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Recent advances on water-in-water emulsions in segregative systems of two water-soluble polymers

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The present paper reviews the most recent knowledge on water-in-water (W/W) emulsions formed in aqueous two-phase systems based on incompatibility between two polymers. The interfaces of these systems are ill-defined, relatively thick, and interfacial tensions are extremely low. Consequently, small molecules do not adsorb in W/W interfaces and emulsions are inherently unstable and the main challenge is achieving a proper colloidal stability at long times. The most widely used strategy is the addition of particles and/or macromolecules able to adsorb at the W/W interfaces, but often the stability of these emulsions is still not satisfactory in the long term. More recently, stabilization of W/W emulsions has been improved by ionic complexation and/or autoaggregation, forming membranes at the interfaces. The proper colloidal stabilization of W/W is paving the way for novel applications, such as carriers of living cells or the development of new 3D cell cultures and cell organoids.

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Introduction

The term 'Water-in-water emulsions' (abbreviated as W/W emulsions) is commonly used to designate colloidal dispersions of one aqueous solution that forms droplets into another aqueous solution [1–3]. In segregative systems, the two liquid phases are in thermodynamic equilibrium because of the immiscibility between two hydrophilic solutes and each phase is enriched in one of the two components although saturated with the other component [4,5]. A good critical review on segregative phase separation has been published recently [5]. Aqueous biphasic systems are most often denoted as 'Aqueous Two-Phase Systems' (abbreviated as ATPS) [6]. Biphasic aqueous systems are of great importance due to their wide applications in separation, extraction, and purification processes of biomolecules and cells, using the preferential affinity toward one of the two aqueous phases. ATPS are used on an industrial scale for the purification of proteins and antibodies, as well as for the extraction of water-soluble molecules. Interesting examples are the isolation and purification of viruses through extraction and concentration processes in ATPS, to allow their detection in environmental samples [7,8]. Moreover, W/W interfaces offer interesting advantages, such as the high permeability to ions and small hydrophilic molecules, allowing an almost free delivery of active components as well as quick responses to osmotic pressure differences.

Segregative W/W emulsions have been known for a very long time, since first described by M.W. Beijerinck in the late XIX century [9]. ATPS focused great interest when Albertsson in 1959 discovered its potential for extraction and purification processes [10]. Phase segregation is a ubiquitous phenomenon that can occur in aqueous mixtures of polymer/polymer, polymer/salt, salt/salt, and many other combinations of different components, as summarized by Pereira and Coutinho [11,12]. W/W emulsions based on aqueous mixtures of two immiscible polymers are the most widely studied, due to their biocompatibility and mimicry of biological entities. W/W emulsions based on polymer/salt systems have only been sparsely studied [13], and to our knowledge, stable W/W emulsions based on aqueous mixtures of only two salts have still not been reported. However, there is no limitation that prevents the stabilization of these types of emulsions, which are not inherently different from emulsions based on immiscible polymer mixtures, and therefore, polymer/salt and salt/salt systems could be the focus of future studies. The present short review focuses on recent advances in W/W emulsions composed of two immiscible polymers. Microfluidic methods are also not described in detail in the present article, and readers who are interested in this topic can consult the excellent reviews published by Shum and collaborators [5,14].

The interfaces of W/W emulsions in segregative systems have extremely low interfacial tensions, often from 10^{-4} to 10^{-2} mN/m [15], which is between 3 and 5 orders of magnitude lower than in typical oil/water interfaces. The interfacial tension in W/W systems decreases to virtually zero at the critical point [16], where the compositions of the two phases become identical and the interface vanishes. In addition, the interfacial region of W/W systems is relatively wide, thicker than the characteristic length of the polymers. Therefore, small molecules do not adsorb on such 'fuzzy' interfaces since their adsorption is not energetically favored against thermal motion (≈kT).

Consequently, colloidal instability is often the main drawback of W/W emulsions in practical applications. The proper stabilization of these emulsions remains a challenge, and much scientific effort is currently devoted to find new methods to achieve long-term stability. These methods can be classified in two broad types: (i) addition of particles, microgels, or soft entities that are large enough to achieve a significant energy of adsorption; and (ii) self-assembly, ionic complexation, and/or precipitation at the interface to produce interfacial membranes. Recent advances in these two strategies are discussed below.

