Supplementary Material (ESI) for Soft Matter

Polymeric Nanocontainers with High Loading Capacity of Hydrophobic Drugs

Supporting Information

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Experimental Part

Materials

All reagents were used without further purification unless stated otherwise. Solvents were purchased from Biosolve Ltd. (Valkenswaard, The Netherlands). Pentaerythritol, dipentaerithritol, stannous octoate, *ε*-caprolactone, poly(ethylene glycol) methyl ether methacrylate (mPEGMA), copper bromide and *N*,*N*,*N'*,*N'*,*N''*-pentamethyl-diethylenetriamine (PMDETA) were purchased from Aldrich (Oakville, On, Canada).

Instrumentation

GPC was measured on a Shimadzu system equipped with a SCL-10A system controller, a LC-10AD pump, a RID-10A refractive index detector, a SIL-10AD autosampler and a Polymer Laboratories Mixed-D column utilizing a chloroform:triethylamine:isopropanol (93:5:2) mixture as eluent at a flow rate of 1 mL/min and a column temperature at 50 °C. Calibration was performed utilizing linear poly(ethylene glycol) standards.

UV/vis spectra were recorded on a FlashScan S12 (AnalytikJena, Germany) in 96-well microtiter plates (polypropylene, flat bottom) from Greiner (Greiner Bio-One, Germany) in a range from 250 to 700 nm. All spectra were referenced to an empty microtiter plate and measurements were performed with four flashes. The actual time for the measurement of one microtiter plate with 96 full UV/Vis spectra was approximately 40 seconds.

NMR spectra were measured on a Varian Gemini 400 NMR spectrometer in $CDCl_3$ and D_2O. The chemical shifts were calibrated to TMS.

DLS measurements were performed on a Brookhaven Instruments Corp. BI-200 apparatus equipped with a BI-2030 digital correlator and a Spectra Physics He-Ne laser with a wavelength of 633 nm. The scattering angle used for the measurements was 90°. The experimental correlation function was analyzed by the method of the cumulants, as described elsewhere.[1] The Stokes-Einstein approximation was used to convert diffusion coefficient into hydrodynamic diameter (D_h).

General procedure for the synthesis of 4- and 6-arm starPCL-OH

Ring opening polymerizations of ε-caprolactone were performed at 130 °C for 8 h using 20.00 g (20.60 mL, 0.18 mol) of monomer. The initiator amount was calculated according to the M/I ratio of 48:1 and 72:1, respectively. The monomer, ε-caprolactone, and the initiator, pentaerythritol for 4-arm starPCL-OH or dipentaerythritol for 6-arm starPCL-OH, were added to a flask and stirred for 15 min at 130 °C in order to obtain a homogeneous solution. Subsequently, the polymerization was started by adding the catalyst, stannous octoate (n(cat) = 1/20 of n(OH-functional groups)). After the reaction time was elapsed the very viscous reaction mixtures were cooled to room temperature in an ice bath to stop the polymerization. The polymers were purified from residual monomer and catalyst by precipitation from a concentrated dichloromethane solution into ice-cold heptane resulting in powdery products. The yields were in the order of > 95%. The corresponding analytical data are depicted in Table 1.

General procedure for the synthesis of 4- and 6-arm starPCL-Br macroinitiators

To a stirred solution of starPCL-OH (1.0 mmol; 5.6 g of 4-arm starPCL-OH or 8.5 g of 6-arm starPCL-OH) and triethylamine (2.0 mmol/arm in 200 mL dry THF), 2.0 mmol/arm of 2-bromoisobutyryl bromide were added dropwise. The reaction was performed at room temperature for 48 to 72 h. The reaction mixture was filtrated through a short pad of neutral alumina to remove the quaternary ammonium salts. After the solvent evaporation and precipitation from a small amount of THF in cold methanol, white powdery polymers were obtained in quantitative yield.

