Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary information

Insight into the influence of the polymerization time of polydopamine nanoparticles on their size, surface properties and nanomedical applications

Table S1 PDA(EtOH) NP diameters as a function of the NH_4OH concentration used to produced them and the reaction time. Size values are those represented in Fig. 1(a).

NH_4OH (%V/V)	PDA(EtOH) NP diameter (nm)				
	0.16 h	**1 h**	**3 h**	**5 h**	**24 h**
1.19	217.3 ± 52.9	313.3 ± 66.0	325.3 ± 65.6	341.9 ± 87.7	359.6 ± 77.5
1.58	212.1 ± 47.3	278.0 ± 57.0	286.4 ± 57.6	286.9 ± 60.7	293.2 ± 58.9
1.98	204.6 ± 48.1	220.3 ± 45.3	212.8 ± 49.8	219.1 ± 55.5	229.1 ± 45.9
2.60	147.2 ± 55.6	164.8 ± 41.4	166.0 ± 36.4	171.9 ± 36.6	180.0 ± 39.9
3.00	126.1 ± 41.7	138.3 ± 36.9	143.5 ± 36.9	152.7 ± 37.5	157.1 ± 40.7

Table S2 PDA(2-PrOH) NP diameters as a function of the NH_4OH concentration used to produced them and the reaction time. Size values are those represented in Fig. 1(b).

NH_4OH (%V/V)	PDA(2-PrOH) NP diameter (nm)				
	0.16 h	**1 h**	**3 h**	**5 h**	**24 h**
1.19	169.3 ± 54.6	244.8 ± 52.6	254.0 ± 55.4	254.9 ± 53.2	274.9 ± 62.1
1.58	148.4 ± 32.4	179.0 ± 41.9	187.6 ± 41.6	194.2 ± 45.0	209.0 ± 58.2
1.98	128.3 ± 68.1	149.7 ± 35.0	155.5 ± 42.0	157.2 ± 36.2	167.4 ± 36.3

Table S3 Yield of DA polymer as a function of the final polymerization time and the NH_4OH concentration used to produce PDA(ROH) NPs.

ROH	**EtOH**			**2-PrOH**	
Final reaction time	**1.98% (V/V)**	**2.60% (V/V)**	**3.00% (V/V)**	**1.58% (V/V)**	**1.98% (V/V)**
1h	11.7	6.88	3.9	9.9	3.6
3 h	7.8	5.44	3.2	4.36	3.1
5 h	6.9	5.62	3.3	5.32	2.4
24 h	6.9	5.52	2.6	5.22	2.2

Table S4 Values obtained for the parameters of the mathematical model proposed to predict the PDA(ROH) NP diameter as a function of final reaction time and the NH_4OH concentration and ROH used for their synthesis. Only *A* varied as a function of the ROH type because this parameter precisely represented ROH effect on the polymerization process. The rest of the parameters were the same for both types of PDA(ROH) NPs. Average NP diameter data fitting to all experimental data was performed by using the Generalized Reduced Gradient (GRG Nonlinear) tab in Excel ® Solver Options, minimizing the residual sum of squares (RSS).

Equation parameter	EtOH	2-PrOH
A (nm*(% (V/V))^x	637.9	451.9
n (d-less)	0.27	0.27
x (d-less)	0.94	0.94
k (min^{-1})	0.69	0.69

Table S5 Comparison of PDA(EtOH) NP diameter values obtained experimentally and theoretically (Eq.1., *A* = 637.9) as a function of the NH_4OH concentration and the final reaction time.

		PDA (EtOH) NP diameter (nm)	
Final reaction time / [NH_4OH]	Model	Experimental	Difference between model and experimental diameters
1h / 1.98% (V/V)	210	227.1 ± 73.2 (PDI: 0.12)	17.1
3 h / 2.87% (V/V)	151	131.1 ± 35.6 (PDI: 0.047)	19.1
5 h / 2.60% (V/V)	170	165.3 ± 44.2 (PDI: 0.044)	4.7
24 h / 2.76% (V/V)	165	177.4 ± 41.9 (PDI: 0.08)	12.4

Table S6 Comparison of PDA(2-PrOH) NP diameter values obtained experimentally and theoretically (Eq.1., *A* = 451.9) as a function of the NH_4OH concentration and the final reaction time.

