ELECTRONIC SUPPLEMENTARY INFORMATION

(ESI)

Complex interfaces in "phase-change" contrast agents

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METHODS

Critical Aggregation Concentration (CAC) studies on DexMA

DexMA with a degree of substitution of 50 % (percentage ratio between the moles of methacrylated units and the total moles of repeating units) was synthesized as already reported.[1-3] CAC of DexMA was determined by pyrene method. Briefly, from a 10^{-6} M stock solution of pyrene in acetone, 1.8 mL aliquots were transferred in a series of test tubes allowing the solvent to evaporate overnight. A series of polymer aqueous solutions at known concentrations, ranging from 10^{-4} to 10^{2} mg/mL, were added to each tube after 10 minutes of sonication in a water bath. The final pyrene concentration in each tube was $6 \cdot 10^{-7}$ M. The mixtures were left to equilibrate in the dark for 48 h and then the fluorescence emission spectra were recorded between 350 and 500 nm with excitation wavelength of 341 nm using a spectrofluorophotometer RF-5301 PC, Shimadzu Scientific, (Milan, Italy). The ratio of the intensities at 373 nm and at 383 nm relative to the vibrational modes, I_1/I_3, of the monomeric pyrene in the emission spectra was calculated and reported in a semi-log plot vs. DexMA concentration, C_{DexMA}. The CAC of the polymer is obtained in correspondence of the break in the I_1/I_3 ratio vs. Log C_{DexMA} trends.

Interface tension by pendant drop method

Drops of DFP were generated against air to measure surface tension while, for interfacial tension measurements, DFP drops were extruded in an optical glass cuvette containing different liquids: water, surfactants in water, DexMA 0.1 % (w/V) and surfactants in water, DexMA 1 % (w/V) and surfactants in water, crosslinked DexMA 0.1 % (w/V) and surfactants in water, crosslinked DexMA 1 % (w/V) and surfactants in water. The mixtures used were prepared according to the same composition of the microdroplets. The crosslinked DexMA and surfactants mixtures in water were prepared by exposing the samples to a 365 nm light source for 20 minutes in the presence of Irgacure®2959 as photoinitiator, added at 0.3 % weight ratio in respect to the polymer. All the measurements were performed at 25 °C.

Differential Scanning Calorimetry (DSC) measurements

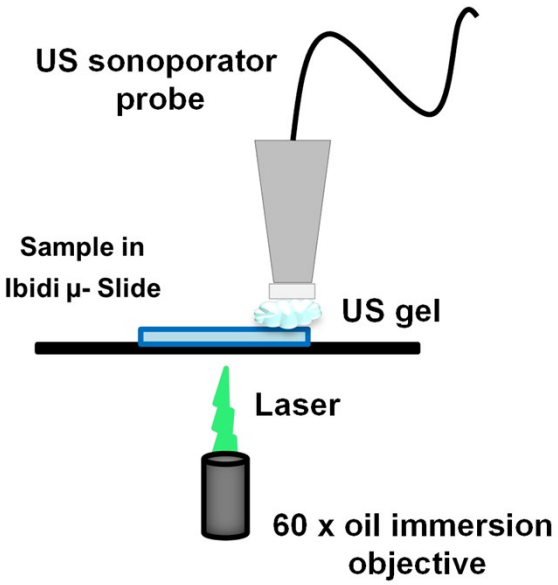
It was preliminary recorded a DSC scan of the liquid DFP and solids PA and Epikuron®200, separately, in order to assess their individual transition temperatures. DSC measurements were carried out by using a differential scanning calorimeter Q-200, TA-Instruments Inc. (Milan, Italy). Then the following samples were analyzed: (**1**) 20 mg of Epikuron®200 and PA mixture in water, prepared by dissolving Epikuron®200 and PA (3:1 weight ratio) in $CHCl_3$ in a round-bottom flask to obtain a thin lipid film by removing the solvent under vacuum. The film was rehydrated adding 500 μL of water at 50 °C followed by vortex stirring; (**2**) 20 mg of the microdroplets of a sample prepared by high speed homogenization (3 minutes at 13000 rpm) of a water mixture having the following composition: Epikuron®200, C = 0.005 % (w/V); PA, C = 0.002 % (w/V); DFP, C = 7 % (w/V); (**3**) 20 mg of the microdroplets of a sample prepared by high speed homogenization (3 minutes at 13000 rpm) of a water mixture having the following composition: Epikuron®200; C = 0.005 % (w/V); PA, C = 0.002 % (w/V); DFP, C = 7 % (w/V), uncrosslinked DexMA, C = 0.1 % (w/V). Measurements were recorded after weighing the pan, closed by a holed lid in order to allow vapors to exit during the heating process. An empty pan was used as reference. Thermograms were recorded under nitrogen atmosphere with a flow rate of 50 mL/min, at 1 °C/min after 1 min of equilibration, starting from 10 °C.

