polymer/salt in water, which leads to a macroscopic liquid phase separation.\textsuperscript{7} Aqueous-two phase systems (ATPS) depend among other factors on polymer concentration, the critical polymer concentration.\textsuperscript{13,17,18} Various studies showed a significant influence of the molar mass to the critical polymer concentration and correspondingly a stable ATPS depend among other factors on polymer concentration, the critical polymer concentration.\textsuperscript{13,17,18} Various studies showed a significant influence of the molar mass to the critical polymer concentration and correspondingly a stable ATPS.

**Introduction**

Water-based polymer systems, especially aqueous-two phase systems (ATPS) are important technologies in areas like medicine,\textsuperscript{1} food industry\textsuperscript{2,3} as well as extraction, purification, and separation of biocompatible aqueous solutions of macromolecules,\textsuperscript{4} proteins,\textsuperscript{5} or metal ions.\textsuperscript{6} An ATPS forms by dissolving two incompatible compounds \textit{e.g.} polymer/polymer or polymer/salt in water, which leads to a macroscopic liquid–liquid phase separation.\textsuperscript{7–10} The most common system, using two polymers is the ATPS formed from poly(ethylene glycol) (PEG) and dextran (Dex).\textsuperscript{5,11} The mixture of PEG and Dex forms an ATPS, when the concentration exceeds the critical polymer concentration. One homopolymer enriches in the upper phase and the second homo-polymers enriches in the lower phase of the two-phase system. The driving force of the demixing process in an all aqueous system is the enthalpy associated with, for example, water–polymer and polymer–polymer interaction opposed by loss of entropy during phase separation. When the entropic contribution favouring mixing becomes smaller relative to the enthalpic contribution opposing it, phase separation occurs.\textsuperscript{12} Phase separation and behaviour in the ATPS depend among other factors on polymer concentration, polymer molar mass, pH, and temperature.\textsuperscript{13–16} One key factor for the critical polymer concentration and correspondingly a stable ATPS is the molar mass of the used compounds. In recen years, various studies showed a significant influence of the molar mass to the critical polymer concentration.\textsuperscript{13,17,18}

Due to the high biocompatibility, water-in-water (w/w) emulsions based on ATPS have received increased attention recently.\textsuperscript{19,20} The dispersion of two thermodynamically incompatible aqueous solutions of macromolecules, \textit{e.g.} two hydrophilic polymers, forms a w/w emulsion. Different polymer mixtures were used in ATPS for w/w emulsion formation, for example, PEG and Dex\textsuperscript{21} or different variations of polyacrylamides.\textsuperscript{17} Nevertheless, the polymer combination PEG and Dex is most commonly utilised for the formation of w/w emulsions. Classic emulsions such as water-in-oil (w/o) or oil-in-water (o/w) emulsions can be stabilised by surfactants or larger particles.\textsuperscript{22} For w/w emulsions, stabilisation based on surfactants is not suitable, due to lower interfacial tension of the ATPS and a very broad interface between the aqueous phases where small surfactant molecules cannot align properly.\textsuperscript{23,24} Thus, various types of particles were introduced for the stabilisation of w/w emulsions \textit{e.g.} latex particles,\textsuperscript{25} polydopamine particles\textsuperscript{26,27} or aggregated double hydrophilic block copolymers.\textsuperscript{28} One strategy for the stabilisation of w/w emulsions is to work with external triggers, for example, temperature\textsuperscript{28,29} or pH-value.\textsuperscript{30,31} That avenue leads to sensitive w/w emulsions, depending on a defined temperature or pH-value. For example, Nicolai and co-workers introduced linear polyelectrolytes, such as diethyl aminoethyl dextran, to stabilise a PEG and Dex w/w emulsion using different pH-values.\textsuperscript{30} Freitas and co-workers reported a pH-switchable aqueous emulsion of xyllohexan and amylopectin stabilised \textit{via} polysaccharide-coated protein particles.\textsuperscript{31} Pavlović \textit{et al.} showed temperature sensitive w/w emulsions employing a double hydrophilic block copolymer that featured a thermo-responsive block and thus thermo-responsive aggregation.\textsuperscript{28} So far, the literature showed that w/w emulsions can be designed to be responsive to external triggers. Especially the use of defined polymer-based stabilisers with integrated pH or temperature switchable blocks enables a considerable control of the emulsion state.