		PDA (2-PrOH) NP diameter (nm)	
Final reaction time / [NH_4OH]	Model	Experimental	Difference between model and experimental diameters
1h / 1.56% (V/V)	180.5	200.7 ± 39.29 (PDI: 0.1)	20.2
3 h / 1.98% (V/V)	154.8	158.3 ± 56 (PDI: 0.086)	3.5
5 h / 1.81% (V/V)	173	170.4 ± 58.7 (PDI: 0.012)	3.9
24 h / 1.42% (V/V)	223	237.8 ± 67.4 (PDI: 0.058)	14.8

Table S7 Viability (%) of BT474 cells after treatment with the PDA(ROH) NPs (0.035 mg/mL) obtained after different reaction times. These values correspond to those represented in Fig. 9.

BT474 cells	PDA (EtOH) NPs			PDA (2-PrOH) NPs		
Final reaction time	24 h	48 h	72 h	24 h	48 h	72 h
1h	79.6 ± 2.4	74.6 ± 1.3	71.2 ± 0.6	98.4 ± 6.8	78.1 ± 3.0	66.6 ± 1.3
3 h	78.0 ± 3.9	73.7 ± 1.2	72.5 ± 0.19	96.6 ± 0.8	70.2 ± 2.6	60.3 ± 1.8
5 h	77.0 ± 6.9	73.3 ± 6.0	71.8 ± 1.5	94.7 ± 7.0	71.6 ± 1.1	59.5 ± 1.8
24 h	78.3 ± 9.5	72.5 ± 3.1	70.9 ± 3.2	93.8 ± 9.0	69.7 ± 1.8	60.1 ± 0.7

Table S8 Viability (%) of HS5 cells after treatment with the PDA(ROH) NPs (0.035 mg/mL) obtained after different reaction times. These values correspond to those represented in Fig. 9.

HS5 cells	PDA (EtOH) NPs			PDA (2-PrOH) NPs		
Final reaction time	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
1h	94.9 ± 5.5	87.6 ± 2.4	75.3 ± 0.8	92.3 ± 3.2	87.2 ± 3.4	84.2 ± 4.3
3 h	92.5 ± 6.1	88.7 ± 2.7	77.0 ± 1.2	96.9 ± 4.8	88.9 ± 5.4	82.2 ± 2.7
5 h	96.4 ± 3.6	86.7 ± 4.3	75.4 ± 5.3	97.1 ± 2.6	86.7 ± 1.6	82.2 ± 1.9
24 h	95.0 ± 4.0	86.3 ± 6.5	74.0 ± 1.0	95.9 ± 8.0	88.5 ± 3.9	84.5 ± 0.2

