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Tuning the bis-hydrophilic balance of microgels: A tool to control the stability of W/W emulsions

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ABSTRACT

Hypothesis: The stability of purely aqueous emulsions formed by mixing incompatible polymers, can be achieved through the Pickering effect of particles adsorption at the interface. However, there is, as yet, no guideline regarding the chemical nature of the particles to predict whether they will stabilize a particular W/W emulsion. Bis-hydrophilic soft microgels, made of copolymerized poly(*N*-isopropylacrylamide) (pNIPAM) and dextran (Dex), act as very efficient stabilizers for PEO/Dextran emulsions, because the two polymers have an affinity for each polymer phase.

Experiments: The ratio between both components of the microgels is varied in order to modulate the bis-hydrophilic balance, the content of Dex compared to pNIPAM varying from 0 to 60 wt%. The partition between the two aqueous phase and the adsorption of microgels at the W/W is measured by confocal microscopy. The stability of emulsions is assessed via turbidity measurements and microstructural investigations under sedimentation or compression.

Findings: The adsorption of particles and their partitioning is found to evolve progressively as a function of bis-hydrophilic balance. At room temperature, the stability of the resulting W/W emulsions also depends on the bis-hydrophilic balance with a maximum of stability for the particles containing 50%wt of Dex, for the Dex-in-PEO emulsions, while the PEO-in-Dex become stable above this value. The thermo-responsiveness of the microgels translates into stability inversion of the emulsions below 50 wt% of Dex in the microgels, whereas above 50 wt%, no emulsion is stable. This work paves the way of a guideline to design efficient and responsive W/W stabilizers.

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1. Introduction

Water-in-water emulsions, i.e. droplets of one aqueous phase dispersed within another aqueous phase, have attracted much attention for many years [1,2], especially for their potential for extraction and purification processes [3,4]. They have gained significant interest over the past years, partly due to their possible contribution to the origin of life [5], as suggested in the 1920s by Oparin [6] and to the recent discoveries about their role in compartmentalization of living cells [7]. Phase separation of binary polymer mixtures occurs through two pathways: the associative route, called coacervation, which leads to a phase rich in both polymers dispersed in a dilute phase, and the segregative route, also called aqueous-two phase separation (ATPS), leading to two phases each enriched with a different type of polymer [2,8]. Phase separation is ubiquitous and concerns a broad range of components including also polymer/salt, salt/salt, etc. [9]. Many of them concern food sci-

ence ingredients such as proteins and polysaccharides [10]. An intensively-studied ATPS system is the ternary system containing dextran (Dex) and poly(ethylene oxide) (PEO) in aqueous solution [11].

Unlike oil-water (O/W) emulsions, which can be easily stabilized by surfactants, obtaining a metastable dispersion of aqueous droplets in an aqueous phase is not trivial. Their interfacial region is much thicker due to the characteristic length of the polymers and their interfacial tension is orders of magnitude lower decreasing to zero at the bimodal [12,13]. Therefore, surfactants are not efficient stabilizers, because they are too small and present an adsorption energy that is too low compared to the thermal motion. Conversely, several examples of particles or self-assembled systems could successfully stabilize these emulsions [14]. Particles of different chemical compositions, softness and shapes were found to stabilize W/W emulsions upon sole adsorption, i.e. without the need of further interfacial cross-linking or complexation [15]. They include organic particles such as synthetic spherical polymeric particles

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[14,16], and microgels [17,18]. rod-like cellulose nanocrystals [19], starch platelet-like particles [20], protein aggregates [21–24], and fat particles [25], as well as inorganic particles [26,27]. Self-assembled systems, such as amphiphilic block copolymers micelles [16,28,29] liposomes [30,31] and even recently lipidic bilayers [32] have also been reported to stabilize W/W emulsions.

In order to stabilize the emulsions, the particles need to adsorb at the W/W interface. The Pickering effect leads to a reduction of the Gibbs energy because of the change of interfacial energy resulting from a decrease of the contact area between the two aqueous phases replaced by the contact area between the particle and each phase [33]. For a spherical particle, it is given by

$$\Delta G = -\pi R^2 \gamma_{AB} (1 - |\cos \theta|)^2 \quad (1)$$

where θ is defined as the contact angle of the particle at the interface, given by

$$\cos(\theta) = \frac{(\gamma_{PA} - \gamma_{PB})}{\gamma_{AB}} \quad (2)$$

It is expected that, even if the interfacial tension is low, the adsorption energy is sufficient to overcome thermal energy using sufficient particle radii and adapted wetting contact angles. However, it was also demonstrated that adsorption of particles cannot guarantee the stability of the corresponding emulsion [14]. In some cases, two contacting droplets were observed to create a connecting narrow channel that enlarged and lead to coalescence while the particles were ejected from the interface [14]. The surface coverage is not a sufficient argument to explain emulsion stability, since some particles do not fully cover the droplet surface, but nevertheless stabilize emulsions [21]. It suggests that the interaction between the particles at the interface plays an important role.

Most often, the two types of emulsions, whether phase A is dispersed in phase B or vice versa, are not equivalent regarding their stability although the interfacial tension is the same. For W/O emulsions, the well-known Bancroft rule [34], relates the solubility of surfactants to the type of preferred emulsion, whether oil-in-water or water-in-oil. The solubilizing medium generally becomes the continuous phase. The hydrophilic-lipophilic balance (HLB) [35], linked to the solubility, allows predicting the type of preferred emulsion in a more predictive and quantitative approach. In fact, the HLB turns out to be related to the curvature of the surfactant monolayer, that governs emulsion stability [36]. Regarding Pickering W/O emulsions, Finkle [37] proposed an equivalent rule, stating that particles having a better affinity for the aqueous phase, or equivalently a contact angle below 90°, would enhance the stability of oil-in-water emulsions, and vice-versa. Although rare counter examples were reported [38], this rule was generally verified and allowed to adapt the particle chemistry to the selected emulsion type, thanks to the control of the wetting contact angle. This was achieved for instance, using hydrophobically-modified silica nanoparticles with controlled wettability [39]. By analogy to O/W emulsions, it was believed that the type of W/W emulsions would depend on the partitioning of particles preferentially in the continuous phase [23]. To date, most examples are in agreement with this statement, but, as for W/O emulsions, some counter-examples were encountered, in which particles preferentially partitioned in the dispersed phase [18]. Attempts to establish a link between the particle chemistry, the wetting contact angle and emulsion stability were reported for hydrophobic silica particles with varying hydrophobicity [21]. Unfortunately, they were unsuccessful to predict the most stable emulsion type, probably because of the *in situ* modification of the particles by adsorption of a polymer from the phase diagram. To date, predicting the W/W emulsion type and stability according to a selected chemistry is still lacking.

Among the different types of particles, our recent findings on bis-hydrophilic microgels seemed to pave the first step to establish a relation between the chemical composition and the adsorption of microgels

at the W/W interface [18]. Indeed, these polymeric network particles, made of two polymers having an affinity for each aqueous phase, poly (*N*-isopropylacrylamide) (pNIPAM) and dextran (DEX), were found to adsorb at the interface and promote emulsion stability when a sufficient amount of DEX was copolymerized with pNIPAM. This work shed light on the role of bis-hydrophilicity as a possible mean to design emulsion stabilizers. Moreover, the thermo-responsiveness of pNIPAM was leveraged to tune the stability of the emulsions. At low temperature, in the swollen state, microgels stabilized DEX-in-PEO (D/P) emulsions, whereas PEO-in-DEX (P/D) emulsions were more stable when the temperature was raised above the volume phase transition temperature (VPTT), i.e. when the microgels were collapsed. Besides, adding charges to these microgels, through the introduction of a charged comonomer, inhibited their adsorption at the W/W interface in the swollen state and their ability to stabilize the resulting emulsions, whereas D/P emulsion stability was strengthened when salt was added in the solution, thereby screening electrostatic interactions [40].

Pursuing our efforts to rationalize the link between the microgel chemical structure and their stabilizing efficiency, we now investigate the role of bis-hydrophilic balance in analogy of the HLB for O/W emulsions. To this end, a series of microgels with different compositions of pNIPAM and DEX was synthesized, by changing the ratio of DEX, its functionalization degree, as well as its molecular weight. The bis-hydrophilic balance could thus be tuned to a large extent, which, in turn, controlled the affinity for each aqueous phase and their adsorption at the W/W interface. The stability of W/W emulsions was modified accordingly for both types of W/W emulsions. The effect of temperature was also investigated.