**Aqueous two-phase system**

Aqueous-two phase systems (ATPS) and water-in-water (w/w) emulsions are a major topic in chemistry and biology, mainly due to their biocompatibility and various applications. In here, the ATPS formed from poly(N,N-dimethylacrylamide) (PDMA) with three different molar masses (24k – 10\textsuperscript{5} g mol\textsuperscript{−1}) and commercial pullulan is investigated. Additionally, the ATPS with ultra-high molecular weight PDMA was transformed into a w/w emulsion using a pH-responsive block copolymer as stabiliser. The w/w emulsion could be destabilised or stabilised, depending on the current pH. The novel pH sensitive w/w emulsion will open up new pathways for example for the encapsulation of biomolecules.
In order to investigate novel ATPS systems and w/w emulsions including new polysaccharides, pullulan (Pull) is a viable choice.\textsuperscript{43,44} Pullulan consists of maltotriose units coupled via 1,6-\alpha-bonds\textsuperscript{44} and is well-known for its application in biomedicine,\textsuperscript{15,16,17} e.g. as a carrier for gene delivery\textsuperscript{16,17}, and to form polysaccharide nanoparticles.\textsuperscript{38,39} Moreover, Nicolai and co-workers used Pull to form a w/w emulsion with amylopectin using protein particles to stabilise the emulsion.\textsuperscript{31} Pull shows an interesting aggregation behaviour as part of block copolymers in combination with various polymer blocks, e.g. poly(N, N-dimethylacrylamide) (PDMA).\textsuperscript{40,41} These studies on block copolymer self-assembly are an indication that polyacrylamides such as PDMA might be a good choice for an ATPS and the formation of w/w emulsions in combination with Pull.\textsuperscript{17,42} PDMA featuring different molar masses can be synthesised via reversible addition-fragmentation chain transfer (RAFT) polymerisation techniques e.g. photo-iniferter (PI)-RAFT polymerisation,\textsuperscript{43,44} which gives a handle towards tailored ATPS and w/w emulsion formation.

Herein, the ATPS formation of commercial Pull and PDMA different PDMAVs were synthesised via RAFT polymerisation and molar masses were varied between lower molar mass of 24 kg mol\textsuperscript{-1} and ultra high molecular weight (UHMW) > 1 × 10\textsuperscript{6} g mol\textsuperscript{-1}. The mixtures of PDMA with Pull were analysed and revealed ATPS formation. Additionally, the ATPS formed by UHMW PDMA and Pull was used to form w/w emulsions stabilised with poly(styrene) (PS) nanoparticles or the pH responsive block copolymer poly(2-(dimethylamino)ethyl methacrylate)-b-poly(oligo(ethylene glycol) methyl ether methacrylate) (PDMAEMA-b-POEGMA).\textsuperscript{45} The emulsions were further analysed via bright-field microscopy and confocal laser scanning microscopy (CLSM). In order to localise the polymer in the emulsion, Rhodamine B (Rhb)- and Fluorescein-labelled polymers were employed. The w/w emulsions stabilised with PDMAEMA-b-POEGMA showed a pH sensitive stability enabling to turn the emulsion state on or off via pH adjustment (Scheme 1).

**Scheme 1** Overview of the pH sensitive water-in-water emulsion using the ATPS of PDMA and Pull stabilised with pH sensitive PDMAEMA-b-POEGMA (POEGMA block shown in blue; pH sensitive PDMAEMA block shown in red).

### Experimental

**Materials**

Acetone (Fisher, analytical grade), acetic acid (1.0 N, VWR), azobis(isobutyronitrile) (AIBN, 99%, Sigma Aldrich, recrystallised from methanol), dichloromethane (DCM, analytical grade, VWR), 2-bromoisobutyric acid (98.5%, Sigma Aldrich), carbon disulphide (CS\textsubscript{2}, 99%, Sigma-Aldrich), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (Sigma Aldrich), 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%, Sigma Aldrich, passed over a column of basic aluminium oxide), dimethyl sulfoxide (DMSO; Merck Millipore, Emsure\textsuperscript{®}, ACS), N,N-dimethyl formamide (DMF, SLS), N,N-dimethylacrylamide (DMA, Sigma Aldrich, passed over a column of basic aluminium oxide), ethanethiol (98%, Alfa Aesar), ethyl acetate (99.5%, VWR), Fluorescein isothiocyanate (FITC, Sigma Aldrich), n-hexane (95%, Sigma Aldrich), hydrochloric acid (conc., Fisher), Millipore water (obtained from a Sartorius Arium pro ultrapure water system), oligo(ethylene glycol methyl ether) methacrylate (OEGMA, Sigma Aldrich, \( M_\text{w} = 500 \text{ g mol}^{-1} \), passed over a column of basic aluminium oxide), poly(styrene) (PS) latex nanoparticles (0.1 µm, negatively charged, Sigma Aldrich, 10% aqueous suspension), potassium phosphate (Sigma Aldrich), pullulan (Pull, TCI), Rhodamine B isothiocyanate (RITC, Sigma-Aldrich), sodium acetate (anhydrous, 98%, Fisher), sodium hydroxide (NaOH, Fisher), sodium sulfate (anhydrous, SLS) and tetrahydrofuran (THF, 99.85%, Acros Organics) were used as received unless otherwise noted. 2-(((Ethylthio)carbonothioylthio)-2-methylpropanoic acid (EMP),\textsuperscript{46,47} RITC-PDMA\textsuperscript{17} and FITC-Pull\textsuperscript{46,49} were synthesised according to the literature.

