Article:
Sarkar, A orcid.org/0000-0003-1742-2122, Zhang, S, Holmes, M et al. (1 more author) (2019) Colloidal aspects of digestion of Pickering emulsions: Experiments and theoretical models of lipid digestion kinetics. Advances in Colloid and Interface Science, 263. pp. 195-211. ISSN 0001-8686

https://doi.org/10.1016/j.cis.2018.10.002

Colloidal Aspects of Digestion of Pickering Emulsions: Experiments and Theoretical Models of Lipid Digestion Kinetics

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Abstract

Lipid digestion is a bio-interfacial process that is largely governed by the binding of the lipase-colipase-biosurfactant (bile salts) complex onto the surface of emulsified lipid droplets. Therefore, engineering oil-water interfaces that prevent competitive displacement by bile salts and/or delay the transportation of lipase to the lipidoidal substrate can be an effective strategy to modulate lipolysis in human physiology. In this review, we present the mechanistic role of Pickering emulsions *i.e.* emulsions stabilised by micron-to-nano sized particles in modulating the important fundamental biological process of lipid digestion by virtue of their distinctive stability against coalescence and resilience to desorption by intestinal biosurfactants. We provide a systematic summary of recent experimental investigations and mathematical models that have blossomed in the last decade in this domain. A strategic examination of the behavior and mechanism of lipid digestion of droplets stabilised by particles in simulated biophysical environments (oral, gastric, intestinal regimes) was conducted. Various particle-laden interfaces were considered, where the particles were derived from synthetic or biological sources. This allowed us to categorize these particles into two classes based on their mechanistic role in modifying lipid digestion. These are '*human enzyme-unresponsive particles*' (*e.g.* silica, cellulose, chitin, flavonoids) *i.e.* the ones that cannot to be digested by human enzymes, such as amylase, protease and 'human enzyme-responsive particles' (*e.g.* protein microgels, starch granules), which can be readily digested by humans. We focused on the role of particle shape (spherical, anisotropic) on modifying both interfacial and bulk phases during lipolysis. Also, the techniques currently used to alter the kinetics of lipid digestion using intelligent physical or chemical treatments to control interfacial particle spacing were critically reviewed. A comparison of how various mathematical models reported in literature predict free fatty acid release kinetics during lipid digestion highlighted the importance of the clear statement of the underlying assumptions. We provide details of the initial first order kinetic models to the more recent models, which account for the rate of adsorption of lipase at the droplet surface and include the crucial aspect of interfacial dynamics. We provide a *unique decision tree* on model selection, which is appropriate to minimize the difference between experimental data of free fatty acid generation and model predictions based on precise assumptions of droplet shrinkage, lipase-binding rate, and

nature of lipase transport process to the particle-laden interface. Greater insights into the mechanisms of controlling lipolysis using particle-laden interfaces with appropriate mathematical model fitting permit better understanding of the key lipid digestion processes. Future outlook on interfacial design parameters, such as particle shape, size, polydispersity, charge, fusion, material chemistry, loading and development of new mathematical models that provide closed-loop equations from early to later stages of kinetics are proposed. Such future experiments and models hold promise for the tailoring of particle-laden interfaces for delaying lipid digestion and/or site-dependent controlled release of lipidic active molecules in composite soft matter systems, such as food, personal care, pharmaceutical, healthcare and biotechnological applications.

Keywords: Pickering emulsion; Particle-laden interface; Lipid digestion; Lipase; Mathematical model

1. Introduction

Emulsions are ubiquitous in nature (*e.g.* milk, butter) and in engineered systems (*e.g.* cosmetics, processed food, pharmaceuticals, paints). Emulsions are intimate dispersions of two immiscible liquids, such as oil and water. In general, the liquid, which is present as micron- or sub-micron-sized droplets in the other liquid is known as the dispersed, discontinuous or internal phase [1-3]. And, the liquid into which the droplets are dispersed is known as the dispersion medium, continuous or external phase. From a thermodynamic perspective, emulsions are metastable *i.e.* they are in a state far from the equilibrium. Thus, emulsions tend to separate into their individual phases to attain an equilibrium configuration over a period of time. This thermodynamic instability is commonly represented using Gibbs free energy change, ΔG, during emulsification. Emulsification at a constant temperature of T alters both the configurational entropy of the droplets, S_{config}, and the contact area between the immiscible phases, A, such that ΔG is given by:

$$\Delta G = \Delta H - T\Delta S_{config} + \gamma_{ow}\Delta A \quad (1)$$

where, ΔH is the enthalpy change, which is almost zero for emulsification, and γ_{ow} is the interfacial tension between the oil and water phases. The change in interfacial free energy ($\gamma_{ow}\Delta A$) is always net-positive as the interfacial area increases after the homogenization process, and is generally much larger than the term containing the configurational entropy change, ΔS_{config}, and thus the latter can be ignored [3]. Hence, ΔG is mainly governed by $\gamma_{ow}\Delta A$. In the presence of an emulsifier, the magnitude of $\gamma_{ow}\Delta A$ is diminished due to reduction of the interfacial tension between the two phases enabled by adsorption of the emulsifier at the oil–water interface, consequently reducing the free energy. Though interfacial energy is the key driving force for emulsion formation, the properties of emulsifiers, such as surface charge and steric hindrance are crucial for providing kinetic stability to the emulsions. Emulsifiers, such as mono or di-acylglycerols, proteins, modified starches play an important role in imparting this kinetic stability to the oil droplets, however they tend to phase separate over a period of weeks to few months.

Besides conventional surfactants, emulsions can also be stabilised by rigid or soft solid particles that form a mechanical barrier *via* the Pickering stabilization mechanism [4, 5]. In fact, such Pickering emulsions are often encountered in the crude oil refining process [6], homogenized milk stabilised by casein micelles and aggregated milk proteins [7] and are engineered for soft material applications [4, 8]. In comparison to classical surfactant-stabilised emulsions, Pickering emulsions that are stabilised by solid particles are distinctively more stable to coalescence and Ostwald ripening [4, 5]. This is due to the large amount of desorption energy (ΔE) required to dislodge the particles from the interface [9]. In other words, for emulsions to destabilize, the free energy (ΔG) of the system has to significantly increase at first, as the particles move from their preferred positions on the interface to one of the two phases. The adsorption energy for a single particle can be expressed using equation (2) [5, 10, 11]:

$$\Delta \boldsymbol{G} = -\Delta \boldsymbol{E} = -\boldsymbol{\gamma_{ow}} \boldsymbol{\pi} \boldsymbol{r_p}^{\boldsymbol{2}} (\boldsymbol{1} - |\boldsymbol{cos}\, \boldsymbol{\theta}|)^{\boldsymbol{2}} \quad (2)$$

where, r_p is the radius of the particle and θ is the equilibrium three-phase contact angle [5, 12] highlighting the wettability of the particles by oil or water phases. The actual energy required to dislodge a particle from the surface has been shown to be even higher, at several times that given by equation

(2). This is due to dissipation of energy stored in the liquid neck, formed between the particle and interface, as the liquid relaxes back once the particle has departed from the surface [9, 13]. Hence, even nano-sized solid particles with $r \approx 50$ nm at a contact angle of 90 ° at the oil-water interface (typical value of $\gamma_{ow} \approx 50$ mN m^{-1}) will have $\Delta E \approx 10^5\, k_B T$, where k_B is the Boltzmann's constant. This is several orders of magnitude higher than that for classical surfactants, which typically have $\Delta E \approx 5\, k_B T$ and therefore tend to continuously "hop on and off" from the interface [14]. Thus, it is almost certain that solid particles once adsorbed under partial wetting conditions by the two phases, will remain irreversibly anchored to the interface making Pickering emulsions highly resilient to coalescence, when compared to similar droplets stabilised by typical molecular surfactants. Theoretically, wetting is represented by the classical Young's equation (equation 3), which is related to the balance of interfacial forces per unit length of the contact line at particle–oil (γ_{po}), particle–water (γ_{pw}), and oil–water (γ_{ow}) surfaces:

$$\cos\theta = \frac{\gamma_{po} - \gamma_{pw}}{\gamma_{ow}} \tag{3}$$

If one of the liquids wets the solid particles more than the other, the better wetting liquid becomes the continuous phase and the other one becomes the dispersed phase. For instance, if θ measured in the aqueous phase is smaller than 90° (Fig. 1a), then particles are preferentially wetted by the aqueous phase (i.e. $\gamma_{pw} < \gamma_{po}$), with a large fraction of such particles residing in the continuous phase and stabilizing an O/W emulsion. Such hydrophilic biocompatible particles include protein microgels, zein particles and chitin nanocrystals [15-18]. However, if θ is greater than 90° (Fig. 1b), the particles tend to be preferentially wetted by oil phase and stabilizes a W/O emulsion, as demonstrated using zein particles in presence of lecithin [19]. Particles wetted equally by oil and water have contact angle of 90° (Fig.1c). It is noteworthy that when θ is relatively close to 90°, then particles effectively act as a Pickering stabilizer as particles tend to remain dispersed in either phases if they are too hydrophilic (θ<<<90°) or too hydrophobic (θ>>>90°) [20]. In order to overcome this latter issue, many studies have modified natural particles to varing degrees in order to make them partially wetted by both the polar and non-polar phases. For example, modified starch granules, prepared via reaction with octenyl

succinic anhydride [21] or modified cellulose prepared using stearoyl chloride [22] have been used as effective Pickering stabilizers of oil-in-water or water-in-oil emulsions, respectively.