2. Materials and methods

All reagents were purchased from Sigma-Aldrich. Dextran from Leuconostoc spp. with different molecular weights (Mw = 450000–650000 g/mol, Mw = 40000 g/mol, and Mw = 6000 g/mol), methacrylic anhydride (AMA, purity 94%), potassium persulfate (KPS purity 99%) and poly(ethylene oxide) PEO (Mw = 250000 g/mol) were used as received. Before being used in synthesis, *N*-isopropylacrylamide (NIPAM, purity 97%) was recrystallized and dried overnight under vacuum. Methacrylated dextran (DexMA) macromers were synthesized as reported in reference [18]. Details are given in SI. For all the solution preparation and reactions Milli-Q water was used.

2.1. Microgel synthesis

Aqueous free radical precipitation polymerisation was used for the synthesis of the microgels. DexMA macromers (from 27 to 60 wt% compared to NIPAM) and NIPAM monomers (70 mM) were dissolved in 48 mL of milliQ-water in a 100 mL three-neck round-bottom flask surmounted by a reflux condenser. The solution was stirred under nitrogen bubbling and heated to 70 °C, using an oil bath equipped with a thermometer for temperature control. After one hour, 0.5 mL of a 2 mg/mL solution of fluorescein methacrylate was added in order to graft fluorescent labels in the microgels. The reaction was initiated with 2.5 mM of KPS dissolved in 2 mL of water and then left for 4 h at 70 °C, stirred and purged with nitrogen. The solution was then dialysed for one week (MWCO 100 000 g/mol). The chemical composition of the microgels was determined by ¹H NMR (See SI).

The microgels were named pNIPAM-Dex-xx, where xx represents the wt% of the dextran chains compared to the NIPAM monomer units. They were further characterized by Transmission electron microscopy (TEM), Dynamic Light Scattering (DLS). Detailed procedures are described in SI.

Of note, hydrogel particles with a diameter below 100 nm should be termed ‘nanogels’, whereas ‘microgels’ is preferred for diameters larger

than 100 nm [41]. The present particles exceed 100 nm in the swollen state. They can swell even more in their charged version [40], so the term ‘microgel’ is preferred.

2.2. Emulsification and emulsion characterization

Two types of emulsions were prepared from concentrated PEO and Dextran solutions: P/D when the fraction of PEO phase was 25 %, D/P when the fraction of Dex phase was 25%. The total volume of emulsion was 2 mL. The solutions were mixed for 1 min using a minishaker to create the emulsion. Detailed procedures are described in SI. The properties of the emulsions did not depend on the order of mixing or after longer mixing. The stability of the emulsions was then studied 1) by visual inspection to observe the presence of a turbid layer due to the light scattering of droplets, 2) by confocal microscopy to observe the microstructure and the droplet size, after the samples were kept in a vertical position to observe the effect of gravity on aging, 3) by turbidity measurements (wavelength 870 nm) along the height of the emulsion, under normal gravity or centrifugation (470 G) (Lumisizer®/Lumirreader®, LUM GmbH), to assess the occurrence of coalescence and define the characteristic time t_c of the emulsion.

3. Results

3.1. Synthesis of the microgels

A series of bis-hydrophilic microgels was synthesized according to the previously established methodology, consisting in the incorporation of dextran units as cross-linker in the pNIPAM microgel structure [18]. To this end, multifunctional polymerizable dextrans were prepared by grafting methacrylate groups on the dextran chains, which were further copolymerized with NIPAM through a precipitation polymerization process. The goal of the present study was to explore the role of bis-hydrophilicity, therefore the largest range of dextran-to-NIPAM ratio was targeted. However, the large molecular weight of dextrans induced a practical limitation to high dextran contents, since the macromer solution became highly viscous at high concentrations. Moreover, upon radical polymerization, the formation of a coagulum was observed as soon as the solid content exceeded a given concentration, which depended on the molecular weight of dextran (MW) and on its substitution degree by methacrylate (DS). This was more pronounced when the DS and MW were high (Table 1). For instance, it was not possible to prepare microgels with a DS 20 and MW of 40 kg/mol even with the lowest content of Dex. Therefore, a lower MW or lower DS were privileged. The effect of the DS was also studied since this modifies the cross-

linking degree of the microgels, and thus their softness. Moreover, changing the DS also modifies the size of the dextran blocks between two cross-linking points. Fig. 1 sketches the structure of the dextran macromers, with the assumption of homogeneous distribution of the methacrylate groups along the chains. Different parameters were modulated to investigate the role of microgel structure and composition to stabilise W/W emulsions. Finally, three different DexMA chains were used: Dex of 6 kg/mol, with DS of 24 (chain A), Dex of 6 kg/mol, with DS of 12 (chain B), Dex 40 kg/mol, with DS of 12. The different microgels studied are listed in Table 1. Their TEM images in the dried state are shown in Figure S1.

^1H NMR was used to analyze the chemical composition of the microgels (Figure S2). In this experiment, it was considered that all the monomeric units gave a signal proportional to their composition, meaning that the polymer network was fully swollen and the segments were mobile, which would not hold if the experiments were done above the VPTT [18]. From these measurements, it is observed that the fraction of dextran in the microgels increases linearly with the amount of dextran added in the synthesis (Figure S3). This confirms that increasing the amount of dextran in the reaction medium leads to the formation of dextran-rich particles. The fraction is higher for shorter dextran chains and lower DS, reaching up to 90% of incorporated dextran for Dex 6 kg/mol DS12, whereas only 66% is effectively incorporated for the same molecular weight and higher DS and 77% for the same DS but higher molecular weight. To explain this effect, one has to consider that the lost fraction refers to water-soluble species having a molecular weight smaller than the membrane cut-off 100 kg/mol, i.e. polymer chains that could not attach to the growing insoluble particles during the precipitation polymerization process. The explanation for these effects is not straightforward. Indeed, the polymerization process involves the cross-reactivity of water-soluble dextrans with pNIPAM growing chains, which become insoluble above a critical length of pNIPAM blocks and possibly favour the nucleation of particles. Due to the low amount of methacrylates compared to NIPAM monomers (2–9 mol% according to Table 1), it was not possible to follow their respective polymerization kinetics. The effect of molecular weight could be explained by viscosity effects and slower dynamics of the chains, that could decrease their reactivity. At first glance, the effect of DS seems contradictory, since a higher amount of MA groups should favour the efficiency of grafting of pNIPAM chains. But the simultaneous growth of many pNIPAM chains may also promote shorter pNIPAM blocks, whose length could be smaller than the critical length required for precipitation [42]. A higher reactivity towards other dextran chains or intra molecular reactivity could also explain the increase of water-soluble species.

Table 1
Summary of the microgel composition and main characteristics of the microgels.

Name	Dextran					MA	Hydrodynamic diameter (nm)			
	Mw of Dex (kg/mol)	DS (mol%)	Weight fraction (wt%) in the synthesis	Fraction (mol%) in the synthesis	Fraction (mol%) determined by ^1H NMR		T = 25 °C		T = 40 °C	
							Fraction (mol%) compared to NIPAM	d_h	PDI	d_h
A-27%	6	20	27	19	13	2.9	130	0.17	92	0.19
A-33%	6	20	33	24	16	4	118	0.28	80	0.14
A-43%	6	20	43	32	22	6	135	0.24	103	0.19
B-27%	6	12	27	20	16	2.8	117	0.10	67	0.03
B-43%	6	12	43	33	30	4.9	107	0.22	79	0.15
B-53%	6	12	53	43	40	7.4	111	0.29	95	0.24
B-60%	6	12	60	50	45	9	132	0.58	126	0.42
C-27%	40	12	27	20	15	2	129	0.15	79	0.10
C-43%	40	12	43	33	25	3.8	130	0.23	97	0.18
D-27%	40	24	27	–	–	–	Coagulum		Coagulum	