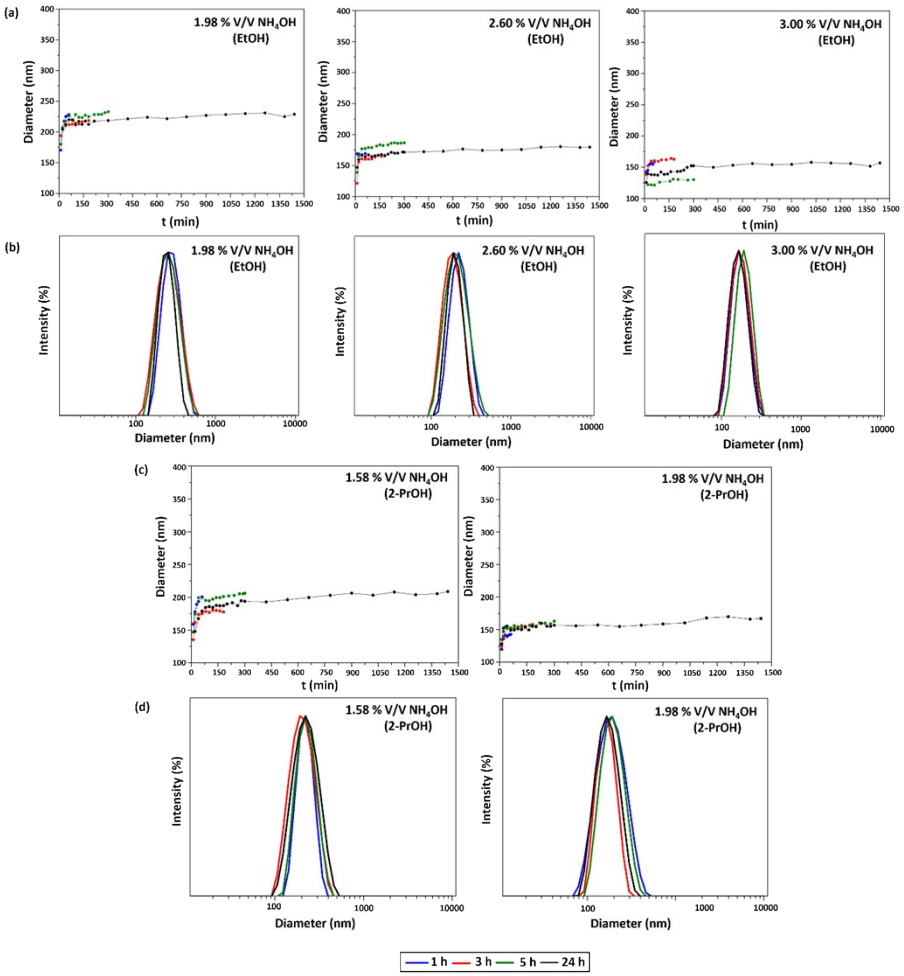

Fig. S1 (a-b) Formation kinetics and DLS intensity distribution of PDA(EtOH) NPs produced with different NH_4OH concentrations (1.98, 2.60 and 3.00% (V/V)), **(c-d)** Formation kinetics and DLS intensity distribution of PDA(2-PrOH) NPs produced with different NH_4OH concentrations (1.58 and 1.98% (V/V)).

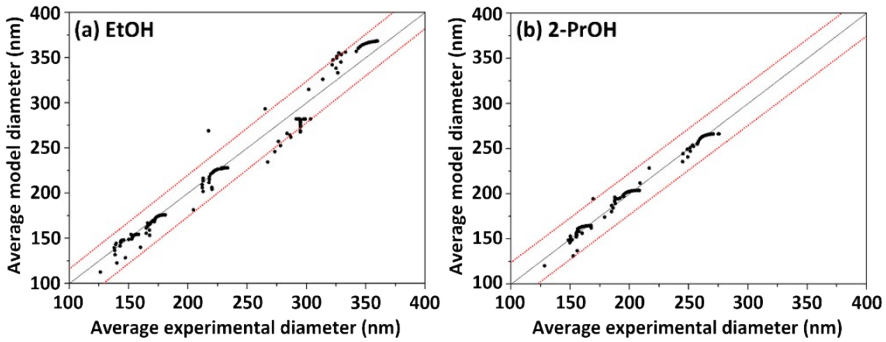

Fig. S2 Parity plot of experimental vs. model average size values of **(a)** PDA(EtOH) NPs and **(b)** PDA(2-PrOH) NPs.

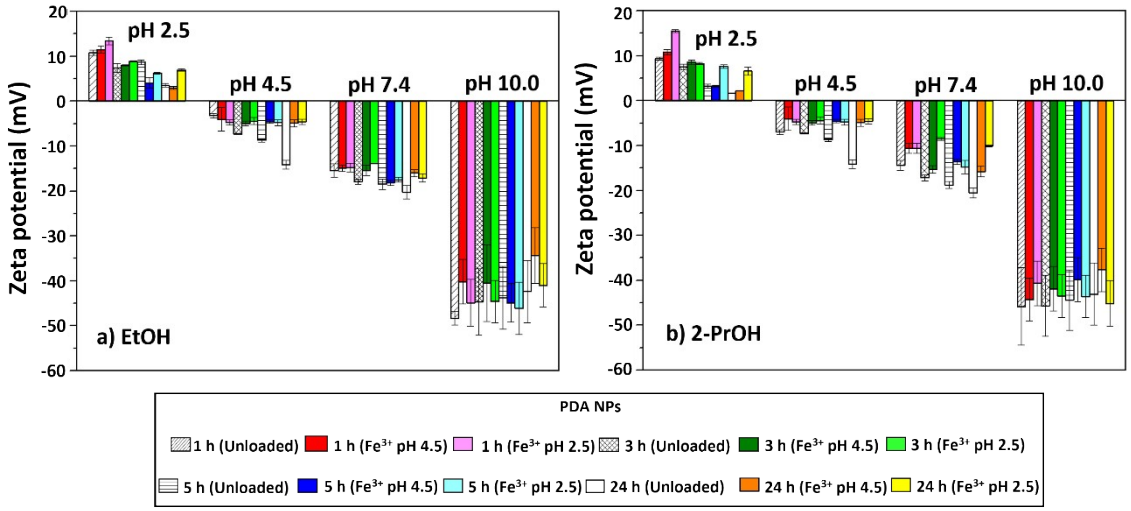

Fig. S3 Comparison of the zeta potential of all PDA(EtOH) (a) and PDA(2-PrOH) NPs at different pH values before and after loading them with Fe^{3+} at pH 2.5 and 4.5.