Stabilization by adsorption of particles at water/water interfaces

The first report of W/W emulsions stabilized by particles (Pickering W/W emulsions) was that of Poortinga et al. [17]. Nowadays, a great variety of different particles has been reported to stabilize W/W emulsions, as reviewed by Dickinson [4] and Chao and Shum [5]. However, because of the low interfacial tension, relatively large particles are required to achieve high adsorption energies that counteract thermal energy. An adsorption energy of 20 kT requires a minimum particle diameter of ≈ 320 nm for spherical rigid particles at 90° contact angle [18]. In the case of rod-like or flat platelet-like particles, the adsorption is favored, thanks to the increased contact area.

The particles can be very varied, and recent examples include cellulose microfibrils or nanocrystals in maltodextrin-in-dextran emulsions [19,20], β-lactoglobulin in dextran and hydroxypropyl methylcellulose aqueous mixtures [21], and platelet-like starch nanocrystals in PEG-in-dextran (P/D) emulsions [22], among other examples [23–25]. Stabilization of W/W requires an accurate control of particle adsorption at the interface (Figure 1).

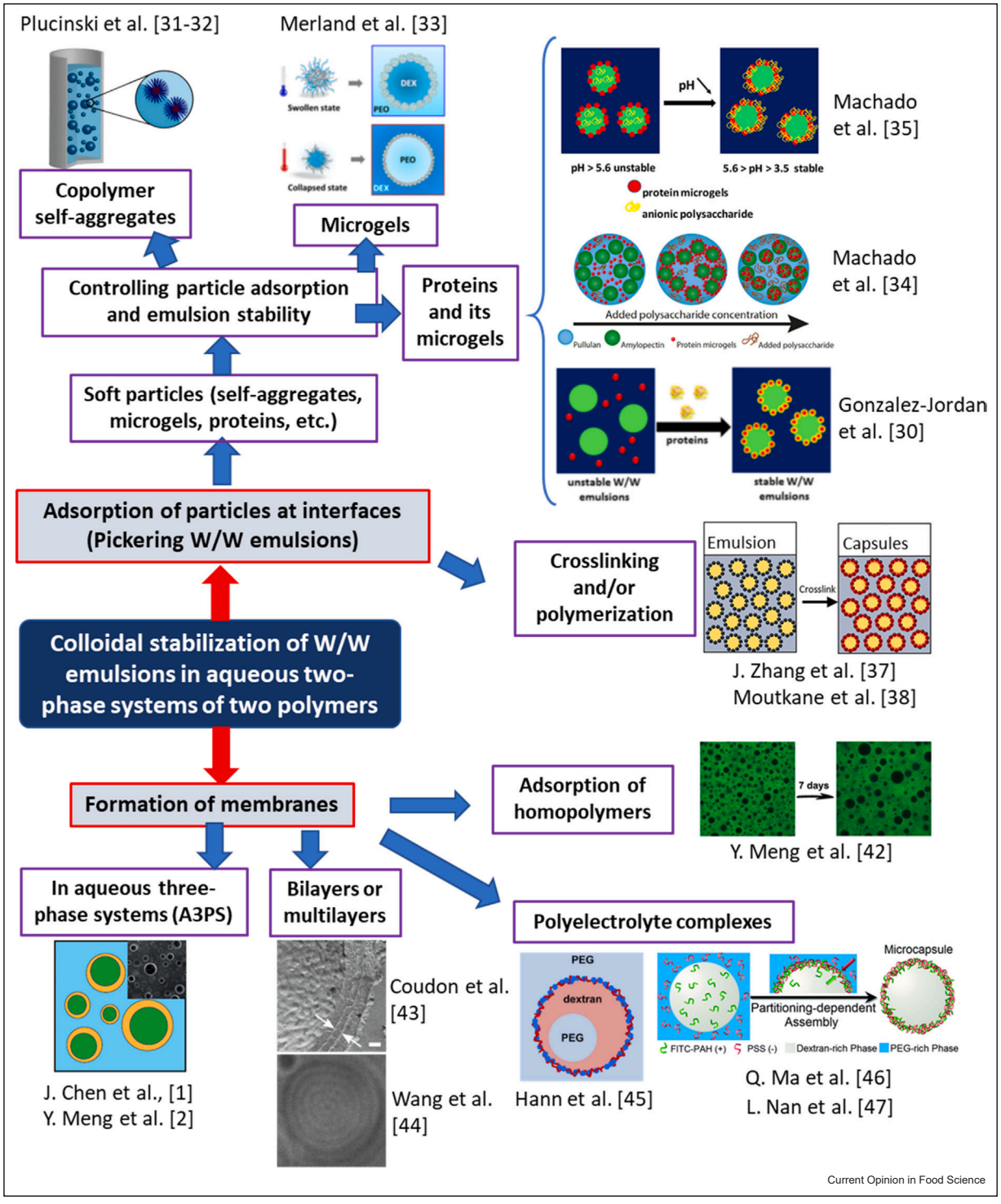
Stimuli-sensitive W/W emulsions can be obtained with microgel particles [26], unilamellar liposomes [27,28], self-assembled polymer micelles [29], or proteinaceous soft particles [30] adsorbed on the interface of emulsion droplets. Nguyen et al. [26] produced pH-responsive W/W Pickering emulsions that changed from stable to unstable with a small variation in pH, which could be modulated by the addition of salts. Dewey, Keating, and coworkers showed that P/D or dextran-in-PEG (D/P) emulsions can be stabilized by the adsorption of PEGylated liposomes (≈130 nm size) that consist of phosphatidyl glycerol [27]. The stabilization mechanism is mainly electrostatic, as the results indicated that effective stabilization occurs with interfaces that are not completely coated with liposomes. D/P emulsions, stabilized by unilamellar liposomes, were used as microreactors for the synthesis of $CaCO_3$ materials in biomimetic mineralization processes [28].