Pickering stabilization of colloids using particles is a century-old concept, first introduced in 1904 by Ramsden [23] and proven experimentally by Pickering few years later [24]. The earliest theoretical treatment, demonstrating how fine particles can stabilize emulsions and foams, seems to be developed by Levine *et al.* dating back to 1989 [25]. Nevertheless, the study of particle-laden interface has revived through a renaissance of research attention in the last two decades. This can partly be attributed to the growing demands for designing ultra-stable emulsions using surfactant-free and 'clean-label' emulsifiers. In part, such a resurgence of research interests in particle-stabilised interfaces are also associated with tremendous progress in mining biocompatible particles from natural sources and laboratory-scale synthesis of relatively low-cost biocompatible colloidal particles with tunable size (micron-to-nanometers) [11, 26, 27] for use in food, pharmaceutical, cosmetics and other allied soft matter applications. In such applications sustainability and biocompatibility are key requirements. Last but not the least, particle-laden interfaces have been lately recognized as promising templates by colloid scientists to address key biological processes, such as delaying lipid digestion [16, 28-30], drug delivery [31], nutraceutical delivery [32] and controlled release of lipophilic molecules via oral and topical administration routes [33, 34].

In particular, lipid digestion is a fundamental biological process that represents a major bottleneck in the pathway to controlled delivery of lipophilic drugs and nutrients when administered through oral routes in human physiology. Lipid digestion is an interfacial process and the kinetics of lipid digestion is governed by the binding of enzymes (lipases, proteases, amylases), biosurfactants (bile salts) and other cofactors onto the surface of emulsified droplets [35]. Simplistically, one might expect lipid digestion to be controlled by tailoring and/or tuning the interfacial network. Hence, research efforts have been directed in recent years to alter the kinetics of lipid digestion by modification of the interfacial structures [1, 16, 29, 36-38]. In particular, solid particles have shown encouraging outcomes in this direction in the past decade by a variety of interfacial mechanisms, which necessities this review. Progress on Pickering emulsion research has been well-described in the excellent reviews by Binks [4, 5, 39], Dickinson [7, 26, 27, 40, 41] and few other research groups [10, 11, 42-46], who have discussed

the physical chemistry, stabilization principle as well as design of elegant Pickering emulsions stabilised by organic (biologically derived) particles. However, to our knowledge, there exists no review that has discussed the mechanistic role of Pickering emulsions in modulating the important fundamental biological process of lipid digestion.

Hence, the purpose of this review is to focus on the experimental investigations and theoretical models on lipid digestion of Pickering emulsions stabilised by nano-to-micron-sized particles within the last decade. In this review, only O/W emulsions are considered. We start with briefly discussing the key physiological players in the biophysics of lipid digestion in the oral, gastric and intestinal regimes. The ultimate step of conversion of lipids into self-assemblies, such as micelles, vesicles, and liquid crystals that are essential for lipid absorption in the lower intestines is beyond the scope of this review. A description of the colloidal aspects of digestion sets the scene for understanding how parameters associated with the particle, such as size, shape, concentration, charge, packing density affect the resilience of the particle-covered droplets to harsh physiological conditions. We have then summarized the current knowledge of how such particles on their own or via suitable physical/ chemical tuning at the interface play an integral role in modifying the kinetics of lipid digestion. Attention is then directed towards reviewing the mathematical models in literature, highlighting the early first order kinetic models to the more recent modelsd attempting to account for the adsorption kinetics of enzymes at the droplet surface and the associated role of interfacial structure. We provide a decision tree on model selection appropriate to likely to represent a given digestion behavior (*e.g.* droplets shrinking, enzyme binding rate, nature of enzyme transport process to the surface of droplets). Finally, we provide suggestions for future work in both theoretical and experimental domains to maximize the potential of Pickering emulsions in order to address fundamental biological and biochemical challenges associated with emulsified lipid digestion. Such knowledgebase can enable us to rationally design particle-laden interfaces for site-dependent controlled release of lipid soluble active molecules in composite soft matter systems, such as food, personal care and pharmaceutical applications.

2. Key players in colloidal structuring of lipids in human physiology

In this review, we are concerned mainly with the physiological re-structuring or resilience-to-destabilization of Pickering emulsion during the trajectory of lipid digestion. Nevertheless, we provide a concise summary of the three sequential regimes (Fig. 2) *i.e.* oral, gastric and intestinal, where complex colloidal structuring might occur with the eventual conversion of lipids to free fatty acids (FFAs) monomers. In particular, we highlight the interfacial role of the key enzymes and biosurfactants, such as mucin (oral), amylase (oral, intestinal), pepsin (gastric), trypsin and chymotrypsin (intestinal), bile salts (intestinal) and most importantly, lipase (gastric and intestinal. This is by no means to underestimate the roles of the inorganic ions (Na^+, K^+, Ca^{2+}, HCO_3^-), pH (full spectrum effects from saliva to gastrointestinal juices), and a range of shear-to-surface forces (oral shear, peristalsis, mixing regimes, interactions with mucus-coated surfaces) throughout the aqueous oral-to-gastrointestinal tract on colloidal structuring, aggregation of droplets and phase separation. Detailed information about colloidal aspects of lipid digestion in surfactant stabilised systems can be found in number of review articles [1, 2, 35, 38, 47-49].

2.1 Oral phase

Saliva is the first complex fluid that an emulsion encounters upon oral consumption. Emulsions reside in the oral cavity for only a few seconds to minutes, depending upon the oral viscosity and coating abilities. Nonetheless, during tis short time period, the emulsion droplets can undergo a diverse palette of destabilization routes from flocculation (bridging, depletion) to coalescence and phase separation depending upon their interactions with salivary components [50-53]. Human saliva has a neutral pH and contains a range of ions, proteins (*e.g.* mucin, immunoglobulin, statherins, proline-rich proteins, lysozymes, serum albumin), enzymes (amylase) and bacterial cells that are dispersed in 99% aqueous phase [54].

Highly glycosylated salivary mucin (MUC5B), which contributes to 10–25% of total salivary proteins is deemed as one of the main components in the dilute saliva that can modify an emulsion's dispersion state. Structurally, salivary mucins are 20% polypeptide core and 80% carbohydrates and have a high molecular weight ($\geq 10^6$ Da) [55]. Emulsion structuring for ionic surfactant-stabilised or

protein-coated droplets in the mouth can occur by virtue of electrostatic interactions with mucin, which is negatively charged at oral pH. The charge density of the mucin is associated with the sulphates and sialic acid (*N*-acetylneuraminic acid) parts of the glycosylated constructs. Hence, positively charged emulsions, such as, lactoferrin-coated droplets may undergo bridging flocculation with simulated or real saliva whereas weakly negatively-charged emulsions, such as, β-lactoglobulin-coated droplets undergo depletion flocculation in oral phase [51, 56-59]. The second important component that can have a serious impact on colloidal instability is the enzyme*, salivary* α*-amylase*, which initiates the hydrolysis of α-1-4 glycosidic bonds in starch in the oral phase. Therefore, α-amylase can rupture the interfacial layer and lead to oil droplet accretion, conditional to the scenario that the interfacial layer contain starch as structural motifs [60, 61]. Besides mucin and α-amylase, salivary ions may induce oral flocculation of droplets via electrostatic charge screening and/or ion binding effects [50, 51, 56]. Oral shear-, surface- and air- [60, 62, 63] induced interactions may also result in droplet coalescence or partial coalescence, with the latter being dependent on the proportion of solid fat content in the droplets at oral temperature.

2.2 Gastric phase

After this relatively short oral residence, emulsions travel through the oesophagus and are subjected to harsh acidic environments (pH 1-3), ions, digestive enzymes (*pepsin* and *gastric lipase*) and mixing vortexes induced by antral contractions as well as shear forces when they reach the stomach [1, 38, 47] (Fig. 2). The physicochemical conditions (pH, ionic strengths) in the stomach may lead to aggregation of the emulsion droplets in the stomach [64]. From a biochemical perspective, proteins are significantly hydrolysed by pepsin to smaller peptides, lipids are only partially digested by acid stable gastric lipase and carbohydrates see almost no breakdown in the gastric regime.

