Supporting information

Matching drug and polymer for efficient delivery of anti-inflammatory drugs: PLGA, polyesteramides and acetalated dextran

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2 Synthesis of polymers

2.1 Materials

(*S*)-3-*Iso*-propylmorpholine-2,5-dione (ValG) and (*S*)-3-benzylmorpholine-2,5-dione (PheG) were synthesized according to Dirauf et al.[1] and Göppert *et al.*,[2] respectively. 1,3-Bis(3,5-bis(trifluoromethyl)phenyl)thiourea (TU) was synthesized according to Pratt *et al*.[3] 1,8-Diazabicyclo(5.4.0)undec-7-ene (≥ 99.0%, DBU), benzyl alcohol (99.8%, BnOH), and benzoic acid (≥ 99.5%) were purchased from Sigma-Aldrich. Dextran from Leuconostoc mesenteroides (average molar mass 9.000–11.000 g mol^{-1}), 2-methoxy-propene (97%), Pyridinium p-toluenesulfonate (PPTS, 98%) and dimethylsulfoxide (DMSO, anhydrous ≥99.9%) were obtained from Sigma-Aldrich. Triethylamine (99%) was purchased from Thermo Scientific. Dichloromethane (CH_2Cl_2) was dried in a solvent purification system (MB SPS-800, MBRAUN). All other chemicals and solvents were purchased from common suppliers and used without further purification.

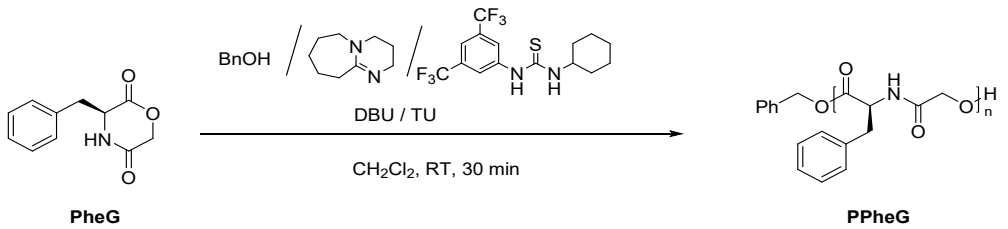
2.2 Instrumentation

Proton (^1H) nuclear magnetic resonance (NMR) spectra were measured on a Bruker AC 300 MHz spectrometer in $CDCl_3$ at room temperature using the residual non-deuterated solvent signal for chemical shift referencing.

Size exclusion chromatography (SEC) measurements were conducted on an Agilent 1200 series system equipped with a PSS degasser, a G1310A pump, a G1329A autosampler, a Techlab oven set to 40 °C, a PSS GRAM guard/30/1 000 Å column (10 µm particle size) and a G1362A refractive index detector (RID). *N,N*-Dimethylacetamide (DMAc) with 0.21 wt% LiCl was utilized as an eluent at a flow rate of 1 mL min^{-1}. The molar masses were estimated using PMMA standards (400 to 1 000 000 g mol^{-1}) from Polymer Standard Services (PSS).

Ring-opening polymerizations (ROP) were performed in a glovebox (MBRAUN) under nitrogen atmosphere.

2.3 Synthesis of PPheG



Scheme S1: Schematic representation of the ring-opening polymerization of **PheG** yielding poly((*S*)-3-benzylmorpholine-2,5-dione) (**PPheG**).

According to a molar [PheG]:[BnOH]:[DBU]:[TU] ratio of 100:1:1:10 and an initial monomer concentration of 0.5 mol L^{-1}, PheG (1.2 g, 5.85 mmol) was dissolved in 11.5 mL of CH_2Cl_2. A solution of benzyl alcohol (6.1 µL, 58 µmol), DBU (8.7 µL, 58 µmol) and TU (217 mg, 580 µmol) in 100 µL of CH_2Cl_2 was added to start the polymerization. The solution was stirred at room temperature for 40 min, and subsequently quenched by addition of a 10-fold excess of benzoic acid (71 mg, 0.58 mmol). A sample was withdrawn to determine the monomer conversion by means of ^1H NMR spectroscopy. The polymer was precipitated from diethyl ether (-80 °C) twice and dried in oil pump vacuum. Yield: 0.9 g. ^1H NMR: Conversion: 90 %, $M_{n,theo}$ = 18 kg mol^{-1}. SEC: M_n = 26.5 kg mol^{-1}, Đ = 1.46.

Characterization data (^1H NMR, SEC) are depicted in **Figure S1**.

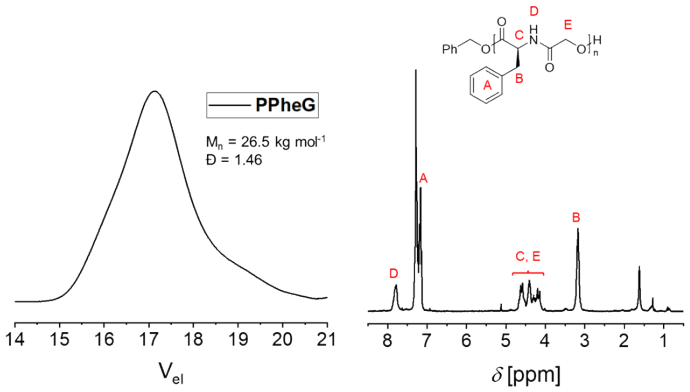
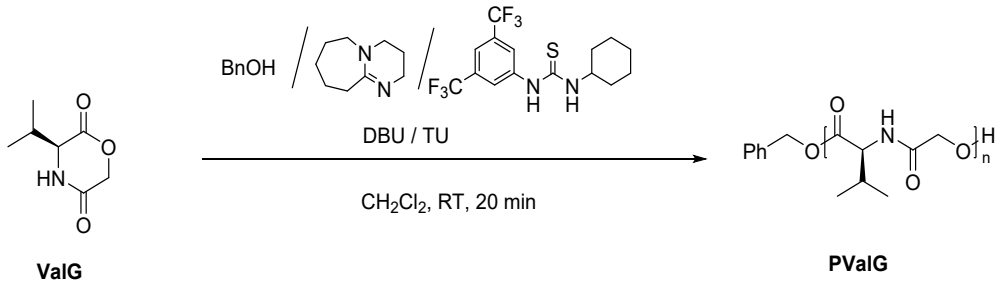


Figure S1: Left: SEC Elugram of **PPheG** (Eluent DMAc, RID, PMMA calibration). Right: ^{1}H NMR spectrum of **PPheG** (300 MHz, $CDCl_3$) and assignment of the signals to the schematic representation of the structure.

2.4 Synthesis of PValG



Scheme S2: Schematic representation of the ring-opening polymerization of ValG yielding poly((*S*)-3-isopropylmorpholine-2,5-dione) (**PValG**).