More recently, Schmidt, Plucinsky, and coworkers have studied the great possibilities offered by temperature-sensitive polymer micelles [29]. They synthesized a water-soluble double- block copolymer that consisted of two hydrophilic chains, poly(N,N-dimethylacrylamide)-b-poly(N,N-diethylacrylamide), which self-aggregates forming polymer micelles. These micelles had a preferential affinity for the polyethylene glycol (PEG) and consequently stabilized D/P emulsions that are highly sensitive to temperature, switching on/off the stability of emulsions [29]. In a more recent work, they designed pH-sensitive W/W emulsions by using pullulan and poly (N,N-dimethylacrylamide) (PDMA), as two incompatible polymers [31,32]. Pullulan-in-PDMA emulsions were stabilized with poly(2-(dimethylamino)ethyl methacrylate)-b-poly(oligo(ethylene glycol) methyl ether methacrylate), which forms polymer micelles (aggregates of polymer chains) as a function of pH [31].

The adsorption of particles in W/W interfaces can be controlled by various methods (Figure 1). Gonzalez-Jordan, Nicolai, and Benyahia demonstrated that the stability of Pickering W/W emulsions greatly depends on the balanced affinity of particles between the two liquid phases [30]. The adsorption of particles can be modulated by varying its surface properties, and thus, emulsion stability can be controlled. Polystyrene particles (PS) were coated with various amounts of whey proteins and used to stabilize both P/D and D/P. The most stable emulsion (P/D or D/P) depends on the preferential affinity of the modified PS particles, improving stability when preferentially wetted by the continuous emulsion phase [30].

Accurate control of emulsion stability, switching on/off as a function of temperature, can be achieved using microgels based on poly(N-isopropylacrylamide) (pNIPAM) derivatives. Ravaine, Nicolai, and coworkers obtained both P/D and D/P emulsions with pNIPAM microgels chemically functionalized with dextran [33]. These microgels stabilized D/P emulsions at low temperatures, but these emulsions inverted to P/D at temperatures higher than the transition point of pNIPAM

Figure 1



Schematic representation of significant recent contributions to the stabilization of W/W emulsions in segregative systems of two polymers. (All figures and schemes have been reproduced and/or adapted with copyright permission).