**Analytical methods**

\textsuperscript{1}H-NMR spectra were recorded in deuterium oxide (D\textsubscript{2}O, Aldrich) at ambient temperature at 400 MHz with a Bruker Ascend\textsuperscript{400}. Size exclusion chromatography (SEC) of UHMW PDMA was conducted in 0.1 M aqueous NaNO\textsubscript{3} buffer at 25 °C using a column system with a PL Aquagel-OH Guard and PL Aquagel-OH MIXED-H and Viscotek VE 3580 RI detector and Viscotek SEC-MALS 20 for the molar mass determination. The system was calibrated with Pull standards. SEC of low molar mass PDMA was conducted in NMP with 0.005 mol L\textsuperscript{-1} LiBr and methyl benzoate as internal standard at 70 °C using a column system with PSS GRAM VS, PSS GRAM 7 µm 100 A, PSS GRAM 7 µm 1000 A columns and PSS SECurity Refractive Index-1260 RID detector and calibration with PS standards. SEC of PDMAEMA and PDMAEMA-b-POEGMA was conducted in DMF at 25 °C using a column system of two Agilent PLgel 5 µm Mixed-D 300 × 7.5 mm columns and an Agilent PLgel Guard 50 × 7.5 mm column, a Shimadzu RID-20A refractive index detector and a calibration with PEG standards. A Brookhaven differential refractometer was used for the determination of \( dn/dc \). Confocal laser scanning microscopy (CLSM) and bright field microscopy were performed on a Zeiss LSM710 confocal microscope (Zeiss, Göttingen, Germany) and software Carl Zeiss ZEN 2011 v7.0.3.286. LD EC Epiplan...
Destabilised DMA (1.0 g, 10 mmol, 1.0 eq. from a stock of 1 mg mL$^{-1}$ DMSO), and acetate buffer (1 mL, 0.2 M, and pH = 5) were mixed in a vial (7 mL) containing a stirring bar and sealed with a septum. The solution was bubbled with nitrogen for 30 min and the polymerisation was initiated using a UV lamp (nail lamp, 4 × 9 W, $\lambda \approx 365$ nm). The polymerisation was stopped after 24 h. Subsequently, the polymer was dialysed against deionised water (Spectra/Por 3500 Da) for 3 days. Finally, the sample was freeze-dried and a white solid (780 mg, $M_n = 1.07 \times 10^6$ g mol$^{-1}$, $D = 1.40$) was obtained.

### Preparation of ATPS and phase diagram

PDMA (50 mg) was dissolved in deionised water (450 mg) to obtain a 10 wt% solution. A 10 wt% solution of pullulan was prepared in the same way. Afterwards both solutions were mixed to receive a 5.0 wt%/5.0 wt% mixture. Subsequently, the solution was equilibrated at ambient temperature in order to demix, investigated and diluted (100 mg of deionised water each cycle). The process was repeated, until no phase separation was observed, which was recorded as the data point of the binodal curve. All other concentration combinations were conducted in a similar way.

### Preparation of w/w emulsions with additional labelled PDMA and Pull

PDMA (12 mg), RITC–PDMA (3 mg), pullulan (12 mg), and FITC-pullulan (3 mg) were dissolved in water (470 mg, pH = 5 or 9) to form a 3.0/3.0 wt% solution. PDMAEMA-b-POEGMA (10 mg) were dispersed in water (490 mg, pH = 5 or 9) to generate a 2.0 wt% dispersion. Both mixtures were combined to obtain a concentration of PDMA/Pull 1.5/1.5 wt% and 1.0 wt% PDMAEMA-b-POEGMA in the mixture. The mixture was subjected to ultrasonic treatment for 2 min, shaken by hand for 1 min, and subsequently analysed via CLSM. After 24 h, phase separation was observed and the sample was analysed again via CLSM.