Table 1 Analytical data of the synthesized star-shaped polymers

M_n (GPC) obtained using linear poly(ethylene glycol) calibration.

polymer	M/I	units of CL per arm	M_n (GPC)	M_n (^{1}H NMR)	PDI	yield
4-arm starPCL-OH	48	12	4100	5600	1.18	98%
6-arm starPCL-OH	72	12	6600	8500	1.18	96%
4-arm starPCL-Br	-	12	4600	6200	1.17	~100%
6-arm starPCL-Br	-	12	7000	9300	1.17	~100%

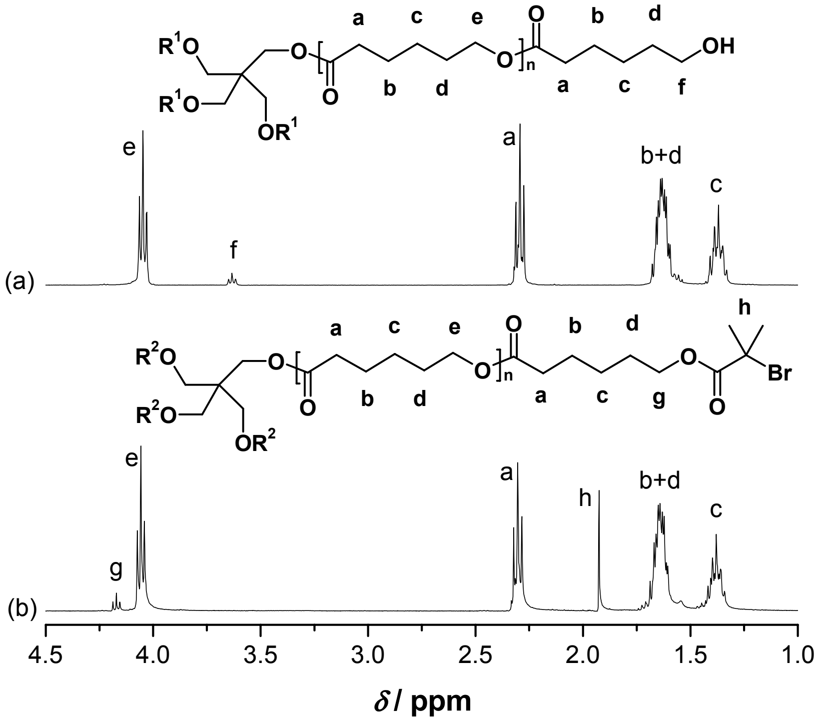

Fig. S1 ^{1}H NMR spectra of starPCL-OH (a) and star-PCL-Br (b).

General procedure for the synthesis of 4- and 6-arm starPCL-*p*(mPEGMA)s

The catalyst CuBr (0.05 mmol) and the ligand PMDETA (0.05 mmol) were added to 5 mL of toluene previously purged with argon for 10 minutes. A solution of macroinitiator (0.05 mmol) and monomer mPEGMA in 15 mL of toluene was purged with argon for 10 minutes and then added to the catalyst suspension. The amount of monomer was calculated according to the M/I ratios. The reaction mixture was stirred for 3 to 4 h at 70 ºC under an inert atmosphere. The reaction was stopped by cooling to room temperature and dilution with toluene in air. The content was then passed through a short pad of alumina to remove the copper catalyst. After solvent evaporation, the polymer was precipitated from a small amount of toluene in diethyl ether, filtered and dried under vacuum for several hours. The conversions (48-85%) were determined by ^{1}H NMR spectroscopy. A typical ^{1}H NMR spectrum and GPC traces of star PCL-*p*(mPEGMA)s are depicted in Figure S2 and S3, respectively.

