16 Pickering emulsions and biomass

Loïc Leclercq, Véronique Nardello-Rataj

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Loïc Leclercq[a] and Véronique Nardello-Rataj[b]

[a] Université de Lille, Cité Scientifique, F-59655 Villeneuve d'Ascq, France.
loic.leclercq@univ-lille.fr

[b] Centrale Lille Institut, Cité Scientifique, F-59655 Villeneuve d'Ascq, France.
veronique.rataj-nardello@univ-lille.fr

16.1 Introduction

In 1903, Walter Ramsden reported for the first time the use of solid particles to stabilize emulsions (▶Figure 16.1, left) [1]. This finding was subsequently taken up by Percival Spencer Umfreville Pickering in 1907 (▶Figure 16.1, right) [2]. Though Ramsden was the first to describe this phenomenon, Pickering is generally associated with this discovery because it brought answers to Ramsden's questions. His work has shown that solid particles, having a higher affinity for the aqueous phase than the oil phase, are more advantageous alternatives than the use of surfactants to obtain very stable oil-in-water emulsions.

Figure 16.1: Prof. Walter Ramsden (left) and Prof. Percival Spencer Umfreville Pickering (right).

Pickering emulsions are surfactant-free dispersions of two immiscible liquids kinetically stabilized by solid particles. For almost a century, Pickering emulsions have been the subject of numerous physicochemical investigations which have made it possible to understand and control their behavior and formation. However, it is only more recently that researchers and industry have taken an interest in their potential applications.

Unlike surfactant molecules which continuously adsorb and desorb from the surface of droplets, solid particles irreversibly anchor to the water/oil interfaces. Accordingly, very high stability of the resulting emulsions up to several years can be obtained due to their high resistance towards coalescence, thus offering multiple possible applications in various domains. Stability is an essential property for industrial products which require a certain shelf life. In addition, the use of surfactants is increasingly criticized because of their possible toxicity for Humans and Environment. The use of colloidal particles to replace surfactants therefore appears as an interesting alternative.

As a consequence, an increasing interest in Pickering emulsions has emerged over the last 15 years, mainly related to their very attractive properties compared to conventional emulsions. Pickering emulsions are present in a wide variety of application fields such as the cosmetics, pharmaceutical and food industries to stabilize emulsions and encapsulate active ingredients, but also in the petroleum industry to stabilize water/petroleum emulsions. They are also found in coatings such as bitumen, paints and adhesives. Finally, Pickering emulsions can be used as matrices in order to prepare porous materials, composite materials or even to carry out emulsion polymerization. More recently, they have received a growing interest in the field of catalysis, insofar as the interfacial reactions which they implement are much more efficient than those carried out in biphasic systems.

In this book chapter, we first describe the main key parameters governing the physicochemistry of the Pickering emulsions. Then, we focus on the one hand on the utilization of biomass as stabilizing particles of Pickering emulsions and on the other hand, on the use of catalytic Pickering emulsions to convert biomass. The interest of these versatile systems is illustrated in pharmaceutical, cosmetic and food applications notably as delivery systems as well as in catalysis for the conversion of glycerol and the production of biodiesel.

16.2 Biomass-based particles for the formulation of Pickering type emulsions

16.2.1 Key parameters of particles

Since the first observation made by Ramsden and Pickering, it is clear that colloidal particles allow the stabilization of droplets by the formation of a dense and rigid film that acts against coalescence [1,2]. Thus, the stabilization of Pickering emulsions results primarily from the decrease of free energy accompanying particle adsorption at the interface. However, in contrast to molecular

surfactants, the colloidal particles do not need being amphiphilic: only a partial wetting of the particles by water and oil allows the strong anchoring of particles at the oil/water interface [3]. In order to characterize the wettability, the three-phase contact angle of particles to the interface, θ (measured through the aqueous phase), can be used to predict the type of emulsions (*i.e.* oil-in-water or water-in-oil). Therefore, relatively hydrophilic particles (θ < 90°) form oil-in-water emulsions [4]. The opposite holds for hydrophobic particles (θ > 90°) (►Figure 16.2). For sake of clarity, spherical particles are used as model in the following discussion, before to be extended to non-spherical particles.

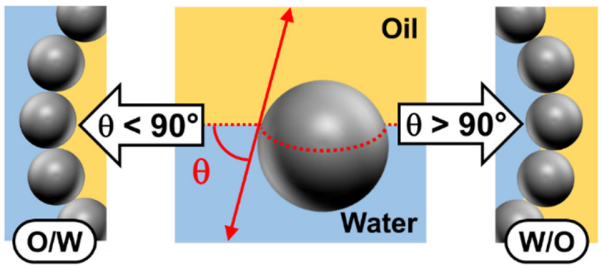

Figure 16.2: Definition of the three-phase contact angle, θ, and its relation with the type of Pickering emulsions (oil-in-water, O/W, or water-in-oil, W/O).

From a theoretical point of view, the desorption energy (E_{des}) is related to the interfacial tension (γ), the radius of the spherical particle (R), and the contact angle (θ) according to the following equation:

$$E_{des} = \pi R^2 \gamma \left(1 - |cos\,\theta|\right)^2 \qquad (1)$$

For spherical particles of 10 nm of radius at γ = 50 mN/m, the particles is most strongly held in the interface for θ = 90° with E_{des} = 3822 k_BT (►Figure 16.3). Either side of 90°, E_{des} falls rapidly. For this reason, the stable Pickering emulsions are theoretically obtained for θ = 90°. However, at this peculiar value, the wettability defines the transition from oil-in-water to water-in-oil emulsions. As consequence, no stable emulsion can be obtained due to a curvature close to zero and only a "bipolar"-like behavior is observed [5]. However, when θ tends to 90°, desorption energies of nanoparticles can easily reach several thousands of k_BT and the nanoparticles are "irreversibly" anchored at the interface. This difference explains the peculiar properties of Pickering emulsions against the coalescence phenomenon. However, very hydrophilic or hydrophobic particles (θ tends to 0 or 180°) are inefficient Pickering stabilizers (►Figure 16.3).

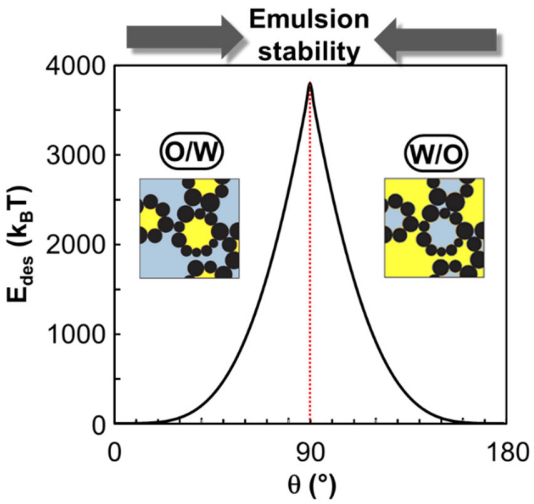

Figure 16.3: Desorption energy, E_{des}, as a function of three-phase contact angle, θ, and its relation with the type of Pickering emulsions and stability (calculated according eq. 1 with R = 10 nm, γ = 50 mN/m at 25 °C; oil-in-water, O/W, or water-in-oil, W/O).

The interfacial thickness also differs from those stabilized by conventional surfactants. Indeed, the thickness is much larger for solids-stabilized emulsions: it is at least equal to the particle size for a monolayer. In addition to the interactions between particles and the dispersed and continuous phases, the mechanical properties of particle-based interfacial layers depend also on the inter-particle interactions within the film: attractive interactions provide mechanical strength (rigidity) to the adsorbed layer [6]. Consequently, some authors have even been compared to an egg shell [3]. In addition, the presence of lateral attractive capillary forces, which results from the deformation of the fluid interface around the particles, contributes to the mechanical stability of the interfacial layer [6-9]. As consequence of desorption energy barrier and inter-particle interactions, the coalescence is limited. Indeed, if the energy required to form Pickering emulsion is too important, for a given oil/water ratio and particle concentration, the surface coverage (τ, proportion of oil-water interface covered by the particles) is partial (τ < 1). In this condition, the droplets coalesce until to obtain a compact monolayer (τ = 1). This limited-coalescence is observed immediately after emulsification and stopped when the droplets interface become densely coated with particles (►Figure 16.4a). However, it is noteworthy that the emulsion destabilization can be produced "on demand" with the use of appropriate external stimuli (*e.g.* centrifugation, dilution, pH, temperature variation, *etc.*) [6]. It is noteworthy that the limited-coalescence is generally observed for spherical particles.

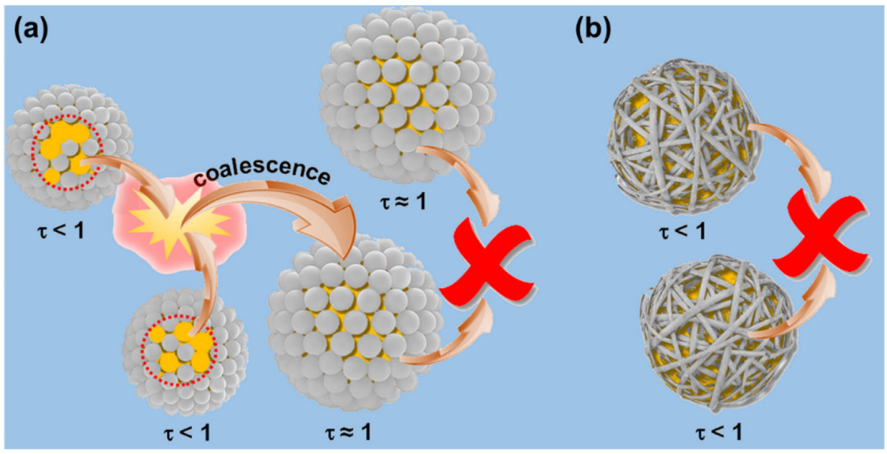

Figure 16.4: Schematic representation of: (a) the limited-coalescence process, observed for spherical particles, until a surface coverage, τ, close to 1, and (b) arrested coalescence in the case of non-spherical particles (needles).

Therefore, neutral or slightly charged monodispersed particles with a three-phase contact angle very close to 90° form a tight hexagonal monolayer. Although it is assumed that monolayer ($\tau = 1$) or multilayer coverage ($\tau > 1$) is often required to form an effective barrier against droplet coalescence, the particle characteristics (*e.g.* size, shape, charge, *etc.*) and conditions (pH, salt concentration, *etc.*) are more essential parameters. For instance, it is possible to form Pickering emulsions with crystalline materials: typically needles. In this case, a rougher and rippled interface is obtained due to the presence of irregularly shaped and/or oriented nanocrystals. Although the droplets are prone to the limited-coalescence phenomenon, crystal reorientation is more complex than for spherical particles. Therefore, a restriction of the internal dynamics ("jamming") due to surface roughness is observed: the individual droplets cannot slide one against each other because of surface roughness (▶Figure 16.4b). Consequently, a partial surface coverage ($\tau < 1$) does not necessarily mean poorer stability. Similarly, highly charged particles with a long-range dipolar moment are packed less compacted compared to neutral ones. Furthermore, particles may form bridging monolayers, by embedding themselves within the interfaces of two droplets, thus keeping droplets at finite distance, while stabilizing the liquid film between droplets. The droplets may then even be stable to coalescence when the entire interfacial layer is closely enough packed. The particles may also form a three-dimensional network in the continuous phase, which greatly enhances the emulsion stability.

As mentioned, the stabilization of Pickering emulsions with particles is related to their ability to adsorb strongly at the liquid/liquid interfaces to sterically hinder coalescence, as well as to slow down the diffusion by structuring in the continuous phase. Indeed, all the basic parameters reviewed in this section (*e.g.* particle adsorption, wettability, surface coverage, shape and size) are essential to govern the properties of Pickering emulsions. However, other parameters linked to the particles (*e.g.* concentration, surface roughness or charge) as well as environmental parameters such as the homogenization process (*i.e.* rotor-stator or high-pressure homogenization, ultrasonic, membrane or microfluidic emulsifications), the oil phase, the oil/water ratio, the temperature, the salt concentration and pH, are also extremely important (►Figure 16.5). Unfortunately, although these parameters make it theoretically possible to finely adjust the emulsion characteristics in order to meet the requirements of specific applications, it is very complicated to study their contribution independently since all these parameters are interrelated and can influence the wetting of the particle and the properties of the Pickering emulsions.