According to a molar [ValG]:[BnOH]:[DBU]:[TU] ratio of 100:1:1:5 and an initial monomer concentration of 0.7 mol L^{-1}, ValG (1.3 g, 8.28 mmol) was dissolved in 11.5 mL of CH_2Cl_2. A solution of benzyl alcohol (8.6 µL, 83 µmol), DBU (12.4 µL, 83 µmol) and TU (153 mg, 414 µmol) in 250 µL of CH_2Cl_2 was added to start the polymerization. The solution was stirred at room temperature for 48 min, and subsequently quenched by addition of a 10-fold excess of benzoic acid (100 mg, 0.83 mmol). A sample was withdrawn to determine the monomer conversion by means of ^{1}H NMR spectroscopy. The polymer was precipitated from diethyl ether (−80 °C) three times and dried in oil pump vacuum. Yield: 1.1 g. ^{1}H NMR: Conversion: 93 %, $M_{n,theo}$ = 14.6 kg mol^{-1}. SEC: M_n = 26.8 kg mol^{-1}, Đ = 1.12.

Characterization data (^{1}H NMR, SEC) are depicted in **Figure S2**.

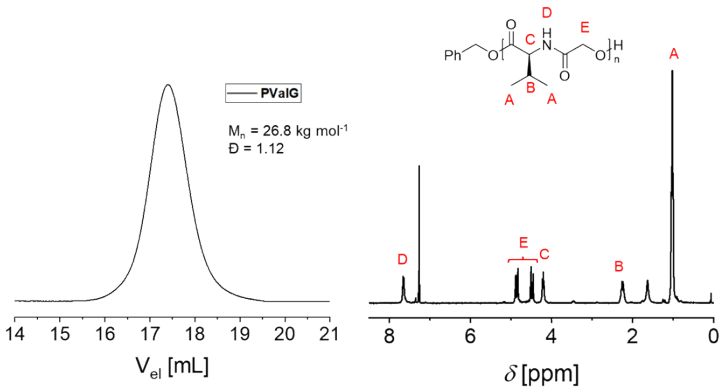
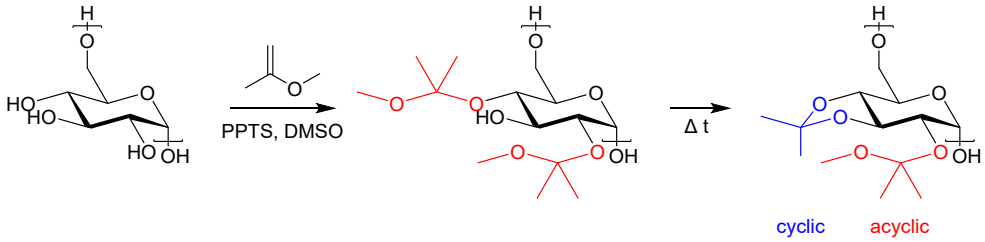


Figure S2: Left: SEC Elugram of **PValG** (Eluent DMAc, RID, PMMA calibration). Right: ^{1}H NMR spectrum of **PValG** (300 MHz, $CDCl_3$) and assignment of the signals to the schematic representation of the structure.

2.5 Synthesis of Ac-Dex



Scheme S3: Schematic representation of the acetalization of dextran yielding Ac-Dex.

Ac-Dex was synthesized from 10 kDa dextran according to an adapted procedure.[4] The final degree of substitution (DS) as determined via 1H NMR spectroscopy of the degradation-products (Fig. S5 Right),[5] was 2.06 (DS cyclic acetal = 1.44, DS acyclic acetal = 0.62).

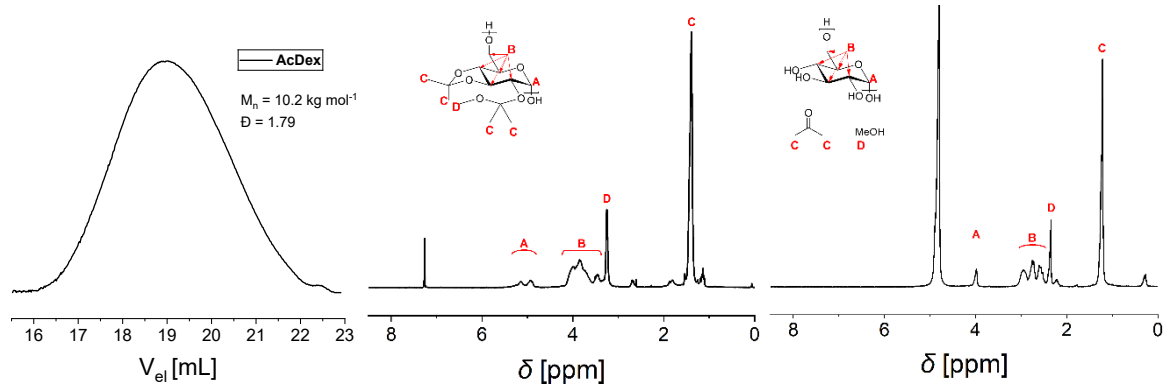


Figure S3: Left: SEC Elugram of **Ac-Dex** (Eluent 0.21 w% LiCl in DMAc, RID, PMMA calibration). Middle: ^{1}H NMR spectrum of **Ac-Dex** (300 MHz, $CDCl_3$) and assignment of the signals to the schematic representation of the structure. Right: ^{1}H NMR spectrum of **Ac-Dex** (300 MHz, D_2O with DCl) and assignment of the signals to the schematic representation of the structure of its degradation products.

3 Formulation details

Table S1: Formulation parameters for nanoprecipitation of NPs. Solvent to water ratio for all formulations was 1 to 8. The water phase was a 0.3% PVA solution. All formulations performed with n = 3.

Sample	$c_{Polymer}$ [mg mL^{-1}][a]	Drug feed [%][b]	Solvent
PValG	15	-	acetone
PValG [MF-15]	15	5	acetone
PValG [BRP-201]	10	5	acetone
PLGA	15	-	acetone
PLGA [MF-15]	15	5	acetone
PLGA [BRP-201]	10	5	acetone
Ac-Dex	15	-	acetone
Ac-Dex [MF-15]	15	5	acetone
Ac-Dex [BRP-201]	10	5	acetone
PPheG	15	-	DMSO
PPheG [MF-15]	15	5	DMSO
PPheG [BRP-201]	10	5	DMSO

a) $c_{Polymer}$ in organic solvent.
b) Drug feed in wt% referred to polymer mass.

4 Formulation characteristics

Table S2: Determined characteristics of formulations.

