Electronic Supplementary Material (ESI) for Polymer Chemistry

PEG-pHPMAm-based polymeric micelles loaded with doxorubicin-prodrugs in combination antitumor therapy with oncolytic vaccinia viruses

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Supporting information

Reagents

Acetone, ethyl acetate, acetonitrile, dichloromethane, diethyl ether and dimethylformamide were purchased from Biosolve Ltd. (Valkenswaard, The Netherlands). $HPMAmLac_2$ was obtained from Syncom BV, Groningen, The Netherlands. The $(mPEG_{5000})_2$–ABCPA (4,4´-azobis(4-cyanopentanoic acid)) macroinitiator was synthesized as reported previously [1]. 2-bromoethanol, magnesium sulfate, copper sulfate, hydroquinone monomethyl ether (MEHQ), methacryloyl chloride, sodium ascorbate, sodium azide, sodium chloride, sodium bicarbonate and triethylamine were purchased from Sigma-Aldrich Co. (Zwijndrecht, The Netherlands). β-glucuronidase from bovine liver (type B-10, ~10,000 units/mg solid) was purchased from Sigma-Aldrich Co. (Steinheim, Germany).

Synthesis of 2-azidoethanol

Sodium azide (23.4 g, 360 mmol) and 2-bromoethanol (27.0 g, 216 mmol) were dissolved in a mixture of acetone (50 mL) and water (10 mL) and refluxed at 75°C for 3 days. After removing acetone under vacuum, the product was 5 times extracted with 100 mL of ethyl acetate. The organic phase was dried using anhydrous $MgSO_4$ and the solvent was removed under vacuum, resulting in 10.4 g of 2-azidoethanol in the form of slightly yellow oil (55% yield).

^{1}H NMR ($CDCl_3$) δ (ppm) 1.8 (O*H*, br), 3.4 (C*H*$_2$N$_3$, t), 3.8 (C*H*$_2$OH, t).

Synthesis of 2-azidoethyl methacrylate (AzEMA)

2-azidoethanol (2.0 g, 23 mmol) and triethylamine (3.8 mL, 27 mmol) were dissolved in 40 mL dichloromethane and cooled in an ice bath. Next, methacryloyl chloride (2.6 g, 25 mmol) dissolved in 15 mL dichloromethane was slowly added for 20 min. The reaction was allowed to proceed for 2 h at room temperature. Next, 30 mL of a saturated $NaHCO_3$ aqueous solution was added and the mixture was stirred for 30 min in an ice bath to deactivate methacryloyl chloride. The organic phase was washed with 30 mL of a saturated NaCl solution once, dried using anhydrous $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash silica gel chromatography (Acros silica gel 60 A, 0.030−0.075 mm, Geel, Belgium) with dichloromethane as an eluent, resulting in 2.59 g of AzEMA as a slightly yellow oil (73% yield). The monomer was stabilized by 400 ppm of MEHQ to avoid premature polymerization and stored at −20°C.

^{1}H NMR ($CDCl_3$) δ (ppm) 2.0 ($CH_2C(CH_3)CO$, s), 3.5 (C*H*$_2$N$_3$, t), 4.3 (C*H*$_2$CH$_2$N$_3$, t), 5.6 and 6.2 (C*H*$_2$C(CH$_3$)CO, t). IR ν_{max} 2106 cm^{-1} (N_3).

Synthesis of mPEG-b-p(HPMAmLac$_2$-co-AzEMA)

The synthesis of azide functionalized copolymer (20% of AzEMA)) was performed by free radical polymerization using HPMAmLac$_2$ and AzEMA as monomers and (mPEG$_{5000}$)$_2$-ABCPA as radical macroinitiator (ratio of monomer/initiator was 150:1), according to a previously published procedure [2]. Briefly, the starting materials were dissolved in anhydrous acetonitrile in airtight glass vials at a total concentration of 300 mg/mL. After flushing with nitrogen for at least 10 min at room temperature, the solution was heated to 70°C and stirred for 24 h at this temperature. Next, the formed polymers were precipitated by dropwise addition of the solution to an excess of diethyl ether. The supernatant was discarded and the precipitated polymers were dissolved in water, dialyzed (membrane cutoff 12−14 kDa) against water for at least 24 h, and finally recovered by freeze−drying.

