Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supplementary information

Nanocapsules with Stimuli-Responsive Moieties for Controlled Release Employing Light and Enzymatic Triggers

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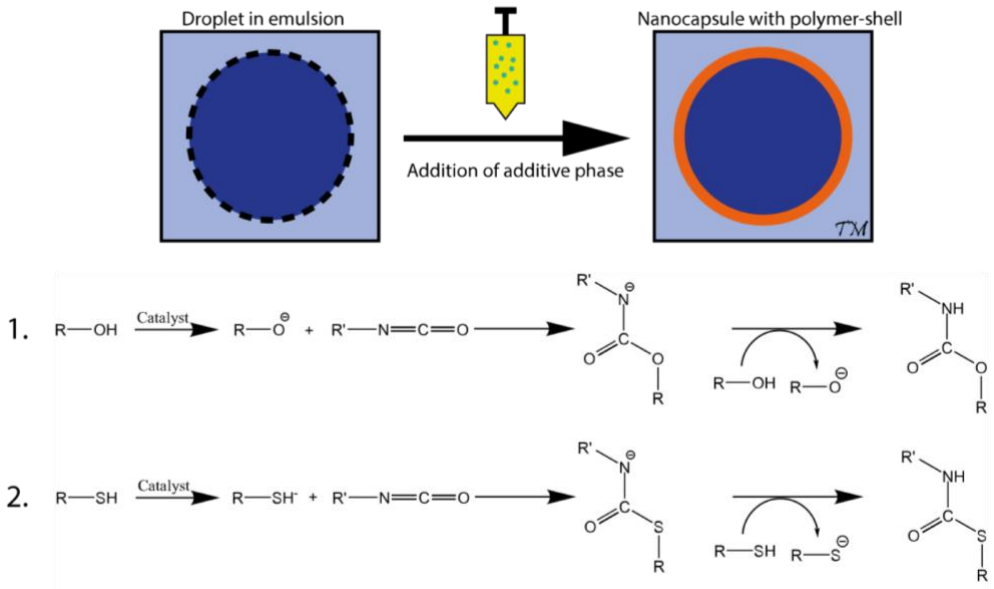
1. Additional experimental results

This section provides additional experimental results on the stimuli-responsive nanocapsules developed in this work. ***Scheme S1*** depicts the different reaction mechanisms between the functional monomers to prepare dual-responsive nanocapsules. ***Table S1*** provides an overview of the dispersion characteristics of stimuli-responsive nanocapsules containing payload.



Scheme S1. The stimuli-responsive nanocapsules were obtained by an interfacial hydroxyl-isocyanate (1) and thiol-isocyanate (2) reaction executed at the droplet interface using the inverse miniemulsion technique incorporating the functional monomers ethylene glycol bis(3-mercaptopropionate) and 4,4'-azobis(phenol).

Table S1. Characteristics of stimuli-responsive nanocapsules encapsulating hydrophilic payload.

Sample	Monomer(s) dispersed phase	Additive phase	Size [nm] /PDI (cyclohexane phase)	Size [nm] /PDI (aqueous phase)	Encapsulation efficiency
1’	3.2 mmol BDT, 0.8 mmol ethylene glycol bis(3-mercaptopropionate), 0.2 mmol PETMP, 2 mg rhodamine B	4.1 mmol TDI, DBU	185/0.14	193/0.19	84%
2’	3.2 mmol BDT, 0.8 mmol 4,4’-azobis(phenol), 0.2 mmol PETMP, 2 mg rhodamine B	4.1 mmol TDI, DBU	169/0.12	178/0.26	91%
3’	2.4 mmol BDT, 0.8 mmol ethylene glycol bis(3-mercaptopropionate), 0.8 mmol 4,4’-azobis(phenol), 0.2 mmol PETMP, 2 mg rhodamine B	4.1 mmol TDI, DBU	176/0.06	198/0.21	86%
4’	2.4 mmol BDT, 0.8 mmol ethylene glycol bis(3-mercaptopropionate), 0.8 mmol 4,4’-azobis(phenol), 0.2 mmol PETMP, 2 mg doxorubicin	4.1 mmol TDI, DBU	183/0.06	203/0.26	93%
5’	2.4 mmol BDT, 0.8 mmol ethylene glycol bis(3-mercaptopropionate), 0.8 mmol 4,4’-azobis(phenol), 0.2 mmol PETMP, 2 mg geranyl acetate	4.1 mmol TDI, DBU	167/0.06	186/0.16	87%

It can be observed that a small amount (~ 15%) of dye from the initially incorporated amount was detected outside of the nanocapsules in the supernatant. It is not unlikely that during redispersion and centrifugation steps, when the nanocapsules were subjected to mild mechanical forces, small leakages of dye (or molecules that are incorporated into the shell) can occur. However, in general the results indicate a good impermeability and resistance of the shell towards undesired release of payload.