Widefield Fluorescence and Confocal Laser Scanning Microscopy (CLSM)

Captured images were analyzed by NIS Element AR software version 4.3, Nikon (Florence, Italy) and Nikon EZ-C1 software version 3.9 for fluorescence and confocal microscopy, respectively. RBITC fluorescence staining[3] of the microdroplet was detected exciting the sample with the He-Ne at 543 nm. 100 μL ($3 \cdot 10^8$ particles/mL) of a RBITC-labeled microdroplets sample was placed in an Ibidi "μ-Slide $I^{0.4}$" Luer flow chamber, Ibidi (Munich, Germany) and monitored by fluorescence microscopy during ultrasound (US) irradiation for the evaluation of the MB diameters immediately before switching off the US probe. In order to monitor the radial shrinking, CLSM was used as

described in Scheme S1: after switching off the US pressure field, a delay of 30 s was necessary to assure that the residual turbulence in the surrounding liquid was expired. The time evolution of the diameters of 16 single MBs was recorded by capturing confocal images every 30 s with an integration time of 1 s by manually refocusing the MBs on each equatorial plane. This procedure, although slowing down the frame capture rate, allows an accurate measure of the particle size after the core condensation and the simultaneous repositioning and refocusing of the relaxing MBs.

Scheme S1. Experimental set-up to follow MBs during and after Acoustic Droplet Vaporization (ADV).



RESULTS

1. Critical Aggregation studies of DexMA

CAC studies were performed to assess the amphiphilic nature of DexMA and its ability to stack in the surfactant layer. The characteristic dependence of the fluorescence vibrational fine structure of pyrene can be used to determine surfactants critical micelle concentration (CMC) and polymers CAC.[4, 5] Figure S1a shows the fluorescence intensity profile obtained for DexMA aqueous solution as a function of the DexMA concentration. The I_1/I_3 fluorescence intensities ratio were plotted against the polymer concentration. In Figure S1b the intersections of I_1/I_3 vs. Log C linear trends allowed the identification of CAC in DexMA solutions, corresponding to about 0.1 % (w/V).

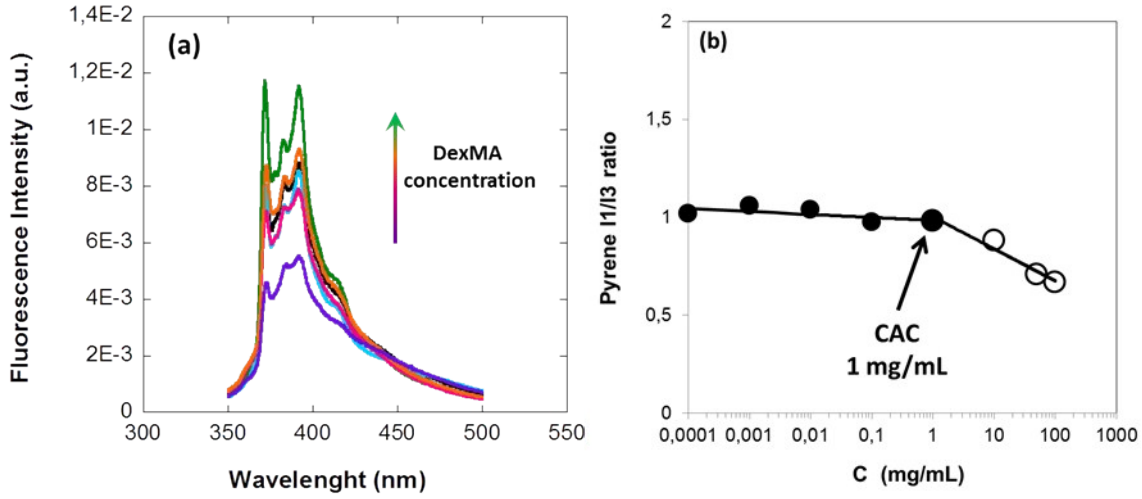


Figure S1. (a) Fluorescence intensity profile of pyrene in DexMA solutions (λex= 341 nm) at different DexMA concentrations, i.e. C, and (b) I_1/I_3 pyrene fluorescence intensities ratio as a function of C.