Sample	d_H [nm] purified[a]	PDI purified[a]	ζ in water [mV][b]	c_{NP} [mg/mL][c]	c_{PVA} [mg/mL][e]	$c_{NP\ w/o\ PVA}$ [mg/mL][f]	LC [%][d]	EE [%][d]	Yield [%][g]
PValG	136 ± 8	0.11 ± 0.03	−29.8 ± 2.4	5.79 ± 0.13	0.156 ± 0.002	5.64 ± 0.13	-	-	74.2 ± 1.9
PValG [MF-15]	146 ± 3	0.07 ± 0.01	−31.8 ± 2.1	6.60 ± 0.13	0.165 ± 0.007	6.43 ± 0.13	3.71 ± 0.01	74.3 ± 0.23	77.5 ± 2.2
PValG [BRP-201]	159 ± 3	0.23 ± 0.03	−18.0 ± 1.7	2.6 ± 0.22	0.139 ± 0.014	2.52 ± 0.21	1.53* ± 0.00	26.1* ± 0.03	34.5 ± 16.5
PLGA	184 ± 1	0.03 ± 0.01	−37.3 ± 1.1	6.62 ± 0.18	0.141 ± 0.009	6.48 ± 0.18	-	-	81.5 ± 3.9
PLGA [MF-15]	184 ± 3	0.08 ± 0.02	−26.5 ± 13.7	7.02 ± 0.13	0.146 ± 0.009	6.87 ± 0.13	3.73 ± 0.05	74.5 ± 0.90	83.0 ± 2.6
PLGA [BRP-201]	175 ± 9	0.12 ± 0.04	−23.5 ± 2.3	4.22 ± 0.32	0.154 ± 0.007	4.07 ± 0.33	4.08* ± 0.11	81.6* ± 2.24	67.3 ± 1.1
Ac-Dex	133 ± 9	0.06 ± 0.00	−19.3 ± 9.0	6.11 ± 0.21	0.144 ± 0.009	5.96 ± 0.22	-	-	73.1 ± 1.7
Ac-Dex [MF-15]	178 ± 8	0.04 ± 0.01	−31.0 ± 1.1	6.09 ± 0.19	0.128 ± 0.016	5.96 ± 0.18	4.18 ± 0.04	83.6 ± 0.77	73.5 ± 1.2
Ac-Dex [BRP-201]	192 ± 14	0.06 ± 0.00	−29.6 ± 3.1	3.57 ± 0.33	0.092 ± 0.002	3.48 ± 0.32	2.98* ± 0.19	59.6* ± 3.70	64.4 ± 4.7
PPheG	110 ± 4	0.05 ± 0.00	−22.8 ± 2.4	5.13 ± 0.73	0.182 ± 0.018	4.95 ± 0.75	-	-	61.1 ± 11.1
PPheG [MF-15]	120 ± 4	0.03 ± 0.01	−29.9 ± 0.9	6.41 ± 0.47	0.179 ± 0.008	6.23 ± 0.46	2.82 ± 0.01	56.3 ± 0.12	76.3 ± 6.9
PPheG [BRP-201]	150 ± 6	0.04 ± 0.01	−22.3 ± 0.6	4.50 ± 0.22	0.122 ± 0.013	4.38 ± 0.23	3.12* ± 0.03	62.3* ± 0.69	80.4 ± 2.3

a) Hydrodynamic diameter (d_H, z-average) and polydispersity index (PDI) determined by DLS.

b) Zeta potential (ζ) determined by ELS.

c) Particle concentration after purification determined by weighting the freeze-dried particles.

d) Loading capacity (LC) and encapsulation efficiency (EE) determined by HPLC.

e) Polyvinyl alcohol (PVA) concentration in whole formulation determined by UV-Vis spectroscopy.

f) Determined PVA amount subtracted from particle concentration to determine the c_{NP} without (w/o) PVA.

g) Yield after purification determined by dividing the actual obtained particle mass by the theoretically possible particle mass multiplied by 100.

All formulations performed with n = 3, LC and EE values marked with an * only determined for n = 2.

Table S3: NP characteristics after resuspension.

Sample	d_H [nm] resus. w/o PVA[a]	PDI resus. w/o PVA[a]	d_H [nm] resus. w/ PVA[b]	PDI Resus. w/ PVA[b]
PValG	563 ± 71	0.41 ± 0.04	172 ± 13	0.18 ± 0.05
PValG [MF-15]	286 ± 66	0.28 ± 0.05	170 ± 7	0.15 ± 0.05
PValG [BRP-201]	367 ± 210	0.27 ± 0.11	152 ± 5	0.08 ± 0.02
PLGA	221 ± 19	0.10 ± 0.02	215 ± 26	0.08 ± 0.02
PLGA [MF-15]	196 ± 6	0.10 ± 0.02	195 ± 2	0.09 ± 0.03
PLGA [BRP-201]	203 ± 13	0.08 ± 0.02	197 ± 22	0.07 ± 0.02
Ac-Dex	210 ± 38	0.21 ± 0.02	215 ± 8	0.21 ± 0.03
Ac-Dex [MF-15]	184 ± 8	0.07 ± 0.01	185 ± 7	0.06 ± 0.02
Ac-Dex [BRP-201]	215 ± 12	0.08 ± 0.04	190 ± 6	0.05 ± 0.01
PPheG	430 ± 75	0.26 ± 0.05	241 ± 22	0.27 ± 0.08
PPheG [MF-15]	430 ± 136	0.33 ± 0.10	285 ± 79	0.28 ± 0.07
PPheG [BRP-201]	352 ± 72	0.27 ± 0.02	202 ± 29	0.16 ± 0.05

a) Hydrodynamic diameter (d_H, z-average) and polydispersity index (PDI) determined by DLS after resuspension of the freeze-dried particles without (wo) the addition of PVA.

b) Hydrodynamic diameter (d_H, z-average) and polydispersity index (PDI) determined by DLS after resuspension of the freeze-dried particles with (w) the addition of small amounts (**PLGA**, **Ac-Dex** 1 µL, **PValG**, **PPheG** 10 µL) of 3% (w/v) PVA aqueous solution before freeze-drying.

All resuspensions were performed with n = 3 different formulations.

4.1 DLS

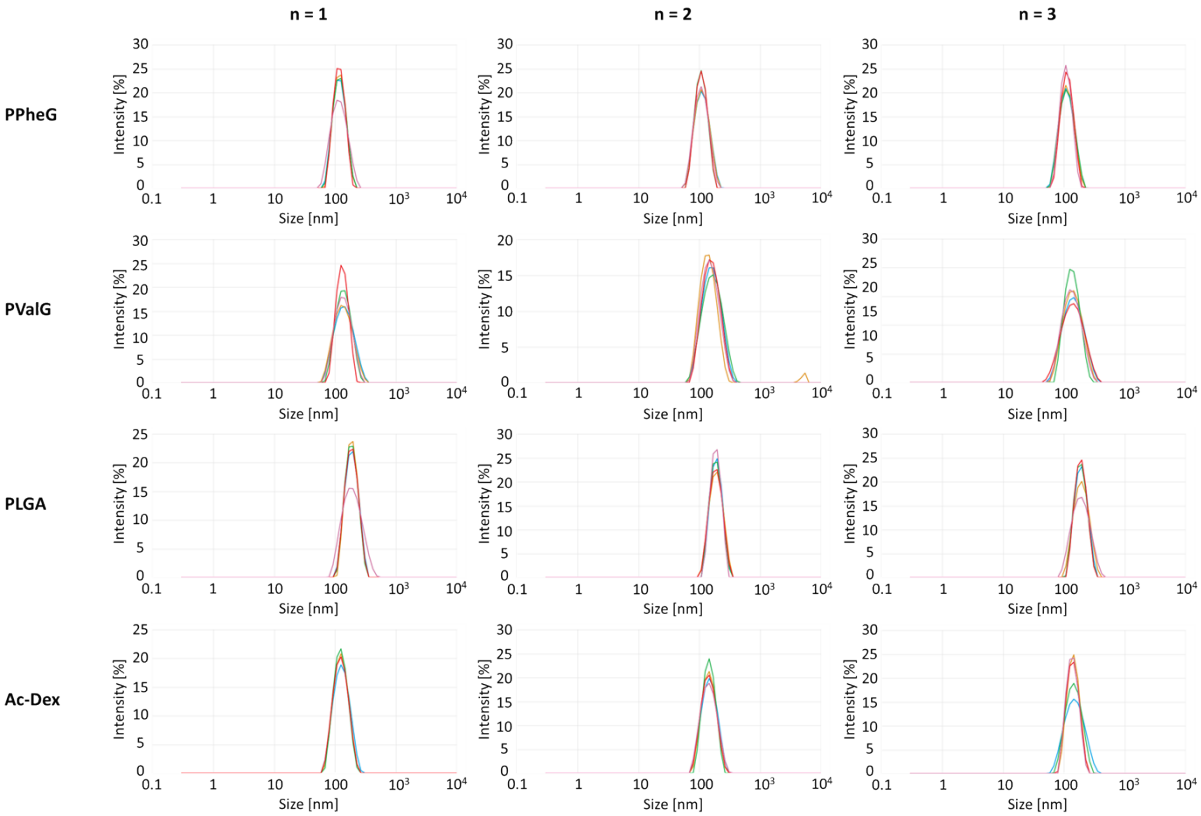


Figure S4: Intensity-based hydrodynamic diameter distributions from DLS measurements of purified blank particles (five measurements each of n = 3 individual formulations).