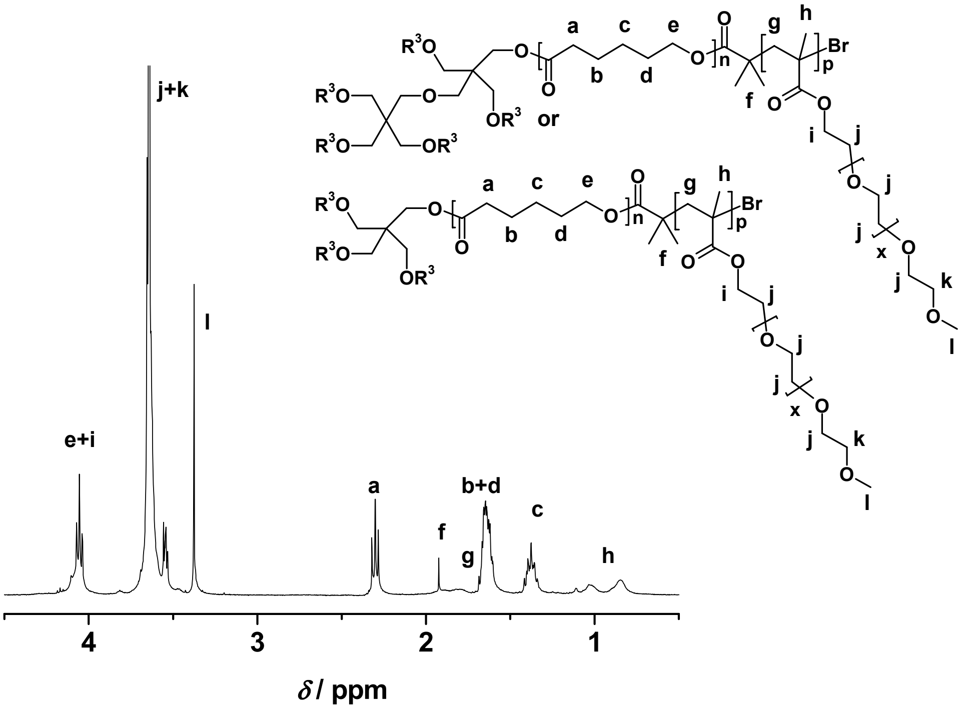

Fig. S2 Representative ^{1}H NMR spectrum for 4- and 6-arm star-PCL-*p*(mPEGMA)s.

The molar masses of all star-shaped polymers were calculated according to the DP of the PCL, DP of the *p*PEGMA shell and the number of arms. The degree of polymerization (DP) of mPEGMA related to the DP of PCL (12/arm) was determined by ^{1}H NMR spectroscopy from the integrated ratios of the signal at 4.1 ppm corresponding to the resonances of the methylenic protons from the PCL core and the signals at 3.4 ppm assigned to the methoxy protons from the starPCL-*p*PEGMAs.

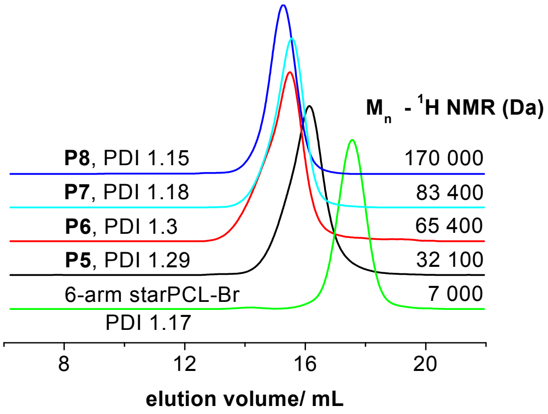

Fig. S3 GPC traces of starPCL-*p*(mPEGMA), **P5**-**P8**.

The semilogaritmic plot of the conversion versus time of the polymerizations at 90 °C and 70 °C are linear, except a fast activation step, suggesting that at the beginning of polymerization, elevated temperatures induce a fast monomer consumption as well as radical terminations.[2] This fast initiation step could be diminished by reducing the temperature (Fig. S4).

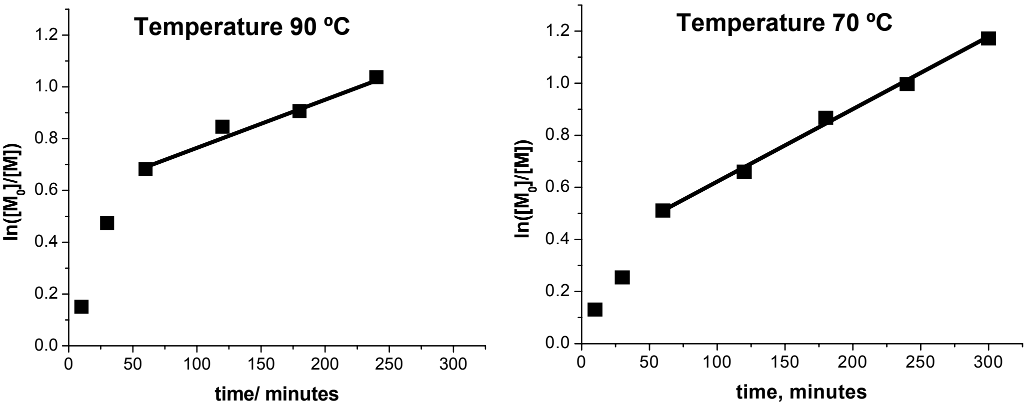

Fig. S4 Semi-logarithmic kinetic plot for ATRP of mPEGMA at different temperatures.