### Results and discussion

#### Synthesis of various poly(N,N-dimethylacrylamides) via RAFT polymerisation

For ATPS formation, PDMA with various molar masses was synthesised at first. RAFT polymerisation is a superb avenue for the synthesis of polyacrylamides such as PDMA. For the lower and medium molar mass range, PDMA was synthesised via classical RAFT polymerisation. EMP was used as the chain transfer agent and the reaction was performed at 65 °C with AIBN as initiator in DMF. In the case of the UHMW PDMA, EMP was used as photoiniferter in acetate buffer via UV light (nail lamp, $\lambda = 365$ nm). Conversions were determined by $^1$H-NMR (Fig. S1–S3†) revealing quantitative monomer conversion for low molar mass PDMA, 75% monomer conversion for medium molar mass PDMA and quantitative monomer conversion for UHMW PDMA. The low and medium molar mass PDMA were analysed via GPC in NMP against PS standards (Fig. S4a and Table S1†) indicating in molar mass of $M_n = 23 \ 900 \ \text{g mol}^{-1}$ and $M_n = 80 \ 000 \ \text{g mol}^{-1}$ and a dispersity of $D = 1.11$ and $D = 1.06$, respectively. UHMW PDMA was obtained with a molar mass of $M_n = 1.07 \times 10^6 \ \text{g mol}^{-1}$ and a dispersity of $D = 1.40$, as analysed via SEC with MALS detection in 0.1 N NaNO$_3$ (Fig. S4b and Table S1†).

#### ATPS of PDMA and pullulan

In order to study the phase behaviour of PDMA and pullulan in water, ATPS formation of pullulan and PDMA of different molar mass in aqueous solution was investigated. For that, phase diagrams were elucidated for three different molar masses of PDMA (24k, 80k and 1 × 10$^6$ g mol$^{-1}$) with commercial pullulan.

To develop the ATPS phase diagram, start solutions of total 10 wt% polymer (PDMA/Pull) concentration (9/1, 7.5/2.5, 5/5,
2.5/7.5, 1/9 wt%, respectively) were prepared. Subsequently, the solutions were mixed, equilibrated at ambient temperature to demix, investigated, and diluted to find the concentration at which only one phase is observed (Fig. 1a). The last concentration with visible phase separation, was used as a data point for the binodal, which is the line separating the one- and two-phase area in the phase diagram (Fig. 1b and Fig. S7†). A shift of the binodal was observed depending on PDMA molar mass. For the combination with low and medium molar mass PDMA the binodal is located at higher concentration. The lowest concentration for an observed phase separation for an observed phase separation on equal polymer concentrations were at 4.0/4.0 wt% for PDMA24k and 3.1/3.1 wt% for PDMA80k. However, for the ATPS using UHMW PDMA the binodal is located at significantly lower concentrations with a lowest concentration for an observed phase separation on equal concentrations of polymer of 1.25/1.25 wt%.

These results show the significant influence of the molar mass of the polymers for the minimum required polymer content for a phase separation. The higher the molar mass, the lower the required concentration for a stable ATPS, which is an effect known from literature.13,18 In order to quantify the demixing of the individual polymer types in the ATPS, the location and concentration of each polymer was detected via 1H-NMR of each phase (Fig. S5 and S6†) employing DMF as internal standard. The results showed a clear separation of the polymers after 24 h. PDMA was enriched in the upper phase and pullulan was enriched in the lower phase of the ATPS. However, in each phase a residual amount of the opposite polymer was present in the respective depleted phases. After 24 h the partition coefficients (eqn (S1)†) for the ATPS of PDMA106 and pullulan were 17.9 for PDMA106 and 0.067 for pullulan in the upper phase as well as 0.056 and 14.87 for PDMA106 and 0.067 for pullulan in the lower phase.

W/w emulsion stabilised by polymer particles
To form a w/w emulsion, the ATPS of UHMW PDMA and pullulan was chosen due to the low polymer concentration required for formation of a stable ATPS. An ATPS of UHMW PDMA and commercial pullulan was prepared at a concentration of 1.5/1.5 wt%, which was selected because it is placed far enough in the two-phase area of the phase diagram to assure a stable ATPS. To stabilise the w/w emulsion, negatively charged PS latex nanoparticles and PDMAEMA-b-POEGMA aggregate particles were used (Scheme 2).