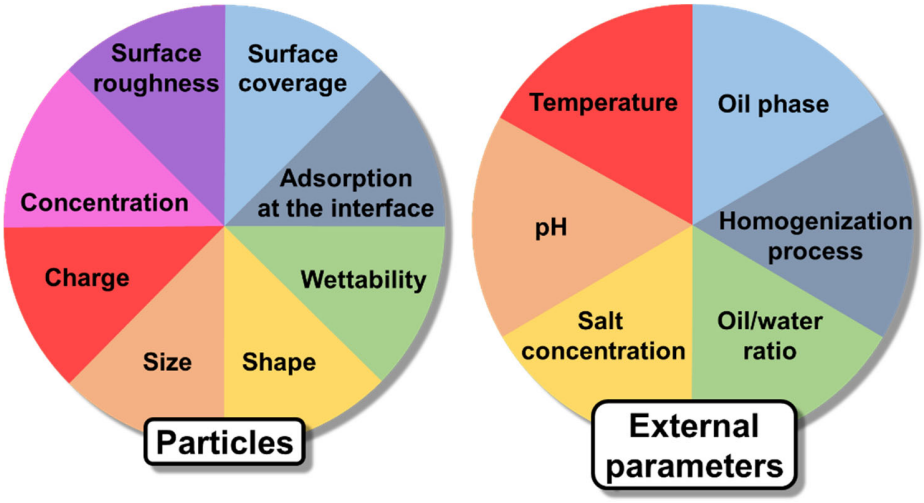

Figure 16.5: The key parameters governing the Pickering emulsion properties.

If the research into Pickering emulsions has been focused on using inorganic particles, their applications are limited due to their biocompatibility and biodegradability. Thus, in the last decade, there has been a shift toward studying materials of biological origin for the stabilization of emulsions. Indeed, many bio-sourced macromolecules exhibit surface activity at liquid/liquid interfaces with tunable properties by changes in pH, temperature, ionic strength, *etc*. Unlike synthetic polymers, bio-sourced polymers are often polydisperse and their chemical functionalities depend on the source. Therefore, learning how to use biopolymers to make particles and materials

with minimal chemical modification is of importance. Due to these advantages for food and biomedical applications, the ability of particles derived from cellulose, lignin, chitin, starch, proteins have been reported in the literature for the stabilization of emulsions. For sake of clarity, some typical families of particles are reported in the following sections. However, it is noteworthy that other types of biological (*e.g.* egg yolk granules, bacterial cells, viruses and spores) are also described to stabilize Pickering-like emulsions. They are described elsewhere in the literature [10,11].

16.2.2 Saccharide-based particles

Saccharides are key biological intermediate in storage energy in the form of polysaccharides. However, they serve also as structural components: *e.g.* cellulose (plants) and chitin (arthropods). In addition, they also play key roles in immune system, fertilization, coagulation, *etc*. Classically, saccharides are classified according to their degree of polymerization: (*i*) sugars (monosaccharides, disaccharides and polyols), (*ii*) oligosaccharides (maltodextrins, *etc*.), and (*iii*) polysaccharides (cellulose, chitin, chitosan, starch). As sugars and oligosaccharides are free soluble or readily dispersible in water, they do not offer good platform to obtain Pickering emulsions. In contrast, polysaccharides, insoluble in water, are widely used to stabilize these emulsions. However, this is subject to an exception in the case of cyclodextrins (cyclic oligosaccharides).

Cyclodextrins

Cyclodextrins, CDs, consisting of a macrocyclic ring of glucose joined by α-1,4 glycosidic bonds, are produced from starch by enzymatic conversion. The natural CDs (α-, β-, and γ-CDs, composed of 6-, 7-, and 8-membered *D*-glucopyranose, respectively) can be used to complex various chemical structures. This property can be used to build up hierarchically oil-in-water Pickering emulsions using the colloidal tectonics concept in which the stabilizers (*i.e.* particles) are formed from the interaction between two tectons: the oil and the CD [12]. Indeed, these Pickering delivery platforms result from the following recognition events and iterations: (*i*) formation of surface-active complexes between CD and oil molecules, (*ii*) emergence of particles *via* dehydration and pseudo-crystallization of the inclusion complexes, (*iii*) particles growth limitation by slower CD and oil molecules transfer rates across the liquid/solid/liquid interface and (*iv*) stabilization of the Pickering emulsion by the particles located in the interfacial layer (▶Figure 16.6) [13]. The particles can be spherical or non-spherical (crystallites). For instance, the

cyclooctene/water biphasic system provides stable oil-in-water emulsions stabilized by 1:1 inclusion complexes agglomerated under the form of spherical nanoparticles (3.6 nm) [14]. Similar observations were made with β-CD/paraffin oil emulsion [15]. Indeed, insoluble β-CD/paraffin oil inclusion complexes formed polydisperse nanoparticles (about 30 to 250 nm). However, the authors pointed out that these nanoparticles can self-assemble in pseudo-crystalline structures (about 1 to 4 μm) for high concentrations of CD. In contrast, the use of octanol, decane or toluene as oil phase seemed to suggest the formation of crystals [16]. These highly flexible systems offer the possibility of obtaining different derivative systems such as cyclodextrinosomes [17] or core-shell nanoparticles [18]. It is noteworthy that the common oils (*e.g.* paraffin or isopropyl myristate) can be replaced by phytochemical oils (*e.g.* carvacrol and terpinen-4-ol) [19]. Additionally, the formation of insoluble α-CD and polyethylene glycol (PEG) polypseudorotaxanes can also be used to stabilize very stable oil-in-water Pickering emulsions *via* the formation of hydrogels prior to the addition of oil and mixing [20]. The emulsion is mainly stabilized by the presence of submicronic nanoparticle-like structures (< 500 nm) made of aggregated PEG/α-CD polypseudorotaxanes aggregated [21].

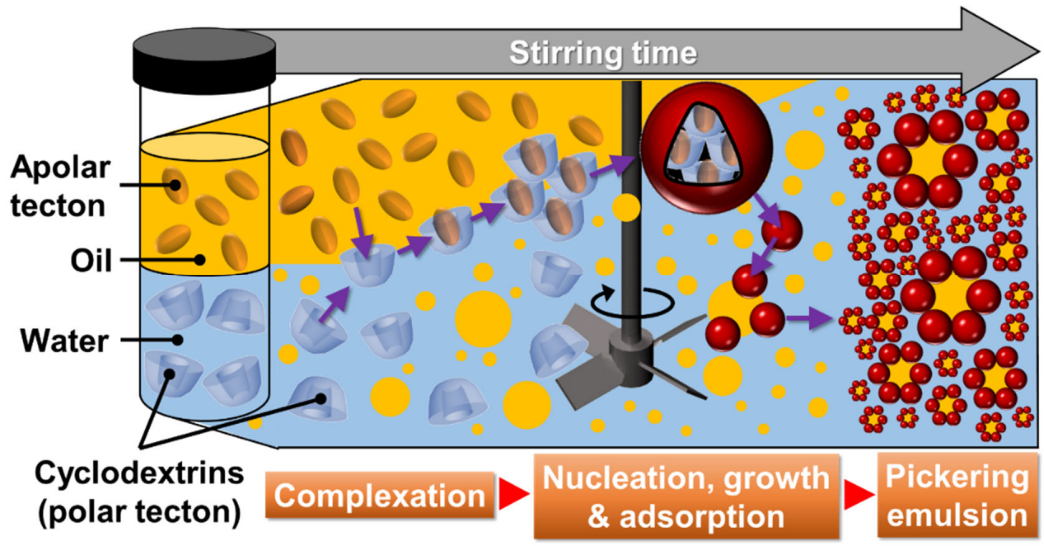

Figure 16.6: Hierarchical construction (purple arrows) of oil-in-water Pickering emulsions by self-assembly of complementary polar and apolar tectons (CD and oil, respectively).

Starch

Starch, the most abundant carbohydrate in the human diet, is a high molecular weight polysaccharide composed of *D*-glucopyranose unit. In addition to its several advantages such as

economical, biocompatible, biodegradable and non-toxic, starch molecules arrange themselves in the plants in semi-crystalline granules that can be used as food-grade Pickering stabilizers due to their size, shape and composition [22]. As all natural products, depending on the source as well as preparation of these granules, the shape and size of these particles can be different [23]. Indeed, each plant species has a unique starch granular size: rice starch is relatively small (~ 2 μm) while potato starches have larger granules (up to 100 μm). Although hydrophilic unmodified starch granules have been shown to stabilize oil-in-water emulsions, native starch particles have the disadvantages of poor hydrophobicity correlated to weak stability and large particle size. Consequently, the stability of Pickering emulsions is not optimal as the particles cannot be well adsorbed at the interface. Many studies use modification to make the particles more hydrophobic with methods as simple as acid hydrolysis, enzymolysis, nanoprecipitation and recrystallization [24]. As starch is a semi-crystalline polymer, its hydrolysis using strong acids allow to obtain crystalline particles. For instance, in 2012, Li and coworkers demonstrated that the sulfuric acid-hydrolyzed waxy maize starch nanocrystals can provide stable paraffin oil-in-water Pickering emulsions (50% v/v, with only 0.02 wt.% of nanocrystal relative to water) even after 2 months of storage [25]. Another more recent example is given by Azfaralariff *et al.* that after an acid hydrolysis method they obtain round and oval-shaped sago starch nanocrystals (between 20 to 100 nm and with a crystallinity about 46%) leading to very stable corn oil-in-water Pickering emulsions with no sign of creaming during two months of storage at room temperature [26]. Alkaline treatment of starch nanocrystals can also be used to prepare Pickering emulsions. In 2020, Wang *et al.* used ammonia to treat normal or waxy maize starch nanocrystals [27]. The droplet size of these emulsions is clearly affected by the structural differences between the two maize starches: the nanocrystals size obtained from waxy maize starch was smaller whereas the ζ-potential was higher than the normal one. The droplet size of sunflower oil-in-water Pickering emulsions (50% v/v) stabilized by 3 wt.% of nanocrystal relative to water was about 5.3 and 70.5 μm for nanocrystals obtained from waxy and normal maize starch, respectively. Consequently, the stability is improved to storage in the case of waxy maize starch nanocrystals which presented stronger gel-like characteristics than other emulsions. The use of octenyl succinic anhydride can also be used to increase the hydrophobicity of starch. For instance, in 2015, Song *et al.* modified indica rice starch using octenyl succinic anhydride esterification to prepare soybean oil-in-water emulsions (50% v/v stabilized by 4.0 wt.% of modified starch particles with a degree of substitution 0.03, pH of

emulsion system between 6.0 and 7.0 [28]. In 2020, a more systematic study has been performed to evaluate the effect of the modification of the rice starch, waxy corn starch, wheat starch or potato starch by the octenyl succinic anhydride. In this study, the authors observed that the oil-in-water emulsion stabilized with rice starch particles (3.85 wt.% of the emulsion) showed the minimum droplet size (83.6 μm) combined with the best physical stability after 30 days of storage [29]. Moreover, based on rheological considerations, the authors claim that the particles allow the formation of wall-like structures around the oil droplets, which prevent them from coalescing. Another typical example, is the nanoprecipitation method employed in 2017 by Ge and coworkers to obtain corn nanoparticles. As reported, the nanoprecipitation promoted the starch nanoparticles adsorption at the interface and formed very stable soybean oil-in-water Pickering emulsions because the three particles (100-220 nm) had a three-phase contact angle, θ, close to 90° [30]. It is noteworthy that physical adsorption of molecules has attracted significant interest due to its simple preparation and efficacy. For instance, BelHaaj *et al.* showed that sulphuric acid-hydrolyzed starch nanoplatelets were not sufficient to stabilize the droplets by themselves, they did provide a synergistic stabilization effect when used together with a cationic surfactant (dodecylpyridinium chloride) but the required surfactant amount was reduced up to a factor 4 [31]. Finally, it is noteworthy that maize starch particles can be modified using a simple media-milling treatment in the presence of different amylose/amylopectin ratios. These particles are able to stabilized stable food-grade Pickering emulsions [32].

