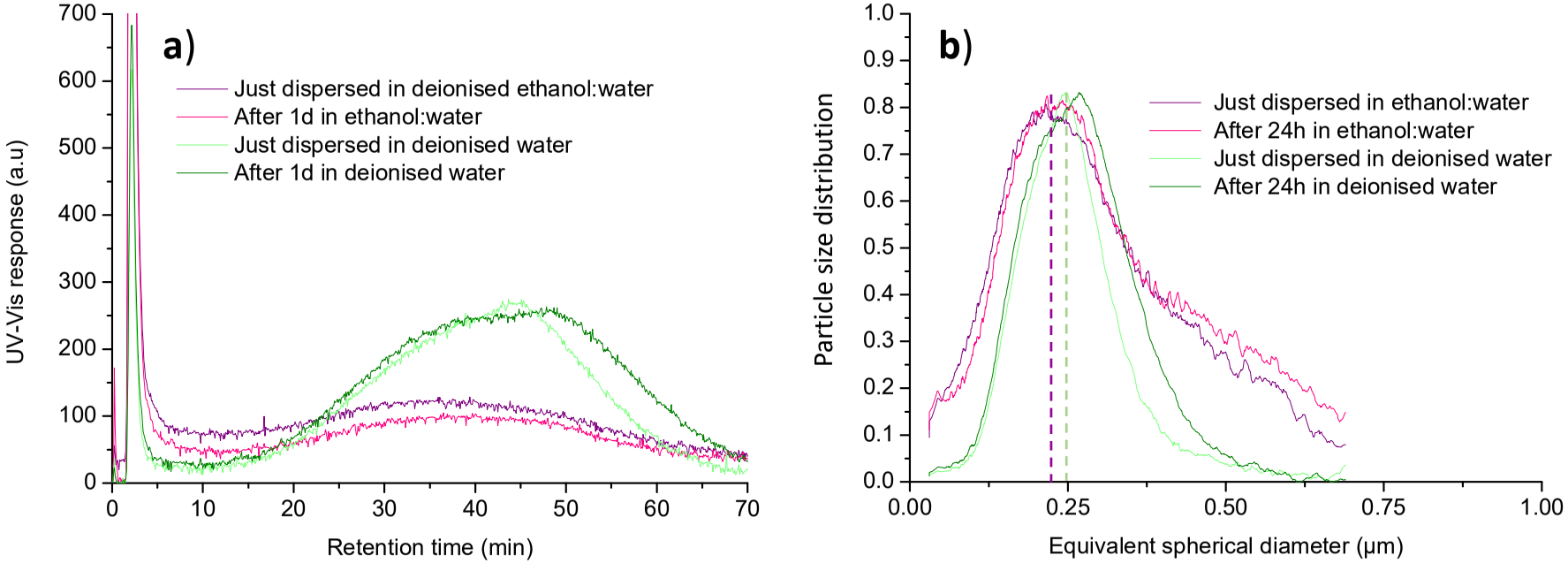
a)
Just dispersed in deionised ethanol:water
After 1d in ethanol:water
Just dispersed in deionised water
After 1d in deionised water
UV-Vis response (a.u)
Retention time (min)
0
100
200
300
400
500
600
700
0
10
20
30
40
50
60
70
b)
Just dispersed in ethanol:water
After 24h in ethanol:water
Just dispersed in deionised water
After 24h in deionised water
Particle size distribution
Equivalent spherical diameter (μm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.00
0.25
0.50
0.75
1.00

Figure S3

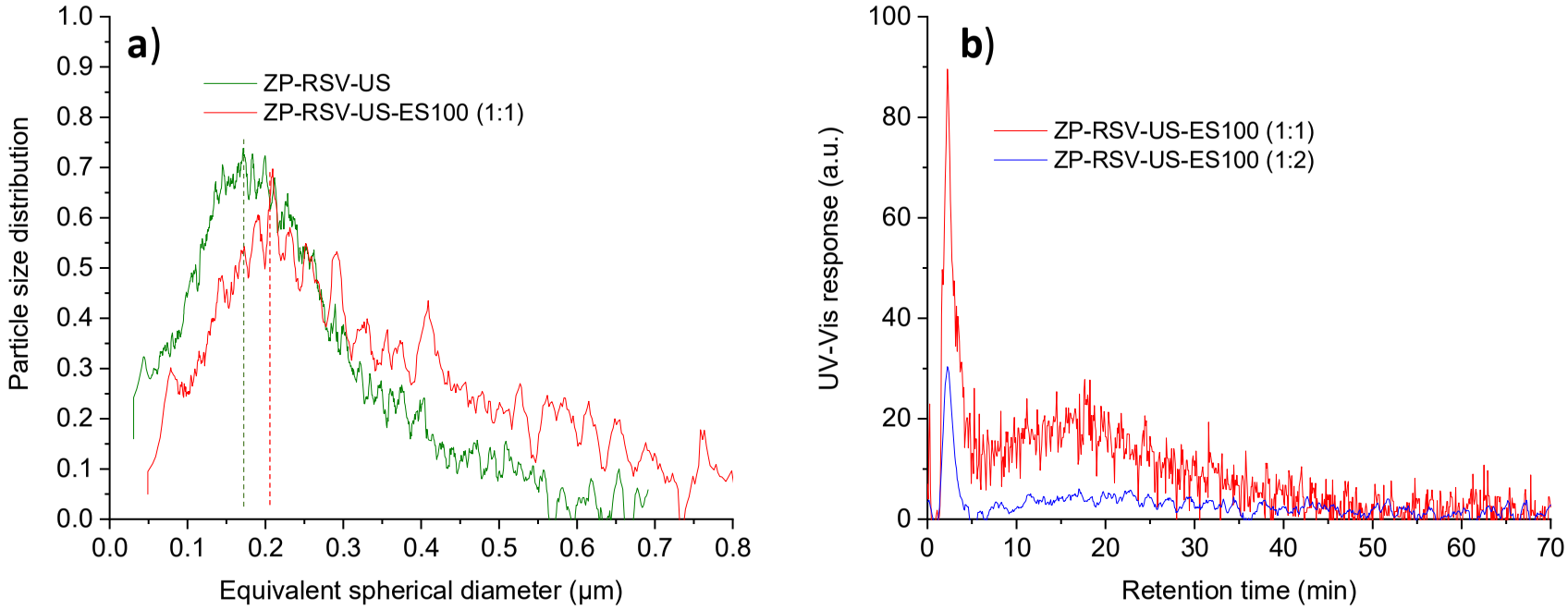
a)
ZP-RSV-US
ZP-RSV-US-ES100 (1:1)
Particle size distribution
Equivalent spherical diameter (µm)
b)
ZP-RSV-US-ES100 (1:1)
ZP-RSV-US-ES100 (1:2)
UV-Vis response (a.u.)
Retention time (min)

Figure S4