Supporting Information

Towards copper-free nanocapsules obtained by orthogonal interfacial "click" polymerization in miniemulsion

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Experimental Part

Materials

Sodium azide (reagent grade, Servay), DMF (Sigma Aldrich, HPLC grade), 2,2-bis(bromomethyl)-1,3-propanediol (Acros, 98%), propiolic acid (Acros, 95%), hexanediol (Sigma Aldrich, 98%), toluene (Acros, 99%, < 50 ppm water content), pentamethyleneethylenetriamine (PMDETA, Sigma Aldrich, 99%), tin(II)2-ethylhexanoate (Sigma Aldrich, 98%), 0.9% NaCl solution (Fresenius Kabi, Germany), sulforhodamine 101 (SR101) (Sigma Aldrich, 90%) were used as received. P(E/B-*b*-EO) and BA_6TREN were synthesized according to literature[1,2]. *p*-Toluene sulfonic acid (Sigma Aldrich, 98%) was recrystallized from dry toluene.

Instrumentation

Nuclear magnetic resonance (NMR) spectroscopy

For NMR analysis ^{1}H-NMRspectra were recorded on a Bruker Avance 300 Spectrometer operating at 300 MHz frequency. The polymers were dissolved by heating in DMSO-d_6. The spectra were calibrated to the solvent peak.

Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) experiments were performed at 60 °C using a column oven from Waters, a Waters Alliance 2000 auto sampler, Waters 510 HPLC pump and a PSS-SDV 300 x 8 mm column with a particle size of 10 mm and a pore size of 500 Å, 10^5 Å, 10^6 Å. A RI detector from SOMA and a UV detector from ERC were used for detection. Elugrams were calibrated to polystyrene standards. DMF was used as a solvent.

Dynamic scanning calorimetric (DSC)

DSC measurements were carried out in the temperature range from -50 °C up to 20 °C below decomposition onset using either an auto-sampled Mettler DSC 30 or a Mettler DSC 822 device with a heating rate of 10 $K \cdot min^{-1}$.

Thermal gravimetric analysis (TGA)

Measurements for thermal analysis have been carried out under nitrogen atmosphere using a Mettler TGA/SDTA 851 Thermobalance. The samples were heated up from 25 °C to 600 °C with a heating rate of 10 $K \cdot min^{-1}$.

Infrared (IR) spectroscopy

The chemical composition of polymer and monomers was studied by FTIR spectroscopy. The sample powder was obtained by freeze-drying of the capsule dispersion for 48 h at -60 °C under reduced pressure. 3 mg of the dry sample were pressed with KBr to form a pellet and a spectrum between 4000 and 400 cm^{-1} was recorded using an IFS 113v Bruker spectrometer.

Scanning electron microscopy

Scanning electron microscopy (SEM) images were taken by a 1530 Gemini LEO from Zeiss with accelerating voltages between 100 V to 30 kV. For the SEM studies, the samples were diluted in water, placed onto a 5 x 5 mm silicon wafer and dried under ambient conditions.

Dynamic light scattering (DLS)

The average capsule size and the size distribution were measured using a PSS Nicomp Particle Sizer 380 (Nicomp Particle Sizing Systems, USA) equipped with a detector at 90 ° scattering mode at 20 °C.

Mass Spectroscopy

ESI mass spectra were obtained from a Q-ToFUltima 3 spectrometer with Lock Spray™-interface from micromass (waters). Sample was dissolved in methanol with a concentration of about 1 $mg \cdot mL^{-1}$.

Synthesis

2,2-Bis(azidomethyl)-1,3-propanediol (BAP)

15 g of 2,2-bis-(bromomethyl)-1,3-propanediol (57.3 mmol) were dissolved in 200 mL of dimethylformamide and 15 g of sodium azide were dispersed in the solution. The mixture was heated for 24 h to 120 °C and formed sodium bromide after cooling. The excess sodium azide was filtered off. DMF was removed using a rotoevaporator and the resulting liquid was

dissolved in 200 mL of ethyl acetate. The organic phase was washed twice with 200 mL of water and dried over magnesium sulfate. After filtration the solvent was removed using a rotoevaporator. After extensive drying in an oil pump vacuum for 2 days, the obtained colorless oil solidified into white crystals upon standing. 10.1 g (95%, 55.5 mmol) of a white solid were obtained (95%).

^{1}H-NMR ($CDCl_3$, 300 MHz, δ in ppm): 4.74 (t, 2H, ^{3}J= 4.9 Hz), 3.28 (m, 8H).