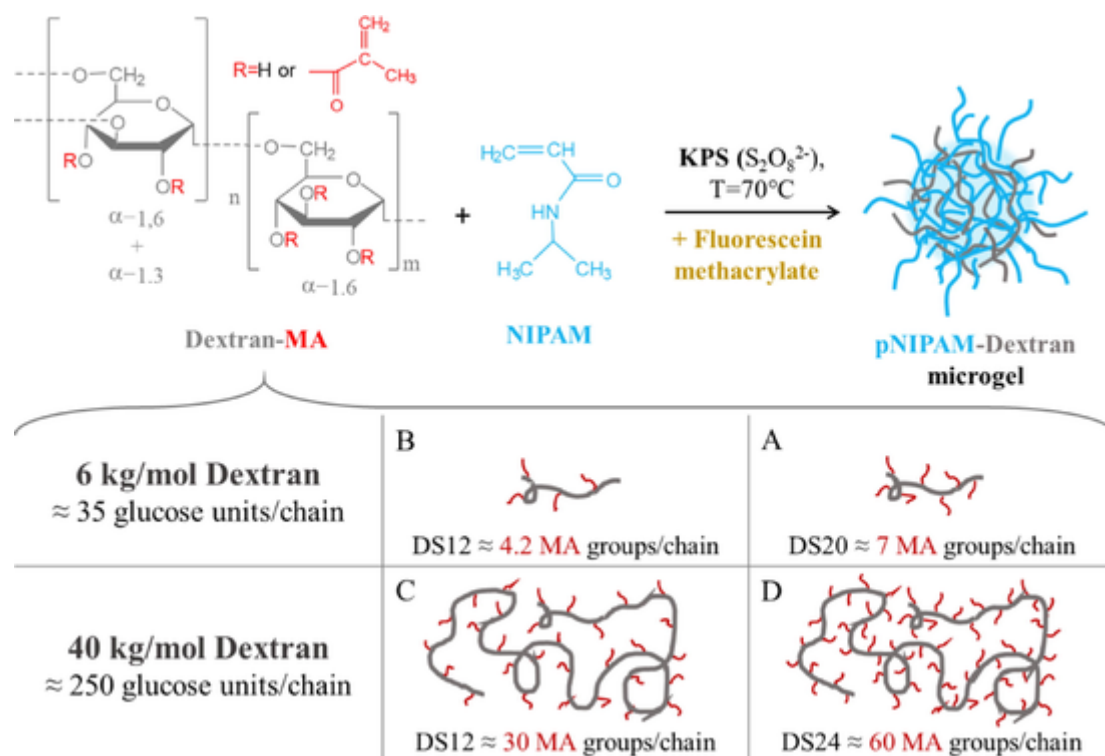


Fig. 1. Scheme of the microgel synthesis and representation of the various dextran macromers.

3.2. Swelling behavior of microgels in suspension

The apparent hydrodynamic diameter of the microgels was measured by DLS at various temperatures (Fig. 2A and Figure S4). All of them were thermoresponsive and present a volume phase transition temperature (VPTT), except the one with the highest amount of dextran, for which the VPTT is almost invisible. As already pointed out for low Dex contents [18], the VPTT was that of pNIPAM and did not depend on the Dex content, although Dex is hydrophilic. Usually, the copolymerization of NIPAM with hydrophilic monomers leads to an increase of the VPTT [43], except when the spatial distribution of the pNIPAM blocks and the hydrophilic ones are spatially separated [44, 45]. This observation implies that dextran and pNIPAM blocks belong to separated domains, or at least that pNIPAM have a sufficient length to keep the same transition temperature. This observation is similar to pVCL-dextran microgels [46].

Above the VPTT, the microgels were in their lowest swollen state, because the pNIPAM segments were in bad solvent condition, hydrophobic and probably aggregated within the microgels. Their diameter was found to enlarge by increasing the fraction of Dex-MA (Fig. 2B and Figure S5). This could be due to two contributions: on the one hand, dextran is hydrophilic and probably increases the hydration of the particle, even in their most unswollen state. On the other hand, the state above the VPTT is the state reached during the synthesis, so the diameter is also ruled by the synthesis conditions. Usually, during the precipitation polymerization process, the monomers are water-soluble and after initiation, the growing chains reach a point at which they become insoluble, which creates the homogeneous nucleation step [47,48]. They continue to grow and possibly, to recombine, until they reach a colloiddally stable state, which is controlled by the amount of stabilizer. The higher the amount of stabilizer, being either charges for electrostatic repulsion or polymer chain in good solvent for steric repulsion, the smaller the particles. Adsorbed hydrophilic polymers can also act as steric stabilizers and thus can decrease the particle size. In the present case, the particle size at the end of the synthesis was 2–3 times smaller the size that is achieved with classical pNIPAM microgels, despite their

possible higher hydration. This is in agreement with the stabilizing role of dextran, which is achieved even for the lowest contents of Dex-MA. As demonstrated earlier, the presence of dextran segments at the surface of the particles brings a steric stabilization since it prevented their aggregation at high ionic strength [18].

The effect of Dex-MA content on the swelling of the particles is also shown, in particular because it acts as a multifunctional macromer and plays the role of cross-linker by increasing the fraction of MA. The swelling ratio Q_v , is defined as the cubic ratio between the diameter in the swollen state and that in the most collapsed state. It is plotted as a function of Dex-MA content in the particles (Fig. 2C and Figure S6). As expected, the swelling ratio progressively decreases, when the amount of Dex increases. This decrease has two origins: on the one hand, increasing the amount of Dex-MA restricts the microgel swelling because it acts as a cross-linker and increases the microgel elasticity; on the other hand, the pNIPAM/Dex ratio decreases, thus the swelling ratio becomes more and more governed by the solubility parameter of dextran. When pNIPAM moieties become insoluble above the LCST, they create hydrophobic domains, that may even act as physical cross-linkers.

3.3. Effect of the microgel composition on water-in water emulsion stability

3.3.1. Stability of w/w emulsions at low temperature

The stability of W/W emulsions was studied in the presence of microgels, whose concentration was kept constant at 0.05 wt%. Two emulsion compositions were studied: one with an excess of PEO phase (75%), which lead to (D/P), the other with an excess of Dex phase (75%) which lead to (P/D). For the sake of simplicity, this study focuses on the microgel B series, which allowed exploring the largest range of Dex content. Series A and C will be used for comparison in given conditions.

First, the stability of W/W emulsions in the presence of microgels was studied by simple visual inspection (Fig. 3). At short times, just after shaking, the emulsions were equally turbid over the total height of the sample (Figure S7). Upon ageing, sedimentation or creaming oc-