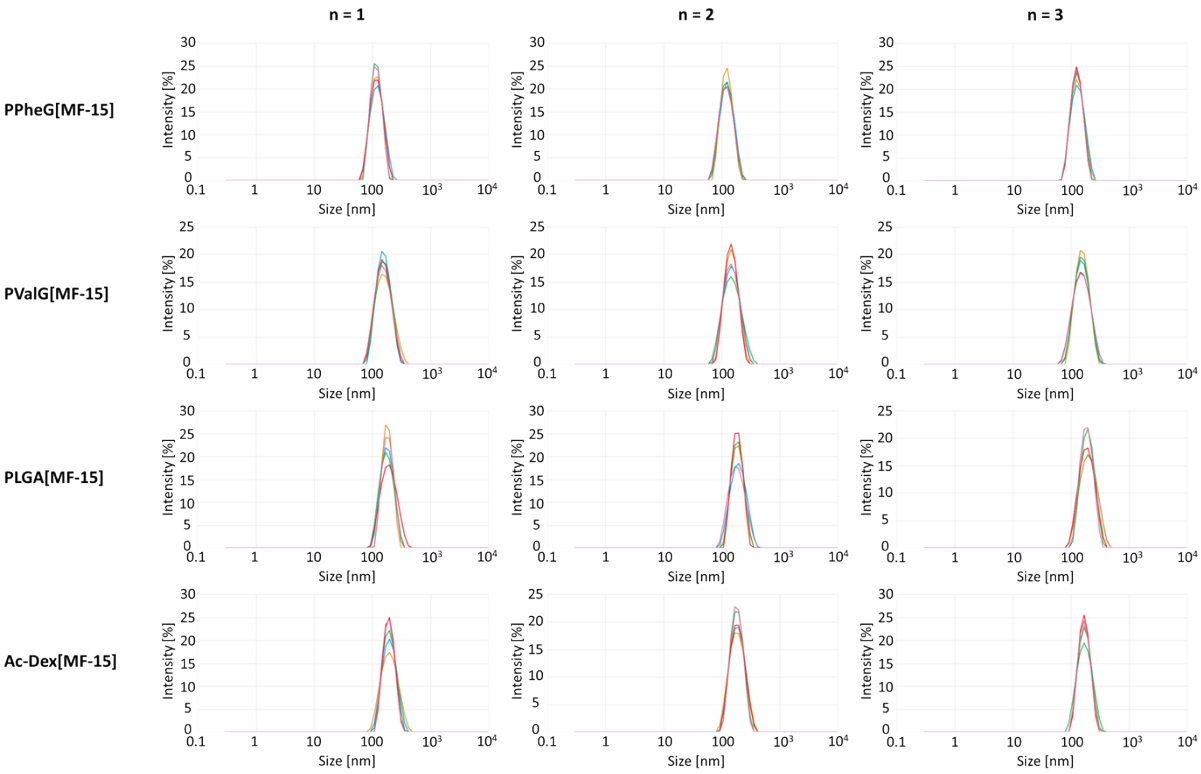


Figure S5: Intensity-based hydrodynamic diameter distributions from DLS measurements of purified MF-15 loaded particles (five measurements each of n = 3 individual formulations).

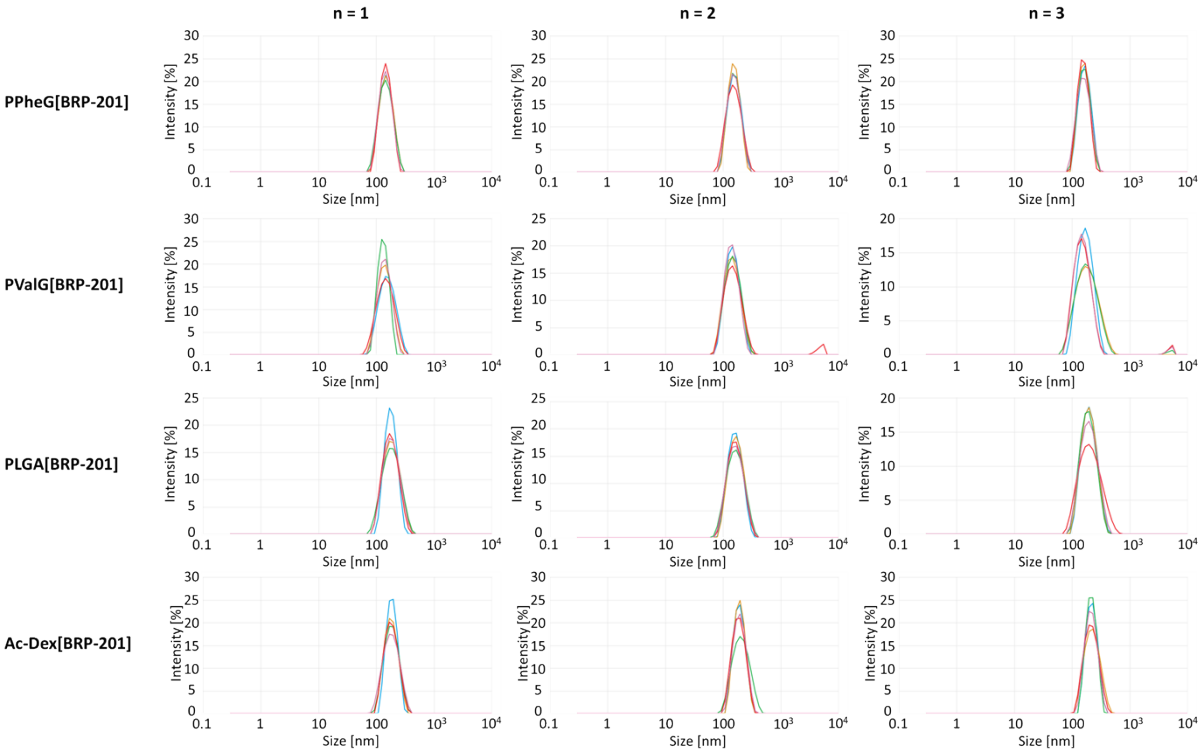


Figure S6: Intensity-based hydrodynamic diameter distribution from DLS measurements of BRP-201 loaded particles (five measurements each of n = 3 individual formulations).