2. Interface tension measurements

Two system configurations were used, as shown in Figure S2.

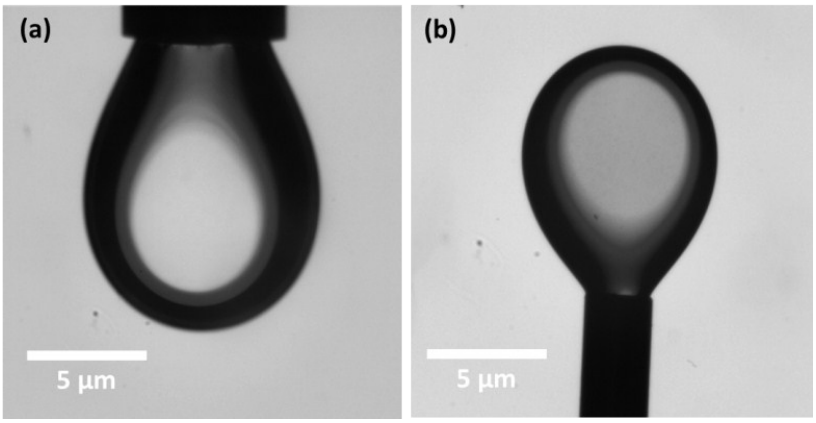


Figure S2. Different configurations for interfacial tension measurements by pendant drop method: (a) classical pendant drop with DFP drop in a medium constituted by crosslinked DexMA 0.1 % (w/V) and surfactants in water and (b) inverse pendant drop method with a drop constituted by a mixture of crosslinked DexMA 1 % (w/V) and surfactants in water, immersed in DFP as medium.

Results of the tensions of different interphases are provided in Table 1:

Table 1. Results from surface and interfacial tension measurements.

Test	**Dispersing phase**	**Drop phase**	**Interfacial tension (mN/m)**
A	Air	DFP	14.00 ± 0.01
B	Water	DFP	37.0 ± 1.9
C	Water + Surfactants	DFP	27.8 ± 0.6
D	DexMA 0.1 % (w/V) + Surfactants + Water	DFP	22.5 ± 0.4
E	DexMA 1 % (w/V) + Surfactants + Water	DFP	20.9 ± 0.5
F	Crosslinked DexMA 0.1 % (w/V) + Surfactants + Water	DFP	26.1 ± 0.7
G	DFP	Crosslinked DexMA 1 % (w/V) +	25.1 ± 0.9

		Surfactants + Water	

3. DSC measurements

In Figure S3, the DSC thermograms of liquid DFP (Figure S3a), solid PA (Figure S3b) and solid Epikuron®200 (Figure S3c) are reported.

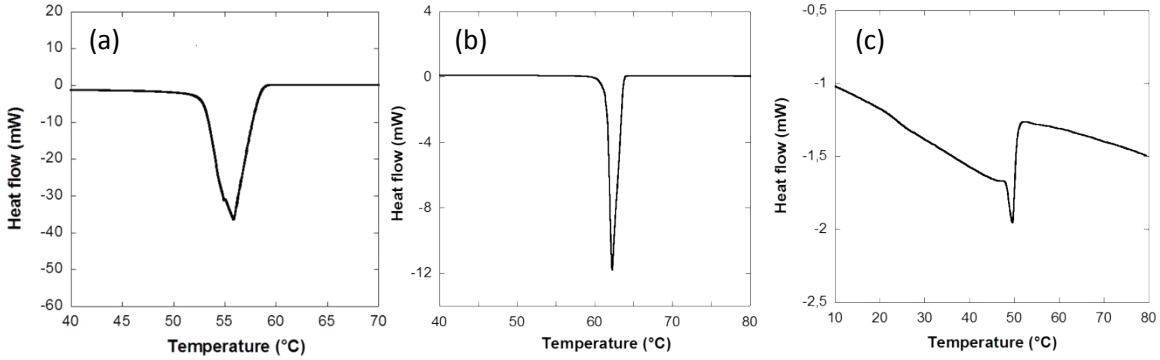


Figure S3. DSC scans of (a) liquid DFP; (b) solid PA; (c) solid Epikuron®200. Exothermic heat: up.

Analogously, Figure S4 shows the DSC thermograms of mixtures of the constituents of the droplets, adding each time a new component as described in the figure caption.