Pepsin is a proteolytic enzyme that breaks down the peptide bonds between hydrophobic groups, preferably aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine. Thus, pepsin-induced cleavage can result in modification of the interfacial structure if the droplet surface that contains proteinaceous materials. Pepsin-induced proteolysis may result in interfacial film drainage eventually leading to droplet coalescence [1, 65-68]. Although the majority of lipolysis occurs in the intestinal

242 phase, gastric lipase can contribute to nearly 10-30% of lipid digestion resulting in generation of FFAs
243 in the stomach [69, 70]. Gastric lipase is active in the pH range of pH 3-6, which suggests that gastric
244 lipase-induced lipid hydrolysis can occur even in the first hours of gastric digestion where the pH of the
245 stomach is still elevated owing to the meal buffering capacity [38]. The gastric lipase-mediated fatty
246 acids released in the stomach may have some surface activity and competitive adsorption behaviour
247 versus the parent material at the interface and thus alter the interfacial composition of lipid droplets.
248 However, studies reported in literature on the interfacial aspects of gastric lipase-mediated lipolysis of
249 emulsified lipids are relatively scarce to enable commenting on the interfacial modification with any
250 certainty. This is because of the unavailability of reliable sources of lipases that behave like human
251 gastric lipases until very recently [71]. Depending on the initial interfacial structure (*e.g.* protein,
252 surfactants), lipid composition (e.g. solid/ liquid lipid ratio) and degree of interactions with organic and
253 inorganic players in the gastric phase, the fate of emulsion structure can vary from remaining intact to
254 flocculated, coalesced, partially coalesced and phase separated [1, 64, 68, 72].
255
256 *2.3 Intestinal phase*
257 The majority of the interfacial alteration of the droplets and lipid digestion (70–90%) occurs in
258 the upper part of the small intestine. The intestinal digestion is a complex process due to the presence
259 of a cocktail of amylotyic, lipolytic and proteolytic enzymes, biosurfactant and inorganic components.
260 As the droplets transcend from the stomach to intestine, the pH of the physiological medium reverts
261 back to nearly neutral [65]. Many, if not most colloid scientists have highlighted the key intestinal
262 players to be *trypsin* and *chymotrypsin* for proteolytic action on protein-coated interfaces [65],
263 *pancreatic amylase* for hydrolysis of starch-stabilised interfaces [73] and biosurfactant (*bile salts*) and
264 *pancreatic lipase* [35] for generating fatty acids, mono and/or diacylgylcerols from the hydrophobic
265 lipid core besides cofactors (colipase) [74, 75] and other enzymes. For instance, trypsin predominantly
266 catalyses the peptide chains at the C-terminal of aliphatic amino acids, mainly lysine and arginine,
267 whereas chymotrypsin favours large aromatic residues, such as phenylalanine, tyrosine and tryptophan.
268 Such interfacial cleavage can have consequences on altering the interfacial composition in the case of

protein-stabilised droplets, such as those stabilised by β-lactoglobulin generating small molecular weight peptides which might not have sufficient viscoelasticity like the parent protein film to prevent droplet deformation [76].

The interfacial dynamics becomes most exciting in the intestinal phase as intestinal lipid digestion is an interfacial process that involves a complex interplay between competitive adsorption of lipase/colipase and bile salts at the O/W interface (Fig. 2). Bile salts are a very unconventional, planar-type of biosurfactant that, unlike classical surfactants, do not have a typical hydrophobic head and a hydrophilic tail group [77]. The facial amphilicity of bile salts originates from the flat steroidal structure, with the polar hydroxyl groups on the concave side and methyl groups on the convex side. Because of their high surface active properties, bile salts play a crucial role in lipid digestion by displacing the initial adsorbed materials from the interface [78, 79] via orogenic displacement if the parent interfacial film is viscoelastic, permitting lipase/colipase complex to act on the bile-coated oil droplets. The role of the initial charge density at the droplet surface determines to a large extent the kinetics of the sequential binding of negatively charged bile salts to the interfacial material or displacement of the initial adsorbed layer by intestinal bile salts [1, 80]. Besides surface activity, bile salts also facilitate the solubilisation of lipid digestion products into lamellar phase or mixed micelles. Often, bile salts can also remove inhibitory surfactants, such as lipid digestion products (fatty acids, mono/ diglycerides) from the interface via competitive displacement mechanism, thus accelerating and enabling continuation of digestion of emulsified lipid droplets [36].

Pancreatic lipase is active approx. between pH 5.5 to pH 7.5 and hydrolyses the emulsified lipids producing two FFAs and a 2-monoacylglycerol [35, 38, 49]. Lipase can only act in the presence of co-lipase and in some cases the presence of co-lipase and bile salts. For lipid hydrolysis to take place, the pancreatic lipase-colipase complex must first adsorb to bile salt-covered interface. Thus, interactions of the adsorbed layer at the droplet surface and lipase are critical in determining the rate of lipid hydrolysis. As lipolysis progresses there is a build-up of fatty acids and monoglycerides at the emulsion interface, which might displace the adsorbed materials. Noteworthy that these lipid digestion products may be surface active but not essentially colloidal stabilizers. Thus, the emulsions generally suffer from droplet coalescence in case of most surfactant-stabilised emulsions in the intestinal stage [1, 72, 76].

297 Nevertheless, bile salts play a key role here in facilitating the desorption of fatty acids and
298 monoglycerides and their solubilisation into the micellar phase promoting further lipid digestion, and
299 some of the bile salts may remain adsorbed to the surface conferring a high negative charge, thus
300 promoting stability [36, 80].
301

302 ## 3. Experimental investigations on colloidal digestion of Pickering emulsions

303 As it is obvious from the biophysical aspects of digestion in human physiology, conventional
304 emulsion droplets prepared using surfactants, such as polysorbates or proteins may eventually
305 shrink as the emulsified lipids in the interior of the droplets undergo easy conversion to FFAs
306 during lipid digestion. Therefore, engineering complex interfacial architecture (*e.g.* interface
307 composition and structural attributes) can be one of the promising solutions to modulate the
308 colloidal aspects of lipid digestion. Indeed, the significant advantage of particle-stabilised
309 emulsions over biopolymers- or other surfactant-stabilised emulsions for altering lipid digestion
310 is their unique resilience against coalescence, dissolution and shrinkage mechanisms [5, 27].
311 The mechanism behind such minimal decrease in the volume of the droplets during lipid
312 digestion is the significant energy barrier for particles to detach from the interface offering an
313 almost impossible exchange by the surface active materials in the physiological regimes (*e.g.*
314 bile salts, lipid digestion products). Moreover, the interfacial thickness and surface load are
315 much greater for particle-laden interface offering barrier properties to physiological
316 restructuring in comparison with conventional surfactant-stabilised counterparts [42].
317 Questions arise as to what happens to such particles and particle-covered droplets, when
318 they traverse the various stages of the human oral-to-gastrointestinal tract. Of course, the fate of
319 these systems might be affected by interference from the competing biochemical processes, such
320 as breakdown of Pickering layers of intact starch-based particles by α-amylase [81] or protein-
321 based particles by pepsin and/or trypsin [16]. However, such factors might not affect
322 polysaccharide-based particle- (*e.g.* chitin nanocrystals) [30], organic crystal-(*e.g.* flavonoids)
323 [82] or inorganic particle- (*e.g.* silica) [83] –covered droplets, which are unaffected by

gastrointestinal enzymes. This section thus summarizes the key colloidal mechanisms from the experimental investigations of *in vitro* digestion of Pickering emulsions carried out in the last decade (see Fig. 2 for the schematic). We have categorised particle-laden interfaces into two discrete classes, namely, 'human enzyme-unresponsive' particles *i.e.* the ones that cannot be digested by human enzymes (*e.g.* amylase, protease) and 'human enzyme-responsive particles', latter that can be readily digested by human enzymes. Characteristics of the Pickering emulsions and their digestion data are listed in Table 1.

3.1 Enzyme-unresponsive particles

Silica nanoparticles. In the case of inorganic particles, silica particles have received much attention not only for their ability to stabilize Pickering emulsions [84] but also for their role in altering the extent of lipid digestion [83]. Using artificial saliva formulation, Ruiz-Rodriguez *et al.* [83] showed that Pickering emulsion droplets of mean size of 3-8 μm stabilised by silica nanoparticles (0.5-5 wt%) had an inter-particle (in other words intra-droplet) aggregation at the interface influenced by the charge screening effects of the salivary salts providing enhanced physical stability to the emulsion droplets. This is unlike the inter-droplet aggregation behaviour observed in ionic surfactant- or protein-stabilised emulsions in presence of artificial or real saliva [56, 58] (Fig. 2).

Interestingly, such silica-stabilised emulsions were stable across the physiological range of pH values from 3 to 7, which is advantageous over many oral emulsion formulations that suffer from pH-induced gastric instability. Finally, Ruiz-Rodriguez *et al.* [83] highlighted that bile salts were unable to displace the practically irreversibly adsorbed silica nanoparticles from the interface, in line with another study on silica-laden interface showing similar results with enhanced curcumin retention [85]. The presence of silica particles at the interface reduced the maximum extent of lipid digestion (φ_{max}, %), but interestingly did not affect the initial rate of lipolysis (k, s^{-1}) [83] (Table 1). This might be explained primarily using the size of the gaps (interfacial pores) in the silica particle-laden interfaces. Such gaps can be expected to be sufficiently large to allow the diffusion of angstrom-sized lipase/bile salts complex to the surface to allow instantaneous onset of digestion, but small enough to allow the migration of the

FFAs generated during lipid digestion into the continuous phase. In addition, one might recognize that for Pickering emulsions, there is ideally no shrinkage of droplets, so the volume of droplets should not alter and a large portion of FFA produced must remain within the droplets. Hence, one might consider not only measuring FFAs using 'gold-standard' titrimetric techniques that measures FFA only after it is released in the continuous phase but also consider measuring the undigested triglycerides together with other digestion products that might have been retained within the dispersed phase or still somehow anchored to the adsorbed phase.