4.2 BRP-201 drug precipitates

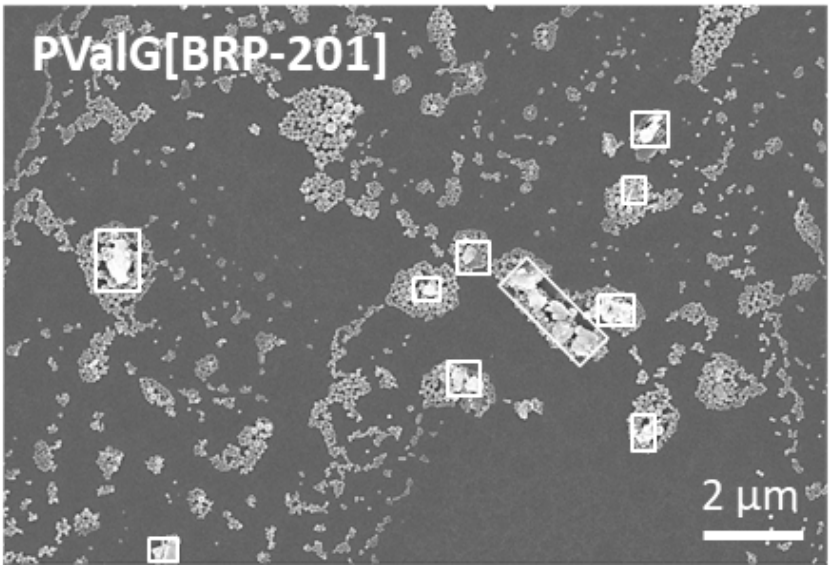


Figure S7: SEM image of BRP-201 loaded **PValG** particles showing free drug precipitates (highlighted with white frames) before additional purification *via* centrifugation.

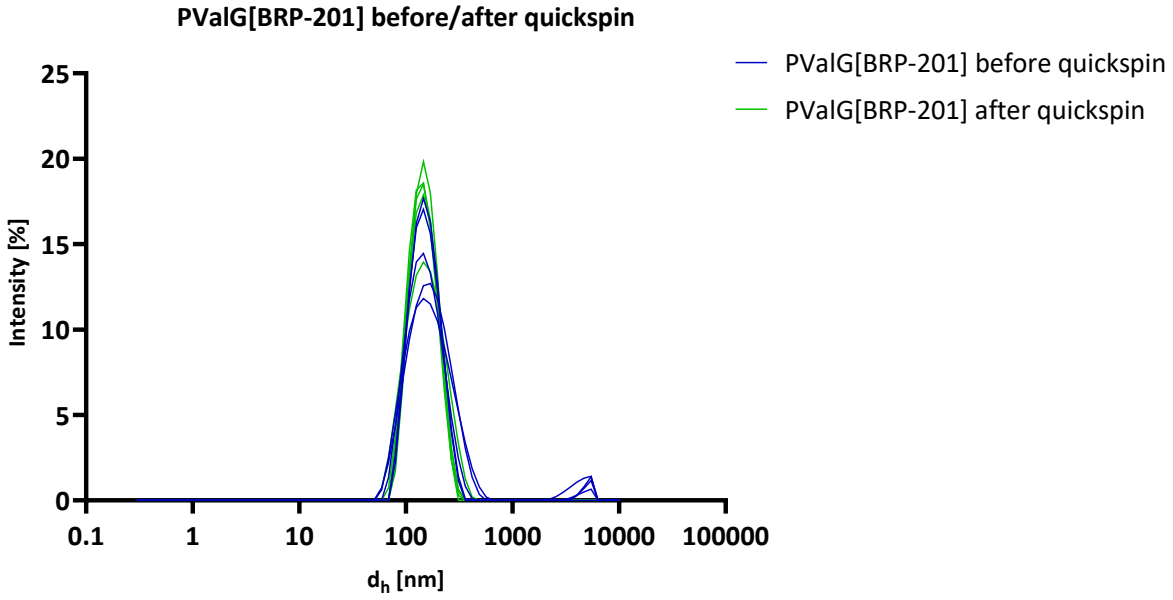


Figure S8: DLS size distributions of the five measurements of **PValG[BRP-201]** before and after additional purification using quick spin to remove the free drug precipitates from the particle formulation.

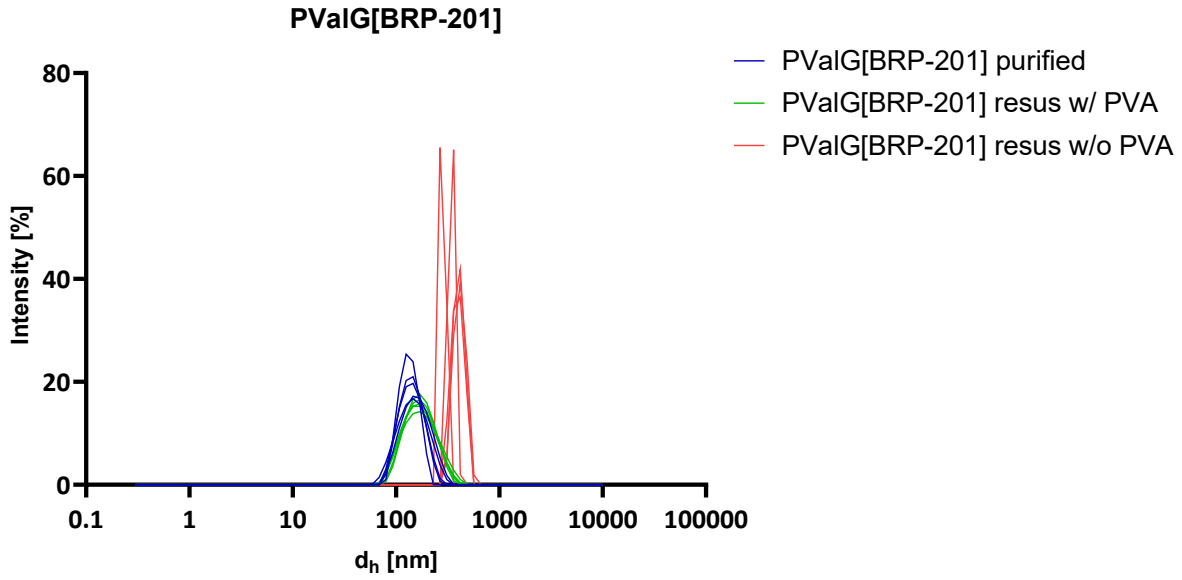


Figure S9: DLS size distributions of the five measurements of **PValG[BRP-201]** after purification and resuspension with (w/) and without (w/o) PVA.