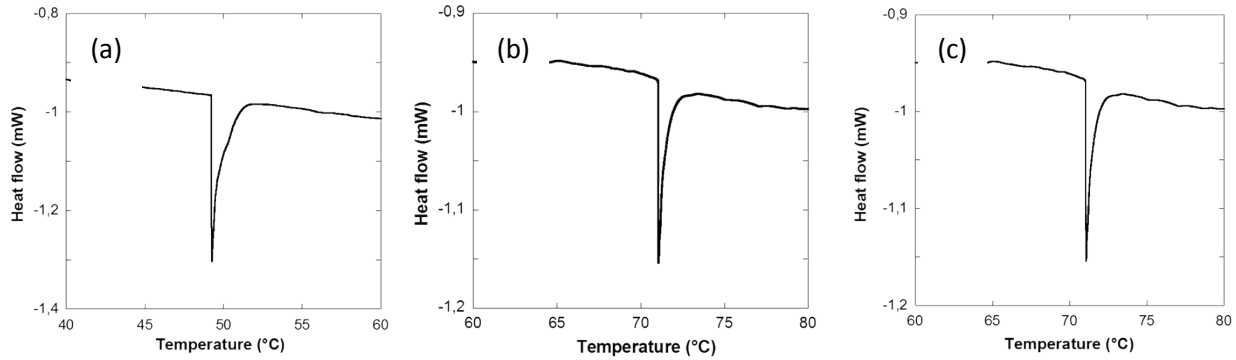


Figure S4. DSC scans of (a) mixture of Epikuron®200 and PA (3:1 weight ratio) in water; (b) water mixture of Epikuron®200, PA and DFP; (c) water mixture of Epikuron®200, PA, DFP and uncrosslinked DexMA, C = 0.1 % (w/V). Exothermic heat: up.

The liquid ↔ gas transition point of DFP encapsulated in a shell of PA and Epikuron®200 dispersed in water displays a temperature increase from 56 to 71 °C, Tests 3 and 6 of Table 2, respectively. When DFP is encapsulated by the surfactants layer and uncured DexMA at 0.1 % (w/V) and 1 % (w/V), the transition temperature raises from 56 °C to 72 and 77 °C, respectively, Tests 7 and 8 of Table 2. The coalescence effect, present in the uncrosslinked samples, hinders a direct correlation between the evaporation temperatures of the DFP phase and the size of the droplets.

Table 2. Summary of the transition temperature of pure components and mixtures.

Test	**Sample**	**Transition Temperature (°C)**
1	PA	61
2	Epikuron®200	47
3	DFP	56
4	Solid Mixture of Epikuron®200 + PA	46
5	Epikuron®200 + PA in water	49
6	Water mixture of DFP + Epikuron®200 + PA	71
7	Water mixture of DFP + Epikuron®200 + PA + 0.1 % (w/V) DexMA (uncrosslinked)	72
8	Water mixture of DFP + Epikuron®200 + PA + 1 % (w/V) DexMA (uncrosslinked)	77
9	Water mixture of DFP + Epikuron®200 + PA + 1 % (w/V) DexMA (UV cured)	72 - 78

4. Widefield Fluorescence and CLSM

In Figure S5 a confocal micrograph of an ultrasound irradiated sample of DexMA droplets is captured after 600 s from irradiation as for the sample illustrated in Figure 7 of the main text. Red fluorescence cluster (see arrow) is due to the RBITC labelling of DexMA polymer chains coming from US destroyed droplet shells. The ring evidences the presence of domains of green fluorescence, FITC, locally suspended in an oil phase domain (black), coming from the collapse of uncured DexMA droplets with DFP core.