^{1}H NMR ($CDCl_3$): δ (ppm) 6.5 (br, CON*H*CH$_2$), 5.0 (br, NHCH$_2$C*H*(CH$_3$)O and COC*H*(CH$_3$)O), 4.4 (br, COC*H*(CH$_3$)OH), 4.1 (br, OC*H*$_2$ of AzEMA), 3.6 (br, PEG methylene protons), 3.5 (br, C*H*$_2$N$_3$ of AzEMA), 3.4 (br, NHC*H*$_2$CH(CH$_3$)), 2.0−0.6 (main chain protons of the HPMAmLac$_2$ block).

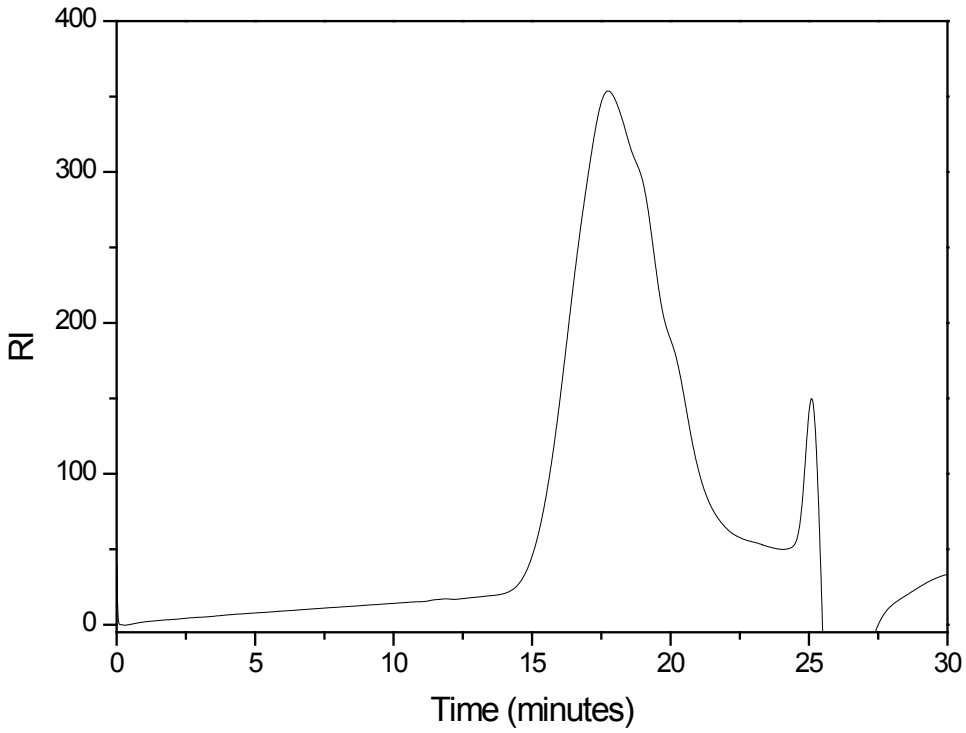

Figure S1. GPC analysis of mPEG-*b*-p(HPMAmLac$_2$-*co*-AzEMA).

References:

1. Van Nostrum, C.F., Neradovic, D., Soga, O., and Hennink, W.E. (2006) Polymeric micelles with transient stability: A novel delivery concept. *ACS Sympos. Ser. 923*, 40-54.
2. Soga, O., van Nostrum, C.F., Ramzi, A., Visser, T., Soulimani, F., Frederik, P.M. Bomans, P.H.H., and Hennink, W.E. (2004) Physicochemical characterization of degradable thermosensitive polymeric micelles. *Langmuir 20*, 9388-9395.