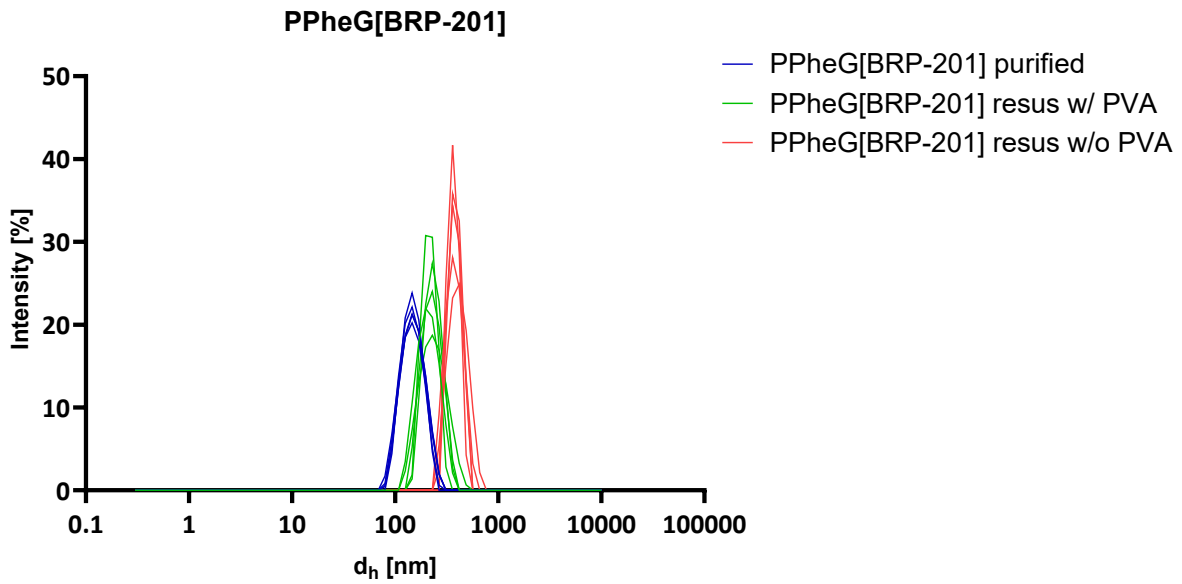


Figure S10: Size distributions of the five measurements of **PPheG[BRP-201]** after purification and resuspension with (w) and without (wo) PVA.

5 HPLC measurements

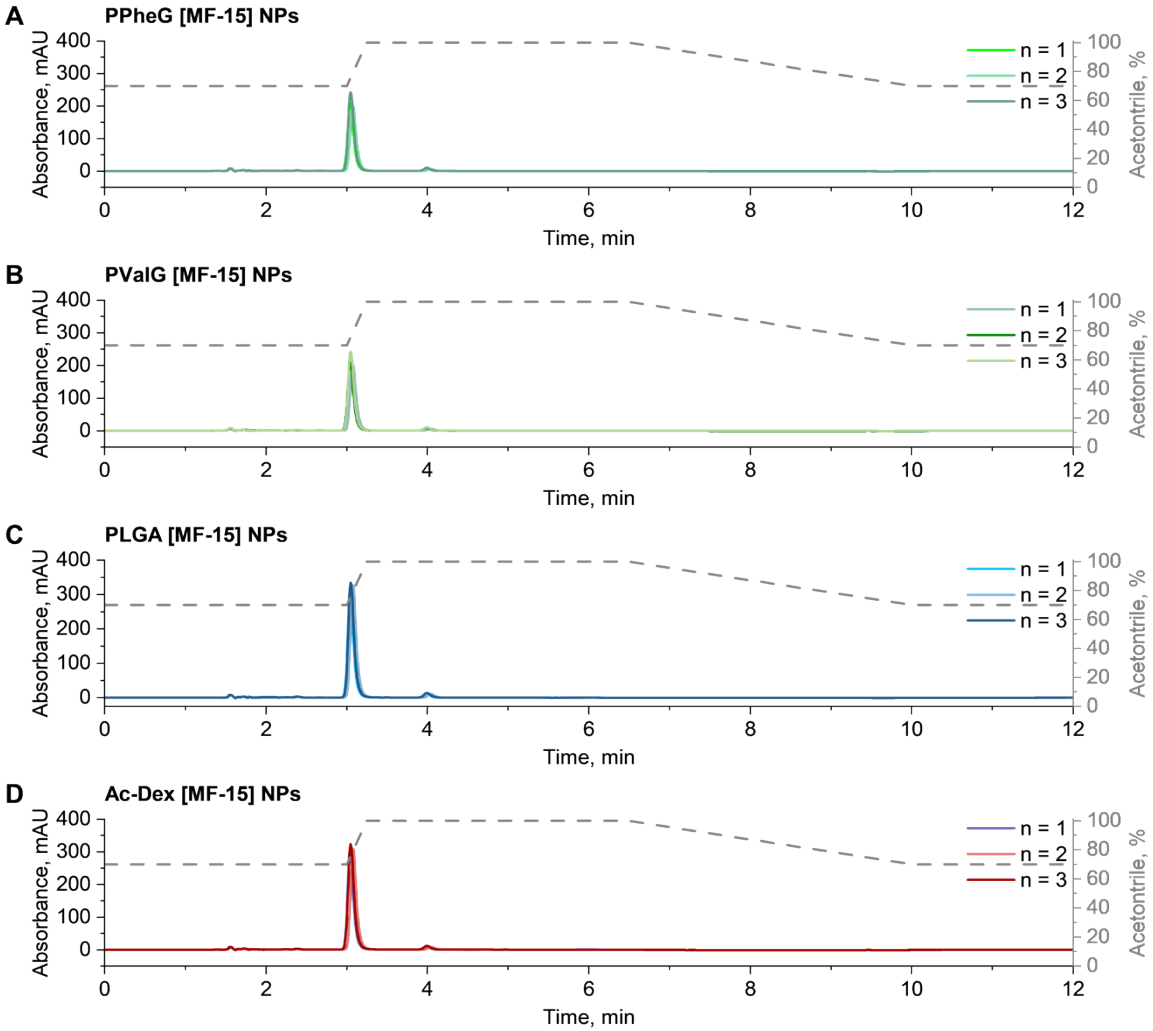


Figure S11: Overlaid elugrams recorded by DAD at 290 nm and received from three repetitive formulations containing MF-15 for each polymer (n = 1, 2, 3). (**A**) **PPheG**, (**B**) **PValG**, (**C**) **PLGA**, and (**D**) **Ac-Dex** nanoparticles. The peak at 3.1 min elution time refers to MF-15. The gray dashed line indicates the CH_3CN gradient elution program. Chromatographic conditions: CH_3CN/0.1 wt% aqueous H_3PO_4 as the mobile phase, flow rate 1 mL min^{-1}.