^{13}C-NMR ($CDCl_3$, 75 MHz, δ in ppm): 59.8, 51.1, 45.5.

IR (ν in cm^{-1}): 3392 (O-H), 2942 ($C_{aliph.}$-H), 2111(-N_3), 1450 (C-H), 1043 (C-O).

R_f (EtOAc): 0.58

1,6-Hexanediol dipropiolate (HDDP)

11.8 g of hexanediol (0.1 mmol, 1.0 eq.), 28 g propiolic acid (0.4 mmol, 4.0 eq.) and *p*-toluene sulfonic acid (1 g, 5 mol%) were charged together with 300 mL of dry toluoene under argon atmosphere into a flame dried three neck flask equipped with a water separator and condenser. The solution was refluxed for 120 h using an oil bath, and the excess of water and propiolic acid was azeotropically distilled out and collected. After cooling, the organic phase was washed with 300 mL saturated $NaHCO_3$ solution and 300 mL water. Organic phase was separated and dried over anhydrous magnesium sulfate, filtered and solvent was distilled out using a rotoevaporator and dried under vacuum. 19.5 g (87%, 0.087 mmol) of a colorless oil was obtained, which solidified upon standing.

^{1}H-NMR ($CDCl_3$, 300 MHz, δ in ppm): 4.15 (t, 4 H, ^{3}J= 6.0 Hz), 2.87 (s, 2H), 1.86 (m, 4H), 1.37 (m, 4H).

^{13}C-NMR ($CDCl_3$, 75 MHz, δ in ppm): 152.9, 74.9, 74.8, 66.3, 28.3, 25.6.

IR (ν in cm^{-1}): 2945 ($C_{aliph.}$-H), 2114 (C_{sp}-C_{sp}), 1702 (C=O), 1249 (C-O).

R_f (Et_2O): 0.76

ESI-MS (m/*z*): 245 $[M+Na]^+$

Copper catalyzed synthesis of nanocapsules using inverse miniemulsion process

200 mg BAP, 1 mg SR101 and 9 mg copper(II)bromide were dissolved in 1 g of 0.9% aqueous NaCl solution. This solution was poured under rigorous stirring into a solution containing 12 g cyclohexane, 120 mg P(E/B-*b*-EO), equimolar amount of diyne, and 37 mg BA_6TREN. Stirring was continued for 1 h and the dispersion was submitted to ultrasonication at 0 °C in order to prevent evaporation of the solvent. For ultrasonication, a Branson Sonifier W450Digital at 90% amplitude for 120 s, equipped with a ½" tip was used. The dispersion was poured into a Schlenk tube, and degassed by bubbling argon through the solution. The reaction was started after addition of 50 µL tin(II)ethylhexanoate and proceeds for 48 h at 25 °C.

Copper-free synthesis of nanocapsules using inverse miniemulsion process

200 mg BAP (1.07 mmol, 1.0 eq.), 1 mg SR101 and 9 mg copper(II)bromide were dissolved in 1 g of 0.9% aqueous NaCl solution. This solution was poured into 12 g cyclohexane, containing 239 mg (1.07 mmol, 1.0 eq.) HDDP and 120 mg P(E/B-*b*-EO). The solution was preemulsified for 10 min at 1000 rpm and ultrasonicated using the procedure mentioned above. Polymerization proceeded for 72 h at 25 °C.

Redispersion of nanocapsules in water

2 g of capsules dispersion in cyclohexane were centrifuged at 4,000 rpm for 15 min. The cyclohexane phase and excess of P(E/B-*b*-EO) were removed and the pallet of capsules was redispersed in the same amount of cyclohexane. The dispersion was poured into 10 g aqueous 0.3 wt% sodium dodecylsulfate solution and stirred at 1000 rpm overnight with open lid to evaporate slowly the cyclohexane. The dispersion was dialyzed in dialysis tubes with a MWCO of 14,000 $g \cdot mol^{-1}$against deionised water for 3 days, changing the dialyzing water daily.