Size distribution of the nanocarriers

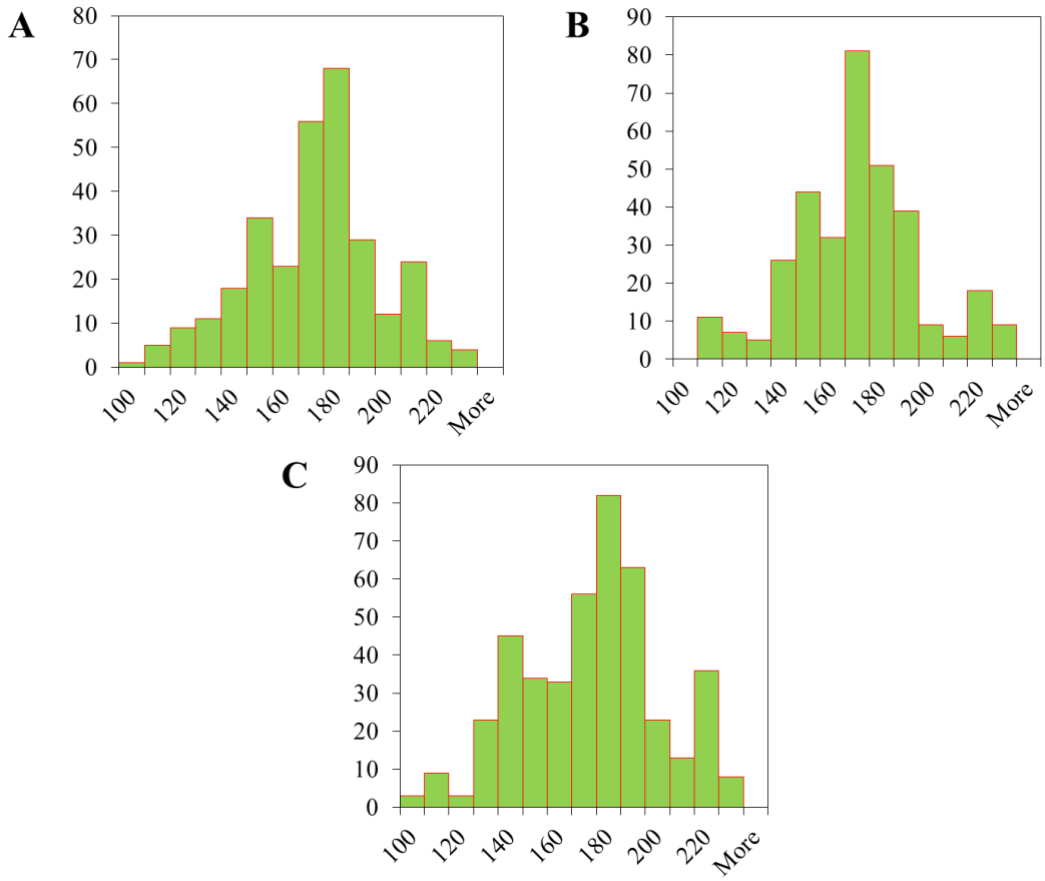


Figure S1. Size distribution of the stimuli-responsive polymeric nanocapsules obtained from TEM images for sample 1 (A), sample 2 (B) and sample 3 (C). HCImage processing and image analysis tools were used to quantitatively analyse more than 300 nanocapsules.

In Figure S1, the obtained average nanocapsules sizes were 171 ± 56 nm, 158 ± 41 nm and 173 ± 76 nm for samples 1, 2 and 3, respectively.