In order to form w/w emulsions, negatively charged PS latex nanoparticles with around 100 nm diameter were added to the ATPS to give a final stabiliser concentration of 0.1 wt%. Subsequently, the mixture was subjected to ultrasonic treatment for 2 min and shaken by hand for 1 min. The mixture turned cloudy, which indicated the formation of an emulsion stabilised with PS nanoparticles. In the following, the emulsion was analysed directly after preparation via bright-field microscopy displaying droplet formation (Fig. S8†). The average droplet size directly after preparation was 32 ± 5 µm at pH = 6 and 76 ± 26 µm at pH = 9. After approximately 3 h the mixture started to phase-separate, which was completed after 24 h. The upper phase remained cloudy, and the lower phase turned clear. Both phases were analysed via bright-field

![Fig. 1](image-url) (a) ATPS of each combination at a total polymer concentration of 10 wt% (5 wt% each). (b) Phase diagrams of the ATPS for all PDMA/Pull combinations employing PDMA24k, PDMA80k and PDMA106 showing the experimental binodal (black, red and blue curve, respectively).
microscopy (Fig. S8†), revealing droplets in the upper phase and no droplets in the lower phase. The average droplet size in the cloudy phase after phase separation was around 49 ± 22 µm at pH = 6 and 118 ± 53 µm at pH = 9. The results revealed droplet size increase during phase separation for the emulsion stabilised by PS. Additionally, the average droplet size of the emulsion in basic solution was significantly higher in comparison to the emulsion in acidic solution. One key factor for the droplet size is the preparation of the emulsion as it was treated ultrasound and shaken by hand only. More defined droplets could be generated e.g. via microfluidics.

To investigate a pH sensitive w/w emulsion stabiliser, PDMAEMA-b-POEGMA was employed. PDMAEMA-b-POEGMA was synthesised via RAFT polymerisation using 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid as a chain transfer agent. The block copolymer was obtained with a molar mass of 108 000 g mol⁻¹ and a dispersity of D = 1.37 (Fig. S9†). The block copolymer PDMAEMA-b-POEGMA was employed due to the pH sensitivity of the PDMAEMA block leading to aggregate formation under basic conditions.45,50 The pKₐ of PDMAEMA is in a range between 7 and 7.5.45,51 Due the tertiary amine in PDMAEMA, charge density can be adjusted via protonating and deprotonating of the amine using different pH.50,51 Dynamic-light scattering (DLS) revealed aggregates with a hydrodynamic diameter of 20 nm in basic aqueous solution (pH = 9) and a hydrodynamic diameter of 3 nm in acidic aqueous solution indicating particle formation and free block copolymer chains, respectively (Fig. S10†).

For emulsion formation, PDMAEMA-b-POEGMA was dissolved at pH = 9 and used at a concentration of 1 wt% in combination with the ATPS formed by PDMA₁₀ and Pull at a concentration of 1.5/1.5 wt%. Afterwards, the mixture was treated like the PS nanoparticle stabilised emulsion employing ultrasound and shaking by hand. In contrast to the PS emulsion, the mixture stayed clear, which might be explained with the difference in stabiliser particle size (100 nm vs. 20 nm). Similar to the PS nanoparticle-stabilised emulsion, phase separation started after around 3 h, which was completed again after 24 h. The mixture was analysed directly after preparation and after 24 h via bright-field microscopy. Bright-field microscopy and CLSM showed droplets directly after preparation and in the upper phase after 24 h (Fig. 2, 3 and S11†). No droplets were observed in the lower phase. The average droplet size directly after preparation was 86 ± 56 µm at pH = 9 and after 24 h, in the cloudy phase the average droplet size was around 107 ± 31 µm at pH = 9. Bright-field microscopy and CLSM images indicated successful w/w emulsion formation from the ATPS formed by UHMW PDMA and pullulan using PS nanoparticles or PDMAEMA-b-POEGMA as stabiliser at pH = 9.

In order to localise the polymers in the emulsion, PDMA was labelled with RITC and pullulan was labelled with FITC. The emulsions were prepared for both stabilisers as described before and analysed via CLSM directly after preparation and after 24 h (Fig. 2). For the system stabilised with PS nanoparticles, PDMA was located over the entire sample directly after the preparation. However, pullulan was only present inside the emulsion droplets. After 24 h, similar to the bright field images, emulsion droplets were observed only in the upper phase of the two-phase system (Fig. 2a and b). In the upper phase, PDMA was located again over the entire sample and pullulan was enriched inside the droplets (Fig. 2e and f).