Cellulose

As cellulose, a linear polysaccharide which consists of β-1,4-linked glucopyranose units, is the most abundant biopolymer in the world (structuring agent of green plant cells), sustainable and biocompatible, it is also used to stabilize Pickering emulsions due to its insolubility in water and organic solvents. It is noteworthy that cellulose can also be produced by algae (photosynthetic eukaryotic organisms), oomycetes (fungus-like eukaryotic microorganisms) and biofilms produced by some bacteria (*e.g. Acetobacter xylinum*). Many properties of cellulose depend on its source, *i.e.* the chain length or the degree of polymerization can vary. For instance, cellulose from wood pulp has typical chain lengths between 300 and 1,700 glucose units in one polymer molecule whereas cotton and other plant fibers as well as bacterial cellulose ranging from 800 to 10,000 glucose units [33]. Additionally, cellulose content of cotton is close to 100% while wood cellulose is only 40 to 50% because it is complexed with lignin and hemicelluloses. In contrast, the bacterial

cellulose not being complexed, it is of a high purity [34]. For instance, bacterial cellulose nanoparticles obtained after acid hydrolysis with HCl are able to stabilize peanut oil-in-water Pickering emulsions because of nanoparticles ideally balanced between hydrophilic and lipophilic domains [35]. Native cellulose exists as macroscopic fibers and microfibrillated cellulose (MFC) in which the polymer contains both crystalline and amorphous domains [36]. However, the degrees of crystallinity vary depending on the source from which the cellulose is obtained: 44% and >80% for cellulose derived from hemp and algae, respectively [33]. Fortunately, the amorphous domains can be cleaved through treatment with various acids (such as H_2SO_4 or HCl) to produce microcrystalline cellulose (MCC) which still contains some amorphous domains. The hydrolysis of MCC is used to obtain nanocrystalline cellulose (NCC). However, cellulose is hydrophilic macromolecule due to the presence of hydroxyl groups on the surface of cellulose. Thus, cellulose are able to stabilize emulsions without the use of additional surfactants. In 2013, Winuprasith and Suphantharika reported soybean oil-in-water Pickering emulsions (30 wt.% of oil at pH = 6.8-7.2) could be obtained by using MFC from mangosteen rind (0.7 wt.% in aqueous phase) without the aid of molecular surfactants [37]. Similar observations are made with NCCs which stabilize oil-in-water emulsions without surfactants due to the amphiphilic nature of NCC [38]. In order to modify the polarity of cellulose, hydroxyl groups can be easily chemically modified by the introduction of methyl, ethyl, hydroxypropyl methyl, carboxymethyl or silyl residues to give methylcellulose (MC), ethylcellulose (EC), hydroxypropyl methylcellulose (HPMC), carboxmethylcellulose (CMC) or silyl cellulose (SC). These chemical modifications are essential to facilitate the formation of stable water-in-oil Pickering emulsions using macroscopic fibers, MCCs, or NCCs. For instance, Andresen and coworkers used silylation to tune the hydrophobicity of cellulose fibers for the stabilization of water-in-oil emulsions [39]. It is noteworthy that the functionalization with poly(*N*-isopropylacrylamide) is also commonly used. In 2012, Zoppe *et al.* use the grafting of poly(N-isopropylacrylamide) on the surface of the CNCs to obtain thermosensitive Pickering emulsions [40]. Additionally, Gong and coworkers oxidized and modified, with phenyltrimethylammonium chloride, cellulose nanocrystals to obtain Pickering emulsions with excellent mechanical and thermal stability against centrifugation and heat due to hydrophobic domains created by the phenyl residues [41]. Obviously, these modified celluloses remain biocompatible and biodegradable like native cellulose and exhibit solubility in some solvents (*e.g.* EC in acetone). Contrary to natural cellulose, this differential solubility can be used to produce easily precipitation into particles of

various shapes and sizes by solvent/anti-solvent exchange or by adjusting solution pH or ionic strength [42]. It is also possible to use cellulose is in the form of insoluble colloidal particles after complexation with other molecules. For instance, tannic acid (a type of polyphenol rich in OH groups) can interact strongly with polysaccharides as well as proteins. In addition, tannic acid as well as polyphenol have antioxidant, antibacterial, and antiviral properties. In respect with this, MC can easily form colloidal particles with tannic acid (56-116 nm) leading to sunflower oil/water emulsions of 35 μm which stayed stable for 3 months of storage [43]. It is noteworthy that the strong interactions of epigallocatechin gallate with MC and HPMC can also be used [44]. Physical treatment of cellulose can also be used. For instance, Sanchez-Salvador *et al*. reported that highly viscous (up to 90 times with respect to the oil phase) sunflower oil-in-water emulsions can be obtained by using cellulose microfibers (1.0 wt.%) produced from cotton cellulose linters by mechanical treatment through a high-pressure homogenizer [45]. Cellulose nanofibrils obtained by aqueous counter collision are also able to stabilize oil-in-water emulsions with excellent stabilities compared to those of cellulose nanofibrils prepared by high-pressure homogenization or other chemical preparation methods [46]. This behavior is attributed to better exposure of hydrophobic surface planes.

Chitin/chitosan

The second most abundant polysaccharide found in nature, after cellulose, is chitin (a long-chain polymer of *N*-acetylglucosamine). It is derived from the exoskeletons of arthropods (*e.g.* shrimp and crab shells), the cephalopod beaks (*e.g.* squids and octopuses) and the radulae of molluscs and beaks), the scales of fish and lissamphibians and the cell walls of fungi [47]. Like cellulose, chitin contains hydroxyl groups along its backbone but the variation of its surface charge with pH is opposite that of cellulose due to the presence of amine groups. It is noteworthy that chitin is the only biodegradable cationic polymer (depending on pH) material in nature. In addition, chitin is insoluble in water whereas chitosan (produced commercially by deacetylation of chitin) is water soluble at low pHs (< 6) but precipitated at high pHs due to the presence of amine residues. Indeed, at low pHs, chitosan is positively charged but at higher pHs, the amine groups is uncharged leading to neutral polymers which aggregate in aqueous solution to form particles [48]. Native chitin is semi-crystalline but rod-like colloidal chitin nanocrystals can be obtained after acid hydrolysis. These chitin nanocrystals are able to form stable oil-in-water emulsions as demonstrated by Tzoumaki and coworkers [49,50]. In contrast, chitosan has pH-dependent solubility in water, and

is not a good stabilizer of Pickering emulsions due to the random presence of amine and hydroxyl groups on the accessible surface of polymer. However, its biocompatibility, biodegradability and antimicrobial properties, are highly valued in biomedicine and pharmaceutics [10]. Fortunately, the hydrophobicity of chitosan and the "emulsifying" activity can be tuned by adjusting pH and the degree of deacetylation. At low pH, the protonation of the amine groups (pK_a of 6.5) leads to strong electrostatic repulsion. The opposite holds under neutral and basic conditions, chitosan precipitates due to the absence of charges. In this context, Wei *et al.* showed that chitosan nanoparticles stabilize oil-in-water Pickering emulsions which can be demulsified by lowering the pH and that the emulsions can be recovered after increasing pH and re-emulsifying [51]. The degree of acetylation is also a key parameter to obtain optimal emulsification (generally observed with moderate deacetylation) as well as the pretreatment of chitosan. For instance, Ho and coworkers reported that the emulsifier property of chitosan was greater before than after ultra-sonication [52]. Additionally, ionic cross-linking between the positively charged amine residues and polyanions (*e.g.* sodium tripolyphosphate) can be used to obtain composite particles and to stabilize oil-in-water Pickering emulsions (oil: medium-chain triglyceride and citral). The chitosan-tripolyphosphate particles form globally stable emulsions at 40 °C for 14 days [53]. It is noteworthy that interaction between chitosan and gliadin (a type of proteins present in wheat and several other cereals) can be used to obtain coacervates able to stabilize emulsions with high viscoelasticity and solid-like behavior [54].

Other polysaccharides

Finally, carrageenan, alginate and xanthan gum are known to improve emulsion stability through modification of the rheological property of the continuous phase [55]. Although the widespread formation of particles with these compounds have not clearly demonstrated in the stabilization process, it is noteworthy that the interaction between xanthan gum and shellac (a resin secreted by the female lac bug) leads to insoluble particles stabilizing pH switchable Pickering emulsions [56]. Similar observations can be made for the shellac/gelatin [57].

X.2.3 Protein-based particles

Proteins, polymers of amino acids covalently linked through peptide bonds, are amphiphilic with a broad range of biological functions. Their primary structure is established by their amino acid sequence which determines their spatial arrangement (*i.e.* the secondary and tertiary structures). The secondary structure describes the arrangement of amino acid residues observed at the atomic

scale (*e.g.* α-helix, β-sheet and turns) whereas the tertiary structure corresponds to the global protein shape. Finally, the assembly of several protein subunits constitutes the quaternary structure. As some amino acids have hydrophobic or hydrophilic side chains, the proteins polypeptides (*e.g.* casein, whey, lactoferrin, soy protein, zein and ferritin) have an amphiphilic nature known to reduce the energy of interfaces and to act as emulsion stabilizers.

Milk proteins (caseins, whey and lactoferrin)

To highlight the mechanism of emulsion stabilization, we present here the milk which is an emulsion of fat globules (0.1 to 15 μm) dispersed in an aqueous environment stabilized by micellar caseins (*i.e.* composed of several thousand associated casein subunits particles from 20 to 600 nm in diameter). In milk, we find four phosphoproteins named α_{S1}, α_{S2}, β, and κ-caseins which present a block distribution of hydrophobic (leucine) and hydrophilic amino acids (glutamic acid and lysine). The strong aggregation of caseins in aqueous solution leads to supramolecular assemblies called casein micelles. Theses insoluble caseins emulsify and stabilize the emulsion by forming thick steric barriers against coalescence in interfacial films [12]. Obviously, micellar caseins can be used to stabilize various Pickering emulsions. In these systems, the emulsion stability depends on the denaturation of proteins which can lose their quaternary, tertiary and secondary structures by application of some external stress (*e.g.* acid or base, inorganic salt, organic solvent, radiation or heat). However, the emulsions can be used to control heat-induced aggregation [58]. In respect with this, in 2019, Silva and coworkers reported that the heat-induced gelation of micellar caseins and plant proteins (soy proteins and pea proteins) sunflower oil-in-water emulsions [59]. These systems can be used to replace milk proteins (micellar caseins or whey proteins) by plant proteins in food formulations for the development of food products (yoghurts, dessert creams and ice creams. Additionally, there is a good potential for use of oil-in-water emulsion stabilized by protein/polysaccharide combination (casein and pectin, respectively) in the delivery vehicles for nutrients and in the protection against enzymatic breakdown [60].

As milk is composed of casein and whey proteins (respectively, 80/20% in cow's milk and 30/70% in human milk), whey protein constitutes a food-grade material used in ice cream and cheeses [61,62]. From a structural point of view, whey protein is the collection of globular proteins isolated from whey (the liquid remaining after milk has been curdled and strained). As during the preparation procedure, whey protein is denature due to the heat treatment, whey protein microgels

are generally used as edible colloidal particles leading to oil-in water high internal phase emulsions (*i.e.* with a volume fraction of dispersed phase above 0.74) [63]. For instance, corn oil-based oil-in-water high internal phase emulsions are reported with higher stability than surfactant-stabilized ones [64]. Whey protein isolate microgels can be used to form grape seed oil-in-water high internal phase emulsions [65]. The authors reported that the encapsulation of *Lactobacillus plantarum* (lactic acid bacterium) within these emulsions increased the cell viability after pasteurization processing. On the other hand, Liu *et al.* reported that glycated whey protein isolate nanofibrils (with glucose, lactose, or maltodextrin) can be used to obtain very stable oil-in-water emulsions due to the modification of the surface charge and hydrophobicity facilitating adsorption and aggregation of nanofibrils on oil droplets [66].