[33]. Nicolai and coworkers finely tuned the adsorption of whey protein microgel particles in pullulan/amylopectin interfaces, by adding small amounts of a third polysaccharide (alginate, pectin, κ-carrageenan, and xyloglucan) [34], inducing a migration of the protein microgels from the pullulan phase to the amylopectin

phase [34]. Interestingly, adsorption of microgels at interfaces occurred even if compositions were near to the binodal line, where the interfacial tension between pullulan and amylopectin is extremely small [34]. The authors concluded that particle adsorption depends not only on the interfacial tension but also on the tension between the particles and each of the phases. In a more recent study of emulsions prepared in pullulan–amylopectin aqueous mixtures, also stabilized by whey protein microgels, Nicolai and coworkers evaluated the influence of pH in the absence and presence of anionic polysaccharides [35]. Decreasing pH produced a change in the preferential affinity of the protein microgels, from the pullulan phase to the amylopectin phase, shifting from amylopectin-in-pullulan emulsions to pullulan-in-amylopectin emulsions. The anionic polysaccharides formed complexes with the protein microgel particles, inducing its partial aggregation, as well as modifying the interfacial tensions [35].

Quite often, the colloidal stability of Pickering W/W emulsions does not extend beyond a range of weeks, and thus, this stability is insufficient for practical applications. Moreover, particle-stabilized emulsions can remain stable at rest for long periods of time, but tend to quickly coalesce upon application of shear. This behavior is common to all types of Pickering emulsions (including O/W, W/O, and W/W), because agitation and droplet deformation generate empty areas at the interface.

Kulkarni et al. have explored stabilization of W/W emulsions by mixing oppositely charged particles (OCP) [36]. These mixtures produced small-particle aggregates that adsorbed at the interface and allowed the preparation of P/D emulsions. However, the colloidal stability was not sufficient to stabilize in the long term, probably because the OCP mixture did not form homogeneous monolayers, but irregular aggregates of particles adsorbed in a patchy distribution [36]. An alternative for extending emulsion stability is cross-linking the particles adsorbed in the interface. J. Zhang et al. cross-linked dopamine particles adsorbed in D/P droplets and interestingly these droplets formed capsules that remained stable upon dilution with water, albeit some swelling was observed [37]. Moutkane et al. also obtained quite stable dispersions of capsules by cross-linking whey protein isolate (WPI) microgel particles [38]. Pullulan-in-amylopectin simple emulsions and P/D-in-amylopectin double emulsions were properly stabilized. The best colloidal stability was obtained when covalently cross-linking with the enzyme transglutaminase. The WPI particles had a higher affinity for the dextran phase and thicker shells could be obtained by cross-linking in the middle phase of double emulsions. Pickering W/W emulsions can be well stabilized by either covalent cross-linking or interfacial ionic complexation, as reviewed by Perro et al. [39]. In these cases, capsules might have the advantage that they remain stable even after diluting with water, below the binodal line where there is no liquid-liquid phase separation.

Stabilization through the formation of membranes at water/water interfaces by polymer adsorption, self-assembly, ionic complexation, and other processes

Colloidal stability of W/W emulsions can also be greatly improved by polymer adsorption, self-assembly, and other processes of interfacial precipitation (Figure 1). An early report example was that of Buzza et al. who used triblock copolymers [40] to stabilize both P/D and P/D emulsions. It is likely that these copolymers produce polymer self-aggregates and thus stabilization was not a consequence of molecular adsorption and formation of a molecular monolayer.

More recently, it has been demonstrated that polymers with high molecular weight can certainly adsorb at W/W interfaces and impart colloidal stability. Nicolai and coworkers [41] have studied the stabilization of D/P and P/D emulsions using homopolymers, showing that chitosan and propylene glycol alginate were appropriate for P/D emulsions, whereas diethyl aminoethyl dextran provided an acceptable stabilization for both emulsion types [41]. More recently, they have stabilized D/P emulsions by addition of xanthan gum [42]. This polymer partitioned preferentially into the dextran phase and greatly increased viscosity of P/D emulsions, stabilizing the emulsions for at least one week. However, it also stabilized D/P emulsions and these results have suggested that xanthan had an additional stabilizing effect by interfacial adsorption.

