

Supporting Information

Melt-Grafting for the Synthesis of Core-Shell Nanoparticles with Ultra-High Dispersant Density

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Contents

1. Experimental section & Results	3
1.1. Synthesis of nitro-dopamine (1) and nitrodopamine-PEG(5000) (2).....	3
1.2. NMR spectrum of nitrodopamine (1)	4
1.3. Mass-spectrum of nitrodopamine (1).....	4
1.4. TEM analysis of synthesized iron oxide-NPs.....	6
1.5. Purification of the Iron oxide-PEG NPs	7
1.6. Additional tests of colloidal stability of nanoparticles in ethanol and PBS	7
2. References	8

1. Experimental section & Results

1.1. Synthesis

Synthesis of nitrodopamine¹⁻³(1)

Dopamine hydrochloride (20 g; 105 mmol) was dissolved in 600 mL water, sodium nitrite (25 g, 362 mmol) was added and the mixture was cooled to 0°C using an ice bath. 200 mL of a 20 v/v% sulfuric acid was slowly dropped into the reaction. After removing the ice-bath the mixture was stirred at room temperature overnight. The crude product was collected by filtering the obtained dispersion. After washing the product with ice-cold water the nitrodopamine hydrogensulfate was dried in high vacuum and stored at 4°C until further use.

Synthesis of MeO-PEG-nitrodopamine (2)

1 g MeO-PEG-COOH (0.2 mmol) was dissolved in 5 mL DMF (headspace grade 99.99%, anhydrous) and cooled to 4°C on an ice bath. COMU[®] (1-Cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate) (95 mg; 0.22 mmol) and *N*-methylmorpholine (0.24 mL; 2.2 mmol) were added and the mixture was stirred for 2 hours. Afterwards a solution of nitrodopamine hydrogensulfate (53 mg; 0.18 mmol) in DMF (0.5 mL; 99,99%) was dropped in during 5 minutes. The reaction was stirred for 2 h at 4°C and 16 h at RT. The solution was poured into 45 mL ice cold acetone and centrifuged (5000 rpm, 4°C, 10 minutes). The precipitate was washed 2 times with acetone and afterwards dissolved in 1 mL water. The crude product was dialyzed against MilliQ-water for 48 hours. Freeze drying gave the pure product (0.95 g; 0.19 mmol; yield 95%).

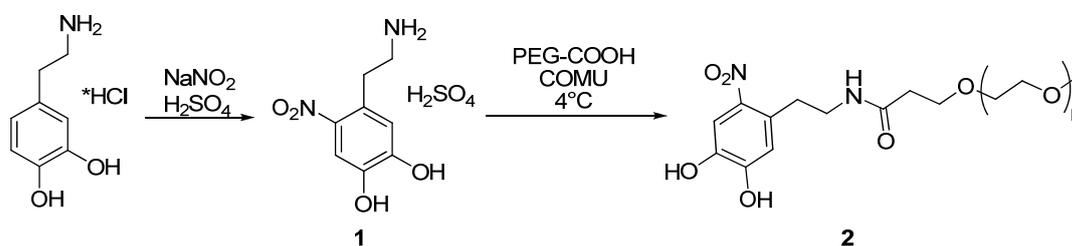


Figure 1: Synthetic pathway for the synthesis of nitrodopamine and the coupling to the PEG5000 using a COMU[®]-activated intermediate step.