Lactoferrin, a multifunctional protein of the transferrin family, has globular shape and is present in various secretory fluids (milk, saliva, tears and nasal secretions). Shimoni and coworkers synthesized multi-component nanoparticles (diameter between 200 to 575 nm) based on lactoferrin/polysaccharide (alginate or carrageenan) complexes *via* the attractive forces between oppositely charged biopolymers [67,68]. Similarly, electrostatic interactions between two oppositely charged globular, lactoferrin and pea protein isolate (a mixture of vicilin, legumin, and convicilin) can be used to form complex coacervates [69]. Protein/polyphenol/polysaccharide non-covalent ternary complexes based on the aggregation of lactoferrin, oat β-glucan and curcumin can also be used as emulsifiers to stabilize soybean oil-in-water Pickering emulsions with enhanced physical stability [70].

Soy protein

Soy protein (isolated from soybean) are largely involved in the stabilization of various emulsions. Due to its nutritional value, soy protein can be equivalent to animal proteins, thus food applications are possible. In addition, soy isoflavones has a cholesterol-lowering function. As glycinin and β-conglycinin (two major components of soy protein) form globular molecules with a hydrophilic shell and a hydrophobic kernel in aqueous solution, soy protein particles have good emulsifying and gelling properties without additional chemical modification [71,72]. Indeed, soy protein nanoparticles obtained after thermal treatment form Pickering emulsions with characteristics similar to conventional particles [73]. As Pickering emulsions can be affected by external conditions (pH, ionic strength or temperature), soy protein are combined with various

other biomolecules. For instance, protein-based particulate stabilizers can be formed *via* co-precipitation with polysaccharides. Recently, Wang *et al.* used soy protein isolate/pectin particle with electrostatic interaction to form by ultrasound treatment very stable emulsion at pH 3.0 [74].

Zein

Zein (a class of protein manufactured as a powder from corn gluten meal) is a natural amphiphilic biopolymer used to form gels in foods, cosmetics and pharmaceutical industry. As native zein can form particles through self-assembly below and above the isoelectric point, the formation of Pickering emulsions has been reported. For instance, de Folter and coworkers used zein particles obtained after nanoprecipitation procedure to stabilize oil-in-water emulsions. The wettability of these particles can be tuned by varying the pH and that stable emulsions were obtained at ζ-potentials above or below the isoelectric point [75]. Therefore, the stability of zein-based emulsions is affected by pH and ionic strength as well as by particle concentration. In addition, combination by electrostatic adsorption of zein with chitosan, caseinate and alginate can be used to obtain biodegradable and edible composite particles [76]. For instance, food-grade sunflower oil-in-water Pickering emulsions can be stabilized by corn fiber gum or xanthan gum/zein complexes *via* precipitation method [77,78]. As previously mentioned for other biomolecules, the formation of tannic acid/zein colloidal particles can be used as stabilizers of Pickering emulsions [79].

Ferritin

Ferritin, a universal intracellular protein storing iron, is produced by numerous living organisms such as archaea, bacteria, algae, higher plants and animals. Ferritin consists of 24 protein subunits forming a globular cage with multiple metal/protein interactions [80]. As exterior modification of the cage is possible using an ATRP-initiator, followed by polymerization of poly(*N*-isopropylacrylamide), PNIPAAm, in combination with a photo-responsive cross-linker 2-(dimethylmaleimido)-*N*-ethylacrylamide, DMIAAm, van Rijn and coworkers obtained particles capable of stabilizing perfluorodecalin-in-water emulsions [81].

16.2.4 Lipid-based particles

If polysaccharides and proteins are able to adsorb at polar/apolar interfaces, fat crystals have been mentioned in the literature to stabilize water-in-oil Pickering emulsions [82]. As reported by Ghosh

and Rousseau, three types of emulsions stabilization can be observed: (*i*) surface-inactive fat crystals *(e.g.* triglycerides) can stabilize emulsions by the formation of 3D fat crystal network linked by van der Waals interactions trapping the dispersed phase (network stabilization), (*ii*) amphiphilic and surface-active fat crystals can formed crystalline monolayers adsorbing onto the interface leading to a steric barrier against coalescence (Pickering stabilization), or (*iii*) a combination of both mechanism [83]. For instance, Hodge and Rousseau investigated the role of continuous-phase fat crystals in the destabilization of water-in-canola oil emulsions [84]. These emulsions were prepared with hydrogenated canola stearine or hydrogenated cottonseed stearine solid fats. Based on pulsed NMR droplet-size analysis, sedimentation and microscopy, the authors reported that addition of either fat prior to emulsification (*i.e.* precrystallized emulsions) or fat quench-crystallized *in situ* following emulsification (*i.e.* postcrystallized emulsions) decreased the degree of droplet coalescence, based on droplet-size analysis. However, postcrystallized emulsions being more stable against coalescence. In addition, sedimentation studies proved that the stability against sedimentation was greatly improved in postcrystallized emulsions. Although both tristearins were under the same crystal structure (β-form), the postcrystallized canola stearine produced slightly more resistant emulsions than did cottonseed stearine. This observation can be related to the surface energies: canola stearine had greater affinity for the oil/water interface. The authors concluded that the emulsions were stabilized *via* the microcrystals adsorbed onto the droplets surface and the formation of crystal networks that reduce the droplets diffusion. In 2016, Pawlik and coworkers produced tripalmitin particles in aqueous solution (> 130 nm) *via* a hot sonication method, with and without the addition of stabilizers: whey protein isolate, soy lecithin, Tween 20 (polyoxyethylene (20) sorbitan monolaurate) and polyglycerol polyricinoleate [85]. Generally speaking, the stabilizers altered the properties of the tripalmitin particles (crystal form, dispersion state and surface properties). The authors proposed two mechanisms: (*i*) the stabilizers allow the formation of tripalmitin crystals with a range of polarities due to the modification of the polymorphic transitions, and (*ii*) the adsorption of stabilizers at the particle interface modifies crystal surface properties. Next, modified fat particle emulsifiers were used to stabilize Pickering emulsions with oil or water continuous one. The polarity of the fat particles decreased as follows: whey protein isolate > soy lecithin > mixture of soy lecithin and Tween 20 > Tween 20 > polyglycerol polyricinoleate > no stabilizer. Consequently, tripalmitin particles stabilized with whey protein isolate formed oil-in-water emulsions whereas the other modified particles formed

water-in-oil emulsions (unstable with soy lecithin, stable with the mixture of lecithin and Tween 20 or highly stable against coalescence with the other stabilizers).

16.2.5 Lignin-based particles

Lignin, an aromatic macromolecule involved in the support tissues of vascular plants and some algae, is the second most abundant biopolymer found in nature and the most abundant natural aromatic molecule [86]. Chemically, lignins are amorphous phenolic 3D cross-linked polymers composed of three monolignols (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol). However, the ratio of these monolignols varies depending on the source. Additionally, depending on the extraction process, the molecular weight as well as the groups accessible at the surface, and therefore the hydrophobicity, can be very different. For instance, we can obtain hydrophobic organosolv lignins and pH dependent water-soluble alkali or sulfonated lignins. Obviously, these lignins present different colloidal properties. Although, lignins are considered as "waste" biomass in the production of pulp and paper industry, their potential use as emulsifiers is of interest in the context of renewable bio-based systems including Pickering emulsions. Various lignins are surface active and can adsorb at the liquid/liquid interface to provide emulsions. It is noteworthy that the adsorption and assembly of lignin particles at oil/water interfaces are described elsewhere in the literature [87]. For instance, alkali lignins (derived from the Kraft pulping process) contain additional hydroxyl and sulfur moieties due to the extraction process. As alkali lignins have pH dependent solubility, their surface activities are obviously affected. Indeed, if alkali lignins are solubilized at high pH, they can aggregate to form particles in solution for pH < 10). In respect with this, Wei *et al.* used these properties to obtain pH responsive emulsions [88]. Based on this work, Pickering emulsions can be obtained at a pH between 3 and 4, but when the pH is increased up to a value greater than 10, the emulsions disappear. Additionally, Li *et al.* reported that oil-in-water fuel emulsions can be stabilized by carboxymethylated lignins [89]. In such systems, the degree of substitution, the salinity and the pH of the aqueous phase are key parameters. In the more appropriate conditions, the authors reported than carboxymethylated lignins can formed stable emulsions with a drop size of approximately 2.5 mm for over 30 days. On the other hand, aromatic lignin colloidal particles, very stable at broad pH range (between 4 and 11) and easily dispersible in organic solvents, can also be obtained using green chemo-enzymatic conversion of the phenol and the hydroxyl groups. For instance, Xiong and coworkers obtained lignin hollow nanospheres (400-600 nm) by slow addition of water into solutions of enzymatically hydrolyzed lignin dissolved

in tetrahydrofuran following removal of the organic solvent by dialysis [90]. The authors proposed that the nanospheres exhibited hollow structure due to the effect of tetrahydrofuran during self-assembly. The hydrophobic outer surface and the hydrophilic inner surface were formed by a layer-by-layer self-assembly process from the exterior to the interior based on π-stacking interactions. These hollow nanospheres presented high load capacity (the surface area up to 25.4 m^2/g). As enzymatic transformations are ecologically and economically viable alternatives to chemical synthetic methods, Mattinen *et al.* showed that various laccases could be used to cross-link colloidal lignin particles [91]. This method increased their stability in organic solvents: the enzymatically cross-linked particles remained stable for several days at pH 12 whereas the non-cross-linked particles dissolved after 4 days. All the approaches involving enzymes for the preparation of lignin nanoparticles are discussed in a recent review [92].

16.3 Conversion of biomass in Pickering emulsions

16.3.1 Pickering-assisted Catalysis and Pickering interfacial Catalysis

The use of colloidal particles as stabilizers provides emulsions with original properties compared to conventional emulsions, microemulsions and micellar systems stabilized by surfactants [6,93-95]. Their application to chemical, biochemical and hybrid catalysis is therefore of particular interest. As mentioned in the introduction, though known for more than a century, these micro-dispersed systems have emerged this last decade as platforms for catalysis resulting in a new concept called “Pickering Interfacial Catalysis” (PIC) which constitutes a very promising field of investigation for biphasic catalytic reactions [96]. Such liquid-liquid-solid microreactors constitute an important avenue of innovation for the conversion of biomass by enabling the production of highly valued chemicals as well as biofuels. In addition, they exhibit improved stability as well as lower toxicity compared to systems stabilized by surfactants. Monodisperse emulsions with controlled size can be easily generated by taking advantage of the “limited coalescence” process as previously discussed. Finally, these micro-dispersed reaction media promote the catalyst recyclability and reuse. The catalyst can be readily separated from the water/oil biphasic system by centrifugation and filtration but though rather efficient, the implementation of this process on an industrial scale is not possible. This is not ideal for an industrial scaling-up as it is time and energy consuming. To circumvent this issue, stimuli-responsive Pickering emulsions have been developed. In such systems, the adsorption/desorption of the stabilizing particle at the interface

induced by a trigger leads to the stabilization/destabilization of the emulsion. Typical triggers are pH, temperature, CO_2 or electrochemical or magnetic responses.

Pickering-Assisted Catalysis (PAC)

Colloidal particles can stabilize water-in-oil or oil-in-water emulsions depending on their wettability by the two immiscible liquid phases resulting in triphasic L/S/L systems as reaction media. As for surfactant-stabilized systems, they allow increasing the interfacial area between hydrophilic and hydrophobic reagents at the mesoscale. Pickering-assisted catalysis (PAC) consists in combining a Pickering emulsifier with a homogeneous catalyst which can be located either in the dispersed or continuous phases (▶ Figure 16.7, left). The reactions proceed at competitive rates with facile phase separation by filtration, centrifugation or volume phase transition temperature.