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**Fig. 2** (a and b, e and f) CLSM images of the w/w emulsion of UHMW RITC-PDMA/PDMA and FITC-pullulan/Pull (1.5 wt%/1.5 wt%) stabilised with PS-nanoparticles (0.1 wt%) at pH = 9: (a and b) after preparation and (e and f) upper phase after 24 h, (c and d, g and h) CLSM images of the w/w emulsion of UHMW RITC-PDMA/PDMA and FITC-pullulan/Pull (1.5 wt%/1.5 wt%) stabilised with PDMAEMA-b-POEGMA (1 wt%) at pH = 9: (c and d) after preparation and (g and h) upper phase after 24 h.
The CLSM images for the system stabilised with PDMAEMA-b-POEGMA displayed, directly after preparation, PDMA located over the entire sample and pullulan enriched inside the droplets. After 24 h emulsion droplets were observed in the upper phase only. Similar to the w/w emulsion stabilised with PS nanoparticles, PDMA was located over the entire phase and the pullulan enriched inside the droplets. Overall, the CLSM results showed that for both stabilisers the polymers PDMA and pullulan are predominately present in different phases. Pullulan enriched inside the droplets and the UHMW PDMA enriched outside the droplets. Nevertheless, the CLSM images of the PS nanoparticle system indicate the presence of PDMA inside the droplets as well. The reason for the increased amount of PDMA in the Pull enriched droplets after phase separation could be the higher concentration of PDMA in the upper phase and shift of the polymer ratio after phase separation. Another reason could be the non-perfect phase separation of the ATPS system PDMA and pullulan (Fig. S6†). Even after a period of 24 h there are approximately 10% of each polymer present in the opposite enriched phase. All CLSM images were prepared with only one dye present at a time. Furthermore, the results indicate the dye functionalisation does not have an influence on the partitioning of the polymers in the emulsion.

**pH-Sensitive w/w emulsions**

In order to prove the pH influence and sensitivity of the emulsion stabilised by PDMAEMA-b-POEGMA, the emulsion was prepared under acidic and basic conditions. The emulsions were prepared with a polymer concentration of 1.5/1.5 wt% and stabilised with 1.0 wt% PDMAEMA-b-POEGMA. Two different emulsions were prepared, one at pH = 5 and one at pH = 9. The mixture was subjected to ultrasonic treatment for 2 min and shaken by hand for 1 min. The emulsion was analysed directly after preparation via bright-field microscopy (Fig. 3a and b). Furthermore, both samples were analysed by bright-field microscopy after 24 h. For the mixture prepared at pH = 5, bright-field microscopy shows the formation of large droplets (>200 µm) directly after preparation. The significant larger droplets indicate that PDMAEMA-b-POEGMA could not successfully stabilise the w/w emulsion at pH = 5. The larger droplets indicate coalescence on the way to a complete phase separation of the mixture. The bright-field images after 24 h showed no droplets at all, which substantiate the unsuccessful stabilisation of the emulsion. The insufficient stabilisation is indicated by DLS showing only small particle diameters of the block copolymer under acidic conditions corresponding to single polymer coils (Fig. S10†) that are not capable of w/w emulsion stabilisation. However, at pH = 9 bright-field images display droplet formation directly after preparation, which indicates a presence of an emulsion stabilised by PDMAEMA-b-POEGMA aggregates.

Furthermore, droplets were only observed in the upper phase after 24 h (Fig. 3d). Overall, the w/w emulsion using the ATPS of UHMW PDMA and pullulan could be stabilised using the block copolymer PDMAEMA-b-POEGMA. The emulsion is only stable in basic aqueous solution (pH = 9), due to the aggregation of the block copolymer under those conditions. In acidic solution however, the emulsion could not be stabilised by the block copolymer PDMAEMA-b-POEGMA. In contrast, non-pH responsive PS nanoparticles were capable of stabilising the w/w emulsion in basic and acidic medium (Fig. S8†).

Our results showed the influence of the pH value on the stabilisation of the w/w emulsion of UHMW PDMA and pullulan stabilised by PDMAEMA-b-POEGMA. In order to prove the sensitivity and switchability of stabilisation using PDMAEMA-b-POEGMA, the pH value was changed in the mixture. The emulsion was prepared with a polymer concentration of 1.5/1.5 wt%, a block copolymer concentration of 1.0 wt% and a start pH value of pH = 10. The mixture was subjected to ultrasonic treatment for 2 min and shaken by hand for 1 min. A small sample was taken for analysis. Afterwards, the pH was changed to pH = 5 using conc. HCl and the sample subjected to ultrasonic treatment and shaking by hand again. A sample was retrieved for analysis and the pH was changed to pH = 10 using NaOH solution and the sample was redispersed. All samples were analysed via bright-field microscopy (Fig. 4). For the first sample at pH = 10 the microscope images showed droplet formation (Fig. 4a). After the pH change to acidic, the displayed droplets were significantly larger due to an unstable w/w emulsion and onset of phase separation in the sample (Fig. 4b). However, after a pH change to basic, the emulsion droplets were stable again (Fig. 4c). Overall the results show that the w/w emulsion of the UHMW PDMA and pullulan, stabilised by PDMAEMA-b-POEGMA is pH sensitive. In basic aqueous solution the emulsion is stable. If the pH value is changed to acidic, the block copolymer is not stabilising the emulsion anymore. The w/w emulsion can be stabilised again with a change of the pH back to basic. However, pH switches are limited by the change in concentration as the sample is diluted by a small amount of acidic or basic medium during
the pH switch. If the polymer concentration will drop under the limit of the ATPS binodal the emulsion is not stable anymore.