Polysaccharide-based particles. Besides inorganic particles, biodegradable polysaccharide-based particles, such as cellulose nanocrystals (CNC) or nanofibrilltaed cellulose (NFC) [28, 29, 86, 87] and chitin nanocrystals (ChN) [30] have been recognized among the most opportune materials as they are not digested by human gastrointestinal enzymes yet are biodegradable. Both cellulose and chitin obtained from plant cell walls and animal sources (sea food wastes), respectively, are essentially naturally-abundant polysaccharides with semi-crystalline architecture containing alternate nanocrystalline and amorphous domains [88-90]. Strong acid hydrolysis can remove the amorphous domains leading to the formation of stiff rod-shaped nanocrystals of high aspect ratio (typically 5–50 nm in width and between 100 nm to several micrometers in lengths). Another favorable aspect of using these nanocrystals at the O/W interface is that these anisotropic particles pack in a more ordered fashion but inhomogeneously [91], thus, providing improved steric hindrance and mechanical strengths at the interface even at lower particle loadings, and thus can bring advantage over spherical particles in modulating lipid digestion.

One of the earliest studies in the field of digestion of Pickering emulsion was conducted by Tzoumaki *et al.* [30] where the authors compared the digestibility of Pickering O/W emulsions stabilised by ChN with that of the conventional emulsions stabilised by milk proteins. Interestingly, ChN at the interface not only decreased the extent of lipid digestion (φ_{max}, %) by two-folds to 33% FFA release (Table 1), as compared to that stabilised by milk proteins, but also significantly reduced the initial rate of digestion (k, s^{-1}). This distinctive reduction in rate of digestion was also shown in studies using NFC [86], which is unlike the behaviour that was observed in the spherical silica-laden interface as discussed previously [83].

In addition to the irreversible adsorption of these rod-shaped particles at the interface and inability to be desorbed by bile salts/ lipid digestion products, two other mechanisms [29, 30, 86, 92] were proposed for this delay in lipid digestion (Fig. 2) - 1) the rod shaped particles formed a rigid inter-particle (i.e. intra-droplet) network at the oil–water interface providing a strong mechanical barrier of considerable interfacial viscosity around the droplets, 2) the rod shaped particles underwent isotropic to nematic phase transition forming highly ordered and densely packed network structures in the *aqueous phase*, which might have mechanically entrapped the emulsion droplets and decreased their access to bile salts or lipase. Mechanism 1) is largely associated with packing of rods that can be much more efficient than that of spheres at the interface. Mechanism 2) is similar to trapping emulsion droplets in a gel-like network to create a tortuous path for lipase/colipase complex to reach the hydrophobic lipid core [93, 94], which is achieved in these ChN and CNC particle-laden interfaces without using an additional gelling agent. This highlights that particles can have interesting interfacial as well as bulk rheology that can be carefully engineered to tailor lipid digestion kinetics and develop controlled delivery applications. For instance, Pickering emulsions stabilised by chitosan tripolyphosphate nanoparticles that are engineered *via* ionic gelation technique with lower extent of FFA release have been applied to enhance bioaccessibility of encapsulated bioactive molecules, such as curcumin as compared to nanoemulsion counterparts [95, 96].

Flavonoid crystals. Another important class of organic particles from biological origin that have been investigated in literature are flavonoid crystals, which are secondary metabolites from plants. Flavonoids, such as tiliroside, rutin and naringin have shown tendency to absorb at oil-water interface [82, 97]. In fact, there has been only one study on flavonoid particles from *Ginkgo biloba* extract that has investigated *in vitro* lipid digestion profile. Yang *et al*. [98] demonstrated that such particles behaved as Pickering stabilizers, however the size and shape of the particles were not mentioned. Such particles reduced both the rate (k = 0.13 μmol s^{-1} m^{-2}) and extent of FFA release (φ_{max} ~ 24%) (Table 1) from the flavonoid covered-lipid droplets by nearly two- and four-folds, respectively, as compared to a surfactant-stabilised emulsion.

406 *3.2 Enzyme-responsive particles*

407 Assiduous research has been devoted to the lipid digestion of Pickering emulsions using digestive
408 particles (Table 1). These particles include starch granules and nanoparticles with or without
409 hydrophobic modification with octenyl succinic anhydride (OSA) and a range of protein particles from
410 animal (whey protein microgel, lactoferrin nanoparticles) sources and more recently plant (karifin
411 nanoparticles, zein protein particles, pea protein microgel particles). Such digestible particles have been
412 widely used in scientific investigation for lipid digestion as they are not only safe in theory as they are
413 digestible by human gastrointestinal enzymes but also perceived as relatively more natural, "clean
414 label" and "green" [99].

415 **Starch particle.** Native starch granules are semi-crystalline [100, 101] and the second-most abundant
416 particle after cellulose. The forms of starch used for making Pickering emulsions reported in literature
417 has ranged across length scales from native to OSA-modified starch granules of mean diameter of 1-50
418 μm [102-104] to starch nanoparticles and nanocrystals of mean diameter of few nanometers that have
419 been engineered physically or chemically [105-107]. Although overwhelming amount of research has
420 been conducted on starch particle-laden interface, investigations are relatively scarce when dealing with
421 lipid digestion of such droplets.

422 In fact, only two systematic studies from the Swedish research group [81, 108] demonstrated
423 lipid digestion behaviour of emulsions stabilised by hydrophobically modified quinoa starch granules
424 [81]. Marefati *et al.* [81] demonstrated that amolytic digestion in oral phase by α-amylase resulted in
425 significant size reduction of the emulsion droplets and release of some free starch particles. However,
426 a major proportion of the modified starch granule-coated droplets still retained their integrity and were
427 resilient to coalescence even after 60 min of salivary exposure, unlike the conventional emulsions
428 stabilised by OSA-modified starch, where the latter underwent dramatic coalescence within seconds
429 [60, 61]. The gastric digestion had no effect on these Pickering emulsions as it can be expected owing
430 to lack of any amylolysis. Interesting conclusions were highlighted about the lipid digestion profile in
431 the intestinal phase [81]. Although the starch granules were not likely to be displaced by bile salts, the
432 gaps between these micron-sized starch granules at the interface allowed rather easy accessibility of

433 bile salts and lipolytic enzymes to the interface in the particle-free area, leading to droplet coalescence.
434 **Protein particles from animal sources.** The acceptability of animal proteins and versatility to create
435 microgel, nanogel or nanoparticles using their heat-sensitivity (*e.g.* whey protein, lactoferrin) [15, 16,
436 109] have enabled creation of laboratory synthesized particle of tuneable size to create Pickering
437 emulsions. The distinctive feature of using protein-based particle is that they can offer both electrostatic
438 stabilization and steric hindrance when present as Pickering layers as opposed to starch granules, in
439 latter, the droplets are only sterically stabilised unless modified.
440 Work carried in our laboratory [16] has demonstrated interesting gastric and intestinal digestion
441 profile of emulsion droplets (mean diameter of 43 μm) stabilised using negatively-charged spherical
442 whey protein microgel particles (WPM, 300 nm). These particles were created using a top-down
443 approach of breaking a 10 wt% whey protein gel in a jet homogenizer as opposed to the bottom up
444 approach used previously [15]. The Pickering emulsions stabilised by WPM showed interesting
445 resilience to gastric coalescence unlike conventional whey protein-stabilised emulsions [68], where
446 dramatic increase in the droplet size has been reported due to pepsin-induced rupture of the interfacial
447 protein layer. Looking at confocal microstructure and polyacryl amide gel electrogram of the protein
448 bands from the adsorbed phase of the Pickering layer of WPM, it was concluded that pepsin was not
449 able to fully access some of the hydrophobic sites due to the reburial of those domains within the
450 microgel particles. Noteworthy is that the interfacial loading (~14 mg m^{-2}) was nearly 12-fold higher
451 than a whey protein monolayer at the interface indicating a substantial increase in substrate required to
452 be digested by pepsin [15] (Fig. 2). Such interesting gastric stability of protein particle versus protein
453 monolayer was also demonstrated in our laboratory also using lactoferrin nanogel particles [110], which
454 was further enhanced when electrostatically complexed with another enzyme-unresponsive
455 polysaccharide particle (inulin nanoparticles). Similar results were obtained by Shimoni *et al.* [109],
456 where kinetic stability to gastric coalescence was imparted when lactoferrin nanoparticles were
457 complexed with polysaccharide, such as alginate and carrageenan.
458 In the intestinal phase, there was stark difference in the digestion profile of protein microgel
459 particle-stabilised interface if the digestion was sequential versus if only bile salts-lipase-catalysed
460 (without any protease) scenario was considered. For instance, if it was a sequential gastric and intestinal

digestion [15], presence of WPM particles versus whey protein monolayer at the interface did not affect the rate or extent of fatty acid release as it was a proteolysis-dominated lipid digestion phenomenon. Similar results were obtained using lactoferrin nanoparticle-laden interface that the extent or rate of FFA release was similar to that of a lactoferrin monolayer at droplet surface [111], which might be associated with the proteolytic effect of the pancreatic lipase used that might have contained proteolytic enzyme residues.