In another work of Nicolai and coworkers [1], they stabilized both guar-in-amylopectin and amylopectin-in-guar emulsions by creating a layer of bovine gelatin on the interfaces. Guar and amylopectin are mutually immiscible, and gelatin is quite insoluble in both at low temperatures. Aqueous mixtures of guar, amylopectin, and gelatin formed biphasic mixtures at high temperatures, but cooling down induced the phase separation of a third phase mainly composed of gelatin, producing a layer at the surface of droplets. Therefore, this system consisted of three immiscible aqueous domains, in which the gelatin layer stabilized W/W emulsions. In a more recent work, Nicolai and coworkers produced aqueous three-phase systems by mixing dextran, PEG, and gelatin; or amylopectin, xyloglucan, and gelatin [2]. Double emulsions were obtained with gelatin forming the intermediate phase. The subsequent gelation of gelatin upon cooling produced hydrogel shells around the droplets, making them stable against dilution and/or agitation. These two works present a new strategy for emulsion stabilization.

Lamellar phases can be used to stabilize W/W emulsions, as shown by Martin, Douliez, and coworkers, who produced bilayers or multilayers composed of sodium oleate and decanol in D/P emulsion droplets, resembling conventional vesicles [39,43]. These lamellar nanostructures not only impart good stability, but are also quite impermeable to large molecules, opening up many possibilities for encapsulating active components. W/W emulsions can also be stabilized by using DNA strands, as reported by Hao and coworkers [44]. DNA adsorbed in the interface and droplet size decreased with DNA length. More interestingly, onion-like multilayer structures were observed at high DNA concentrations.

A very promising method for the stabilization of W/W emulsions is ionic complexation at the interface. Lee, Stebe, and coworkers formed double PEG-in-dextran-in-PEG emulsions by combining positively charged poly (diallyldimethylammonium chloride) (PDADMAC), with negatively charged silica nanoparticles [45]. This process was followed by the diffusion induced by osmotic pressure differences, leading to condensation of PEG droplets inside dextran microcapsules.

Shum and coworkers [46,47] have used four polymers for preparing rigid microcapsules: two nonionics and two ionics, one of which is cationic and the other is anionic. The two nonionic polymers constitute the ATPS, while the two polyelectrolytes with opposite charges form a shell of ionic complex at the interface. The nonionic polymers were PEG and dextran, the cationic polymer was poly(allylamine hydrochloride) (PAH), and the polyanion was poly(sodium 4-styrenesulfonate) (PSS). Microcapsules with rigid shells were obtained by the coprecipitation of the PAH–PSS ionic complex at the interface [46,47].

Emerging systems and possible applications

The stabilization of W/W emulsions that mimic real biological systems allows to develop novel biomedical applications (Figure 2). W/W emulsions produce low-friction coefficients on many surfaces, which is relevant for controlling the mouthfeel of food formulations, as reported by You, Murray, and Sarkar [38], who studied tribological properties of emulsions of gelatinized corn starch in κ-carrageenan, stabilized by whey protein microgel particles [3]. Jingcheng Hao and coworkers reported the rheology and tribological properties of D/P emulsions stabilized with collagen nanofibrils and evaluated its possible suitability for joint lubrication [48], showing the absence of cytotoxicity and protective effects on articular cartilage, in in vivo tests performed on Sprague-Dawley rats. Therefore, the emulsions could serve as a replacement of synthetic synovial fluid for the treatment of osteoarthritis [48].