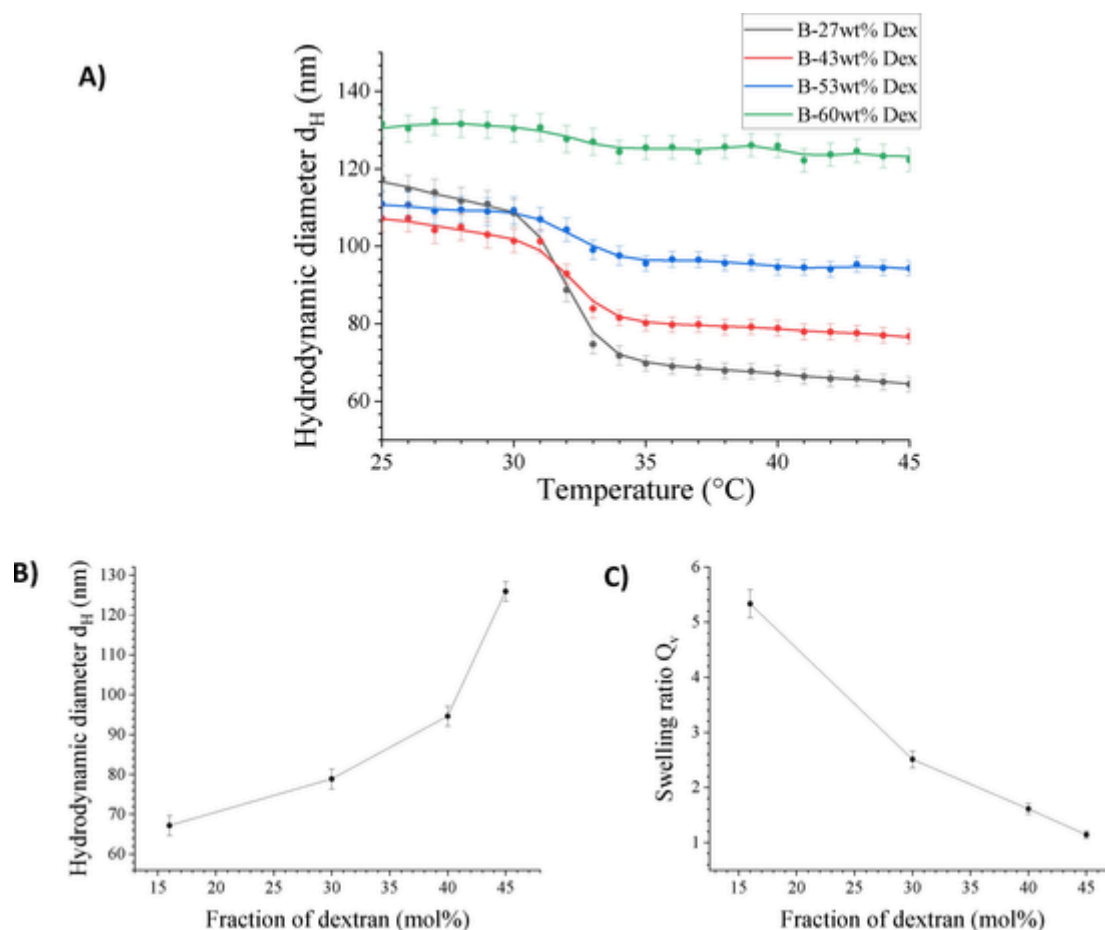


Fig. 2. Behavior of the pNIPAM-Dex microgels (Series B, Dex 6 kg/mol – DS 12) in aqueous suspension: A) hydrodynamic diameter as a function temperature, B) Influence of Dex-MA content on the diameter in the collapsed state; C) Influence of Dex-MA content on the swelling ratio of the microgels.

currred. If the drops remained intact, a turbid layer could be seen at the bottom of the vial for D/P emulsions because dextran-rich droplets sedimented, whereas for P/D, PEO-rich droplets creamed toward the top of the vial. When phase separation occurred due to coalescence, the turbidity was lost. The height profile of the turbidity was also measured using the Lumireader® apparatus (Figure S8). For all series of microgels A, B and C, microgels with low Dex-MA content (below 50 wt%) stabilized D/P emulsions but not P/D emulsions (Fig. 3 and Figure S7–8). No major difference could be observed between the 3 series. Interestingly, the microgels at 60% of Dex-MA was the only one to stabilize both the P/D and D/P emulsion.

To get a more accurate picture of the stability, the emulsions were observed at the mesoscopic scale by confocal microscopy (Fig. 4). At short times after the preparation (less than 1 h), in all the situations, FITC-labelled microgels were visible at the D/P interface, forming a layer around the droplets. There was no significant effect of the composition on the drop size. Obviously, the two phases presented different fluorescence intensities, showing an uneven distribution of the microgels in the medium. At low Dex-MA content, an excess of microgels was visible in the PEO phase, which was more fluorescent. The fluorescence intensity of the PEO phase decreases as the Dex-MA content in the microgels increases. For a more quantitative approach, the partitioning between the two phases, given by the fluorescence intensity ratio between those two phases, was plotted in Fig. 4B. It shows that the affinity for the PEO phase decreases as the Dex-MA content increases. The intensity ratio between the Dex and PEO phases evolves from 0.6 to 1.1 when the Dex-MA content raises from 27 wt% to 60 wt% in the case of D/P emulsions. The trend is the same for P/D emulsions but the ratios are lower. In both cases, there was never a partitioning in favour of the

Dex phase. None of the microgels were in excess in the Dex phase. A sharp increase of the intensity at the interface was observed caused by the adsorption of microgels. The relative intensity between the interface and that of PEO phase is plotted in Fig. 4C. An excess of fluorescence intensity at the interface is observed, which rises with an increasing Dex-MA content. It should be noted that the relative fluorescence intensity at the interface was larger for the D/P emulsions than for P/D ones.

The evolution of the emulsions structure during ageing was also monitored by confocal microscopy, by keeping the samples in sealed cells, which were kept vertical and placed horizontally for observations (Fig. 5). For the P/D emulsion with microgels at low Dex-MA contents, a POE phase is visible at the top after 2 h. When increasing the Dex-MA content to 50 wt%, coalescence occurred more slowly and the phase separation was observed after one day. Upon further increase to 60 wt%, coalescence was even slower. Droplets grew to reach 65 μm after 1 week but no phase separation was observed. D/P emulsions were more stable than P/D. The Dex phase started to phase separate after 24 h for 27 wt% Dex-MA content, and after 4 days for 43 wt%. Droplets were still visible after one week for the two highest contents of Dex-MA, but the drop size was bigger for the 60 wt% content, suggesting an optimal stability for 50 wt%. The kinetic evolution of the drop diameter is given in Figure S10.

The stability of the different emulsions was also compared during centrifugation (470 G), by measuring the turbidity profiles as a function of height in the centrifugation tubes (Figure S11). Centrifugation accelerates creaming or sedimentation of the droplets and also may accelerate coalescence by compressing the droplets. For both P/D and D/P emulsions, the addition of microgels increased the stability. With B-

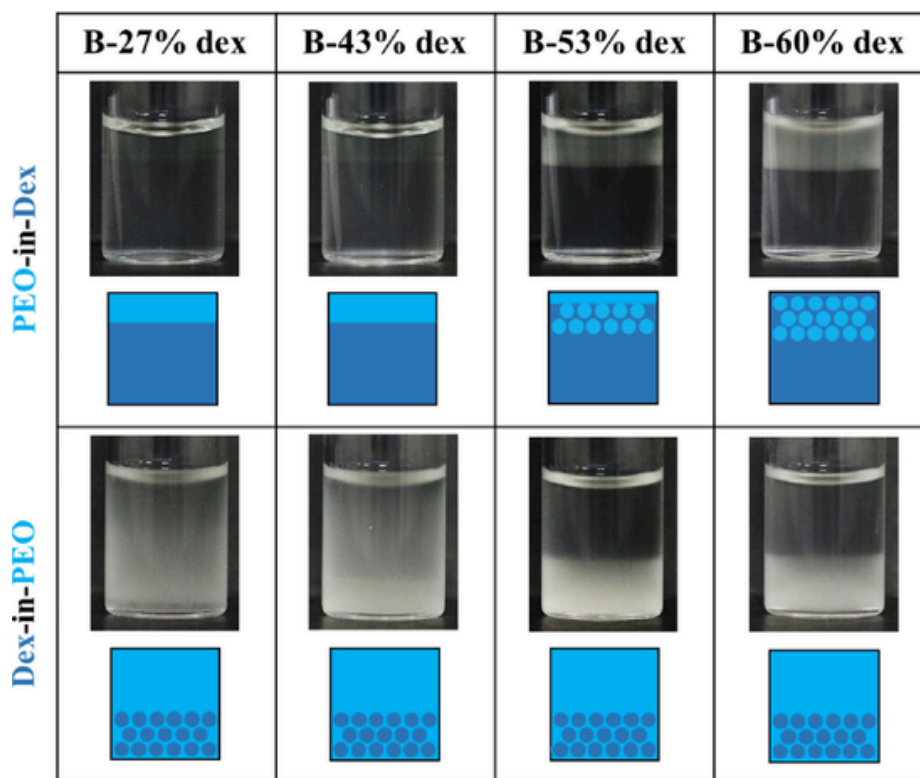


Fig. 3. Macroscopic stability, depicted with schematic representation, after 48 h of W/W emulsions in presence of different microgels (series B). Top row: D/P, Bottom row: D/P.

27 wt% microgels, the P/D emulsion phase separated after 10 min compared to 1 h with the B-43 wt%. For the emulsion stabilised with B-53 wt% and B-60 wt%, the phase separation was not observed after 12 h. A turbid zone corresponding to emulsion droplets could be observed close to the P-D interface. Similarly to observations under 1G, the D/P emulsions were more stable in the presence of microgels. With B-27 wt% microgels, two transparent phases were visible after 2 h. For the emulsions stabilized with the three other microgel candidates, the phase separation was much slower. Maximum stability was observed with the addition of the 53 wt% dextran microgels.