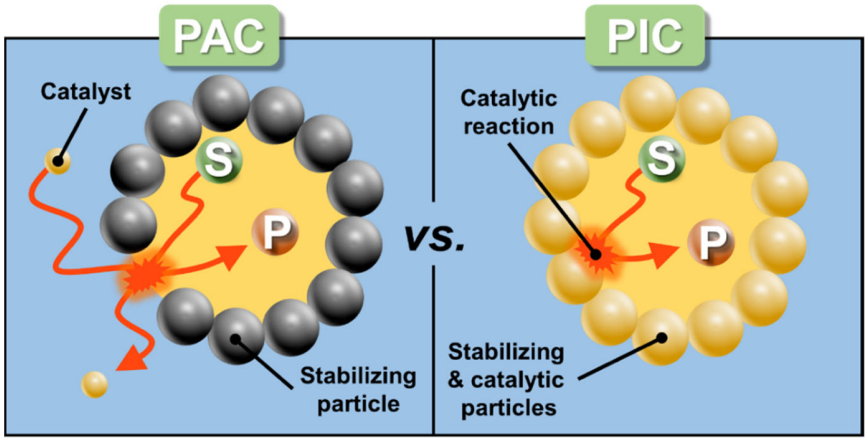

Figure 16.7: Oil-in-water Pickering catalytic emulsions operating transformation by Pickering-assisted (on the left) or Pickering interfacial (on the right) catalysis (PAC or PIC, respectively, S = substrate and P = product).

As described above, cyclodextrins (CDs) can be used as stabilizers of emulsions leading to efficient reaction media. Indeed, oil and CD form an insoluble inclusion complex which adsorb at the water/oil interface owing to its partial wettability by both water and oil. In 2013, Leclercq *et al.* reported some prospective catalytic applications of such emulsions for the oxidation of alkenes, alcohols and organosulfides using the water-soluble catalytic polyoxometalate $[Na]_3[PW_{12}O_{40}]$ and H_2O_2 as the oxidant [14]. The same year, Potier *et al.* published the Rh-catalyzed hydroformylation of higher alkenes in Pickering emulsions based on a mixture of native α-CDs and high-molecular-

weight polyethylene glycol (PEG) [20]. In this case, the formation of a hydrogel constituted of α-CD/PEG nanocrystallites stabilize oil-in-water Pickering emulsions increasing the contact between the organic substrate and the water-soluble catalyst.

Pickering emulsions are also efficient platforms for biocatalysis. Indeed, enzymes can display, high chemo-, regio- and stereo-selectivities under mild conditions providing relevant alternatives to traditional chemical catalysts. Furthermore, their encapsulation in Pickering emulsions can improve their catalytic activity. In such systems, the enzyme behaves as a homogeneous catalyst inside the emulsion droplets stabilized by nanoparticles. As an example, Wei *et al.* have reported a water-in-oil Pickering emulsion to carry out enzymatic hydrolysis kinetic resolution of racemic esters [97]. The strength of their system lies in the fact that it requires neither stirring nor immobilization of the enzyme and thus allows a larger number of effective recycling of the enzyme compared to classical biphasic systems which requires strong stirring. This is accounted for by the large reaction interfacial area and the shorter molecule distances in the Pickering emulsion. Another example among many others is that recently reported by Yu *et al.* who describes the hydrolysis of olive oil and the esterification of octanol with oleic acid in a CO_2/N_2-switchable Pickering oil-in-water emulsion stabilized by silica nanoparticles hydrophobized *in situ* by a CO_2/N_2-switchable surfactant (*N*,*N*-dimethyldodecylamine). Once again, the Pickering emulsion displays a higher reaction efficiency compared to biphasic systems [98].

Pickering Interfacial Catalysis (PIC)

In the Pickering interfacial catalysis (▶ Figure 16.7, right), the emulsions are generated by adsorbing at the water/oil interface particles that combine both amphiphilic and catalytic properties. As a consequence, they favor the reaction at the water/oil interface through a greatly increased contact area, the acceleration and the selectivity of the reaction. In other words, they combine the advantages of both the homogeneous and heterogeneous catalyses. The first example of such systems has been reported in 2010 [99]. In this study, the authors described the preparation of single-walled carbon nanotubes-silica hybrid nanoparticles supporting Pd nanoparticles which both stabilize water-in-oil emulsions and catalyze the hydrodeoxygenation and condensation of different substrates of interest in biomass refining.

Pickering stabilizers can be made up of several particles, assembled together at the interface in order to obtain the required emulsifying and catalytic properties. Thus, by using the colloidal

tectonic approach, the mixing of dodecyltrimethylammonium phosphotungstate $[C_{12}]_3[PW_{12}O_{40}]$ and silica functionalized with alkyl and sulfonic acid groups, $[C_n/SO_3H]@SiO_2$, provides stable emulsions which are efficient for the oxidative cleavage of olefins. The interfacial self-assembly of the particles is driven by synergistic interactions resulting from the partial penetration of the alkyl chains of $[C_n/SO_3H]@SiO_2$ into the $[C_{12}]_3[PW_{12}O_{40}]$ supramolecular porous structure constituted of polar and apolar regions (▶ Figure 16.8) [100].

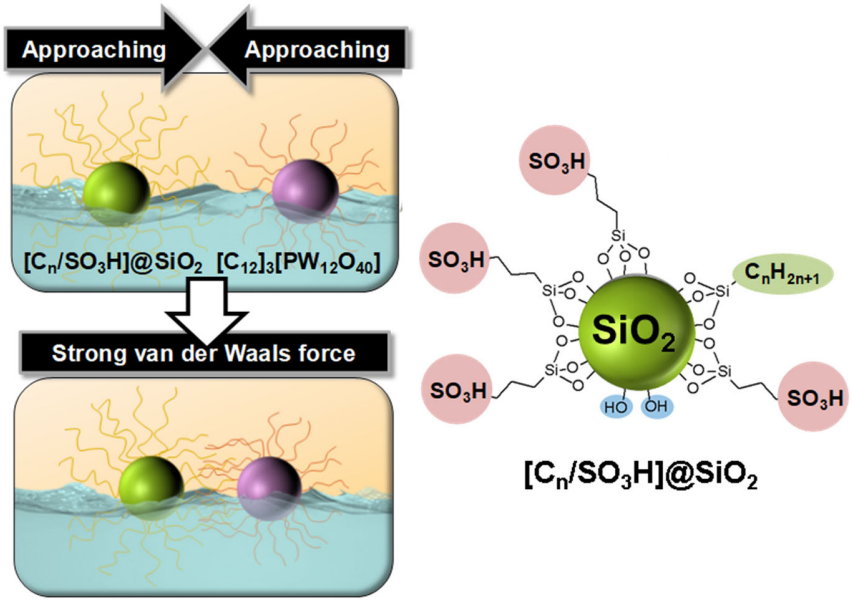

Figure 16.8: Interfacial synergistic interactions between $[C_n/SO_3H]@SiO_2$ and $[C_{12}]_3[PW_{12}O_{40}]$.

Composite materials such as zeolites and macroporous polymers are often encountered in Pickering interfacial catalysis reactions. In 2016, Zhang *et al.* described the use of macroporous hybrid polymers for the conversion of cellulose to 5-hydroxymethylfurfural (HMF). The direct transformation of cellulose to HMF involves several steps: hydrolysis, isomerization and dehydration [101]. Among the different ways of converting cellulose to HMF, chemical transformations *via* catalysis are the most attractive methods in chemical processes. In this work, oleic acid modified zirconium dioxide particles were used as stabilizer to form a high internal phase water-in-oil Pickering emulsion. The synthesis of macroporous carbon solid catalyst is carried out in 3 steps: (*i*) a macroporous hybrid polymer is generated from styrene and divinylbenzene monomers, used as a crosslinking agent, zirconium dioxide modified with oleic acid as a stabilizer, and azodiisobutyronitrile in benzene as a co-stabilizer, (*ii*) the resulting composite is calcined and then treated with sulfuric acid, and (*iii*) functional groups are introduced by a sulfonation process.

Nanohybrid compounds such as functionalized carbon nanotubes are also used for PIC, in particular for oxidation, reduction and hydrogenation reactions. Amphiphilic carbonaceous microspheres-supported Pd catalysts have been prepared and investigated for the hydrodeoxygenation of vanillin. The authors showed that the wettability of the support played a key role in forming Pickering emulsions and that the selectivity of the hydrodeoxygenation reactions was determined by the type of the Pickering emulsion [102]. In another study, carbon nanotubes-supported Ni catalysts were used for the hydrogenation of furfural as a biomass-derived furanic model compound. The reaction takes place at the liquid-liquid interface of a Pickering emulsion [103]. The Ni/CNTox catalyst can convert furfural into cyclopentanone with a highest furfural conversion of 35% and a cyclopentanone yield equal to 25% at 200 °C, 2 MPa of H_2 pressure after 1 h reaction in a water/dodecane Pickering emulsion. Note that the use of these materials in industry is mainly limited to the hydrogenation of vanillin and coupled C-C reactions such as aldocondensation and alkylation reactions.

16.3.2 Conversion of glycerol

The use of glycerol ethers as nonionic surfactants is well known and could represent an alternative to surfactants derived from ethylene oxide, criticized because of their dangerousness for humans and the environment. Currently, the production of these compounds mainly relies on the use of epichlorohydrin, of which the price and danger for the environment is problematic. The development of glycerol ethers through pathways using glycerol is thus of peculiar interest from an environmental and economic point of view. Today, glycerol comes from the fats industry and in particular from the synthesis of biodiesel. Indeed, glycerol is a by-product of the synthesis of methyl esters from vegetable oil (methanolysis of triglycerides). The emergence of biodiesels has dramatically increased the production of glycerol since one ton of biodiesel produces 100 kg of glycerol.

One of the pioneering studies which succeeded in the etherification of glycerol has been published in 2011 by Gaudin *et al.* [104]. In this work, the authors studied new catalytic pathways for the etherification of glycerol, in particular in the presence of aliphatic alcohols. The coupling of fatty alcohols with glycerol is complex and requires taking into account several aspects. The difference in polarity between glycerol and fatty alcohols provides a two-phase reaction medium. Therefore, the catalyst must be active at the glycerol/fatty alcohol interface in order to overcome

the limitations of mass transfer. On the other hand, the formation of glycerol ethers is a thermodynamically unfavorable reaction. In addition, the increased reactivity of glycerol, due to the presence of a hydroxyl group, under acidic conditions can lead to the formation of many undesirable products. In 2014, Fan *et al.* have determined the parameters governing the stability and the catalytic activity of Pickering emulsions based on glycerol and dodecanol in the presence of various modified silica nanoparticles such as SiO_2-C_3 particles, consisting of 18% of acidic propylsulfonic groups and 82% of propyl groups and SiO_2-C_{18} particles, consisting of 16% of propylsulfonic groups and 84% of octadecyl groups [105]. The SiO_2-C_{18} silica particles exhibit a greater hydrophobic character than the SiO_2-C_3 silica particles. The catalytic activity of these nanoparticles has been studied and compared to the performance of paratoluenesulfonic acid (PTSA), a conventional catalyst. A mixture of SiO_2-C_3/PTSA particles was also tested to assess the synergistic effect of these two catalysts. The turnover number (TON), representing the maximum number of substrate molecules transformed per second and per molecule, was calculated for the conversion of dodecanol and glycerol in the presence of the different catalysts. An approximately 30 times higher value was obtained for SiO_2-C_3 compared to the homogeneous PTSA catalyst or the SiO_2-C_3/PTSA mixture. The improved catalytic activity of SiO_2-C_3 is explained by an improved accessibility of the acid sites on the surface of the particles compared to those of SiO_2-C_{18}. The higher hydrophilicity of the SiO_2-C_3 nanoparticles can also explain the increased catalytic activity since the acid groups on the surface of these nanoparticles will preferably be located inside the glycerol droplets. The authors also show that the formation of glycerol/dodecanol emulsions which are stable under reaction conditions (150 °C) is an important parameter for the efficient conversion of glycerol. This work was completed a few years later by a study aiming at understanding the enhanced catalytic activity at the glycerol/dodecanol interface in the Pickering emulsion [106]. By combining dissipative particle dynamics simulations and emulsification experiments, the authors could determine the optimal surface properties of the silica particles in terms of length and density of alkyl chains. In addition, they highlighted an enhanced nanomixing between glycerol and dodecanol near the catalytic acid centers, thus favoring the reaction. It is noteworthy that double emulsions can also be obtained by using polystyrene-grafted silica nanoparticles bearing sulfonic acid centers [107]. Finally, a more recent meso–microscale computational study of the glycerol/dodecanol Pickering emulsions stabilized by sulfonated polystyrene-grafted silica nanoparticles pointed out different emulsification regimes depending on

the length of polystyrene brushes as well as on the surface density, sulfonation degree and distribution of sulfonic acid groups in the brushes [108].