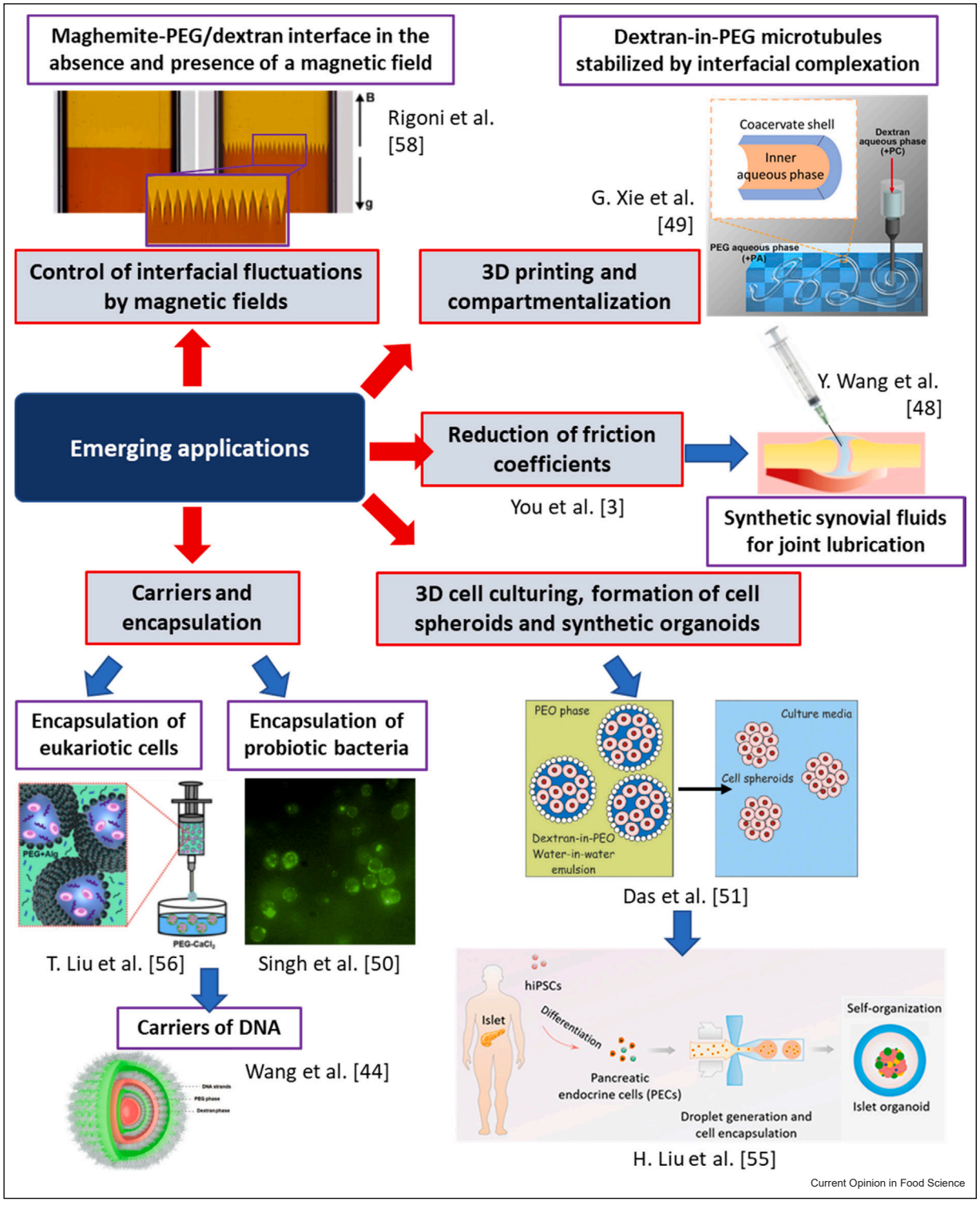
W/W dispersions stabilized by interfacial complexation can be used in 3D printing. Russell, Xie, and coworkers [49] reported the formation of tubular structures based on the combination of the cationic PDADMAC and the anionic PSS previously incorporated to dextran and PEG solutions, respectively. The ionic complexation of PDADMAC and PSS occurred at the dextran/PEG interface, producing stable and relatively rigid coacervate membranes that allowed 3D printing of tubular structures. These membranes were permeable to small molecules, and thus, the tubular structures could be used for cascade reactions that involved confinement of enzymes [49].