Conclusions

ATPS and w/w emulsions are a major topic in current polymer chemistry. In here, a new ATPS consisting of PDMA and pullulan was investigated. The stability of the PDMA/pullulan ATPS is depending on the molar mass of the polymers. PI-RAFT polymerisation allows an easy access to higher molar mass PDMA, which enables a significant decrease in the required concentration for a stable ATPS. In addition, the ATPS was used to form w/w emulsions stabilised by PS nanoparticles or the block copolymer PDMAEMA-

(b) -POEGMA in basic aqueous solution. The emulsion was stable in the mixture and after phase separation in the upper phase. Pullulan was enriched inside the droplets and PDMA was located all over the sample. Furthermore, the w/w emulsion stabilised by PDMAEMA-

(b) -POEGMA was pH sensitive, i.e. depending on the pH, the emulsion could be stabilised or not stabilised by the block copolymer. The development of this novel pH sensitive w/w emulsion will be open new pathways for various applications, for example for the encapsulation of biomolecules or pH sensitive biomolecule purification.

Author contributions

AP: conceptualisation, data collection, writing original draft. BS: conceptualisation, review and editing the draft, supervision, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 R. Haag and F. Kratz, Angew. Chem., Int. Ed., 2006, 45, 1198–1215.
2 J.-W. Rhim, H.-M. Park and C.-S. Ha, Prog. Polym. Sci., 2013, 38, 1629–1652.
3 M. Rito-Palomares, A. Negrete, E. Galindo and L. Serrano-Carreon, J. Chromatogr. B: Biomed. Sci. Appl., 2000, 743, 403–408.
4 C. D. Keating, Acc. Chem. Res., 2012, 45, 2114–2124.
5 J. A. Asenjo and B. A. Andrews, J. Chromatogr. A, 2011, 1218, 8826–8835.
6 P. da Rocha Patricio, M. C. Mesquita, L. H. M. da Silva and M. C. H. da Silva, J. Hazard. Mater., 2011, 193, 311–318.
7 J. F. Pereira, M. G. Freire and J. A. Coutinho, Fluid Phase Equilib., 2020, 505, 112341.
8 P. Albertsson, Biochem. Pharmacol., 1961, 5, 351–358.
9 R. Hatti-Kaul, Mol. Biotechnol., 2001, 19, 269–277.
10 Y. Chao and H. C. Shum, Chem. Soc. Rev., 2020, 49, 114–142.
11 J. Ryden and P.-a. Albertsson, J. Colloid Interface Sci., 1971, 37, 219–222.
12 Å. Gustafsson, H. Wennerström and F. Tjerneld, Polymer, 1986, 27, 1768–1770.
13 D. Forciniti, C. Hall and M.-R. Kula, Fluid Phase Equilib., 1991, 61, 243–262.
14 L. H. M. d. Silva, J. S. Coimbra and A. J. d. A. Meirelles, J. Chem. Eng. Data, 1997, 42, 398–401.
15 W. J. Frith, Adv. Colloid Interface Sci., 2010, 161, 48–60.
16 A. P. Constantino, A. Tall, Q. Li and T. K. Georgiou, J. Polym. Sci., 2022, 60, 188–198.