1.2. NMR spectra of nitrodopamine (1)

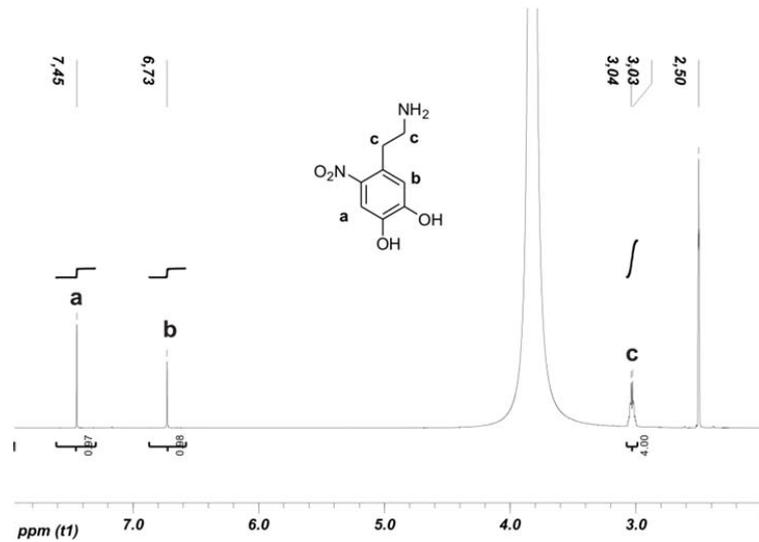


Figure 2: NMR-spectrum of nitrodopamine (1) in DMSO-d₆. Due to the high water content of the NMR-solvent the primary amine and the two hydroxyl-groups cannot be seen.

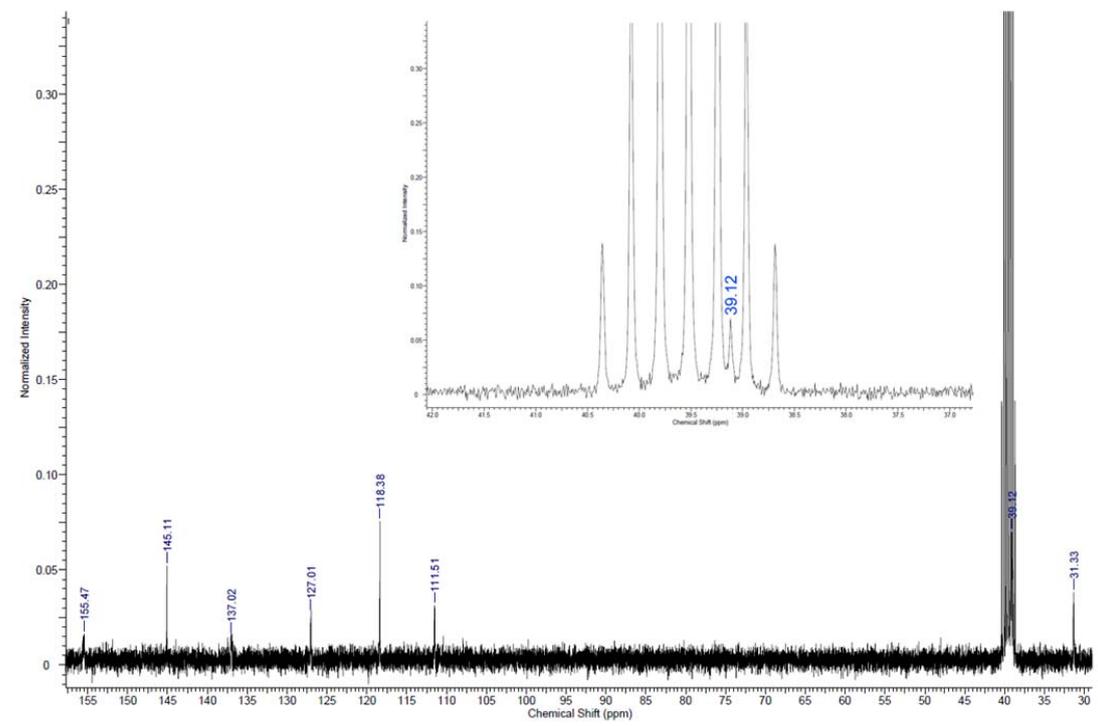


Figure 3: ¹³C-NMR-spectrum of nitrodopamine (1) in DMSO-d₆.