W/W emulsions are fully biocompatible dispersions that can be used to encapsulate living cells. Sodium carboxymethyl cellulose-in-gelatin emulsions were used to encapsulate Lactobacillus rhamnosus GG [50], increasing its viability in simulated gastric fluids. Thus, W/W emulsions have a great potential for the preparation of 3D cell cultures and/or organoids. One example was described by Paunov and coworkers [51], who obtained cell spheroids inside D/P emulsion droplets. The increase of PEG concentration in the continuous phase caused osmotic shrinking of the dextran droplets and formation of cell spheroids.

Jinhau Qin and coworkers [52] have extensively studied the formation and manipulation of W/W droplets using microfluidic devices, encapsulating cells inside the droplets and forming cell organoids. They reported the formation of multicore hydrogel capsules that were used as multicompartment carriers to coencapsulate two different types of cells, hepatic and endothelial, in the same 3D cell culture [52]. They also reported the high-throughput generation of D/P emulsion droplets that were gelified with calcium alginate [53]. More recently, they have also described the production of hydrogel fibers as cell organoid carriers [54]. Pancreatic endocrine progenitor cells were encapsulated into the droplets, retaining high viability and preserving the function of insulin secretion. In another work, pancreatic endocrine cells were encapsulated inside D/P emulsions that were stabilized by ionic complexation of the oppositely charged sodium alginate and chitosan [55]. These encapsulated cell spheroids exhibited insulin secretion stimulated by glucose. Another recent example of encapsulation of living cells using W/W emulsion droplets was described by Y. Wang and coworkers [56]. Cells were introduced inside D/P droplets in the presence of sodium alginate in the continuous phase. These droplets were stabilized by particles and encapsulated into alginate beads by introducing the emulsions into $CaCl_2$ baths and forming calcium alginate shells.

Shum and coworkers reported the formation of multiple W/W/O emulsions by microfluidics, stabilizing the W/W interface by ionic complexation [47]. One aqueous phase

Figure 2



Selection of novel applications of W/W emulsions prepared in segregative systems.
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contained dextran with PAH and the other aqueous phase had PEG in the presence of PSS. Removal of the volatile oil produced W/W emulsions with a membrane of PAH–PSS ionic complex. W1/O/W2 multiple emulsions have been prepared by Zhou et al [57]. The W1 inner phase was an aqueous solution of either dextran or PEG, the middle phase was immiscible oil, and the W2 most external phase was either PEG or dextran.

A very interesting new subject is the formation of ferrofluidic ATPS, studied by Timonen and coworkers [58] in dextran–PEG aqueous mixtures, incorporating maghemite superparamagnetic nanoparticles into the dextran phase where these nanoparticles have a preferential affinity. This ATPS showed superparamagnetism, and remarkably, the typical properties of ATPS were preserved, obtaining a biphasic system with extremely low interfacial tension and highly responsive to external magnetic fields. The very low interfacial tension allows to form new patterns at the interface, with strong deformations and motions that can lead to new magnetically responsible materials [58].

Concluding remarks

The current great development of W/W emulsions is due in part to recent innovations in their stabilization. During the last years, a great scientific interest has been focused on stabilization by adsorption of particles at the W/W interface, and numerous studies have reported rather stable Pickering W/W emulsions. In addition, in recent years, the formation of membranes at the W/W interface, such as by ionic complexation, has opened a new path for the stabilization of W/W emulsions with very much improved colloidal stability. This has allowed the emergence of new applications with great potential, such as preparation of 3D cell cultures and/or organoids of living cells with specific metabolic functions. The study of new W/W emulsions will allow achieving unforeseen novel applications, such as magneto-responsive interfaces.

Data Availability

Data will be made available on request.

Conflict of interest statement

The author declares no conflict of interest.

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- •• of outstanding interest

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The adsorption of whey protein microgel particles in W/W interfaces was controlled by the addition of small amounts of a third component. Particle adsorption was maximum at certain intermediate concentrations of that component. This work is highly interesting because particle adsorption can be switched on/off depending on the composition of the emulsion external phase. This finding can be generalized and might be applying to many other systems.

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