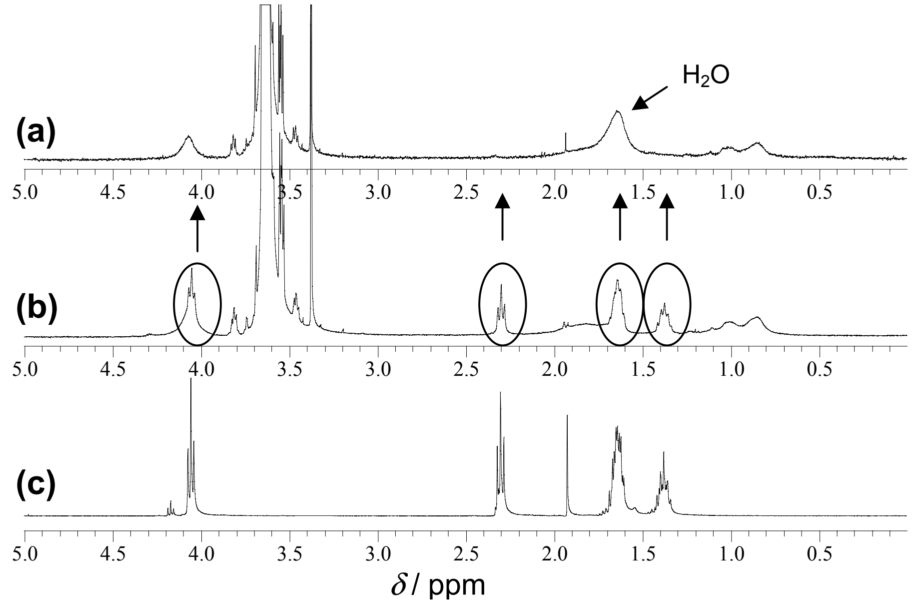

Fig. S5 ^{1}H NMR spectra of (a) *p*(mPEGMA) from the acid-catalysed hydrolysis of 6-arm starPCL-*p*(mPEGMA), (b) 6-arm star-PCL-*p*(mPEGMA) (**P8**), (c) 6-arm starPCL-Br.

The degradation of the PCL core was also performed using a lipase from *Rhizopus arrhizus* (enzyme/polymer 1:5 (w/w)), in phosphate buffer solution (PBS, *p*H = 7.0). Every 24 h, new amounts of enzyme were added, to enssure that the enzyme present in the PBS solution is catalytically active. The starPCL core was completely degraded after 4 days of stirring at 37 °C, as determined by GPC measurements.

General procedure for the UV/Vis polymer titrations

For the preparation of a [1]:[1] molar ratio [dye]/[polymer] aqueous solution, 4.84 mg, (0.02 mmol) of disperse orange 3 were added to a solution of polymer, 0.02 mmol, in 200 mL of demineralized water. After ultrasonication for 2 minutes at 40 ºC when the dye was completely dissolved and 10 mL of this solution were placed into a separated vial. To the rest of the solution 4.35 mg of dye were added, corresponding to a [dye]/[polymer] ratio of [2]:[1] and after ultrasonication at 40 ºC for 2 minutes, another 10 mL were placed into a separated vial. The procedure has been repeated for all 10 or 15 solutions of different ratio [dye]/[polymer] ratios. The solutions were then diluted with 30 mL of water and their UV/Vis absorptions were measured. A control UV/Vis spectrum was recorded for a suspension of 1.21 mg of dye in 10 mL of water, (0.5 mM) in the absence of polymer (see Figure S6, S7).

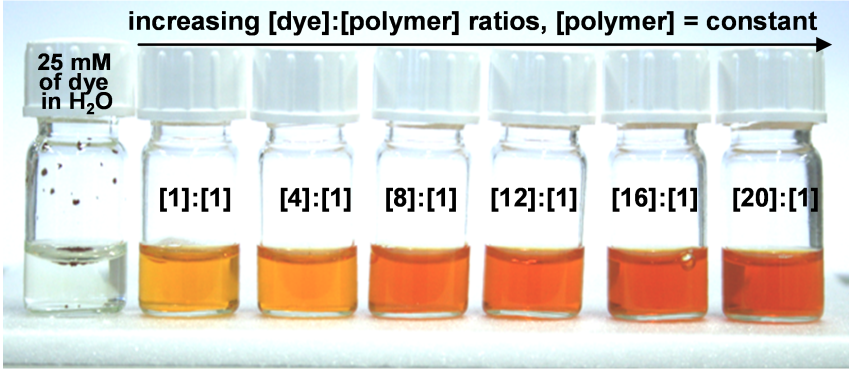

Fig. S6 Different aqueous solutions for the titration of polymer with guest **1**.