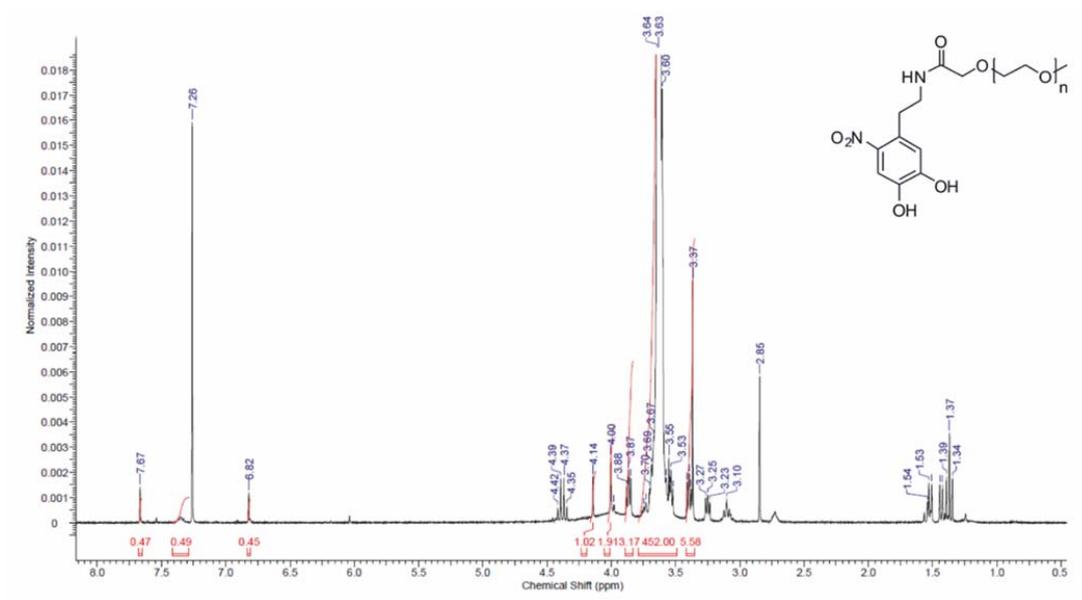


Figure 4: NMR-spectrum of nitrodopamine-PEG (2) in CDCl_3 .

^{13}C NMR (300 MHz, D_2O): 172.3, 144.2, 142.7, 131.7, 121.2, 116.7, 116.3, 69.0-71.1, 58.2, 40.4, 33.9

1.3. ESI-TOF-spectrum of Nitrodopamine (1)

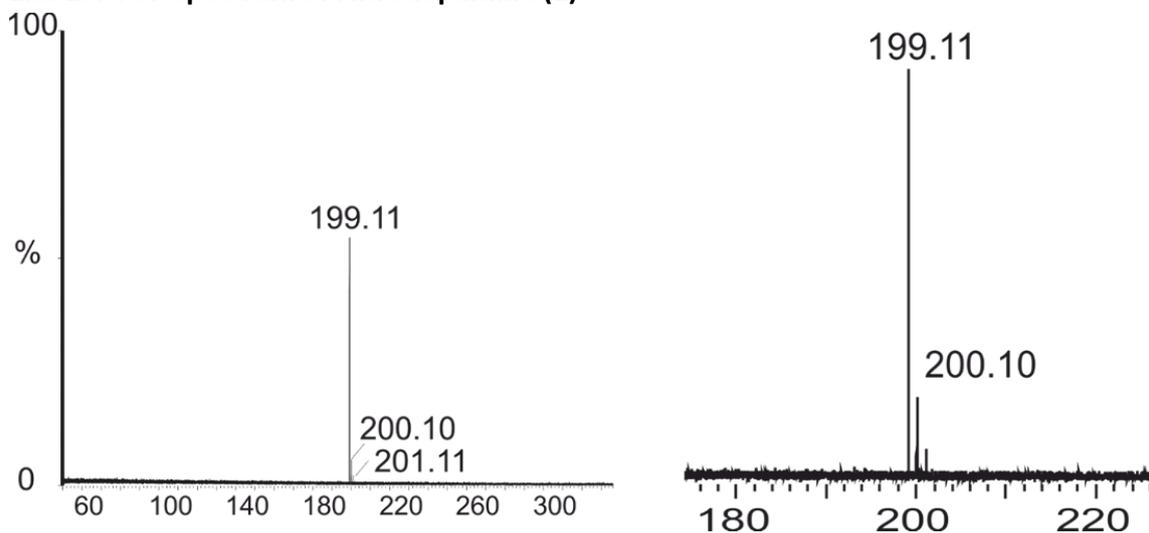


Figure 5: Mass-spectrum of pure nitrodopamine (1); the mass peak at 199.10 can be correlated to the nitrodopamine ($m=198.06+1$ (proton))=199.06). Higher mass-peaks can be assigned to oligomeric structures of nitrodopamine and associates with salts.