Transmission electron microscopy

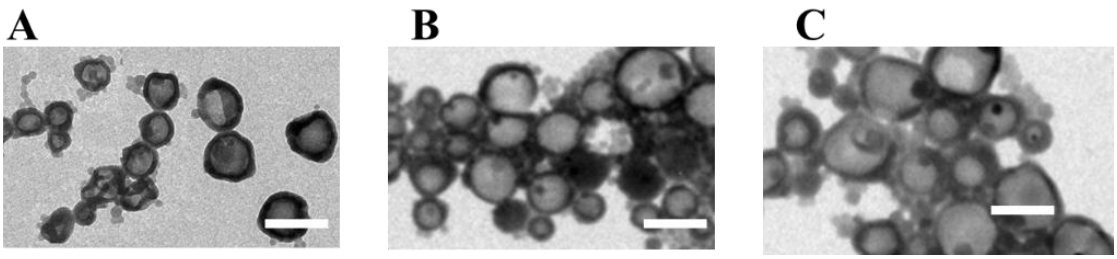


Figure S2. TEM images of sample 1 (A), 2 (B) and 3 (C) from the aqueous phase. Scale bar corresponds to 300 nm.

To be useful for biomedical applications, the nanocapsules dispersed in cyclohexane were transferred into an aqueous solution consisting of 0.1 wt.% SDS. To remove excess of surfactant molecules, the nanocapsule dispersions were washed by repetitive centrifugation. After redispersion in the aqueous phase, the core/shell structure of the polymeric nanocapsules and the presence of salt crystals (black dots within the nanocapsules) can be clearly observed, indicating a successful transfer of nanocapsules into the aqueous phase.

Stimuli-dependent release kinetics

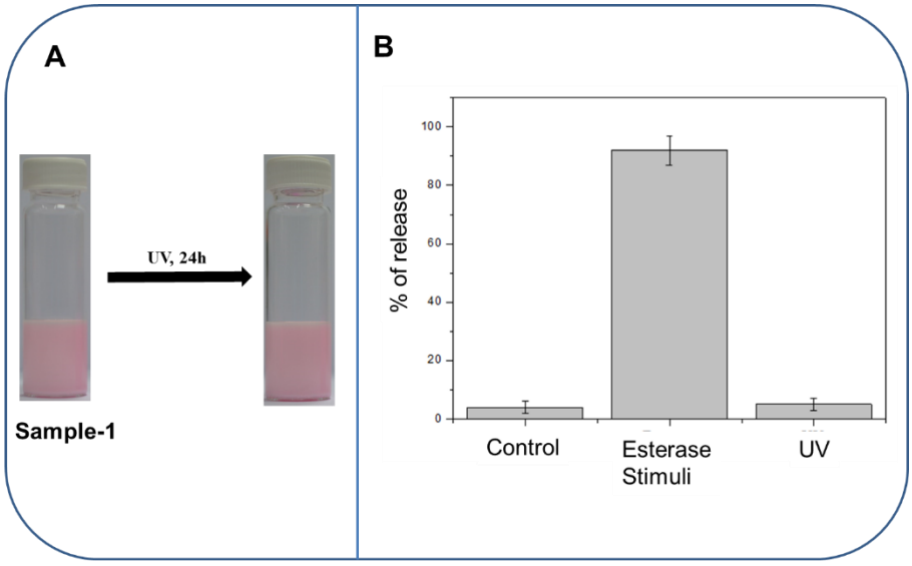


Figure S3. (A) Color change of aqueous dispersed sample 1' nanocapsules after exposure to UV-light. (B) Release profile of rhodamine B from sample 1' nanocapsules after enzymatic treatment (with esterase from porcine liver) and exposure to UV-light.

Sample 1’ nanocapsules contain only ester-linkages that are susceptible to degradation by the enzyme esterase, using ethylene glycol bis(3-mercaptopropionate) as functional monomer to form the capsule shell.