16.3.3 Biodiesel production

Among the various routes for upgrading biomass, the production of biofuel is one of interest since these products can be used as an alternative to fossil fuels, such as petroleum, in engines. Indeed, it can be blended with conventional diesel fuel to reduce the quantity. It has gained increasing attention in recent years because of its environmental benefits as a renewable source of energy. There are two types of biofuels: (*i*) bioethanol, obtained from sugars contained in plants such as beets and sugar cane, and from starch, present in corn, potatoes or wheat. The first step is to ferment the sugars, then, the "juice" from this fermentation is then distilled to provide the alcohol. By dehydration, the pure ethanol obtained is then incorporated into the gasoline; (*ii*) biodiesel, produced by the reaction between vegetable oils and alcohol. The feedstock is mainly issued from plants rich in oil, such as rapeseed and sunflower flowers, or soybeans. The seeds are pressed to extract the oil which is then refined for use. Switching from crude vegetable oils to biodiesel requires several steps. In the last stages of the process, the oil is refined in a purification step before chemical treatment, usually a transesterification reaction with short-chain alcohols in the presence of a catalyst. This step produces alkyl esters, which are used for the production of biodiesel, as well as glycerol as the major by-product.

Due to their low cost and good catalytic activity, homogeneous alkaline catalysts such as potassium or sodium hydroxides are often used for the transesterification reaction but the processes display many drawbacks. The catalysts dissolved in the reagents are difficult to recycle and produce large amounts of wastewater upon treatment. The environmental issues of these catalysts therefore force manufacturers to turn to more environmentally friendly processes. In this context, Pickering emulsions have emerged as a relevant alternative (▶ Figure 16.9). Research on solid catalysts for transesterification reactions for the production of biodiesel is quite recent. Different types of particles have been examined such as mesoporous organosilica particles containing lipases [109] amphiphilic silica particles grafted with alkyl chains and propylsulfonic acid groups [110], as well as silica particles functionalized with guanidine groups [111,112].

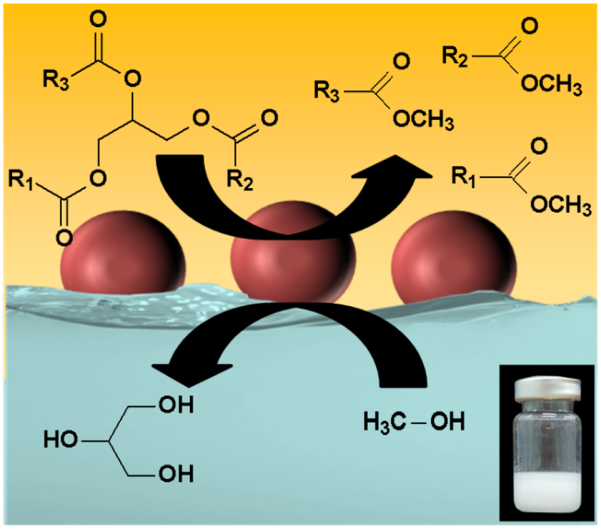

Fig 16.9: Transesterification of triglyceride with methanol in a Pickering emulsion stabilized by catalytic nanoparticle.

Indeed, a lipase-containing periodic mesoporous organosilica has been developed as a biocatalyst for biodiesel production in Pickering emulsion. A maximum biodiesel yield was obtained for the esterification of oleic acid with ethanol reaching a yield of 95.8% while the synthesis of biodiesel from *Jatropha curcas* oil could reach 87.1% *versus* 73.0% after 10 cycles [109]. In another work, Mangas-Sanchez and Adlercreutz converted triolein into ethyl esters using *Thermomyces lanuginosus* lipase as the catalyst in the presence of silica nanoparticles which favoured faster mass transfer due to the formation of smaller and monodisperse emulsion droplets [113]. A yield of 96% could be obtained in 5 h for the ethanolysis of rapeseed oil.

In 2017, Yang *et al.* reported the use of the PIC concept for the biodiesel production in methanol/triglyceride Pickering emulsions stabilized with silica nanoparticles on which alkyl chains (C_3, C_8 and C_{18}) and propylsulfonic acid residues were grafted [110]. After optimization of the ratio between the inert alkyl chain and the active surface functional groups, excellent catalytic could be obtained with C_{18}-SiO_2-SO_3H (C_{18} : SO_3H molar ratio = 54 : 46 with a degree of grafting of 48%) since more than 92% conversion of triglycerides was reached at 90 °C after 12 h.

In 2018, Tang and coworkers explored the effectiveness of Pickering magnetic interfacial catalysis for the transesterification reaction of soybean oil [111]. Fe_3O_4 silica nanospheres (PS) were functionalized with chloromethyl groups. On these supports, tetramethylguanidine (TMG) groups were added. Soybean oil and methanol can adsorb and enrich themselves on the surface of

Fe_3O_4 @PS-TMG particles. Compared to a conventional two-phase reaction with homogeneous catalysts, solid catalysts are more efficient at speeding up reactions. This is accounted for by the fact that Pickering emulsions can be likened to uniformly dispersed microreactors, with a larger interfacial area and smaller distances between molecules, thus promoting reactions. More recently, surface-modified SiO_2 nanoparticles were prepared by combining a guanidine group (1,1,3,3-tetramethylguanidine [TMG]) as the base catalytic functionality and *n*-alkyl chains as the hydrophobic functionalities (C_4, C_8, C_{12} and C_{16}) [112]. They were shown to stabilize soybean oil-in-methanol Pickering emulsions and were used as interfacial catalysts in the transesterification reaction for biodiesel production. The C_8-SiO_2-TMG catalyst provided the highest conversion of 66.7% at a catalyst concentration of 7 wt % after 5 h at 70 °C.

16.4 Bio-based Pickering emulsions as delivery systems

16.4.1 General point of view

Numerous possible applications of delivery systems based on Pickering emulsions has emerged over the last decades due to their very attractive properties compared to conventional emulsions (including their excellent stability, sometimes up to several years, and the possibility to use biopolymers as stabilizers). As emulsions are widely used in pharmaceutical, cosmetic and food applications (creams, as well as some gels, ointments, pastes or vaccines), they hold great promise for encapsulated active ingredients while increasing its solubility and/or bioavailability. Depending on the solubility of the active ingredients the use of simple emulsions can be very useful: hydrophilic molecules are encapsulated in the aqueous droplets of a water-in-oil emulsion whereas hydrophobic ones are incorporated in the oil droplets of an oil-in-water emulsion. According to their applications, water-in-water or oil-in-oil Pickering emulsions as well as multiple water-in-oil-in-water or oil-in-water-in-oil or oil-in-oil-in-oil emulsions can also be prepared to obtain multiple encapsulation, protection, controlled and sustained release. Consequently, all these systems are particularly attractive as they exhibit a simple preparation process, with sometimes with the possibility to be prepared in a single step, and a long-term stability that are challenging to obtain when using surfactants.

On the other hand, the possibility to obtain stimuli-responsive emulsions using particles sensitive to pH, ionic strength or temperature is also very promising. Indeed, an emulsion disruption with extrinsic stimuli can induce: (*i*) enhanced stability during storage if the emulsion is

only destabilized with an external stimulus which can be controlled during storage, and (*ii*) a targeted and controlled release of the active molecule during its use. As the use of inorganic particles in Pickering emulsions can be seen as an issue since possible health concerns, bio-sourced emulsions obtained from biodegradable and biocompatible particles (including cellulose, chitosan, chitin, starch, *etc*.) and oils appear particularly attractive. If the key parameters to obtain particles able to stabilize Pickering emulsions have already been previously described, the specification of the delivery systems based on Pickering emulsions depends on the applications (*i.e.* the routes of administration for pharmaceutical emulsions: injection, oral administration, topical application, *etc.*). For instance, the droplet size should usually be smaller than 5 µm for the injection route.

From a general point of view, an active ingredient encapsulated in the droplets of Pickering emulsions exhibits the same advantages than classical emulsions stabilized by synthetic surfactants: protection, solubility and bioavailability increase, taste and/or texture modification [114]. In addition, Pickering emulsions could help to reduce the toxicity of synthetic surfactants with a very good physical stability. In addition, various observations are made for delivery systems based on Pickering emulsions compared to conventional ones which are said to improve: (*i*) the protection of the encapsulated molecule due to the solid barrier of particles around the droplets (in particular from oral and gastric digestion), (*ii*) the skin absorption and accumulation of the active molecule, and (*iii*) the global efficacy and bioaccessibility. It is noteworthy that the active molecule can also be encapsulated within the particles or grafted onto their surface [115]. However, active molecules can be used directly as stabilizing particles [116].

In this section, typical references taken from the literature are used to illustrate the advantages of bio-based Pickering emulsions as delivery systems in foods and pharmaceuticals. However, it is worth mentioning that the separation of the advantages in given sections is purely fictive. Indeed, the combination of benefits is often reported.

16.4.2 Main advantages

Water-solubility and stability of active ingredients

As commonly active ingredients have a low water-solubility, the use of Pickering emulsions can be seen as a very attractive tool to modify this physicochemical property. Moreover, the Pickering emulsions can also be used to improve their shelf-life (*e.g.* stability to heat, light, oxygen, *etc*.). Numerous examples are available in the literature for food applications with biocompatible oils.

For sake of clarity, only three of them are presented here. Tzoumaki and coworkers used vegetable oil-in-water Pickering emulsions stabilized by chitin nanocrystals (sunflower and corn oils) [50]. Interestingly, during *in vitro* enzymatic protocol, these Pickering emulsions slowed down the lipid digestion. Based on these results, the authors proposed to use these systems to treat obesity by reducing caloric intake and promoting satiety. In 2015, Xiao *et al.* used Pickering emulsions stabilized by kafirin nanoparticles to encapsulate curcumin (used as herbal supplement, cosmetics ingredient, food flavoring and food coloring). In this system, the authors reported that the curcumin as well as the oil molecules were protected against photo-oxidation and lipid oxidation, respectively [117]. In 2019, Dai and coworkers solved the problems of light-unstability and low-water solubility of *trans*-resveratrol by the formation of Pickering emulsions stabilized by functionalized lignin-based nanoparticles [118]. The nanoparticles were based on the self-assembly of a thermo-responsive lignin copolymer obtained by grafting poly(*N*-isopropylacrylamide) onto industrial waste lignin *via* atom transfer radical polymerization. The nanoparticles are able to stabilize palm oil-in-water emulsions which can loaded with *trans*-resveratrol (used as dietary supplement and studied for its potential therapeutic use). The light stability of *trans*-resveratrol was improved by the protecting role of the nanoparticles layer stabilizing the droplets. This protection occurs thanks to the chromophoric groups of lignin. In addition, the emulsion properties and release behavior are strongly influenced by the temperature as well as the nanoparticles size. Indeed, the temperature decrease induces the deformation of the nanoparticles at the interface leading to an increase in droplet size and to a fast release of *trans*-resveratrol.