Fig. 4 (a–c) Bright-field microscopy images of the pH-sensitive w/w emulsion of UHMW PDMA and commercial pullulan (1.5 wt%/1.5 wt%) stabilised with PDMAEMA-b-POEGMA (1 wt%) (a) at pH = 10, (b) at pH = 5 after pH change with HCl and redispersion as well as (c) at pH = 10 after pH change with NaOH and redispersion.
17 A. Plucinski, M. Pavlovic and B. V. K. J. Schmidt, Macromolecules, 2021, 5366–5375.
18 Y.-T. Wu, D.-Q. Lin and Z.-Q. Zhu, Fluid Phase Equilib., 1998, 147, 25–43.
19 J. Esquena, Curr. Opin. Colloid Interface Sci., 2016, 25, 109–119.
20 T. Nicolai and B. Murray, Food Hydrocolloids, 2017, 68, 157–163.
21 D. M. A. Buzzal, P. D. Fletcher, T. K. Georgiou and N. Ghasdian, Langmuir, 2013, 29, 14804–14814.
22 J. W. Kim, D. Lee, H. C. Shum and D. A. Weitz, Adv. Mater., 2008, 20, 3239–3243.
23 D. Forciniti, C. Hall and M. Kula, J. Biotechnol., 1990, 16, 279–296.
24 E. Scholten, J. E. Visser, L. M. Sagis and E. van der Linden, Langmuir, 2004, 20, 2292–2297.
25 H. Firoozmand, B. S. Murray and E. Dickinson, Langmuir, 2009, 25, 1300–1305.
26 J. Zhang, J. Hwang, M. Antonietti and B. V. K. J. Schmidt, Biomacromolecules, 2018, 20, 204–211.
27 J. Zhang, B. Kumru and B. V. K. J. Schmidt, Langmuir, 2019, 35, 11141–11149.
28 M. Pavlovic, A. Plucinski, L. Zeiningger and B. V. K. J. Schmidt, Chem. Commun., 2020, 56, 6814–6817.
29 T. Merland, L. Waldmann, O. Guignard, M.-C. Tatry, A.-L. Wirotius, V. Lapeyre, P. Garrigue, T. Nicolai, L. Benyahia and V. Ravaine, J. Colloid Interface Sci., 2022, 608, 1191–1201.
30 L. Tea, T. Nicolai and F. Renou, Langmuir, 2019, 35, 9029–9036.
31 R. A. de Freitas, T. Nicolai, C. Chassenieux and L. Benyahia, Langmuir, 2016, 32, 1227–1232.
32 J. P. Machado, L. Benyahia and T. Nicolai, J. Colloid Interface Sci., 2021, 603, 633–640.
33 J. P. Machado, I. Capron, R. A. de Freitas, L. Benyahia and T. Nicolai, Food Hydrocolloids, 2022, 124, 107320.
34 I. W. Sutherland, Trends Biotechnol., 1998, 16, 41–46.
35 R. S. Singh, N. Kaur, M. Hassan and J. F. Kennedy, Int. J. Biol. Macromol., 2021, 166, 694–706.
36 M. Rekha and C. P. Sharma, Trends Biomat. Artif. Organs, 2007, 20, 116–121.
37 H. Hosseinkhah, T. Aoyama, O. Ogawa and Y. Tabata, J. Controlled Release, 2002, 83, 287–302.
38 A. Plucinski, Z. Lyu and B. V. K. J. Schmidt, J. Mater. Chem. B, 2021, 9, 7030–7062.
39 L. T. Carvalho, R. M. Moraes, A. J. R. Teixeira, D. B. Tada, G. M. Alves, T. M. Lacerda, J. C. Santos, A. M. Santos and S. F. Medeiros, J. Appl. Polym. Sci., 2021, 138, 51344.
40 A. Plucinski, J. Willersinn, R. B. Lira, R. Dimova and B. V. K. J. Schmidt, Macromol. Chem. Phys., 2020, 2000053.
41 J. Willersinn, A. Bogomolova, M. B. Cabré and B. V. K. J. Schmidt, Polym. Chem., 2017, 8, 1244–1254.
42 B. V. K. J. Schmidt, Macromol. Chem. Phys., 2018, 219, 1700494.
43 R. N. Carmean, T. E. Becker, M. B. Sims and B. S. Sumerlin, Chem, 2017, 2, 93–101.
44 R. N. Carmean, M. B. Sims, C. A. Figg, P. J. Hurst, J. P. Patterson and B. S. Sumerlin, ACS Macro Lett., 2020, 9, 613–618.
45 D. de Morais Zanata and M. I. Felisberti, Eur. Polym. J., 2022, 111069.
46 B. V. K. J. Schmidt, M. Hetzer, H. Ritter and C. Barner-Kowollik, Macromolecules, 2011, 44, 7220–7232.
47 J. Skey and R. K. O’Reilly, Chem. Commun., 2008, 4183–4185.
48 A. De Belder and K. Granath, Carbohydr. Res., 1973, 30, 375–378.
49 J. Liu and Y. Tabata, J. Drug Targeting, 2010, 18, 602–610.
50 V. Büttün, S. Armes and N. Billingham, Polymer, 2001, 42, 5993–6008.
51 Z. Dong, H. Wei, J. Mao, D. Wang, M. Yang, S. Bo and X. Ji, Polymer, 2012, 53, 2074–2084.