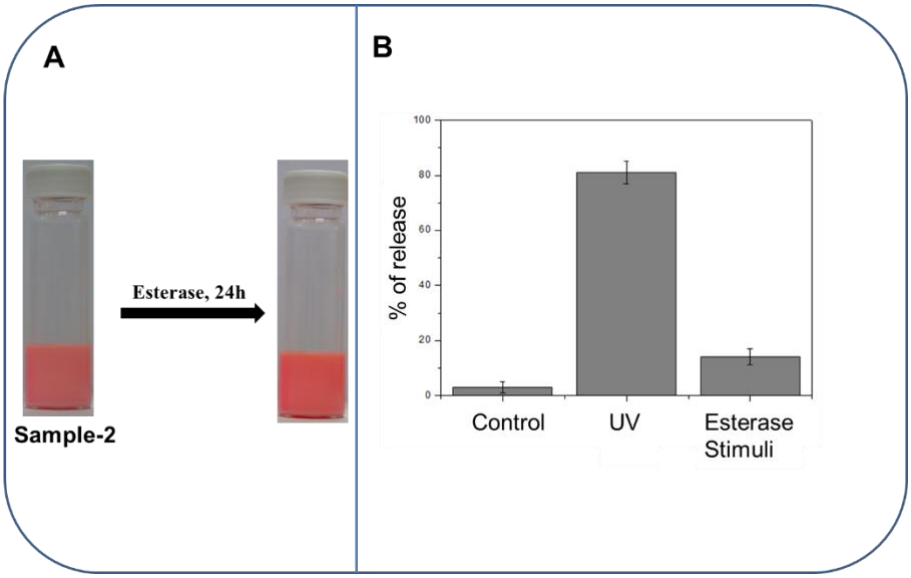


Figure S4. (A) Color change of aqueous dispersed sample 2’ nanocapsules upon enzymatic treatment with esterase (from porcine liver). (B) Release profile of rhodamine B from sample 2’ nanocapsules after enzymatic treatment (with esterase from porcine liver) and UV-light exposure.

Sample 2’ nanocapsules were synthesized using 4,4’-azobis(phenol) as a functional monomer. To this extent, these nanocapsules contain azo-linkages that undergo photoisomerization upon exposure to UVlight. Consequently, no release of dye is expected upon exposure to the enzyme esterase. However, dye release (± 17%) was observed upon enzymatic treatment for 24 hours which can be attributed to cleavage of the ester linkages present in PETMP (which is used as a crosslinker to impart post-surface grafting possibilities).

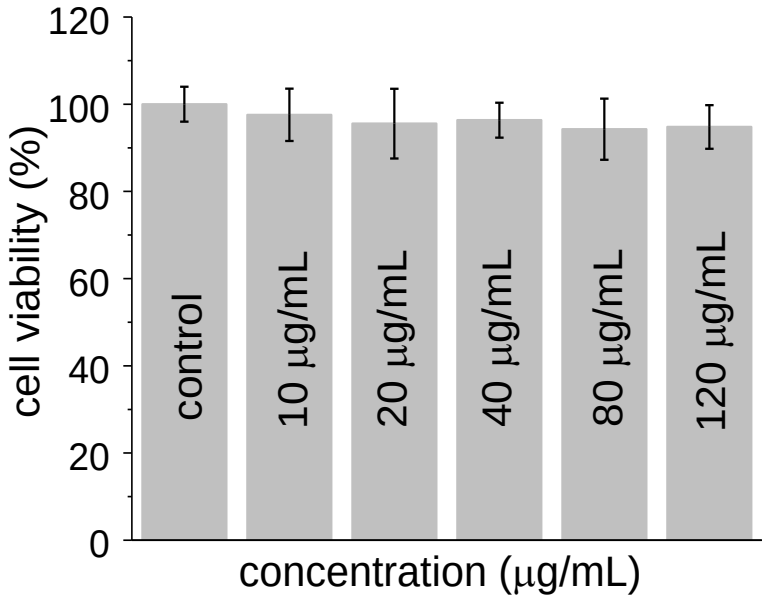


Figure S5. Cell viability of MCF 7 after 24 h exposure to a concentration range of nanocarriers, determined using the MTT assay. Data represent mean ± standard deviation of three replicates.

Figure S5 shows that cell viability levels remained stable as compared to the control group, no decrease in cell viability below 97 % was observed after exposure (24 h) to different concentrations of nanocapsules. This confirms that the synthesized nanocapsules are fully biocompatible. Here, the concentrations used are notably higher than the range used in medical applications. One-way Anova statistical analysis was used to determine if a concentration effect was present for the sample. At $p < 0.01$ no statistically significant difference between the concentrations used was present, thereby indicating no dose-dependent effect for the used concentration range.