Synthesis of polytriazole polymers in solution

300 mg BAP (1.61 mmol, 1.0 eq.) and 171 mg OD or 202 mg DEB (1.61 mmol, 1.0 eq.) were dissolved in 10 g DMF containing 10 mg $[Cu(PMDETA)]Br_2$ (0.025 mmol). Argon was bubbled through the solution and 10 cm of Cu-wire (0.15 mm average diameter, 99.9% metal content) was wrapped around a magnetic stirring bar and added. The green color of Cu(II) vanished after 30 min and the solution was heated to 60 °C under argon atmosphere in an oil bath for 24 h. Polymers were subsequently precipitated in 100 mL methanol, dried and used for the analysis. In the case of copper-free “click” polymerizations, 300 mg BAP (1.61 mmol,

1.0 eq.) and 357 mg HDDP was dissolved in 10 g DMF and the solution was stirred for 72 h at room temperature. The polymer was precipitated in 100 mL of cold methanol and after drying used for the analysis.

DEB:

^{1}H-NMR (DMSO-d6, 300 MHz, δ in ppm): 7,81 (s, 2H), 4.30 (s, 4H), 3.10 (m, 4H), 2.65 (m, 4H), 1.64 (m, 4H). 3.29-3.24 (m, 4H), 1.71-1.58 (m, 4H), 1.43-1.30 (m, 4H).

HDDP:

^{1}H-NMR (DMSO-d6, 300 MHz, δ in ppm): 8.63-8.58 (m, 1H), 4.97 (m, 2H), 4.26 (m, 2H), 4.13 (m, 2H).

Reaction monitoring by FT-IR

As stated above, FT-IR spectra were recorded of the reaction mixture between BAP and OD before and after the reaction took place. The results are shown in Figure S1.

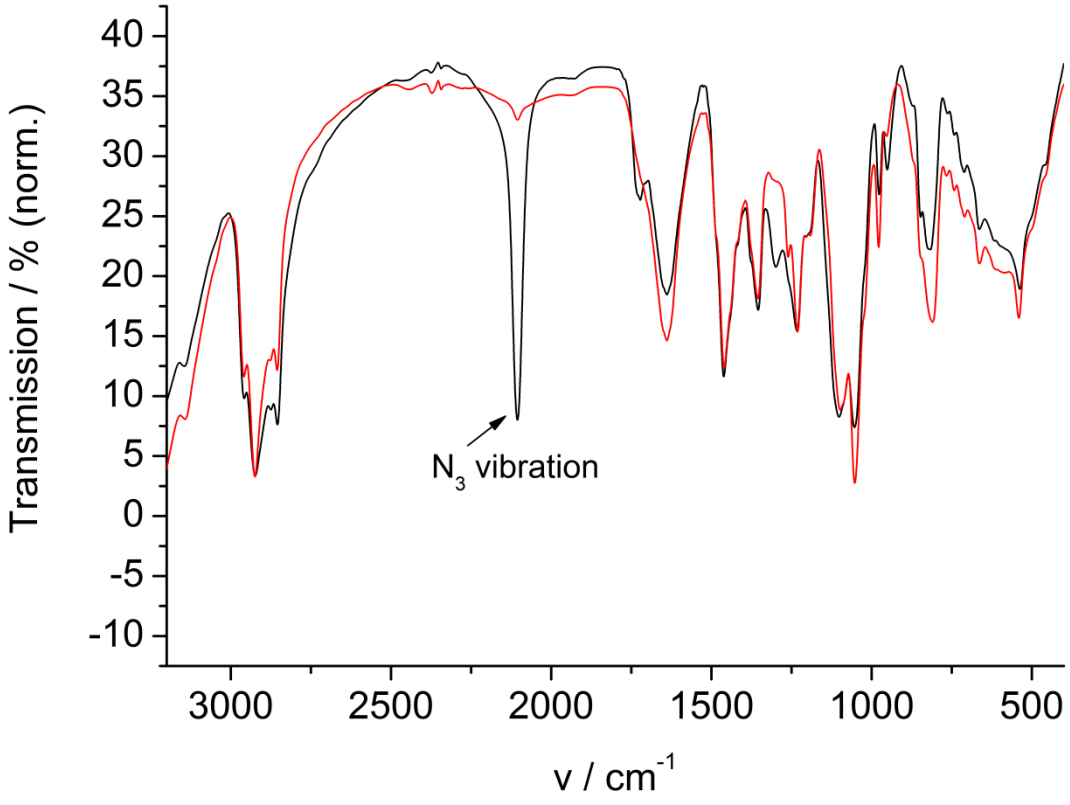

Figure S1: Comparison of the FT-IR spectra of a reaction mixture of BAP and OD before the reaction (black line) and the spectra of the resulting product (red line) obtained directly from the dried samples.