Bioavailability, bioaccessibility and controlled-release

As mentioned above, the improvement of the water-solubility increases the bioavailability (amount of a compound reaching the systemic circulation) or bioaccessibility (amount of a compound that is released in the gastrointestinal tract) of oil-soluble bioactive compounds. In addition, Pickering emulsions can also be useful to obtain slow and controlled-release. As an example, Cossu *et al.* showed the potential of starch-based extra virgin oil-in-water Pickering emulsions, alone or incorporated in an alginate film, to treat the fungal infections of the surface of the gastrointestinal tract such as candidiasis [119]. Two antifungals were used: thymol, and amphotericin B. It is noteworthy that the amphotericin B existed in the form of an oral preparation but is not widely available due to an increased cytotoxicity in the oral cavity (replaced by other antifungals such as miconazole). Indeed, the amphipathic nature of amphotericin along with its low solubility and

permeability has posed major hurdles for oral administration given its low bioavailability. Therefore, their encapsulation and release in a controlled manner from the Pickering emulsion during *in vitro* digestion with α-amylase as well as the comparison between the antifungal activity against *Candida albicans* of encapsulated thymol or amphotericin B is very interesting. The results showed that the emulsions were stable even after storage for 3 weeks whereas upon the digestion of the emulsion by α-amylase led to rapid coalescence of emulsion droplets and phase separation. Additionally, the antifungal activity of encapsulated thymol or amphotericin B was enhanced upon incubation with α-amylase. Finally, the authors highlighted that the emulsions dispersed in alginate films are efficient to inhibit *C. albicans* and that the addition of α-amylase to the alginate films resulted in a decreased inhibitory effect. In 2016, Shah *et al.* encapsulated curcumin in the oil droplets of Pickering emulsions stabilized by chitosan nanoparticles crosslinked with tripolyphosphate (prepared by the ionic gelation technique) [120]. The results showed that Pickering emulsions offered better protection of curcumin against degradation during storage and slower release rate compared to classical emulsions. Additionally, a sustained release as a function of pH was also obtained with the loaded Pickering emulsions. Indeed, 40% of curcumin was released at pH 7.4 whereas about 55 % were released at pH 2 (gastric environment) after 24 h. In 2017, Tan and coworkers encapsulated β-carotene (used as food coloring) in the oil droplets (sunflower or medium-chain triacylglycerol oils) of Pickering high-internal phase emulsions stabilized by gelatin particles [121]. The emulsion droplets from a few to tens of micrometers were able to stabilize the β-carotene more strongly than in dispersion in bulk oil, even after storage for 27 days. Additionally, the release of β-carotene during *in vitro* digestion of the Pickering emulsion showed that its bio-accessibility was improved by a factor of 5 compared to the β-carotene solubilized in oil. In 2019, Marto and coworkers performed *in vitro* and *in vivo* (with mice) experiments with aluminum starch octenylsuccinate-based emulsions loaded with minocycline hydrochloride [122]. It is noteworthy that minocycline hydrochloride, a tetracycline antibiotic used to treat a number of superficial bacterial infections, is particularly indicated for the topical treatment of inflammatory lesions of non-nodular moderate to severe acne vulgaris. The water-in-oil emulsions (oil: liquid paraffin or caprylic/capric acid triglyceride) provided a sustained release of the minocycline hydrochloride. Indeed, the drug did not permeate through the entire skin layer probably due to its hydrophobicity and charge, leading to an appropriate accumulation of the drug in the *stratum corneum* promising for the efficient topical treatment of superficial skin infections.

Eco- and bio-toxicity

As mentioned earlier, classical emulsions, based on molecular surfactants, can cause irritations or adverse allergic responses such as hemolysis, protein denaturation, contact urticarial, pruritus, irritation, pain, burning, itching, erythema, *etc*. [123]. These allergies to surfactants even if they are rare events should be considered and can be seen as an aggravating factor when they are in combination with various drugs. In addition, the use and synthesis of surfactants is generally harmful to the environment and the human health. For instance, ethoxylated surfactants, produced by reacting various alcohols (natural or synthetic) with ethylene oxide, allows the production of various detergents with a wide range of molar ratios of ethylene oxide. Even if ethylene oxide can be obtained from bioethanol *via* bioethylene, its handling remains hazardous because it is a carcinogenic, mutagenic, irritating flammable and anaesthetic gas. In addition, the partial biodegradability of ethoxylated surfactants can also be problematic. Consequently, a smart solution to replace the molecular surfactants is to use Pickering emulsions stabilized by modified colloidal silica nanoparticles. Unfortunately, they present some risks human health even if inconsistent results were obtained [124]. However, in the context of green pharmacy, consisting in the design of products and processes that address answer to economic, environmental and social issues along the whole lifecycle of medications, Pickering emulsions stabilized by biocompatible and bio-sourced particles can be very useful to replace the synthetic or semisynthetic molecular surfactants.

Pickering emulsions can directly be stabilized by drug particles. In respect with this, Aditya and coworkers stabilized sunflower oil-in-water Pickering emulsions with curcumin particles (about 220 nm) [116]. These amorphous curcumin nanoparticles, obtained by anti-solvent precipitation technique and nanoization, were able to stabilize emulsions with small droplets (close to 1 μm). For their part, Yi and coworkers stabilized glyceryl monocaprylate oil-in-water emulsion using silibinin nanocrystals (a flavonoid used in a number of pharmacological effects, particularly in the two type of hepatic steatosis: non-alcoholic fatty liver disease, NAFLD, and alcoholic liver disease steatohepatitis, NASH) [125]. As silibinin has a very limited aqueous solubility associated with a poor oral bioavailability, emulsions are a very suitable platform to deliver it. The flavonoid particles of about 300 nm were obtained from high-pressure homogenization treatment. The emulsion droplet (27 μm) showed high stability over 40 days. The *in vitro* release, *in vivo* oral bioavailability of this emulsion were investigated in rats. As expected, the authors observed a faster *in vitro* dissolution and released of the silibinin from the emulsion than from the control (*i.e.* the

nanocrystal suspension). This observation is directly related to the partial dissolution of silibinin in the oil phase of the emulsion. In addition, the blood concentration of silibinin increased with the Pickering emulsion compared to the control experiment. These emulsions directly stabilized by active particles are undoubtedly promising for pharmaceutical applications as they could enhance the bioavailability of a poorly soluble active molecules especially in the context of green and sustainable pharmacy. Unfortunately, the generalization of this concept to many other drugs remains to be overcome before potential industrial applications.

A more attractive concept based on the colloidal tectonics approach, allowing the construction of colloidal systems from molecular tectons, has emerged in the last decade (see above). Indeed, as biocompatible CDs are able to emulsify oil (paraffin and isopropyl myristate oils) and water mixtures due to the formation of nanoparticle-like structures made of aggregated insoluble oil/CD inclusion complexes [15]. Unlike common surfactants or silica nanoparticles, these self-aggregated systems can easily be dissociated without harmful side effects. In respect with this, the formulation of Pickering emulsions stabilized by self-assembled nanoparticles can be used to encapsulate antifungal medication of the azole class in the oil droplets of these emulsions. Their antifungal and antimicrobial activities against *Candida albicans* and *Staphylococcus aureus* were evaluated *in vitro*, showing an efficiency at least as important as a surfactant-based commercial product (Pevaryl®) form but without the risk associated with the synthetic or semisynthetic surfactants. Unfortunately, despite their high stability, the physicochemical properties as well as the biocidal activity of these emulsions have been shown to be influenced by the size of the CD. For instance, the γ-CD-stabilized emulsions by have no or very weak antimicrobial properties due to the encapsulation of azole antifungal drug inside the γ-CD. To minimize the antifungal/CD interactions, the use of polypseudorotaxane-stabilized emulsions using a pre-assembled system built on low toxicity substances such as α-CD and PEG (guest) was recently studied [21]. As mentioned above, in aqueous solution, the PEG/CD crystallites act as physical cross-links leading to the formation of a hydrogel and the Pickering emulsions can be easily obtained from this hydrogel with the introduction of an oil phase (*e.g.* liquid paraffin). These emulsions can be used as surfactant-free systems to encapsulate azole-based antifungal drugs (*e.g.* miconazole and econazole). The antifungal and antimicrobial effects of such Pickering emulsions were evaluated *in vitro*, showing an activity at least comparable of two surfactant-based commercial references (Micatin® and Pevaryl®). Finally, the authors mentioned that Pickering emulsion droplets are

suitable templates to obtain microcapsules (cyclodextrinosomes) with potential applications in drug delivery.

Synergistic effect

The versatility of the colloidal tectonics concept can be employed to achieve green pharmaceutics but also synergism in terms of antimicrobial activity. Indeed, a boosted antimicrobial could then be highly helpful for clinical purposes (faster and larger broad-spectrum eradication, shorter treatment time and a reduction of the dose-related toxicity). For instance, self-assembled Pickering emulsions containing biocidal phytochemical oils (*e.g.* carvacrol and terpinen-4-ol) and β-CD were able to potentiate the antimicrobial and antibiofilm activity of miconazoctylium bromide (a potential new drug obtained from the *N*-alkylation of miconazole) [19]. However, it is noteworthy that carvacrol is approved by the Food and Drug Administration and the Council of Europe as a food additive whereas terpinen-4-ol can only be used externally to avoid allergic adverse reactions. The authors reported that the carvacrol/miconazoctylium bromide emulsion was two-fold more sensitive against *Candida albicans* and methicillin-resistant *Staphylococcus aureus* and highly efficient against *Escherichia coli*, compared to a commercial reference containing miconazole nitrate (Monistat Derm^{TM}). Moreover, this emulsion provided a synergism against *C. albicans* (30% more efficient than the additive effect) but only additive responses are obtained against *S. aureus* and *E. coli*. These effects were associated with a remarkable staphylococcal biofilm activity. These results were ascribed to the following cumulative damages in the microorganisms: membrane permeabilization, enzymes inhibition and accumulation of reactive oxygen species. These Pickering emulsions can be probably useful for clinical applications due to their broad-spectrum and fast action against bacteria and fungi, resistant strains and biofilms.

Skin absorption

Using oil-in-water Pickering emulsions stabilized by starch particles, Marku *et al.* evaluated their possible use as vehicles for topical drug delivery [126]. All these emulsions were highly stable against coalescence, even after storage for 8 weeks. A sensory analysis was performed on the uncharged Pickering emulsion containing 214 mg/ml of starch and 56% oil (miglyol, paraffin and sheanut oils). The following attributes have been evaluated: visual appearance of the formulation, feel of the cream (thick, sticky, slippery and watery), skin feel during application (spreadability, permeability) and skin feel/appearance after absorption (glossy, residues). All panelists' scores

reveal that emulsions are found to give acceptable appearance, tactile feel and texture even if the sheanut oil to be thicker and stiffer, requiring more force during spreading. On the other hand, the *in vitro* skin penetration of methyl salicylate (used as flavoring agent, fragrance and rubefacient and analgesic), encapsulated in these emulsions, were nearly twice higher drug penetration rate with Pickering emulsions than with the control (*i.e.* the methyl salicylate solution). This observed greater accumulation of the drug in the *stratum corneum* is promising for topical drug delivery.

Dried Pickering emulsions

Pickering emulsions can be used to form colloidosomes which are microcapsules whose shells are composed of colloidal particles (*i.e.* dried Pickering emulsions). These structures are produced through the particle assembly into on the droplets surface which retain their stability after the removal of the solvents (*i.e.* water and oil). These microcapsules could find applications in pharmaceutical formulations as microencapsulation and drug delivery vehicles. As mentioned above, CD-stabilized oil-in-water emulsions can be used as a template for preparation of colloidosomes [17]. However, cyclodextrinosomes can be easily obtained by vacuum treatment to obtain fully dried colloidosomes or by air-drying to avoid the removal of the oil core [21]. Indeed, in some cases, the stabilizing particles are able to maintain the integrity of the droplets after removal of the external phase only. For instance, Marefati and coworkers investigated the possibility to produce novel powder materials based on chemically modified starch granule stabilized Pickering oil-in-water emulsions [127]. The effect of partial starch gelatinization, oil phase type, freezing method and thawing, and freeze-drying and rehydrating were also studied on the properties of the emulsions. The authors demonstrated the feasibility of the production of oil based powders, through combination of heat treated or even non-heat treated starch Pickering emulsions and freeze-drying. The freeze-drying of oil-in-water Pickering emulsions stabilized with starch granules resulted in oil powder (oil concentration up to 80 wt.%). This oil powder can easily be rehydrated to reconstitute the Pickering emulsion. This technique can be a valuable strategy for Pickering emulsion storage especially when the degradation of the particles involves a hydrolysis mechanism.