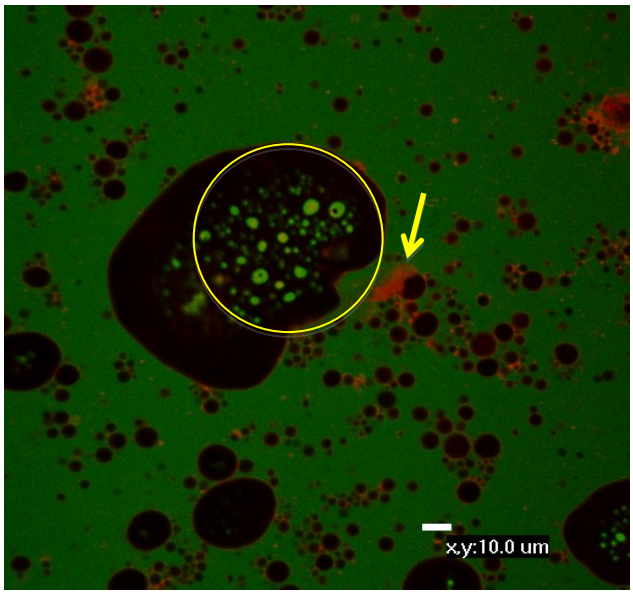


Figure S5. Confocal microscopy image of DexMA UV uncured droplets after 600 s from US irradiation. Yellow arrow indicates DFP spillage, evidenced by nile red (red fluorescence); yellow ring contours FITC labeled water droplets inside a large DFP domain.

In Figure S6, it is shown a time lapse experiment to monitor the radial shrinking of RBITC-labeled MBs with hybrid shell. Time t = 0 is taken at the switching off of the US probe in the experimental configuration described in Scheme S1.

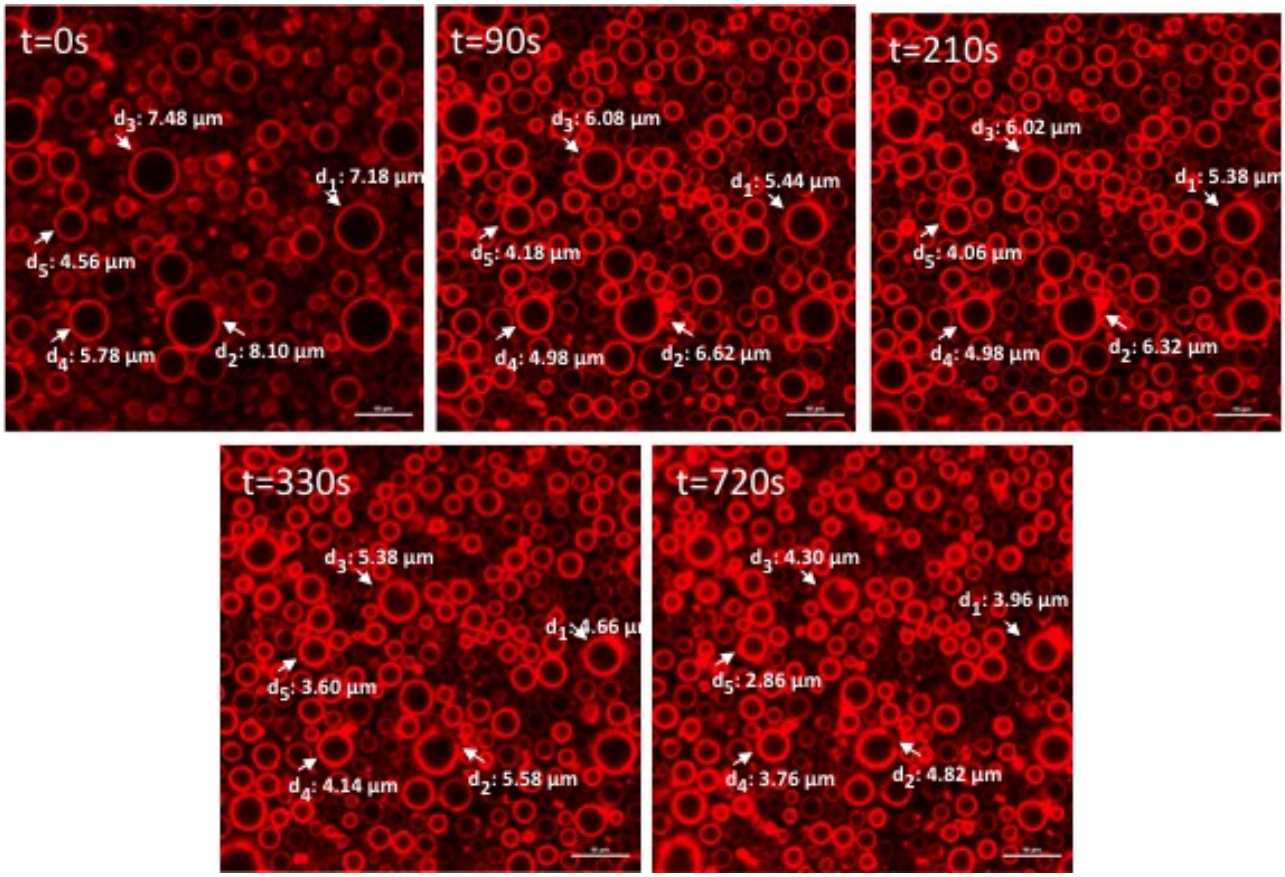


Figure S6. Radial shrinking of individual RBITC- labeled MBs monitored by a time lapse experiment in CLSM modality.

