Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

Reconstitution properties of biologically active polymersomes after cryogenic freezing and freeze-drying process

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Content of ESI

Experimental

Synthesis of the compounds in order to get the block-copolymer

The first step was to synthesize the required block copolymer (**Figure 1-SI**) having methoxy (**BCP1**) end groups at their hydrophilic poly(ethylene glycol) (PEG) segment by using our previous published approach[1,2] through atom transfer radical polymerization (ATRP) and identical use of monomer ratio[3] for the fabrication of **BCP1**. The hydrophobic part of the block copolymers consists of pH-sensitive 2-(diethylamino)ethyl methacrylate (DEAEM) and photo-crosslinker, 3,4-dimethyl maleic imidoethyl methacrylate (PDMIBM).[4,5] The composition of **BCP1** was determined by ^{1}H-NMR and SEC-MALLS. The composition and the number average molecular weight (M_n) of the block copolymer **BCP1** were determined with ^{1}H NMR spectroscopy from the peak integrals of PEG (3.65 ppm), DEAEMA (2.65-2.78 ppm) and DMIBM (3.52 ppm) by taking the PEG block as an internal standard. Additionally, the molar mass distributions (*Đ*) were determined by SEC as described in previous section. **Table S1** shows the corresponding results.

Additional figures and tables

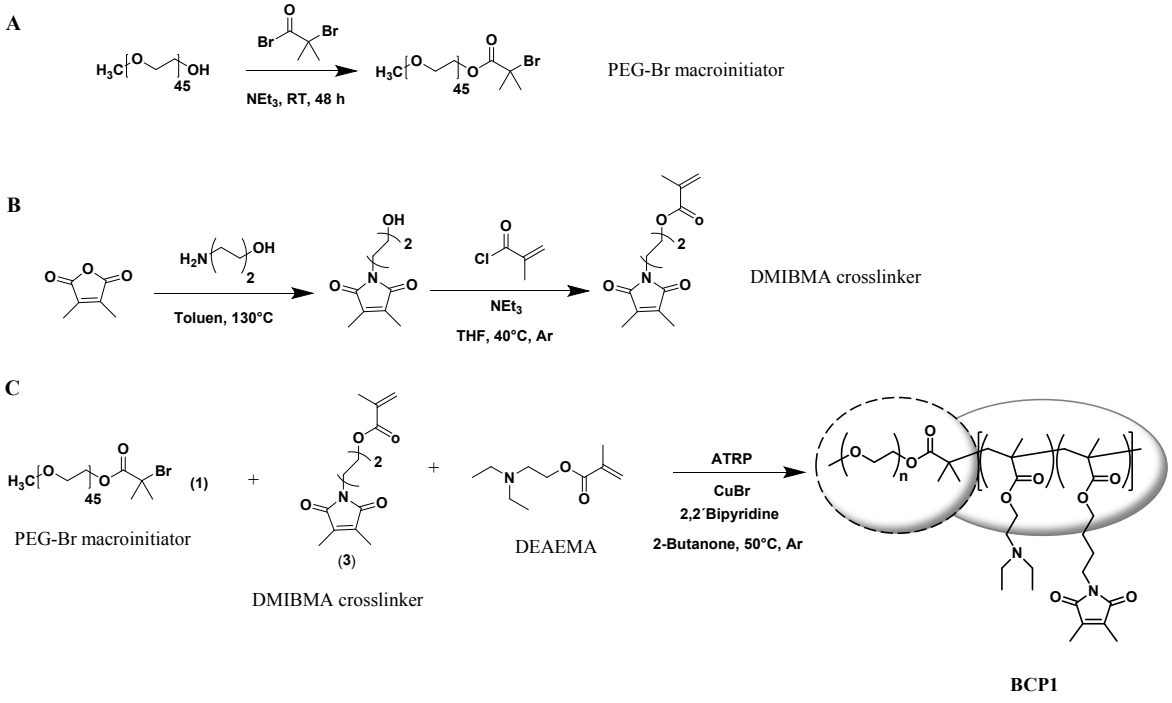

Figure ESI-1 Reaction scheme for the preparation of: A) PEG-Br macroinitiator, B) pre-crosslinker and the crosslinker DMIBMA and C) poly(ethyleneglycol)$_{45}$-*block*-poly(diethylaminoethyl-methacrylate-*stat*-3,4-dimethylmaleinimidobutylmethacrylate)$_{99}$ (PEG$_{45}$-*b*- P(DEAMA-*s*-DMIBM)$_{92}$; BCP1).