To understand this better, we carried out lipid digestion investigations with WPM-laden interface in our laboratory using pure lipase and bile salts without any of the proteolysis that normally occurs during the gastric or intestinal digestion stage [15]. The extent of FFA release was reduced by two folds (20%) and initial rate of digestion was diminished (Table 1) when exposed to just pure lipase as opposed to proteolytic-lioplytic mixture. This suggests the inability of bile salts to displace the intact non-proteolysed WPM from the interface. In other words, a large portion of the surface was not available for the adsorption of the lipase/colipase complex. This reduced the overall rate of FFA generation. However, it is noteworthy that the Pickering layer of particles was not impervious but rather semipermeable. Bile salts and lipase being small molecules could access the interface through the gaps in the microgel-stabilised interface, similar to that discussed in starch granule-stabilised interface [81], but bile salts could not displace the microgel particles, due to the very strong binding of the WPM to the interface.

Protein particles from plant sources. There is burgeoning research interest in designing biocompatible particles derived from plant proteins due to their limited contribution to environmental footprints as compared to the counterparts derived from animal proteins. Filippidi *et al.* [112] took the advantage of water insolubility and slow protease-induced digestibility of zein particles (a prolamin rich protein from corn) to create Pickering emulsions. As discussed in the WPM digestion study [15], the rate-limiting step was again the full or partial hydrolysis of zein particles by gastric/ intestinal proteases, which eventually allowed access of the lipase to the inner lipid core. However, intelligent design of the zein-particulate shell created by solvent precipation to a greater thickness (4 μm versus 1.5 μm in the thinner layer) slowed the rate and extent of digestion remarkably [112]. Another study highlighted that creating particles using zein hydrogen bonded with tannic acid can be an alternative

approach to provide protection to Pickering emulsion droplets against a harsh gastric environment [113], facilitating a reduction in the release of FFA during *in vitro* intestinal digestion. Similar Pickering layer approach has been also used using gliadin, another prolamine-rich protein precipitated with a flavonoid (proanthocyanidins) to create particles that was successful in reducing the extent of FFA release ($\varphi_{max} \sim 40\%$) [114] (Table 1).

Interestingly, the gastric instability of the plant protein particles was also observed in Pickering emulsions stabilised by karifin particles [115, 116] (Fig. 2), another water-insoluble prolamin protein derived from sorghum. In fact the oil droplets lost their integrity at the end of simulated gastric digestion resulting in macroscopic phase separation as the particle-laden interface was readily pepsinolysed. However, to investigate the possible effect of the kafirin particle layer on lipolysis of emulsified oil droplets, the karifin particle-stabilised emulsions were subjected to intestinal digestion bypassing the gastric regime. As expected, karifin-stabilised droplets showed three-fold slower FFA release kinetics and extent of FFA release ($\varphi_{max} \sim 40\%$) (Table 1) as compared to the surfactant counterpart.

Work on Pickering stabilised emulsions created using pea protein nanoparticle aggregates at pH 3 [117] and heated soy glycinin particles [118] have also shown enhanced protection for delivery of a bioactive (β-carotene). However, careful attention needs to be provided in these studies as the reduced extent of lipid digestion and bioactive release was associated with rheological properties of the gel-like emulsion driven by volume fraction of the droplets rather than interfacial architecture of the particles.

4. Future interfacial design strategies to control digestion profiles

Particle-laden interface offer new opportunities to control digestion profile in addition to the obvious energy barrier-associated mechanism *i.e.* almost irreversibly anchored particles are unable to be desorbed by bile salts and other lipid digestion metabolites. We now propose a list of design strategies that can be used to manipulate interfacial architecture and composition of particles to enable them to act as transient or complete mechanical barrier to the diffusion of lipases in both gastric and intestinal phases.

4.1 Particle to droplet size ratio

Particle size of the Pickering stabilizer plays a determinant role in the fate of the lipid digestion kinetics. According to equation (2), the detachment energy for particles is proportional to the square of the radius of the particle. Thus, the larger the size of the particle, the higher the thermal energy required to dislodge them from the interface. However, one has to be careful about the role of gravitational force versus thermal energy in such a scenario. The ratio of gravity forces to surface tension forces for a particle adsorbed at an interface is represented by Bond number as shown in equation (4):

$$B_o = \frac{(\rho_p - \rho_c) g d_p^2}{\gamma_{ow}} \quad (4)$$

where, ρ_p and ρ_c are the densities of the particle and the continuous phase, respectively, d_p is the average diameter of the particle, γ_{ow} is the surface tension and g is acceleration due to gravity. So, it is only for particles with $B_o << 1$, surface-tension forces tends to dominate [119]. Furthermore, if the particles are too small, eventually the desorption energy required to dislodge the particles will be low, specifically in presence of the bile salts, where γ_{ow} is small. Thus, use of particle-laden interface might not be beneficial in such a case.

Also, it is worth to remind that mostly particle-laden interfaces are generally far from possessing a complete monolayer at the interface and even if the Pickering layer is complete, inter-particle gaps remain. These gaps will tend to allow the passage of the lipase-colipase/ bile salts to the bare interfaces, as discussed previously. For instance, for an idealized case of monodispersed spherical particles, the highest surface coverage is achieved when particles on the droplet surface are arranged on a regular 2D triangular lattice. In such a scenario, the typical dimensions of the gaps between the particles will be $\left(\sqrt{3}-1\right) {}^{d_p}/_2 \cong 37\ nm$ for particles of size $d_p = 100\ nm$. This is nearly fifteen-folds higher than the typical dimension of lipase/colipase complex and thus may not have a substantial impact on creating a barrier against the diffusion of lipolytic catalysts to the droplet interface [16]. Even though the enzymes and bile will be able to access the droplet surface through the gaps between the particles,

542 the available amount of interface can be envisaged to be significantly reduced by the presence of the
543 particles, hence limiting the rate of lipolysis.

544 It is noteworthy that size of particles also determine the size of the emulsion droplets and that
545 the particles are significantly smaller than the targeted emulsion droplet size (at least one-to-two orders
546 of magnitude). Thus, lipid digestion kinetics largely depends on the available surface area of the
547 droplets. For example, emulsions with smaller droplet size will present a larger surface area and
548 therefore a greater number of anchoring sites for lipase. Consequently, to reduce the rate and extent of
549 lipolysis, a coarser emulsion with larger droplet size and lower surface area may be preferable. The
550 alternative to reduce these interstitial spaces will be to employ "polydispersity" of size distribution as a
551 tool to increase surface packing. Although colloid scientists have attempted to create monodisperse
552 particles and eventually monodisperse droplets, polydispersity of particles can be an elegant technique
553 to reduce inter-particle spaces and thus lipid digestion rate, provided such polydispersity is well-
554 controlled. Therefore, there exists a delicate compromise for determining the particles of optimum size
555 distribution to achieve the ideal energy barrier, gap dimension and consequently the droplet size.
556 Ultimately, this may govern the rate of diffusion of lipase and surface area available for lipolysis.
557 Finally, it is worth mentioning that it is always important to provide the FFA release data with the droplet
558 size so that a comparison can be made with the literature, as Pickering emulsion droplets are generally
559 larger in size as compared to surfactant-stabilised emulsions. So, it is important to understand whether
560 the reduction in lipolysis kinetics is linked to the size of the droplets or interfacial architecture created
561 by the Pickering layer of particles

562

563 *4.2 Particle charge*

564 Adsorbed charged particles can be useful to provide electrostatic repulsion between the oil droplets of
565 an o/w emulsion in a similar fashion as ionic emulsifiers. Moreover, they may also help to create an
566 electrostatic barrier to the possible approach of negatively-charged bile salts to the vicinity of a
567 negatively-charged particle-laden interface. However, with increasing magnitude of charge of the
568 particles, there will be a corresponding increase in the interstitial separation due to particle-particle
569 electrostatic repulsion at the interface. Such an approach can be useful to create a porous membrane at