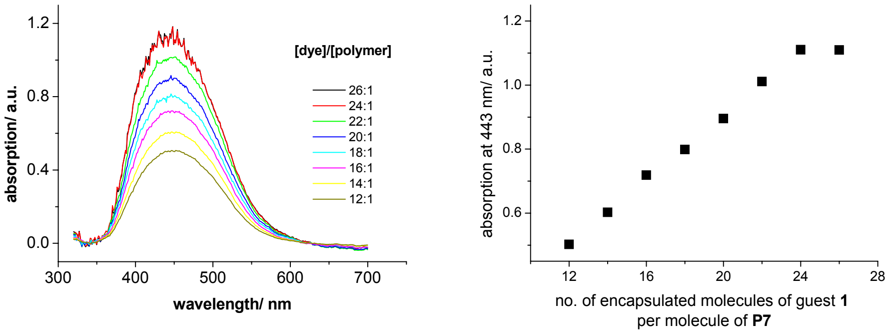

Fig. S7 UV/Vis data obtained from titration of **P7** with guest **1** ([**P7**] = 15 μM).

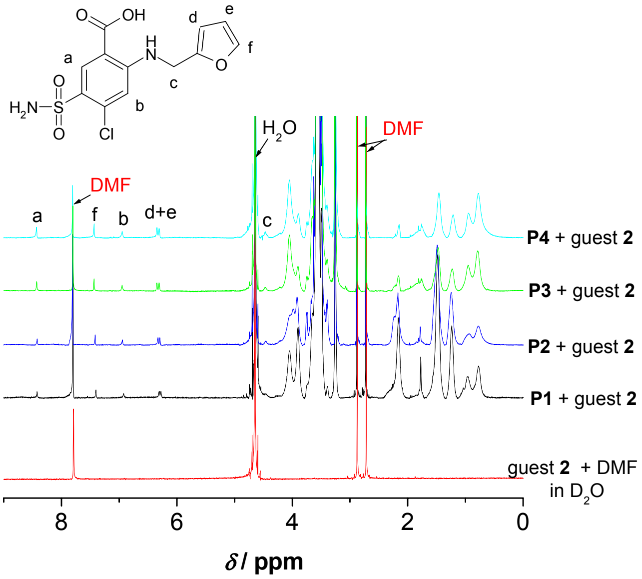

Fig. S8 ^{1}H NMR spectra of polymer encapsulated guest **2** in D_2O (DMF as internal standard).

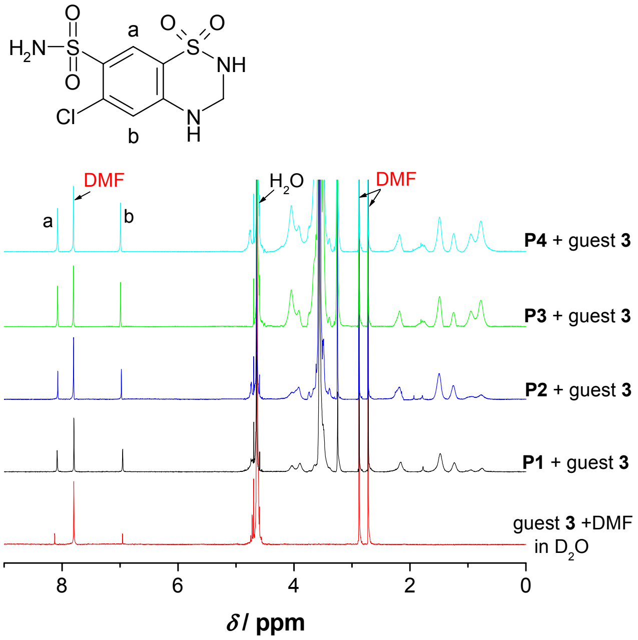

Fig. S9 ^{1}H NMR spectra of polymer encapsulated guest **3** in D_2O (DMF as internal standard).

References

1 B. J. Berne, R. J. Pecora, *Dynamic Light Scattering*; John Wiley and Sons: Toronto, **1976**.

2 K Matyjaszewski, J. Xia, *Chem. Rev.* **2001**, *101*, 2921-2990.