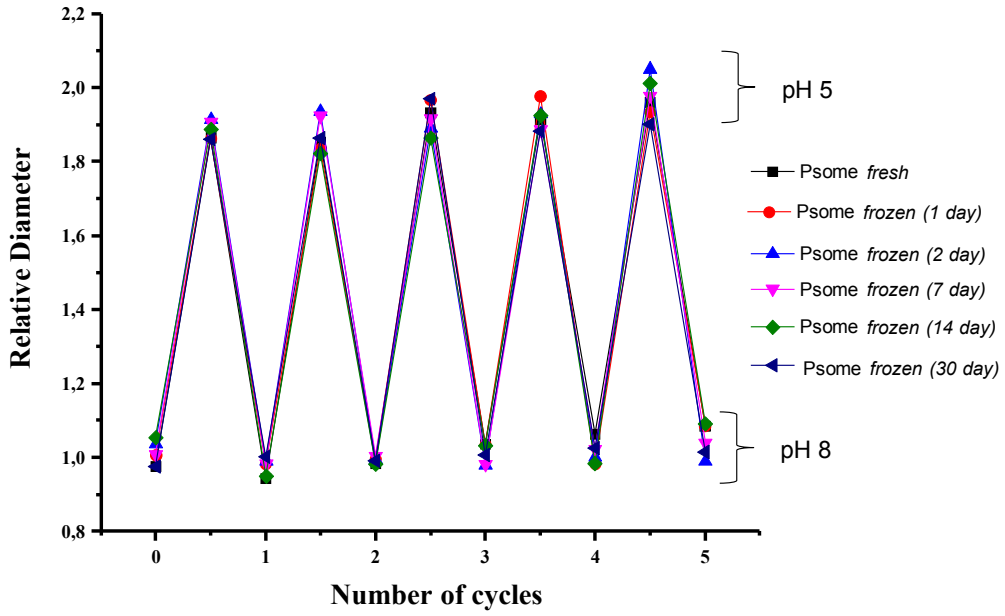

Figure ESI-2 Swelling-shrinking cycles of pH-responsive polymersomes between pH 5 and pH 8 Psome *fresh and* Psome *frozen* storage for different times.

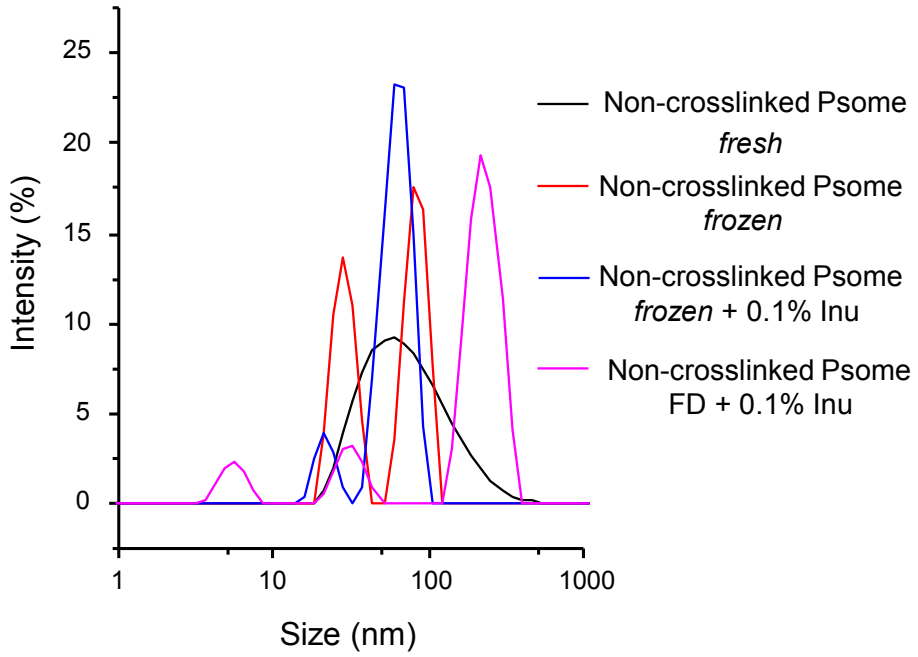

Figure ESI-3 Diameter of the reconstituted non- crosslinked Psomes studied by DLS using SM-1 (stored at -20°C for 8 days) and SM-2 (stored at 4°C for 8 days after freeze-drying process) as storage methods.

Table ESI-1 Specifications of Block copolymers synthesized by ATRP

Code	Polymer Chemical Composition	M_w (g/mol)[a]	M_n (g/mol)[a]	*Đ* (Mw/Mn)[a]	M_n estimated by NMR [b]
BCP1	PEG_{45}-b-($DEAEMA_{73}$-s-$DMIBM_{19}$)	29300	23850	1.22	20800

[a]Molar mass distribution is determined by SEC. [b] Molecular weight is calculated by ^1H NMR.

Table ESI-2 Diameter distribution of polymersomes (Psome) modified with HSA, investigated as (i) freshly prepared sample, (ii) frozen sample at -20°C for 1 day followed upon gentle thawing to room temperature, and (ii) after freeze-drying and direct redispersion in slightly acidic solution. *The DLS measurements correspond to Psome at pH 5.

Modified Psome*	Diameter (nm)	PDI
Psome-HSA *fresh*	128	0.224
Psome-HSA *frozen*	126	0.256
Psome-HSA-FD	116	0.181

References

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(4) Save, M.; Weaver, J. V. M.; Armes, S. P.; McKenna, P. *Macromolecules,* 2002, **35**, 1152.

(5) Vo, C. D.; Kuckling, D.; Adler, H. J. P.; Schönhoff, M. *Colloid. Polymer. Sci,* 2002, **280**, 400.