570 the interface in order to accelerate digestion or enhance release of digestion products. However, if the
571 objective is to slow down digestion, one might attempt to increase the salinity of the system to screen
572 the charge so that the particles aggregate at the interface and provide the interfacial barrier effects to
573 lipolysis as was observed in silica-nanoparticle-stabilised interfaces [111].
574
575 *4.3 Particle fusion at interface*
576
577 One of the approaches that has been used to reduce this interfacial spacing has been to adopt the
578 complementary "interfacial particle fusion" *i.e.* sintering of particles once they have been already
579 adsorbed (Fig. 3a). This helps to take the advantage of the energy barrier of the particles combined with
580 a mechanical barrier effect of a cohesive "single" bulky layer. Interestingly, physical treatments, such
581 as use of heat, has been shown to fuse the particles once they are already absorbed in the interfaces.
582 This approach has been used in two independent studies using starch granules [99, 108] (Fig. 3b) and
583 whey protein microgels [16] (Fig. 3c) and has shown dramatic effects on improving the barrier property
584 and delay in FFA release. Since the gaps are expected to be significantly smaller due to this particle
585 fusion, the approach impedes all aspects of the process *i.e.* the diffusion of lipolysis-limiting digestion
586 products, such as FFA away from the reaction sites, as well as that of the lipase/colipase-bile salt
587 complex to the surface. Such fusion can also be brought about using pH shifts, using mono- and divalent
588 ions and/ or enzymatic crosslinking, depending upon the responsiveness of the particles to these aspects.
589 A study of such aspects will demand future research work.
590
591 *4.4 Particle shape*
592 As expected, the "spherical" particles have primarily been investigated to create Pickering
593 emulsions from inorganic silica nanoparticles to bio-derived protein microgels. However, the influence
594 of particle shape on interfacial packing and emulsion stability has attracted relatively little research
595 attention. The non-spherical particles, such as rods, cubes, peanut-shaped particles (Fig. 4) tend to have
596 more positive effects on the emulsion stability *via* differences in packaging density [120, 121]. Even at
597 a lower concentration, particles with higher aspect ratio, such as rod- and peanut-shaped particles can
598 improve the interfacial loading tremendously by creating interfacial stacking, leading to some sort of

599 interlocking structure at the interface. This is demonstrated by hermatite particles [120] and CNC
600 particles [121] in Fig. 4. For instance, Capron et al. [122] created stable o/w emulsions using <0.1 wt%
601 of CNC, as a result of the entanglements between the CNCs. Recently, lipid digestion work using rod-
602 shaped cellulose nanocrystals either at the surface or as a barrier layer are showing interesting lipid
603 digestion outcomes, as discussed previously [28, 29, 86]. In fact, now the time seems ripe for colloid
604 scientists to explore bio-derived anisotropic shaped particles, considering biocompatible processing
605 routes to create such particles and models to understand their emulsion stability during lipid digestion.
606 Such particles may help to control lipid digestion rate by particle networking attributes, not only at the
607 interface but also in the bulk phase as shown in Fig. 2 [30].
608
609 *4.5 Particle loading at the interface*
610
611 Under given emulsification conditions, the initial particle loading can influence the interfacial packing
612 behavior and structure, furthermore affecting the average droplet size and emulsion stability. Once
613 close-packing is established, the loading ceases to have an effect. For classical emulsions stabilised by
614 surfactants, the average droplet size reduces with increasing surfactant concentration until reaching a
615 critical concentration point and then droplet size reaches a plateau [4]. The decreasing trend of droplets
616 size relates to the process that larger droplets breakdown into smaller droplets creating more surfaces
617 due to the reduction of interfacial tension. Pickering emulsions also follow a similar trend in a classical
618 emulsion [43].
619 Chevalier et al. [45] summrized the three stages of Pickering emulsion formation based on the ratio
620 between solid paticles *(M_p)* and the oil mass *(M_o)*. At the low concentration, coalescence occurs due to
621 insufficient quantity of solid particles to cover the droplets. As the solid particle concentration increases,
622 the larger droplets are broken into smaller droplets, leading to a reduction in the average droplet
623 diameter (D_o) as shown in equation (5):
624
625 $$D_o = \frac{6}{\rho_0 a_s}\frac{M_o}{M_p} \quad (5)$$

626 where, ρ_o is the density of oil and a_s is the interfacial area covered by the solid particles. In the final

627 stage, the average droplet size reaches a steady state where a complete saturation in achieved. With a
628 high particle concentration, the excess solid particles create either a multilayer or do not get absorbed
629 to the interface and remain dispersed in the continuous phase. With fewer particles, a typical interfacial
630 structure can be a single bridging layer stabilizing the interface between two droplets [123]. Such
631 bridged network can be actually useful to create a network and reduce the surface available for lipase
632 to bind. Note that for incorrectly chosen contact angles, such bridging might instead cause coalescence
633 of droplets. Another option can be to increase the mechanical barrier of the interface by creating a
634 multilayered particle architecture [124] and eventually increase the adsorbed particle loading. If
635 carefully controlled, such multilayers of Pickering particles can help to form a tortuous path for the
636 diffusion of bile salts and lipases' ability to anchor to the interface and thus control the kinetics of lipid
637 digestion.
638
639 *4.6 Material chemistry of particle*
640 Of course, the material of the particles has a crucial role in determining their fate during the sequential
641 lipid digestion. In mind of the previous discussion, it is clear that human enzyme-responsive particles,
642 such as unmodified protein-based or starch-based particles might not be suitable to delay lipolysis if
643 sequential three-phase digestion is considered. As the particles are significantly influenced by
644 biochemical interferences due to their responsiveness to physiological enzymes, they lose their
645 particulate integrity before and during the lipolysis step. Hence, enzyme-responsive particle-stabilised
646 emulsions can only offer altered digestion profile if they are administered specifically at the site rather
647 than typical oral administration routes. One way to avoid this is to modify the interfacial thickness of
648 the particles by precipitation techniques [18] or protect the droplets from instantaneous digestion by
649 changing the particle material chemistry. Such modifications in particle chemistry can be obtained by
650 complexation with human enzyme-unresponsive polysaccharides, such as alginate [111, 125, 126], or
651 tannic acid/ flavonoids [113, 114] or binding with or another layer of enzyme-unresponsive particles,
652 such as inulin [110].
653 Interestingly, from an environmental viewpoint plant proteins are preferred for creating emulsifiers.

In fact most plant proteins have limited aqueous solubility [127-130] and are less digestible as compared to animal proteins [18, 130], which restricts their use as classical biopolymeric surfactants. This offers a great window of opportunity for colloid scientists to utilize plant protein aggregates or synthesize particles derived from plant proteins for altering lipid digestion profile. This has indeed started to capture attention of researchers relatively recently. Other opportunities include hydrophobic modification of enzyme-unresponsive polysaccharide particles for tailoring lipid digestion, though careful attention needs to be taken to allow compliance as being "safe" and also "clean-label", if food application is the ultimate goal.

5. Mathematical models for lipid digestion kinetics

In this section, we shift our focus from experimental investigations to review theoretical models which is crucial to quantify lipid digestion kinetics and understand mathematically the similarities and dissimilarities of Pickering emulsions versus a classical surfactant stabilised emulsions. Typically, the pH-stat method is used to monitor the concentrations of FFAs released during the lipid digestion upon introduction of lipase at neutral pH levels. Experimental procedures may commit emulsions to simulated digestion processes by initial incubation in simulated gastric fluid (SGF) and then subsequently in simulated intestinal fluid (SIF). Monitoring and control of changes in pH permit quantification of the concentration of FFA generated during digestion of the emulsified lipids. The percentage of FFA released may be calculated from the number of moles of a base *e.g.* NaOH required to neutralize the FFA that could be produced from the triacylglycerols present in the lipid under the assumption that 2 FFAs per triacylglycerol molecules are generated as can be seen in equation (6) [36, 131]:

$$\%\boldsymbol{FFAs} = \frac{\boldsymbol{V_{Base} \times M_{Base} \times M_w}}{\boldsymbol{2W_w}} \quad (6)$$

where, V_{Base} is the volume (mL) and M_{Base} the molarity (M) of the base respectively, M_w is the average molecular weight of the lipid (kg mol^{-1}) and W_w is the initial weight of the lipid (g). We denote the fraction of converted lipid in the emulsion at time t by $\alpha(t)$, which is equivalent to the fatty acid released $\varphi(t)/\ \varphi_{max}$ where φ_{max} is the maximum fatty acid level attained after digestion.

A major consideration in developing models to capture possible delays in digestion of emulsion droplets is to correctly account for the kinetics of arrival and adsorption of enzymes onto their surface. This is particularly important given that much of the discussion above concerns development of suitable means of slowing down such adsorption process. It is reasonable to assume that at any time *t,* the reaction rate will be a first order one varying linearly as the fraction of unconverted oil in a droplet, namely $(1-\alpha(t))$. This situation is true for relatively small droplets one encounters in most practical emulsion systems. For these, the composition of the droplet remains homogenous throughout, as would be the case if there is rapid diffusion of unconverted/converted oil between the surface and interior of the droplet (i.e. concentration gradients within the fine droplets remain small). Furthermore, since such reactions only occur at the surface of the droplets where the lipase is adsorbed, the reaction rate is expected to be proportional to the coverage of surface by enzyme at any given time t, i.e. $\Gamma(t)$. Accordingly, sub-maximal conversion rate constant (per unit area) at time t may be expressed as:

$$\frac{\boldsymbol{\Gamma(t)}}{\boldsymbol{\Gamma^{Max}}}\boldsymbol{k} \tag{7}$$

where it is assumed that the surface coverage achieves a maximum of Γ^{Max}, whereupon the lipid conversion rate constant also achieves its maximum value. Here, k (mol s^{-1} m^{-2}) is defined as lipid conversion rate per unit area of the droplet surface, occurring at maximum lipase surface coverage. In its simplest form, we may expect the value of k to be proportional to the exposed part of the surface, namely $k=k_o(1 - S_p)$. Here, S_p is the fraction of the surface covered by the particles and k_o represents the rate of hydrolysis per unit area if all the surface of the droplets