Determination of the polymeric nanocapsules permeability

The permeability of the capsules' shells was studied on SR101containing capsules redispersed in water using a fluorescence spectrometer (microplate reader, Infinite M1000, Tecan, Switzerland). The fluorescent dye SR101 absorbs light at 550 nm and emits light at 605 nm. After the encapsulation and redispersion process the polymeric nanocapsules were sedimented by centrifugation (Sigma, 3k-30). The nanocapsules prepared without fluorescent dye, but redispersed in an aqueous SDS solution containing SR101 (the amount is equal to SR101 amount taken in the encapsulation experiments), were used as a reference. The total release of SR101 from the capsule was calculated as a difference between the fluorescent intensities of the supernatant obtained from the sample and the reference. The fluorescence signal of the reference sample was set as 100%. The polymeric nanocapsules were shaken gently for 40 days at 25 °C. After a given period of time the amount of released SR101 was determined in the supernatant of the sedimented capsules and compared with the initial value (see Figure S2). For each sample the encapsulation efficiency was calculated from three single measurements.

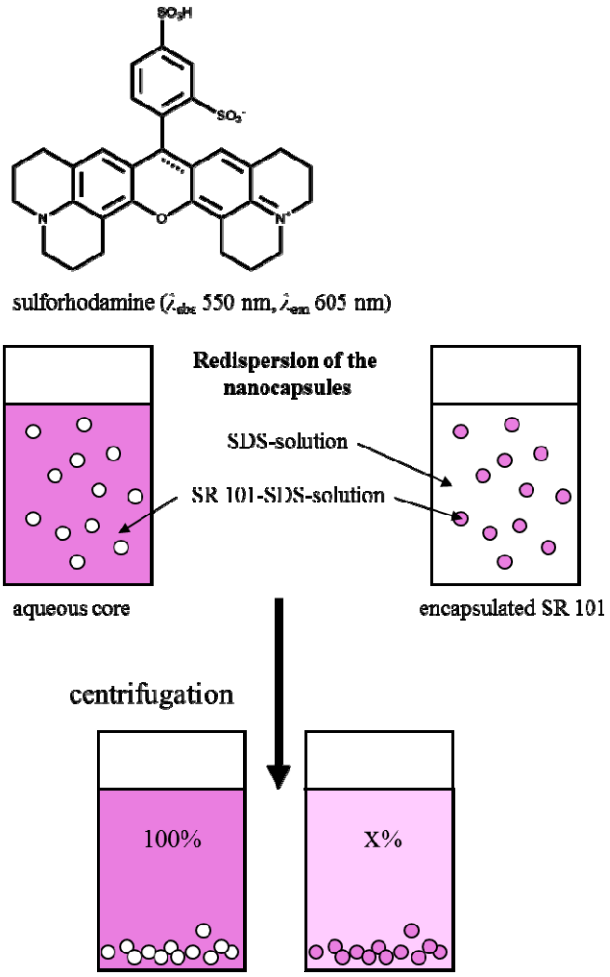

Figure S2: Procedure for determination of leakage of SR101 from the nanocapsules.

Determination of the cell viability by MTS assay

The cell viability was confirmed by MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, USA), performed according to the product insert in 96 well assay plates (Corning Incorporated costar® 3603, Corning, Germany). Each well was populated with 10 000 cells the day before the experiment. Absorbance (490 nm) of this assay was measured with a microplate reader (Infinite M1000, Tecan, Switzerland).

Leakage from the nanocapsules

The leakage from the nanocapsules is shown in Figure S3.

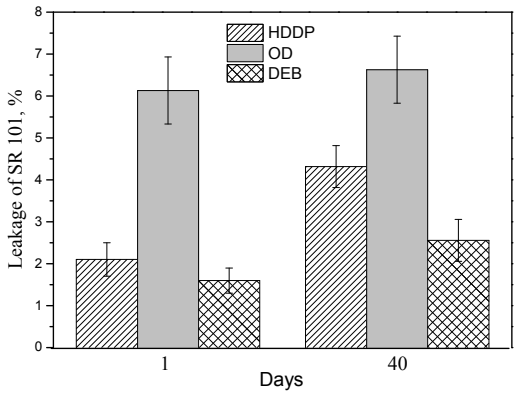

Figure S3: Leakage in % determined by fluorescence intensity of SR101 in the continuous phase after different storage periods at 25 °C.

References

(1) *Macromolecules* **2000**, *33*, 1628.

(2) *Macromolecules* **2001**, *34*, 4302.