These measurements allowed extracting characteristic times t_c , which correspond to the times taken to reach half of the full transmittance of dispersed phase ($d = 0\text{--}25\% \cdot H_{\text{tube}}$ for D/P emulsion, $75\text{--}100\% \cdot H_{\text{tube}}$ for P/D emulsion) (Figure S12), as described in the Method section in SI. The characteristic time t_c is plotted as a function of microgel composition, for each type of emulsions (Fig. 6). This representation highlights the huge importance of the microgel composition on the mechanical stability of the emulsions. Indeed, the timescale spans over 3 orders of magnitude. At low fraction of Dex-MA (27 wt%), the characteristic times were very close for the D/P and P/D emulsions. Increasing the fraction of Dex-MA in the microgels progressively enhanced the stability of the P/D emulsion and further that of D/P, which was maximal close to 50%. Increasing further the fraction of Dex-MA decreased the stability of D/P but continued enhancing that of P/D. DS and Mw also gave a higher t_c at low density of Dex, especially in D/P emulsion, and this effect is no more obvious when increasing the fraction of Dex. The stability mainly depends on the %Dex in the microgel composition.

3.4. Effect of temperature on emulsion stability

In this section, the effect of temperature on the emulsion stability was studied. As previously described, increasing the temperature induces a sudden drop in the microgel diameter due to the collapse of

pNIPAM chains. Previously studied microgels with low Dex-MA contents were found to invert the stability of the W/W emulsion, although the partitioning remained in favour of the PEO phase [18]. It was concluded that the conformational change of the microgels had an influence on their interactions at the interface. The same investigation methodology was applied here to other compositions. Not only ageing at 50 °C was studied, but also the preparation pathway: in one case, the samples were emulsified at room temperature, in the other case, they were heated at 50 °C and then emulsified.

Figure S13 shows the kinetic evolution of the drop structure when submitted to gravitational field in confocal microscopy. This time, the D/P drops coalesced faster than the P/D. This trend was similar for all the microgel compositions. Fig. 7 shows the partition between POE, Dex and the interface. For both emulsions, heating the emulsion that was initially prepared at 20 °C did not change the partition, whereas the microgels gained more affinity for the Dex phase when they were first heated at 50 °C. Increasing temperature had no effect on the relative intensity of the interface when the emulsion was prepared at 20 °C. However, when the emulsion was prepared at 50 °C the relative intensity at the interface was lower at higher contents of Dex-MA. In this case, a few aggregates of microgels were formed which had a tendency to adsorb at the interface, see Figure S14.

The stability of the different emulsions was studied during centrifugation (Figure S15) and the characteristic time t_c was determined (Figure S16), as plotted in Fig. 8. Without microgels, the characteristic time decreased, which can be explained by the diminution of the phase viscosity and therefore faster sedimentation or creaming. After the addition of microgels, the D/P emulsion was much less stable than at 20 °C, whatever the preparation pathway. Concerning the P/D emulsion, heating the sample before or after emulsification led to a better stability in presence of B-27 wt% and B-43 wt%. The emulsion with B-50 wt%, was more stable when heated after emulsification and less stable if heated before the sample preparation. Finally, with B-60 wt%, both heated emulsions were less stable than at 20 °C. Thus, the previ-

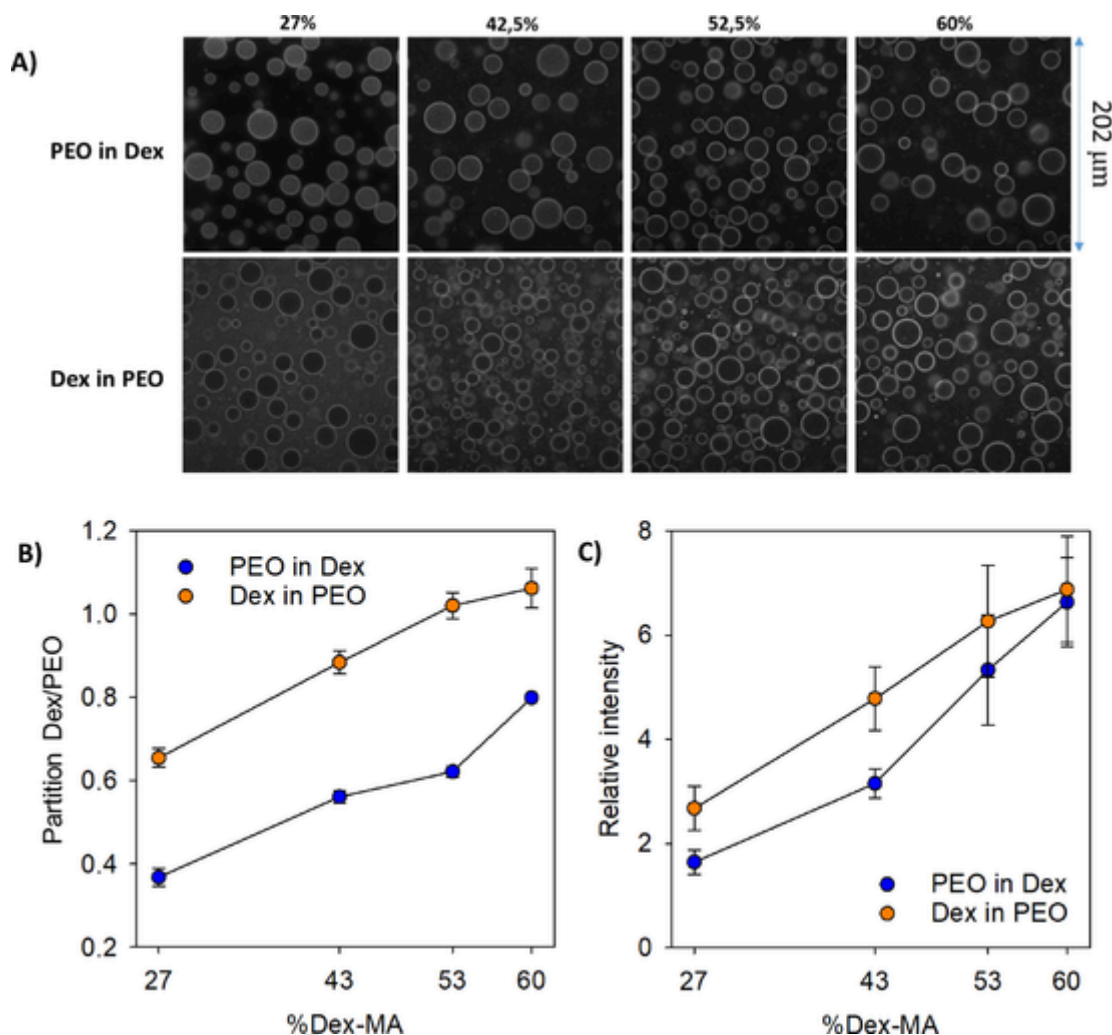


Fig. 4. A) Confocal microscopy images of the emulsions immediately after preparation, B) Partitioning between the PEO and the Dex phases when increasing the %Dex-MA, C) Relative fluorescence intensity ratios at the interface of P/D and D/P emulsions as function of %Dex-MA, extracted from the profile views of the drops. All the emulsions were studied at 20 °C with the B microgels.

ously observed inversion of emulsion stability is confirmed for the Dex-MA contents below 50 wt%. Above 50 wt%, when the stability of P/D is promoted at 20 °C, increasing the temperature decreased the stability.

4. Discussion

In the present study, our objective is to shed light on a possible structure–property relation between the chemical composition of W/W emulsifiers and the stability of the corresponding emulsions. Increasing the Dex-MA fraction in pNIPAM microgels is a means to tune the bis-hydrophilic balance, because both polymers have a different affinity for the two aqueous phases. Indeed, the PEO phase offers a more hydrophobic environment [49] which is more favorable to the amphiphilic pNIPAM. Indeed, this effect is well-known for the partition of proteins and have been modelled by a modified Flory-Huggins approach [50]. The Hansen solubility parameter [51] can be used for the determination of the interaction parameter χ [52]. In the microgels, Dex-MA also acts as a cross-linker, a full series of microgels were prepared with varying dextran contents and degrees of substitution in methacrylate, allowing us to discriminate between the impact of the cross-linking density, in other words the physical structure of the microgels, and that of bis-hydrophilicity, that is to say the chemical composition.