was available for adsorption by lipase. The value of S_p itself is a sensitive function of the degree of polydispersity, the shape of the particles, the contact angle at surface and any possible inter-particle interactions. This makes it rather difficult to calculate S_p for a general case. However, for some specific ideal situations, a value may be given. For examples, if the particles were monodispersed hard spheres, then at maximum packing, obtained for a regular 2D triangular lattice arrangement (see section 4.1), the value of $S_p \approx 0.9$ if the contact angle is 90 °, and ≈0.45 when it is 45 ° [16]. Typically estimates for Γ^{Max} coverage can be calculated for the lipase-colipase complex using the molecular radius of gyration (*e.g.* 25Å providing an estimate of 2.66 x 10^{-7} moles m^{-2}) [132]. Thus, for a droplet of size d_0

$$\frac{\pi d_0^3}{6}\frac{\rho_0}{M_w}\frac{d\alpha}{dt} = \frac{\Gamma(t)}{\Gamma^{Max}} k\pi d_0^2(1-\alpha) \tag{8}$$

where πd_0^2 is the surface area of the droplet and M_w the molar weight of the lipid molecules. The general formal solution to the above equation is

$$\alpha(t) = 1 - exp\left(-\frac{6M_w}{d_0\rho_0}\frac{k}{\Gamma^{max}}\int_0^t \Gamma(t')dt'\right) \tag{9}$$

Different more specific forms of equation (9) now emerge depending on how $\Gamma(t)$ varies with time. Historically, to gain insight into the dynamics of lipid digestion, a first order rate kinetics model was initially introduced by Ye *et al.* [133]. This early model amounts to assuming that the adsorption kinetics of lipase onto the droplet surface is very rapid. In such a case the full lipase coverage is achieved almost immediately and $\Gamma(t) \sim \Gamma^{Max}$ from the onset. This situation would be appropriate for Pickering type emulsions, since the gaps between the adsorbed particles on the interfacial surface are sufficiently large to allow unhindered access of the lipase [16], given that the radius of gyration of the pancreatic lipase/co-lipase complex is approximately 25 Å [132]. Similarly, the long-time behaviour of any adsorption model, once

full coverage has been achieved past a reasonable period of time, increasingly approaches that predicted by the model of Ye *et al.* [133]. With $\Gamma(t)$ set to Γ^{Max} throughout the digestion process, equation (9) simplifies to (10) below:

$$\boldsymbol{\varphi(t) = \varphi_{Max}(1 - exp(-k_1 t))} \tag{10}$$

Here, k_1 (s^{-1}) is the rate of first order kinetics and *t is* the digestion time (s) in Ye et al model and can be related to parameters of the more general model according to $k_1 = 6M_w k/(d_0\rho_0)$. This model has successfully been used by the same authors to explain the effect of calcium concentration in promoting lipase adsorption at the interfacial surface. The model has also proved useful in interpretation of experimental data, allowing an understanding of differences in reaction rates of emulsions stabilised by different interfacial materials (e.g. bile salts, protein, protein-particle composites) [29, 36] to be achieved.

When the dynamics of enzyme adsorption process is not sufficiently fast, it becomes important to take the variation of $\Gamma(t)$ with time into account. If arrival of the lipase to the interface is the limiting factor in determining the rate of adsorption to the surface, then for such a diffusion-limited process, $\Gamma(t) \approx [(2Dt/d_0) + 2(Dt/\pi)^{1/2}]n$ in the early stages of adsorption, where D denotes the diffusion coefficient of the enzyme (typically 10^{-9} – 10^{-10} m^2 s^{-1}) in the continuous aqueous phase and n is their molar concentration in the bulk solution. Substituting this form of $\Gamma(t)$ in equation (9) gives:

$$\alpha(t) = \frac{\varphi(t)}{\varphi_{Max}} = \left[1 - exp\left(\frac{-6knM_w}{\rho_0 d_0^2 \Gamma^{Max}}\left(Dt^2 + \frac{4d_0\sqrt{D}}{3\sqrt{\pi}}t^{3/2}\right)\right)\right] \tag{11}$$

The second term in the above exponential becomes much smaller than the first one for time periods $t \gg d_0^2/D$. For typical values of D and emulsion drops of size say 10 μm, this occurs

very early on, $t \sim 0.1$ to 1 seconds. Thus, while still in rather early stages of digestion, equation (11) can be further approximated to

$$\alpha(t) = \frac{\varphi(t)}{\varphi_{Max}} = \left(1 - exp\left(\frac{-6kM_w Dnt^2}{\rho_0 d_0^2 \Gamma^{Max}}\right)\right) \tag{12}$$

Equation (12) has a more convenient form for fitting to experimental data. The half-life ($t_{1/2}$) *i.e.* the time interval whereby half of the initial amount of lipids has been converted to FFA, for each of the two cases discussed above can be obtained by setting $\alpha(t)=1/2$ in equations (10) or (12). This yields

$$\boldsymbol{t_{1/2} = \left(ln(2)\frac{d_0^2 \rho_0 \Gamma^{Max}}{6kDnM_w}\right)^{1/2}} \tag{73}$$

$$\boldsymbol{t_{1/2} = ln(2)\frac{d_0 \rho_0}{6kM_w}} \tag{14}$$

Utilising the parameters k and half-life ($t_{1/2}$) provide valuable information with which to compare the digestion profiles of different emulsion samples. For example, Sarkar *et al*. [16] successfully used the above models to understand the differences in the digestion behaviour of Pickering type emulsions, stabilised by whey protein microgel particles, before and after their heat treatment. An interesting prediction of equation (12) is a rather slow start to hydrolysis and an initial "convex shaped" curve (Fig. 3c) for the variation of amount of converted lipid with time. Though regularly seen in experiments, prior to the work of Sarkar *et al.* [16], this feature was not fully appreciated and had often been ignored during the fitting of the data.

781 The linear variation of the coverage of interface by lipase with time, can also be applied
782 to analyse the early stages of digestion in systems where the adsorption is barrier limited. For
783 these
784
785 $$\Gamma(t) \cong \lambda n t \qquad (13)$$
786
787 where instead of $\lambda = 2D/d_0$ as for the diffusion limited case, now the value of λ depends on
788 the thickness, the material and porosity of the barrier layer formed around the droplets. Once
789 again it should be noted that equation (13) applies to time frame where the surface of droplets
790 are only scarcely covered and as yet far from reaching their maximum saturation by bile
791 salt/lipase. There are many models for the variation of $\Gamma(t)$, encompassing the entire adsorption
792 period for the barrier limited situation. However, the usefulness of these in the context of
793 digestion of lipid emulsions is as yet to be fully established. Therefore, for now at least, we
794 refrain from discussing these type of models in any greater detail, leaving such discussion to
795 possible future reviews.
796 The models discussed thus far all assume emulsion droplets that more or less maintain
797 their original size, as lipid is converted to FFA. This is a reasonable assumption for Pickering
798 type emulsions, as was discussed earlier. When the emulsions are stabilised by molecular layers
799 of surfactants or proteins, the picture alters significantly. For now such emulsifiers are displaced
800 by bile salt and in turn any generated FFA can favourably partition into the aqueous phase
801 without much difficulty, resulting in a shrinkage of droplet size. At the same time, the decrease
802 in the surface area that such shrinkage entails may result in desorption of bile salt/lipase,
803 depending on the kinetic of desorption and amount of enzyme already accumulated on the
804 surface at any given time. It is quite reasonable to consider droplets as entirely consisting of
805 unconverted lipids throughout the process, given that originally the aqueous phase is devoid of
806 FFA and the strong tendency of fatty acids to partition into water. This is to say that all
807 generated FFA migrate immediately out of oil droplets. With this assumption, and once again