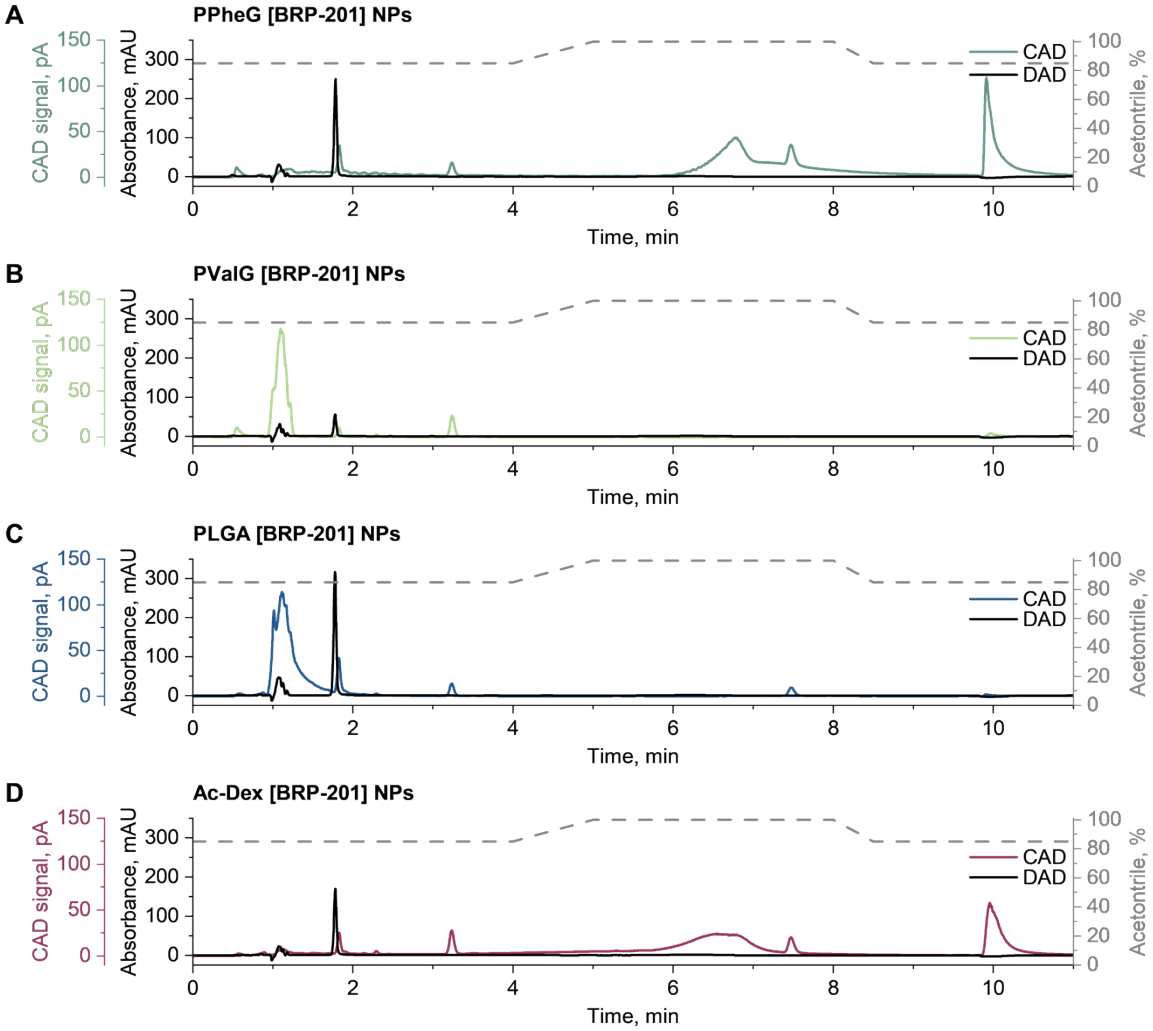


Figure S12: Elution pattern of one exemplified batch of BRP-201-loaded polymer NPs recorded by universal CAD (colored trace) and DAD (black trace) operated at 312 nm. The gray dashed line indicates the gradient elution programming of the acetonitrile/water mobile phase.

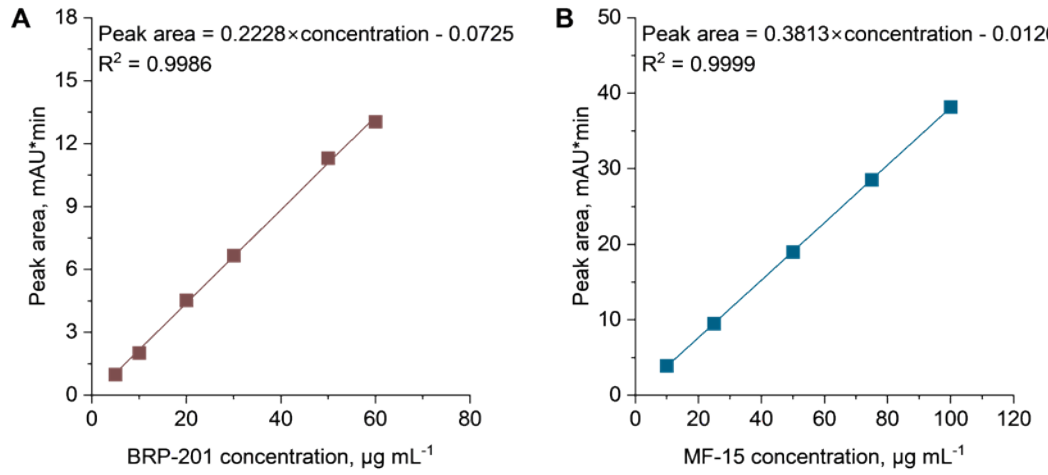


Figure S13: Calibration curves for (**A**) BRP-201 and (**B**) MF-15 presented by plotting peak areas as a function of analyte concentrations. Data were fitted linearly.

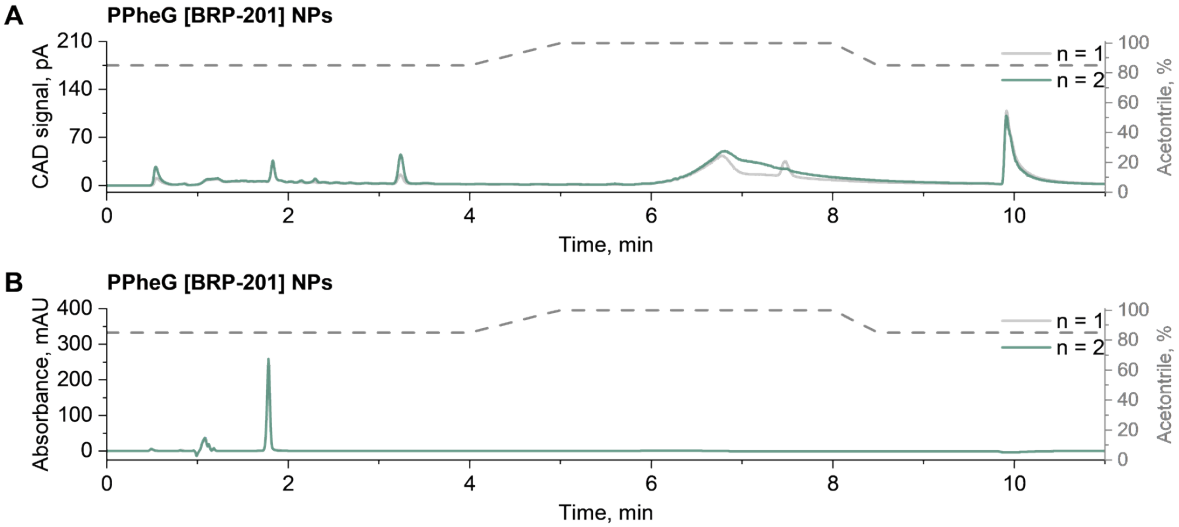


Figure S14: Overlaid elugrams of two repetitive formulations of **PPheG** NPs containing BRP-201 (n = 1, 2) recorded by (**A**) CAD and (**B**) DAD at 312 nm. The peak at 1.8 min elution time refers to BRP-201. The gray dashed line indicates the CH_3CN gradient elution programming. Measurement conditions: CH_3CN/H_2O mobile phase, flow rate 1.5 mL min^{-1}.