Before starting the discussion about the possible stabilizing mechanisms, a few important points need to be reminded. First of all, unlike

O/W emulsions, the type of W/W emulsions is ruled only by the volumic ratio between the phases, the minor one becoming the dispersed one. Therefore, W/W emulsions are always in the dilute state, meaning that the drop are not in contact just after the preparation. The kinetic stability is then followed upon sedimentation/creaming, meaning that the drops are concentrated at the bottom (resp. top) of the vial. The time taken for this step depends on the mismatch between the phases densities, on the square of the particle size and on the viscosity of the continuous phase [53]. After this first step, the drops are close to each other and may coalesce, depending on their mutual interactions, which rule the film thickness and stability of the films, and on the mechanical properties of the adsorbed layer, which rule the resistance to film rupture. In fact, the former concerns the interactions between two adsorbed layers, which need to be repulsive to guarantee the stability, whereas the latter concerns the anchoring energy of the individual particles and the cohesion of the adsorbed layer, which relates to the interactions between adjacent microgels. In the present work, we were able to track the effect of chemical composition on the microgel adsorption at the interface, and its impact on the resulting stability of the different types of emulsions. However, at this stage, it was not possible to distinguish interactions at the interface from interaction between drops. Deciphering the exact stabilization mechanism by these microgels is out of the scope of the present work, especially because microgels are dy-

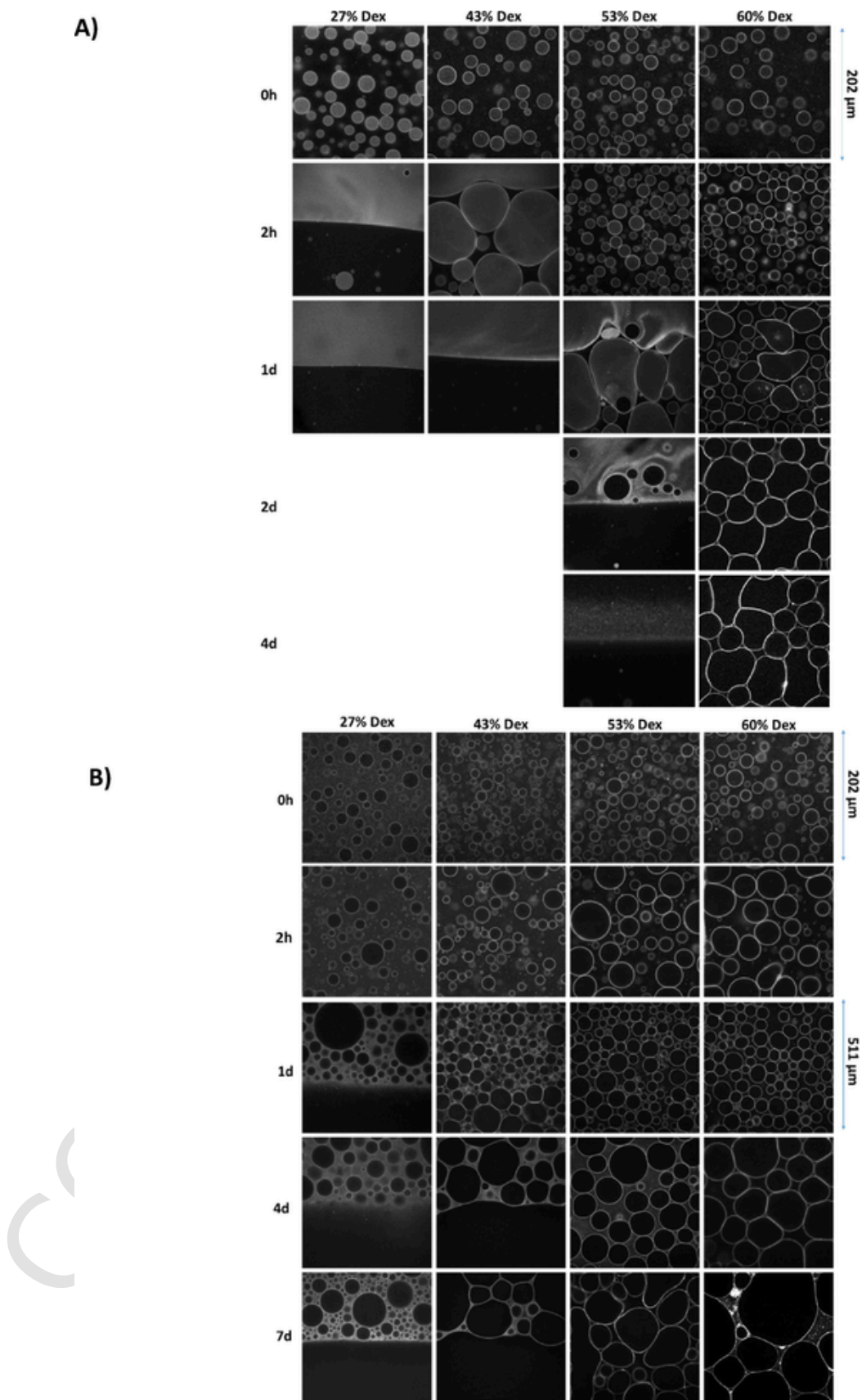


Fig. 5. Evolution of the microstructure of P/D (A) and D/P (B) emulsions containing 0.05 wt% of different microgels (series B).

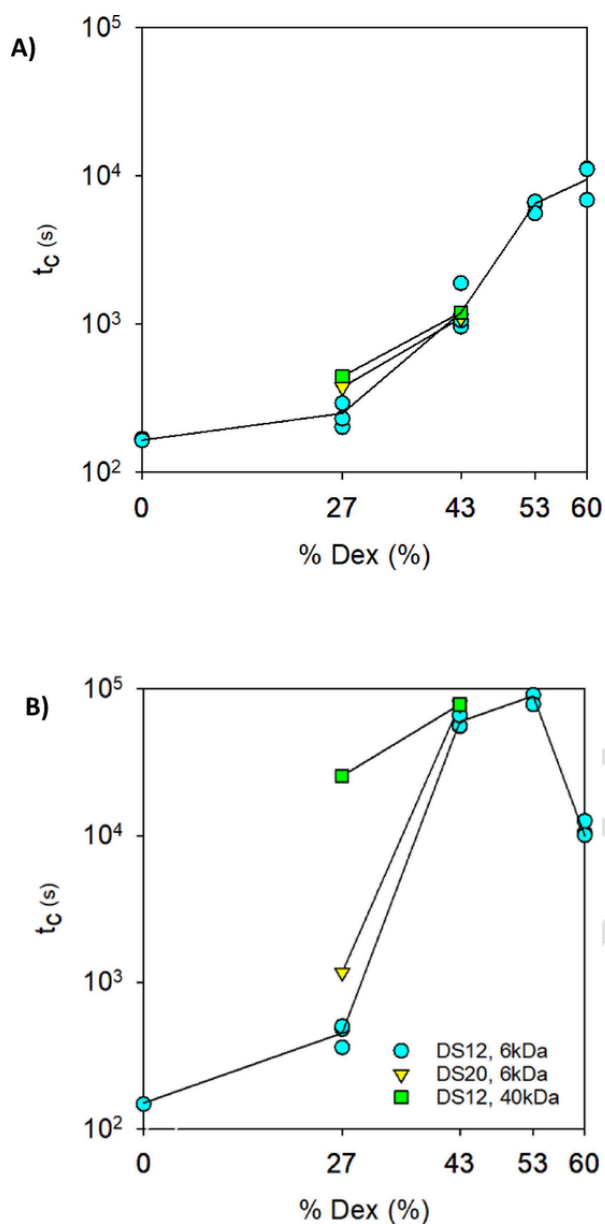


Fig. 6. Characteristic time of the P/D (A) and D/P (B) emulsions in function of the Dex fraction in the microgels. 0% Dex corresponds to the emulsion without particles.

nanic structures which can also undergo different conformational changes upon adsorption [54].