In Figure S7 a typical fluorescence microscopy image captured during US irradiation is shown.

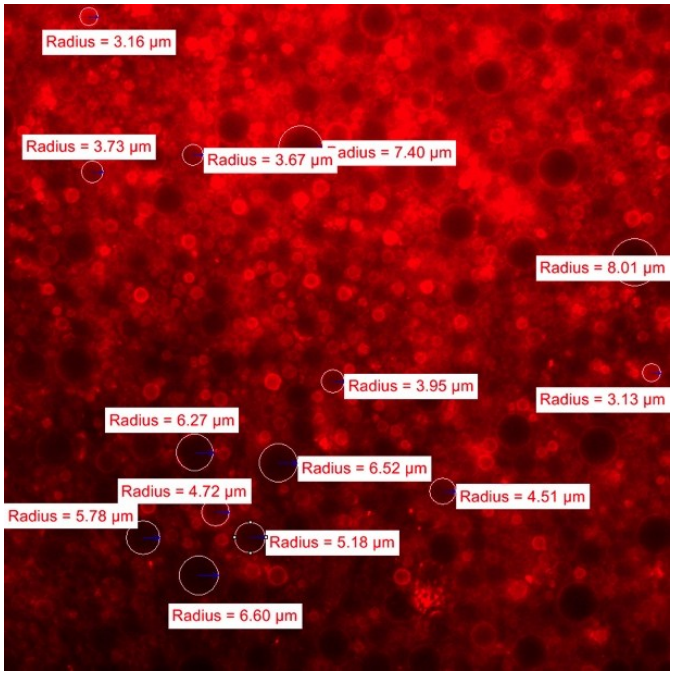


Figure S7. Fluorescence microscopy image captured in the presence of US field. Focused RBITC-labeled MBs are highlighted with corresponding radii. Oil immersion objective magnification: 60×.

5. Modelling of the shell shrinking

An example of the fit obtained for the radial shrinking of hybrid MBs imposing a shear viscosity value of $1 \cdot 10^8$ Pa·s is shown in Figure S8.

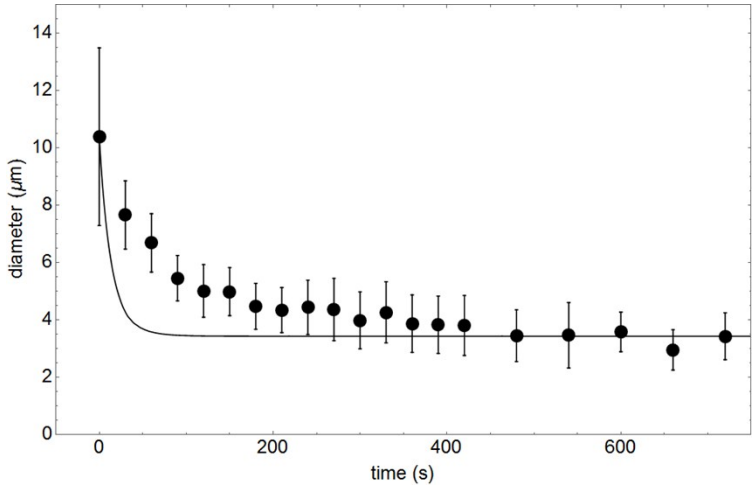


Figure S8. Simulation of the MBs relaxation using a shear viscosity $\mu_S = 1 \cdot 10^8$ Pa·s.

REFERENCES

1 W. N. E. van Dijk-Wolthius, J. J. Kettenes-van den Bosh, A. van der Ken-van Hoff and W. E. Hennink, *Macromolecules*, 1997, **30**, 3411.

2 W. N. E. van Dijk-Wolthius, O. Franssen, H. Talsma, M. Steenberger, J. J. Kettenes-van den Bosh and W. E. Hennink, *Macromolecules*, 1995, **28**, 6317.

3 S. Capece, E. Chiessi, R. Cavalli, P. Giustetto, D. Grishenkov and G. Paradossi, *Chem. Commun.*, 2013, **49**, 5763.

4 K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*,1977, **99**, 2039.

5 N. J. Turro, B. H. Baretz and P. L. Kuo, *Macromolecules*, 1984, **17**, 1321.