1.4. TEM analysis of iron oxide NPs

The statistical size distribution was obtained by the use of the freeware Pebbles⁴ (<http://pebbles.istm.cnr.it>).

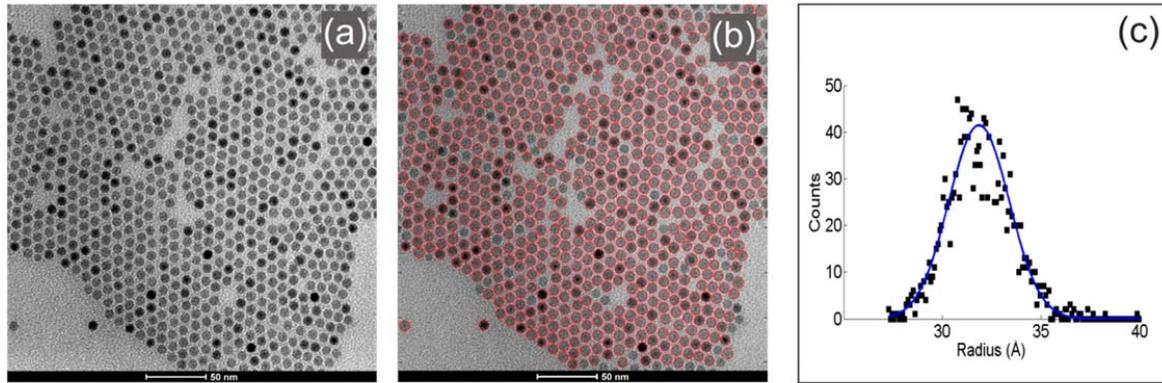


Figure 6: Pebbles⁴ results of a TEM image. (a) TEM image as measured (b) pebbles automatically identifies and marks the nanoparticles and provides (c) the statistical evaluation of the size distribution of the particles in the respective TEM-image given by Pebbles.

Calculation of the grafting densities:

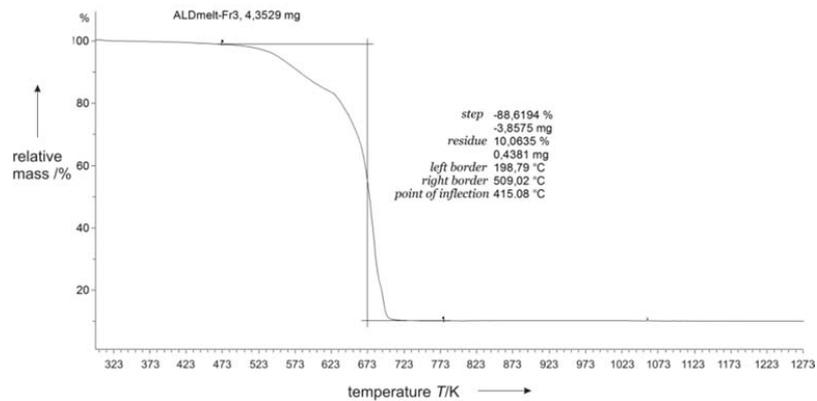


Figure 7: Example for the TGA-curves of the main fraction (here: ALD-melt, table 1, entry 15)

ALD-melt:

organic content: 88.6%

r= 2.0 nm

M= 5200 g/mol

Density(PEG)= 1.12g/cm³

Density (iron oxide)= 5.24 g/cm³

$$V(\text{NP})=33.5 \text{ nm}^3$$

$$\text{Mass (1 NP)}= 1.8 \times 10^{-19} \text{ g/NP (core)}$$

0.886 g PEG per gram product

$$1.7 \times 10^{-7} \text{ mol PEG}$$

$$1.0 \times 10^{17} \text{ molecules PEG}$$

0.115 mg iron oxide per gram product

$$6.5 \times 10^{14} \text{ NP/g}$$

3.1 chains/nm^2

1.5. Purification of the Iron oxide-PEG NPs



Figure 8: Sephadex G75-column purification/separation of the PEGylated products. Pictures were taken over the time of the purification to show separation into different fractions.