We first discuss the effect of the microgel composition on their adsorption at the W/W interface and the stability of the resulting emulsions at ambient temperature. Fig. 4, B and C, show unambiguously that the partitioning and adsorption of the microgels is directly related to their composition. Enriching the microgel composition in Dextran progressively decreases their affinity for the PEO phase and increases their adsorption at the D/P interface, although it never reached the point where the microgels preferred the dextran phase. No maximum nor plateau was reached for the studied composition range. The same trend was found for the partitioning between the two phases, with the remarkable effect that the partitioning was always lower for dextran-rich emulsions. The effect of bis-hydrophilicity on emulsion stability is more complex. As shown in Figs. 5, 6 and S8, the stability at rest (1 G) and under centrifuge acceleration follow the same trends. An optimal stability is found for the D/P emulsion stabilized by microgels with 50 wt%

of dextran, which appear to be very robust even under compression. Below 50 wt%, the D/P emulsions are less stable than the optimal but they are more stable than the corresponding P/D emulsions. The P/D stability evolves similarly to the adsorption of microgels at the interface but the lack of parallel between the evolution of adsorption and emulsion stability, for the D/P emulsions, clearly indicates that adsorption is not the sole criterion controlling the stability. From this observation, one can conclude that the adsorption at the interface is a pre-requisite but not a sufficient condition to guarantee the stability of emulsions. It is likely that interactions between adjacent microgels should affect the mechanical properties of interfaces [55] but interactions between drops should also be considered. Steric repulsion is likely to occur, because the microgels are weakly charged due to the initiator [18].

The bis-hydrophilic balance was tuned by increasing the fraction of Dex-MA in the microgels, which also cross-links the network. Therefore, increasing the content of Dex-MA also increased the network elasticity. For a given fraction of dextran, microgels with the highest DS, or equivalently more cross-linked microgels, lead to D/P emulsions with a slightly higher stability. Thus, it can be concluded that the softness of microgels is not an important parameter to stabilize W/W emulsions, conversely to W/O emulsions for which this parameter is of paramount importance [56]. The role of the molecular weight of the dextran chain was also investigated. It was found that microgels with a higher molecular weight of dextrans, 40 kg/mol, led to a higher stability compared to dextrans of 6 kg/mol with the same degree of substitution. However, the exact role of this parameter is difficult to assess since the microstructure of the microgels is not known at this stage.

Finally, the role of temperature was explored. Temperature impacts microgel conformation, since it induces the deswelling of most microgels, the only exception being those with the highest Dex-MA content. The distribution of microgels between the phases and at the interface was very sensitive to the preparation pathway of the emulsions. It means that the configurations were kinetically trapped. When the emulsions were prepared at room temperature and then heated above the VPTT of the microgels, no change in the distribution could be observed. This is in line with studies at interfaces, which demonstrated that the footprint of pNIPAM microgels at the interface did not evolve with temperature once adsorbed, although deswelling could occur in the direction perpendicular to the interface [57,58]. When microgels and the phases were first heated, the dispersion in the PEO phase decreased but it did not promote the adsorption since the excess of fluorescence also decreased (Fig. 7). Simultaneously, the presence of aggregates was observed. Although the pathway had an important impact on the microgel distribution in the system, the emulsion stability was almost insensitive to the preparation route (Fig. 8). Contrary to their behavior at room temperature, all the D/P emulsions were unstable, whatever the composition and whatever the pathway of preparation. Again, this contradiction points out the difficulty to relate the emulsion stability solely to the microgel distribution. Interactions between microgels at the interface and between drops are likely changed when temperature is raised above the VPTT. The loss of steric repulsion in the PEO phase could explain the loss of stability for the D/P. Regarding P/D emulsions, the stability is promoted for the microgels containing the lowest amounts of Dex-MA (below 50 wt%) thus confirming the previously reported stability inversion [18]. The Dex chains in the particles are in good solvent and may promote repulsion between drops. More surprisingly, the P/D which were stable at room temperature for Dex-MA-rich microgels (above 50 wt%) become unstable above the VPTT (Fig. 8). In this case, it should be noted that the stability change occurred in spite of constant swelling of the microgels. This new trend shows that the very rich tunability of these bis-hydrophilic responsive microgels to be used as W/W stabilizers.

In summary, the systematic modification of the bis-hydrophilicity in microgels has enabled us to modify the ability of microgels to adsorb at the W/W interface below their VPTT. However, no adsorption maxi-

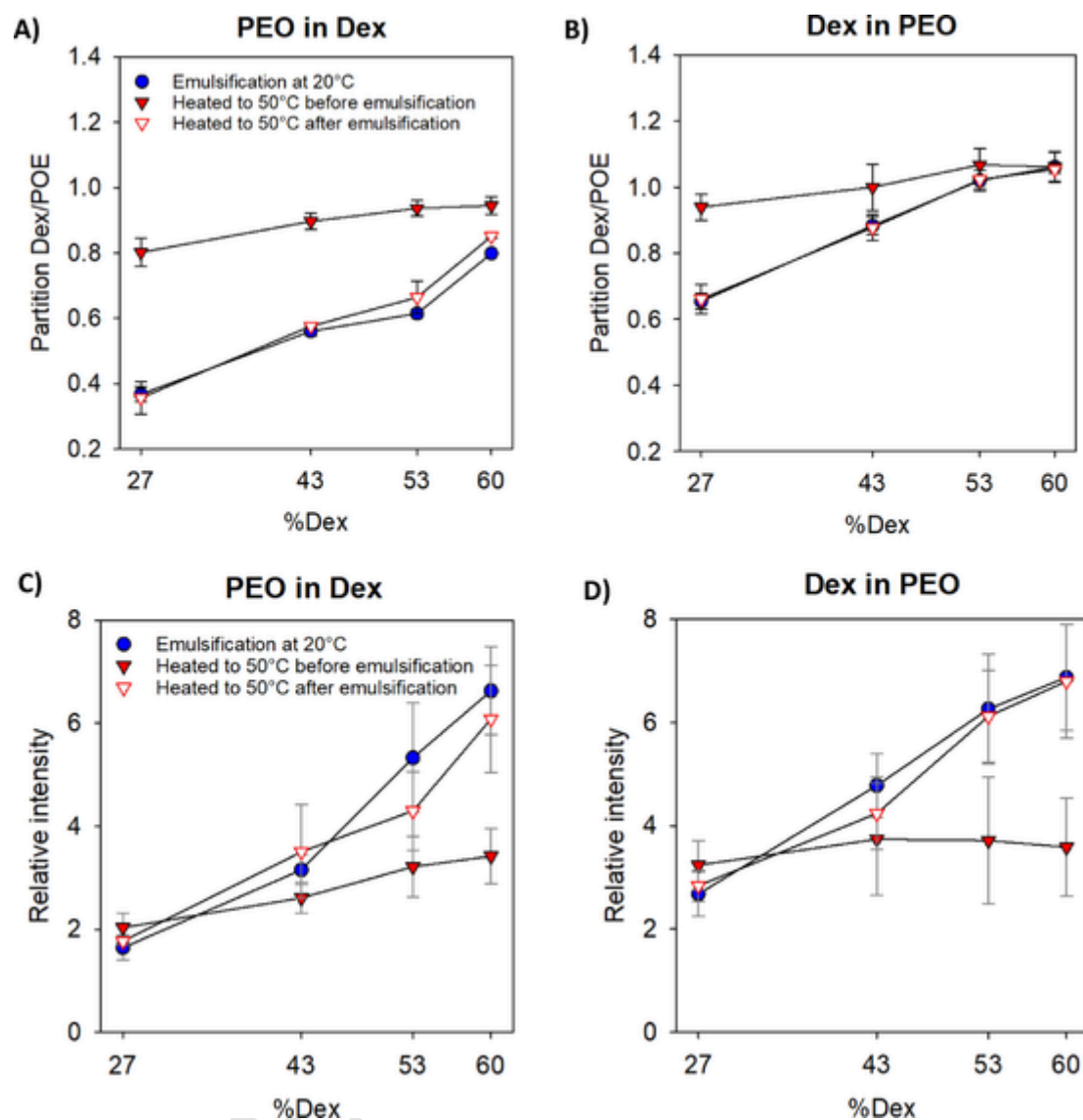


Fig. 7. Effect of temperature on the partition of microgels (series B) between the Dex and PEO phases (A, P/D; B, D/P) and on the normalized intensity at the interface (C, P/D; D, D/P).