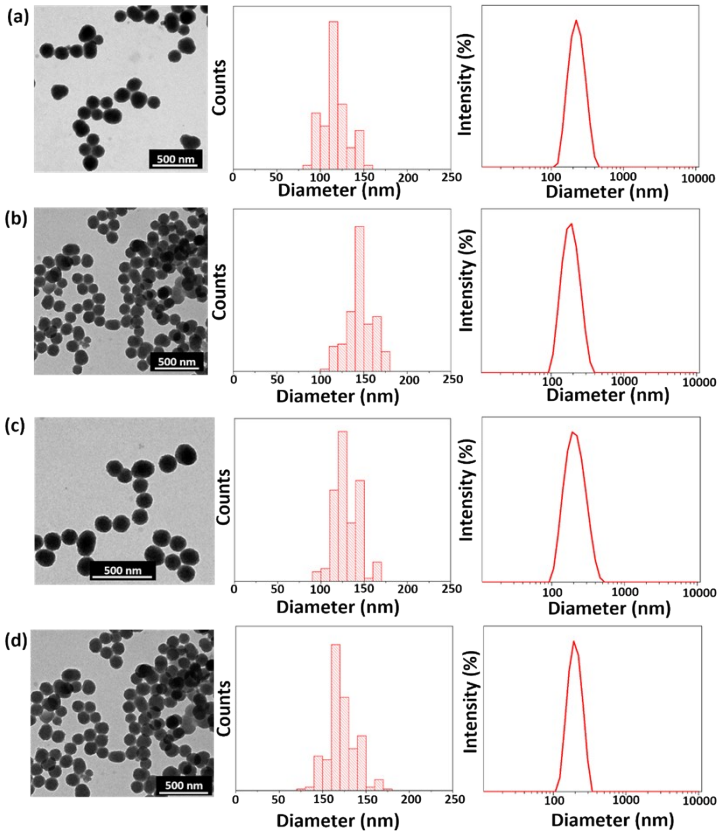

Fig. S4 TEM images, size histograms and DLS intensity distributions of the PDA(EtOH) NPs obtained after different reactions times to perform viability assays: **(a)** 1 h (117.2 ± 15 nm), **(b)** 3 h (145.5 ± 14.6 nm), **(c)** 5 h (129.7 ± 14 nm) and **(d)** 24 h (122.6 ± 16.2 nm).

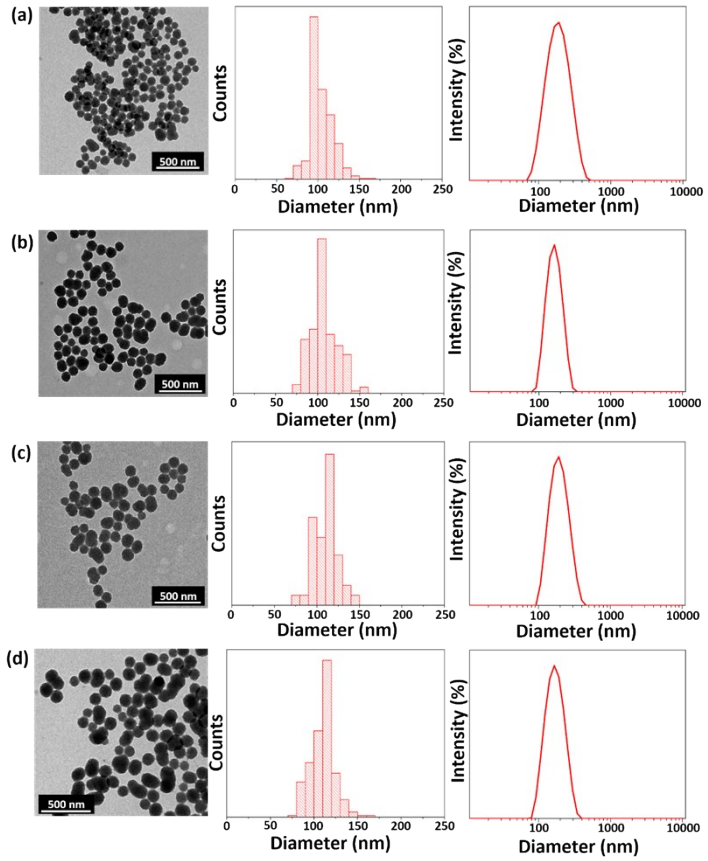

Fig. S5 TEM images, size histograms and DLS intensity distributions of the PDA(2-PrOH) NPs obtained after different reactions times to perform viability assays: **(a)** 1 h (103.7 ± 14.9 nm), **(b)** 3 h (107.6 ± 15.7 nm), **(c)** 5 h (110.6 ± 14.2 nm) and **(d)** 24 h (110.3 ± 14.3 nm).