Stimuli-sensitivity

As the possibility to obtain stimulable Pickering emulsions using responsive particles is very promising, we have selected some examples available in the literature. For instance, Zhang *et al.* reported the *in vitro* use of Pickering emulsions stabilized using pH-responsive hydrophobic

modified calcium alginate nanoparticles [128]. The nanoparticles were synthesized *via* gelation between calcium in emulsion and alginate sodium that reacted with diacetone acrylamide. The authors evaluated the *in vitro* released of curcumin with these pH-responsive Pickering emulsions. Interestingly, the emulsions released curcumin specifically in intestine (37% in 4 hours at pH 6.8). In contrast, the release in the gastric fluid is only of 3% in 4 hours at pH 1.5. In 2019, Sufi-Maragheh *et al.* reported the use of amphiphilic crosslinked starch nanoparticles to stabilize Pickering emulsions [129]. The nanoparticles were prepared through alkali-freezing method followed by crosslinking using citric acid leading to monodisperse objects of about 140 nm. The nanoparticles formed very stable sunflower oil-in-water emulsions. Interestingly, the physicochemical properties of emulsions varied as a function of pH (from 3 to 7.4). Indeed, the emulsion stability increased with pH whereas the droplets size decreased. Moreover, the surface coverage increased from 10.6 to 22.2% with increasing pH. This system appears to be a promising candidate for oral drug with controllable release as i*n vitro* controlled release have shown that the release of encapsulated curcumin increased with pH. The same year, Low and coworkers investigated Pickering emulsions stabilized by magnetic cellulose nanocrystals to improve the bioactive release in the human colon cancer therapy [130]. The authors used superparamagnetic Fe_3O_4/cellulose nanocrystals to stabilize palm olein-in-water Pickering emulsions containing curcumin. The authors observed that around 53% of the initial loading of the curcumin was released for the Pickering emulsions after exposure to an external magnetic field of 0.7 T over a 4-day period. The anticancer activity determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole used for the colorimetric determination of the number of viable cells, colored in purple, in the sample) showed that the curcumin-loaded emulsions inhibited the human colon cancer cells growth in the presence of external magnetic field (up to 18%). Moreover, the nanocrystals were found to be non-toxic to brine shrimp up to a concentration of 100 µg/mL. All these results suggested that the easy preparation of Pickering emulsions stabilized by biocompatible and bio-based responsive solid particles are promising drug carriers to treat various disorders *via* oral drug controllable release.

Bio-functionalized Janus particles for medical uses

As described in the literature, the particles whose surfaces have two or more distinct types of properties are named Janus particles in reference to the two faced Roman god Janus [131]. Their synthesis requires the ability to selectively create each side of a particle with different chemical

properties. Initially, this was a difficult task, but within the last 15 years, Pickering emulsions can be used in the synthesis of Janus particles. From a general point of view, one of the first techniques developed for the synthesis of Janus particles was the "masking" which involves the protection of one side of a particle followed by the modification of the unprotected side and the removal of the protection. Obviously, Pickering emulsions, stabilized by particles which adsorb onto the interface between the two phases, can be used for this purpose [132]. However, the particles can rotate at the interface leading to the chemical modification on more than one face [133]. However, the use of a "crystallizable" oil to fix the particles is very attractive. In the classical method used to produce Janus particles, molten paraffin wax was used as oil phase. In the presence of water, oil and particles, the homogenization allows the formation of Pickering emulsions. When the solution is cooled, the wax is solidified, the particles are frozen trapping half of each particle in the wax droplets, leaving the other half of the particle exposed. The water is then filtered in order to provide colloidosomes (microcapsules whose shells are composed of colloidal particles). Then the exposed surfaces of the immobilized particles are chemically modified with appropriate reagents. The particles are then filtered and the wax was dissolved with an appropriate solvent (chloroform), leaving the other particle surface for further chemical modification to obtain the desired Janus particles. In respect with this, Zhang and coworkers reported that azide-modified silica particles can be selectively modified *via* the formation of molten paraffin wax-in-water Pickering emulsions (▶Figure 16.10) [134].

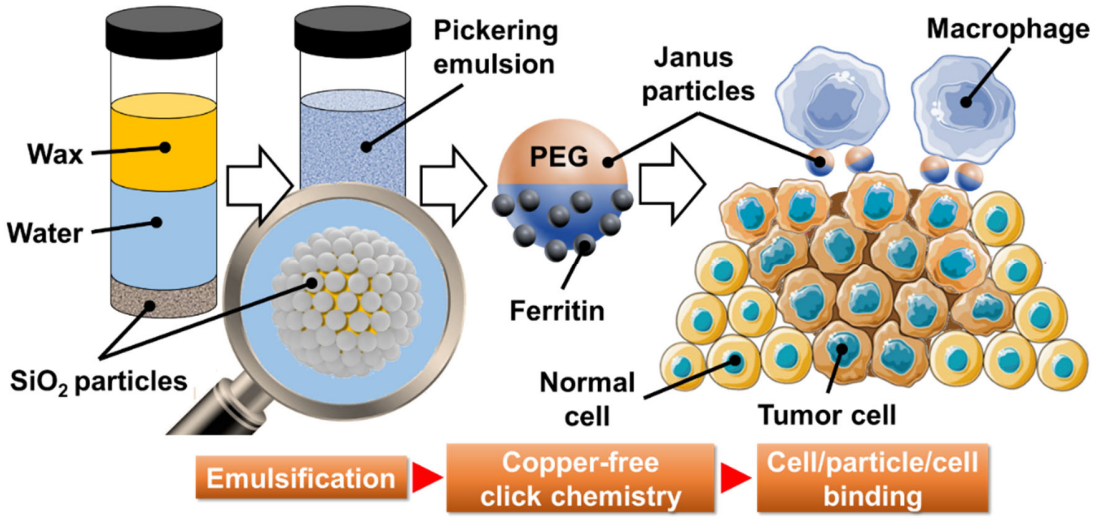

Figure 16.10: Proposed bio-functionalized Janus particles for phagocytosis of tumor cells by macrophages.

For medical uses, the authors used ferritin as well as a variety of biopolymers (bovine serum albumin, transferrin and anti-signal regulatory protein-α) to obtain bio-functionalized Janus particles by the use of a versatile strategy based on the combination of Pickering emulsion and copper-free click chemistry. Applying the general method (see above), azide-modified silica particles were functionalized with polyethylene glycol and ferritin in opposite faces. These PEG/SiO_2/ferritin nanoparticles can be used for cell/particle/cell binding leading to potential cancer immunotherapy (*i.e.* for the selective interactions to either macrophages or tumor cells in order to mediate more efficient phagocytosis of tumor cells by macrophages, ▶Figure 16.10).

16.4.3 Main challenges for industrial application

All these results showed that after optimization, bio-based Pickering emulsions can be obtained with uniform droplet size distribution and high stability against coalescence, pH, salts and temperature leading to high long-term storage stability. Consequently, these bio-sourced emulsions can be an effective route for delivery of bioactive compounds. However, no product based on Pickering emulsions is commercialized yet, despite numerous patents. Indeed, some obstacles to the Pickering emulsions industrialization remained to be overcome (▶Figure 16.11).

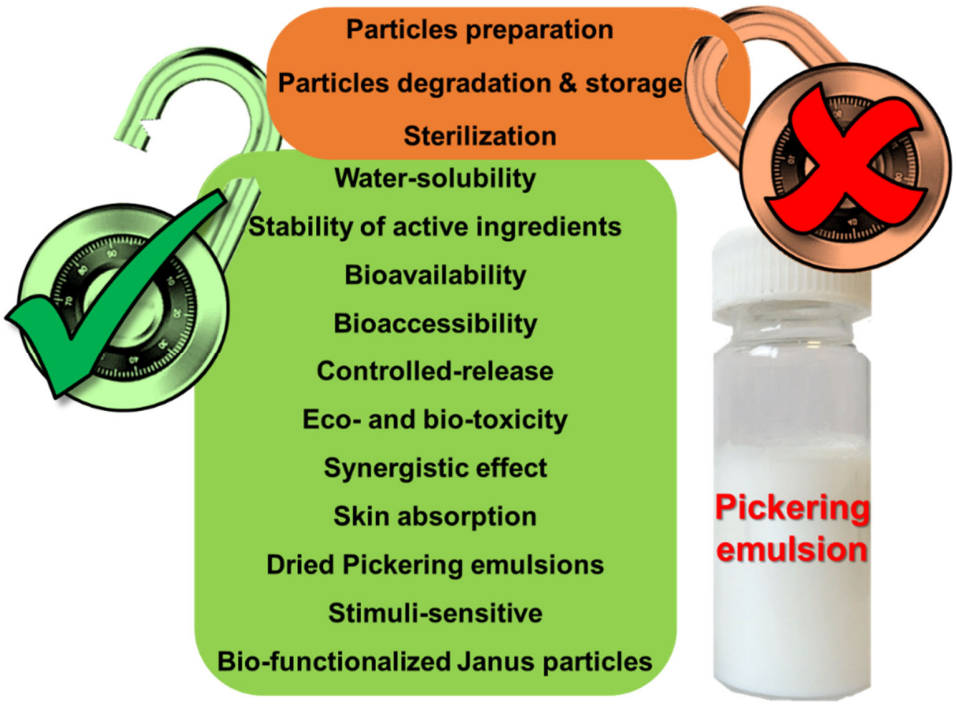

Figure 16.11: Main benefits and obstacles for the use of Pickering emulsions in food and pharmacy.

For instance, the preparation of bio-based particles on a large scale to ensure emulsions with reproducible properties is not obvious. The storage of Pickering emulsions stabilized with biodegradable particles are also difficult in the context of industrial applications. For instance, some biodegradable particles are sensitive to moisture (hydrolysis mechanism). Fortunately, the use of dried Pickering emulsions can be helpful to solve this issue. As they can easily be rehydrated to reconstitute the Pickering emulsion, these dried emulsions could be a valuable strategy for the storage of water-sensible Pickering emulsions (see below). The sterilization of the formulations is also problematic for Pickering emulsions. Obviously, the sterilization by filtration is difficult because the bio-based particles used to stabilize Pickering emulsions are sometimes smaller than the filtration pores. The sterilization by heating is also an issue for high temperature-sensitive particles (*e.g.* proteins). In respect with this, the use of sterilized components to produce Pickering emulsions seems to be appropriate, but the sterilization of particles could be difficult to achieve. Therefore, some work is still needed to solve these problems prior industrial applications.

16.5 Conclusions

Emulsions are widely studied for their numerous potential applications in many fields such as cosmetics, food, pharmaceuticals where emulsions and encapsulation of actives are important. A smart solution to replace the commonly molecular surfactants, which are usually released in the environment, is to use solid particles in order to form the so-called Pickering emulsions. The specificity of these alternative systems is that the particles are irreversibly anchored to the liquid/liquid interface, thus giving emulsions excellent stability. Inorganic particles are widely used to stabilize Pickering emulsions. Indeed, the most commonly studied particles are based on modified silica, clays, calcium carbonate, titanium dioxide or hydroxyapatite. As these particles can use synthetic steps with hazardous products and as the biocompatibility as well as the eco-toxicity and the biodegradability are currently major issues, the industrial applications of these systems remain very low, even if many systems have been patented. Fortunately, other particles, biodegradable, biocompatible and derived from biomass feedstocks, can be used to stabilize Pickering emulsions. These particles present an excellent opportunity to produce fully bio-based Pickering emulsions with the use of food-grade particles (natural proteins, polysaccharides, lipids, *etc*.) in combination with bio- and/or eco-compatible oils. Bio-based Pickering emulsions comes here to initiate a global chain more respectful of the environment. These eco-friendly systems can be applied in the pharmaceutical industry but also in the food, flavor and fragrance, cosmetic,

personal care, advanced materials industries, and even in the processes of the fine chemical industry. Indeed, the conversion of biomass to obtain high added value products is a considerable challenge since it can be used to produce heat by combustion, biogas through anaerobic digestion, as well as biofuels. Crude products require processing before they can be exploited. However, traditional processes present several drawbacks since they use toxic reagents and solvents, which are not very recyclable and reusable, and they produce a significant amount of waste. In a context where sustainable chemistry has become essential, Pickering interfacial catalysis (PIC) appears to be a particularly suitable solution because of its numerous advantages. In these systems, the particles combine both stabilizing and catalytic properties, promoting the reaction between hydrophilic and hydrophobic reagents at the water/oil interface thanks to a greatly increased contact area and favoring the acceleration and the selectivity of the reactions. In addition, the solid catalysts have many advantages over traditional catalysts since they can be recycled and reused over several cycles, thus reducing the generation of pollutants. In addition, they do not require additional separation and purification steps and the solid catalysts greatly reduces production costs. Therefore, the use of bio-based Pickering emulsions in the fine chemical and pharmaceutical industry processes as well as in the many applications mentioned above gives a strong impetus for researchers and is an invitation to creativity and ability to innovation in the face of environmental problems.

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