imum could be found despite D/P emulsion stability presented a maximum at 50%wt of Dex-MA in the microgel. Above this value, P/D emulsion were more stable than D/P. Although interactions between microgels at the interface and between drops are certainly involved in the stabilization mechanism, it is also tempting to draw a parallel with O/W, whose type and stability depend on the wetting contact angle of the particles [39]. In this work, it was not possible to determine the contact angle but it is likely that it evolves continuously with the microgel bis-hydrophilicity, as represented in Fig. 9. The composition with 50 wt% of Dex-MA corresponds to a maximum of stability for the D/P phase, possibly because it corresponds to an optimum in terms of adsorbed amount and of mutual repulsive interactions between the drops. Upon further increase of the contact angle, steric repulsion may decrease due to the decrease of the hydrogel layer in the PEO phase. Obviously, controlling the adsorption at the interface is not sufficient to guarantee emulsion stability since interactions between microgels at the interface can also come into play. The adsorption of microgels above the VPTT of microgels depends much less of the bis-hydrophilic balance (Fig. 7). It appears more difficult to draw a parallel between the composition and the adsorption when pNIPAM moieties are insoluble. However, the

composition affects the resulting emulsion stability, again demonstrating that adsorption does not solely control the stability.

Finally, we point out the limits for comparing W/O and W/W emulsions. Indeed, here we chose to change the adsorption of particles at the interface by tailoring their composition. It is also possible to manipulate the phase diagram of the emulsion itself with slight changes in the composition resulting in important changes in the interfacial behavior. A very simple strategy was achieved recently by Machado et al. [59], who added a third component in the system that had more affinity with one of the phases, to modify the interfacial tension and thus, enhance the adsorption of protein microgels with a given composition.

5. Conclusion

To the best of our knowledge, it is the first time that a chemical parameter, here the bis-hydrophilic balance held by the composition of Dex in the particles, allows controlling the adsorption energy at the W/W interface, likely due to a progressive change of the wettability, and the stability of the W/W emulsions in any given type of dispersed phase (A-in-B or B-in-A). Previous works reported the incidence of the phase diagram itself [41], of particle charges [13,28,19] or of particle disas-

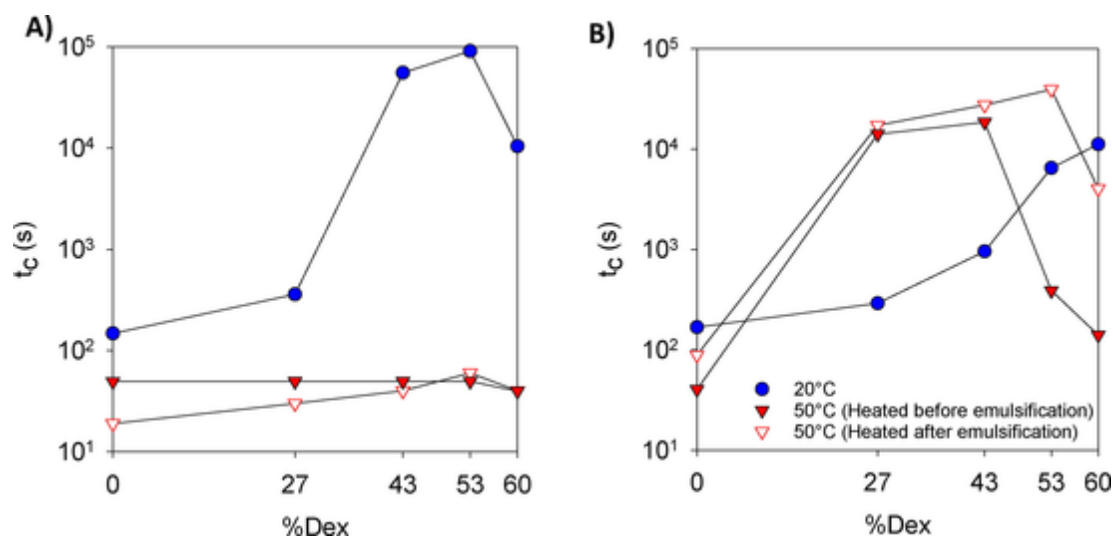


Fig. 8. Characteristic time of the A) P/D, B) D/P as a function of the Dex fraction in the microgels (series B) at 20 and 50 °C, for emulsions prepared in different conditions. 0% Dex corresponds to the blank emulsion without particles.

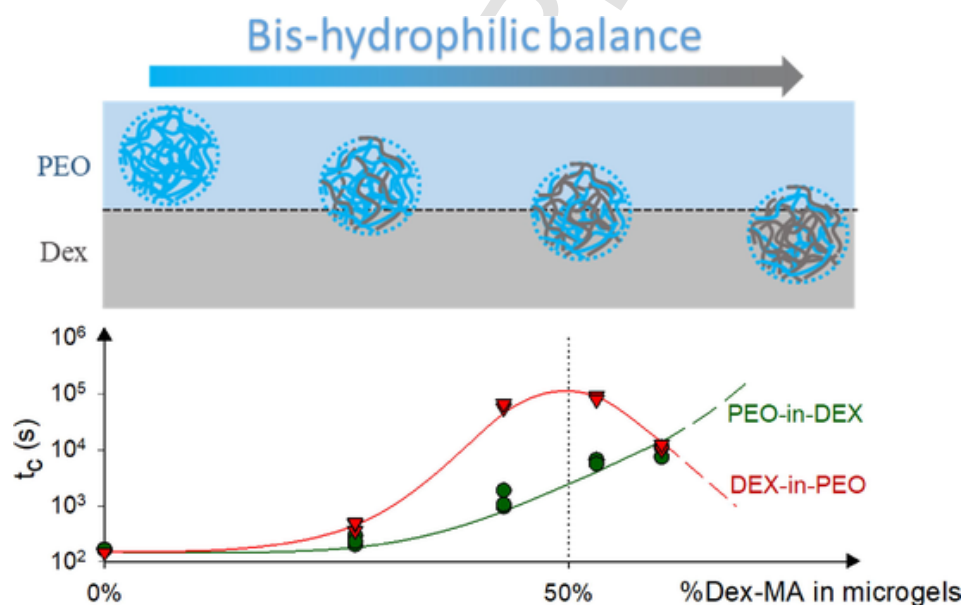


Fig. 9. Schematic representation of microgels at the W/W interface with tuned bis-hydrophilic balance, at low temperature, i.e. below the VPTT and the corresponding emulsion lifetimes (data taken from Fig. 6). The lines are guides for the eye.

sembly [23], for a given chemistry, but no attempt was made to adapt the chemical composition of the emulsifier to that of the aqueous phase diagram. So far, the most systematic approach of the emulsifier structure focused on the hydrophobization of fumed silica particles, but it failed into predicting emulsion stability because polymer adsorption modified *in situ* the surface chemistry of the particles [21].

The experimental results demonstrate the huge potential of these systems to finely tune W/W emulsion stability via chemical composition combined with internal conformation triggers. This point opens rich perspectives for encapsulation applications since the encapsulation of a cargo is related to its partitioning within one of the phases [3]. Not only these findings should give clues to the chemists to design and build efficient particles as stabilizers, possibly incorporating new functionalities such as catalytic ones [60], it should also guide the selection of the best candidates for particles made of natural biopolymers, such as denaturated proteins, glycoproteins or polysaccharides [61], that are naturally bis-hydrophilic. This strategy should not be restricted to one type

of W/W emulsions, it should be transposable to other systems, as soon as the particles gather an affinity for both phases.

Uncited references

[62,63].

CRediT authorship contribution statement

Léa Waldmann : Investigation, Data curation, Visualization, Writing – original draft. **Do-Nhu-Trang Nguyen** : Investigation, Data curation, Visualization, Writing – original draft. **Stéphane Arbault** : Supervision. **Taco Nicolai** : Conceptualization, Methodology, Writing – review & editing. **Lazhar Benyahia** : Writing – review & editing, Project administration, Conceptualization, Supervision. **Valérie Ravaine** : Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Valerie Ravaine reports financial support was provided by French National Research Agency.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcis.2023.09.049>.

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