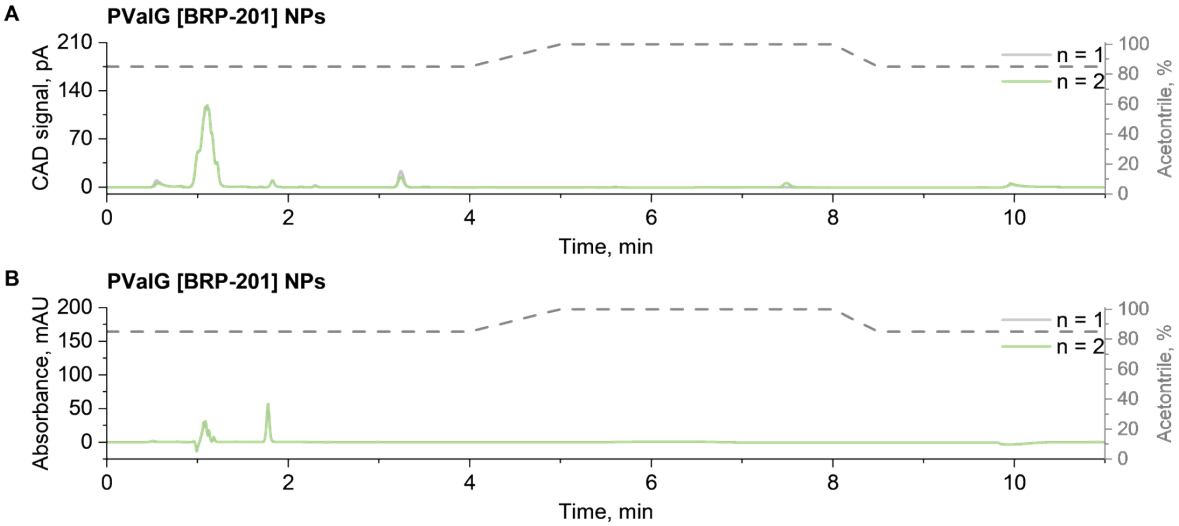


Figure S15: Overlaid elugrams of two repetitive formulations (n = 1, 2) of **PPheG** NPs containing BRP-201 recorded by (**A**) CAD and (**B**) DAD at 312 nm. The peak at 1.8 min elution time refers to BRP-201. The gray dashed line indicates the CH_3CN gradient elution programming. Measurement conditions: CH_3CN/H_2O mobile phase, flow rate 1.5 mL min^{-1}.

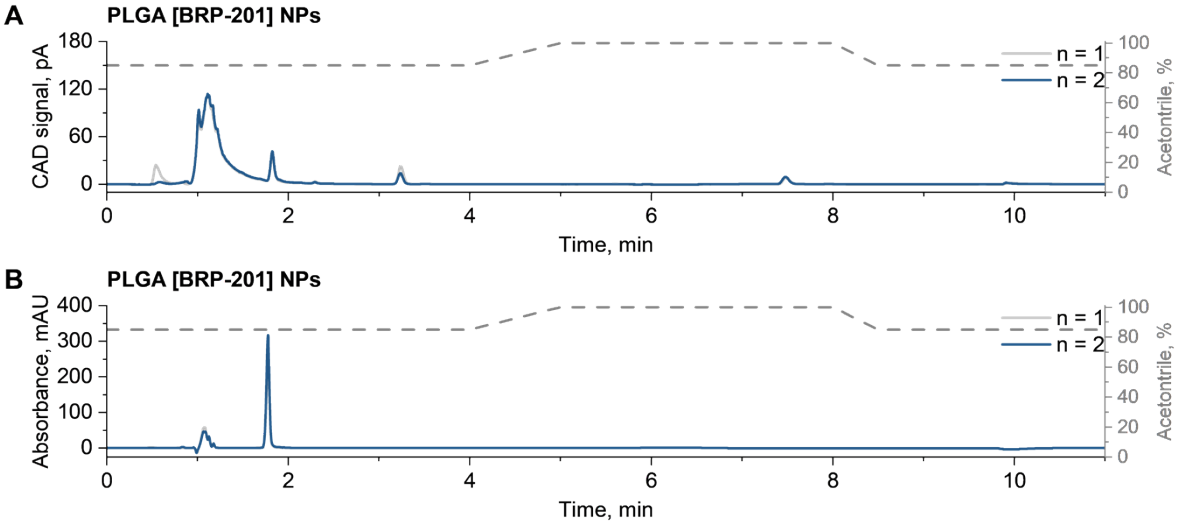


Figure S16: Overlaid elugrams of two repetitive formulations (n = 1, 2) of **PLGA** NPs containing BRP-201 recorded by (**A**) CAD and (**B**) DAD at 312 nm. The peak at 1.8 min elution time refers to BRP-201. The gray dashed line indicates the CH_3CN gradient elution program. Measurement conditions: CH_3CN/H_2O mobile phase, flow rate 1.5 mL min^{-1}.

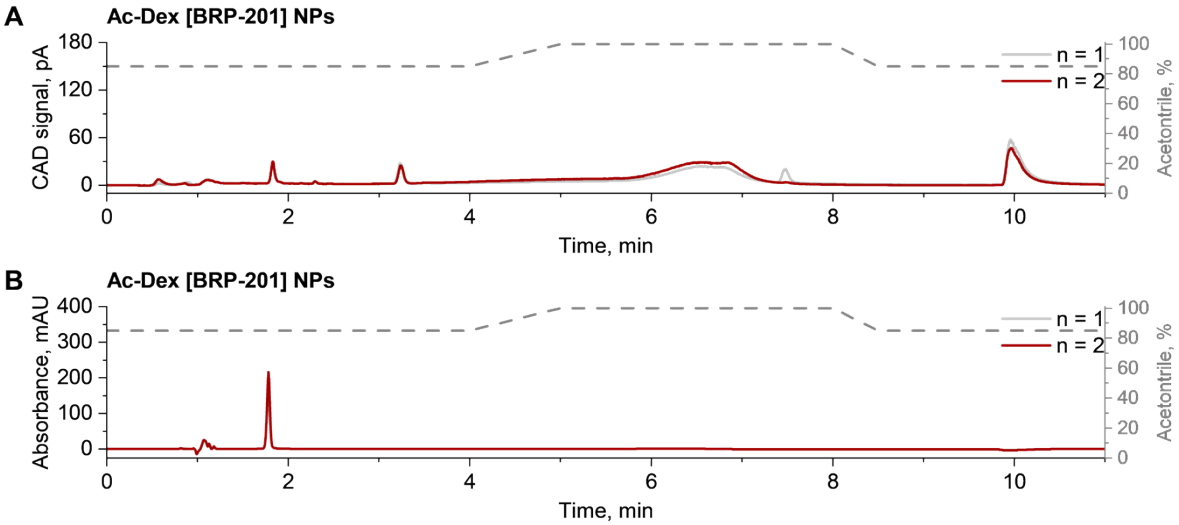


Figure S17: Overlaid elugrams of three repetitive formulations (n = 1, 2) of **Ac-Dex** NPs containing BRP-201 recorded by (**A**) CAD and (**B**) DAD at 312 nm. The peak at 1.8 min elution time refers to BRP-201. The gray dashed line indicates the CH_3CN gradient elution programming. Measurement conditions: CH_3CN/H_2O mobile phase, flow rate 1.5 mL min-^{1}.

6 References

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