1.6. Additional tests of colloidal stability of nanoparticles in ethanol and PBS

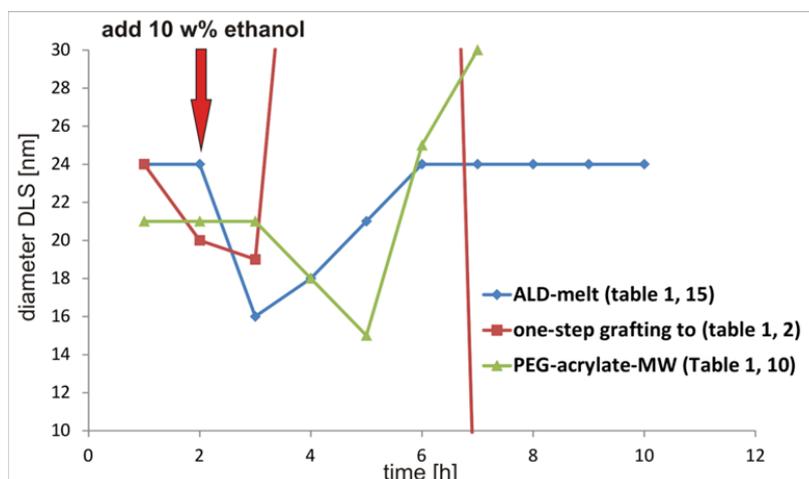


Figure 9: Effective hydrodynamic size of differently grafted core-shell NPs measured by DLS at 25°C over the course of 10 hours. After 2 hours 10 w% of ethanol was added to the solutions; the solution was shaken once and the measurement continued.

Ethanol is expected to partially collapse the PEG shell and induce aggregation and possibly precipitation of poorly stabilized PEG core-shell NPs. The ALD-melt grafted NPs showed a slight decrease in size and Figure 6 indicates this correlation between the grafting density of PEG-chains and the colloidal stability of the core-shell nanoparticles. The particles synthesized via the new method (ALD-melt, Table 1, **15**, blue curve) show a temporary decrease in hydrodynamic size, but a following full “recovery” of the original shell-size. A precipitate was not observed. Particles with a grafting density around 1 chain/nm² (PEG-acrylate-MW, Table 1, **10**) show slow aggregation and particles with a grafting density below 1 chain/nm² (one-step grafting-to, Table 1, **2**) aggregate quickly and precipitate completely.

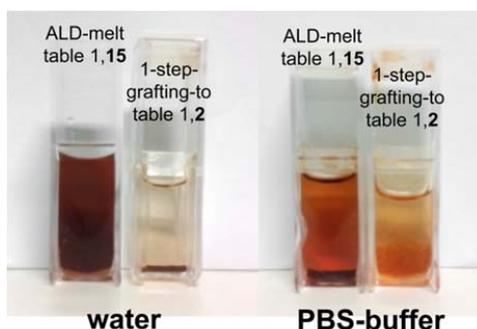


Figure 10: Pictures of the particles grafted with the novel melt method (ALD-melt, Table 1,15) directly compared to particles stabilized through 1-step-ligand exchange (Table 1,2). The pictures were taken after the solutions were heated to 75°C for 15 hours.

At room temperature NPs showed colloidal stability in water and PBS (physiological buffer) over long time scales. After being kept at an elevated temperature of 75°C, differences in the colloidal stability could be observed that depended on the grafting density. The ALD-melt grafted nanoparticles are stable dissolved in water over the investigated period of 15h; they are also predominantly stable in PBS after 15 hours at 75°C, which is known to cause aggregation of weakly stabilized iron oxide nanoparticles. The particles synthesized by the traditional one-step ligand exchange method - and therefore having considerably lower grafting density - precipitated completely in water and *dissolved* in PBS during the heat-treatment.

References

1. E. Amstad, T. Gillich, I. Bilecka, M. Textor and E. Reimhult, *Nano Letters*, 2009, **9**, 4042-4048.
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4. S. Mondini, A. M. Ferretti, A. Puglisi and A. Ponti, *Nanoscale*, 2012, **4**, 5356-5372.