808 considering the fact that hydrolysis only occurs at the surface, the governing equation for the
809 variation of droplet size, d, with elapsed time t becomes:
810
811 $$\frac{\pi\rho_0 d^2}{2M_w}\frac{d}{dt}(d) = -\pi d^2 k\frac{\Gamma(t)}{\Gamma^{max}} \quad (14)$$
812
813 Equation (14) together with the initial condition $d=d_0$ at time $t=0$, admits the following general
814 solution
815 $$d(t) = d_o\left(1 - \frac{2M_w k}{d_0\rho_0\,\Gamma^{max}}\int_0^t \Gamma\left(t'\right)dt'\right) \quad (15)$$
816
817 which in turn gives the amount of converted lipid as
818 $$\varphi(t) = \varphi_{Max}\left[1 - \left(1 - \frac{2M_w k}{d_0\rho_0\Gamma^{max}}\int_0^t \Gamma(t')dt'\right)^3\right] \quad (16)$$
819
820 In general it is quite difficult to theoretically determine the form of $\Gamma(t)$, given the possibility of
821 competitive adsorption occurring between the bile salt/lipase and an already existing protein,
822 particularly where such protein has formed a viscoelastic cross-linked surface layer. This
823 situation is further complicated if any subsequent enzyme desorption kinetics is slow compared
824 to the rate of droplet shrinkage, thus giving rise to the possibility of $\Gamma(t)$ exceeding Γ^{Max} at some
825 point during the shrinkage. However, for situations involving none-film forming proteins, as
826 for example casein, or where the emulsion was stabilised by a relatively low molecular weight
827 surfactants one may plausibly assume that such complications do not arise. In other words the
828 kinetic of adsorption and possible desorption of lipase/bile salt are fast enough for enzyme
829 surface coverage to always be maintained at Γ^{Max}, from the very early stages of digestion all
830 the way to the end of the process. With this reasonable assumption, it is easy to see that the
831 general equation ((16) readily simplifies to (17):
832

$$\varphi(t) = \varphi_{Max}\left[1 - \left(1 - \frac{2M_w k}{d_0\rho_0}t\right)^3\right] \quad (17)$$

where as before ρ_0 and M_w are density and molar weight of lipid, respectively and d_0 the initial droplet diameter. The model encompassing the above assumption was first proposed by McClements and Li [131, 134] and later solved by Gaucel *et al*. to yield equation (17) [135]. The model predict a fixed finite time at which droplets will be completely hydrolyzed (*i.e*. shrank to zero). This time is $\tau = \frac{d_0\rho_0}{2M_w k}$, beyond which equation (17) is no longer physical. Another aspect of the model is that as droplets shrink, the surface to volume ratio increases and hence the model predicts a rather rapid upturn in the rate of hydrolysis towards the end of the process. This is thought to not be all that realistic. A possible modifications to overcome this issue has been discussed by Gaucel *et al*. [135]. Once again if one is interested in the half-life ($t_{1/2}$), then using (17) this is found to be

$$\boldsymbol{t_{1/2} = \frac{\rho d_0}{2kM_w}\left(1 - \frac{1}{\sqrt[3]{2}}\right)} \quad (18)$$

In Fig. 5, a schematic representation of the mathematical models applicable to the pathways relevant to surfactant-stabilised or particle-stabilised emulsions. Herein, we provide the modelling pathways relevant to the various droplet behavior and interfacial dynamics during digestion. Under the assumptions of rapid adsorption/desorption of surface enzymes and permissible reduction in droplet size, equations (16) and (17) are relevant where short time digestion and subsequent asymptotic equilibrium is observable. Under conditions where the droplet size remains stable during digestion, i.e. in the case of Pickering emulsion, equation (9) is the appropriate modelling route and where equation (10) is selected for rapid interfacial adsorption and in the cases where interfacial dynamics are anticipated, equation (12) is appropriate. In the latter case it is notable that interfacial adsorption at short-times results in delayed digestion and that the large-time behaviour (marked in red) of both equations (10) and (12) asymptotically stable, approach a constant plateau value.

In this section, we have discussed several different models currently being used in the literature to fit experimental data on digestion of emulsion systems. We have sought to provide a clearer understanding of the connection between various models, by discussing them as

limiting cases of a more generalised situation. In doing so, we highlight the limits of validity and underlying assumption in each case. It is important that some though be given to the type of model that best captures the situation one is investigating, rather than a forced attempt to fit the data to equations for which the underlying model is not necessarily appropriate. When this is done carefully, these models provide useful means to compare the digestion of emulsion systems that possess different surface properties and will facilitate standard parameters to compare reaction behaviour *e.g.* the case where, particles at interface remain intact or fuse together either with or without heat treatments, respectively. We envisage that the future models will provide closed form equations which will accommodate more complex systems where surface and droplet shrinkage dynamics both occur simultaneously.

6. Conclusions

The biophysical aspects of lipid digestion of particle-stabilised emulsions is a rapidly growing research domain owing to its fundamental importance to human biology and numerous industrial applications in food, pharmaceutical, personal care, biomedical sectors, such as, designing systems allowing sustained release of lipophilic molecules. Recently, there has been a substantial improvement in the understanding the role of particle-laden interface on modifying colloidal aspects of lipid digestion. We have provided the first systematic, balanced and comprehensive summary of experimental investigations as well as mathematical models for lipid digestion of Pickering emulsion droplets within this review. The key benefits of using Pickering emulsions is the ability of these particles to resist any competitive displacement by surface active bile salts by virtue of high desorption energies of these particles. Although the gaps between the particles may provide access to the lipolysis enzymes, but the area available for lipase to bind is significantly reduced due to the presence of the particle on the droplet surface, providing further benefits to reduce the kinetics and extent of digestion. Besides intact particles, some attempts have been made in literature to include particle fusion, such as using heat treatments after thermos-labile particles have been adsorbed at the interface in order to

886 reduce those interstitial pores and consequently, delay lipid digestion. Particle shape anisotropy
887 has also been shown to offer interesting features in altering digestion profile by not only
888 influencing interfacial adsorption and surface packing to reduce interfacial porosity but also
889 impacting bulk interactions with key lipid digestion players. In order to adequately compare
890 different mathematical models ranging from simple first order kinetics to those that account for
891 kinetics of lipase-binding and interfacial dynamics, a decision tree has been proposed based on
892 underlying assumptions and boundary conditions to ensure validity of the models. In summary,
893 in order to adequately manipulate lipid digestion kinetics, we propose specific interfacial design
894 strategies for future investigations focussing on particle physics and chemistry as well as the
895 development of closed-loop mathematical models.
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1233 **Table 1.** Summary of literature reports on Pickering O/W emulsions where *in vitro* lipid digestion kinetics

1234 was followed.

1235

Type of particles (*E = Human enzyme-responsive, U = Human enzyme - unresponsive*)	**Particle Shape & Size Range** (*d_p = diameter, l_p = length, h_p = height, t= shell thickness*)	**Emulsion droplet size** (μm)	***In vitro* digestion regimes**	**Intestinal lipid digestion with kinetic data**			**Reference**
				k	φ_{max} (%)	$t_{1/2}$ (min)	
Silica nanoparticles (*U*)	Spherical, d_p= 50-500 nm	4 – 8	Oral, Intestinal	≈control	~50%*, <control	-	[83, 85]
Nanofibrillated cellulose (NFC) (*U*)	Fibre, d_p= 57 nm l_p = Several μms	9 – 24 μm	Sequential (Oral-Gastric-Intestinal)	7.5-12% FFA/ min*, <control	~78-84%*, < ontrol	-	[86]
Chitin nanocrystal (*U*)	Rod, d_p= 18 nm l_p = 240 nm	5 – 7 μm	Intestinal	2.0×10^{-4} s^{-1}, <control	33%, <control	-	[17, 30, 92]
Chitosan-tripolyphosphate nanoparticles (*U*)	Spherical, d_p= 214–522 nm	19 – 86 μm	Sequential (Oral-Gastric-Intestinal)	-	33%, <control	-	[95, 96]
Flavonoid glycosides from *Ginkgo biloba extracts* (*U*)	-	1 μm	Intestinal	0.13 μmol s^{-1} m^{-2}, <control	33%, <control	9.8, >control	[98]
Starch granules (*E*)	Polyhedral, d_p= 1.8 μm	27 – 32 μm	Oral, Gastric, Intestinal	Relative lipolysis rate: heated < not heated	-	-	[81, 99, 108]
Starch granules with heat treatment (*E*)					-	-	
Whey protein microgel particles (WPM) (*E*)	Spherical, d_p= 300 nm	43 μm	Sequential (Gastric-Intestinal), Only Intestinal step without proteases**	0.31** μmol s^{-1} m^{-2}, <control	42%, 20%**, <control	6.6, 16.5**, >control	[16]
Whey protein microgel particles with heat treatment (HT-WPM) (*E*)				0.35** μmol s^{-1} m^{-2}, <control	42%, 16%**, <control	6.6, 44.4** >control	
Lactoferrin nanoparticles (LFnp)	Spherical, d_p= 200 – 400 nm	1 – 11 μm	Sequential (Oral-Gastric), Intestinal	≈control	~64%*, ≈control	-	[109, 111, 126]
Lactoferrin nanoparticles complexed with alginate (LF-ALG) (*E/ U*)		1 – 56 μm		<control	~50%*, <control	-	
Lactoferrin nanoparticles complexed with carrageenan (LF-CAR) (*E/ U*)		3 – 9 μm		>control	~70%*, >control	-	
Zein particle shell (*E*)	Spherical, t = 1.5 – 4 μm	30 – 40 μm	Sequential (Gastric-Intestinal l)	<control	~40-100%*, <control	-	[112]
Zein + tannic acid (*E*)	-, d_p= 96 –2 03 nm	25 – 45 μm	Sequential (Gastric-Intestinal)	<control	~4-6%*, <control	-	[113]

Gliadin + aproanthocynaidins (*E*)	Spherical, d_p= 87-290 nm	53-94 μm	Sequential (Gastric-Intestinal)	<control	~40%, <control	~40*, >control	[114]
Karifin nanoparticles (*E*)	d_p= 92 – 434 nm		Gastric, Intestinal	<control	~38%, <control	-13.23***, > control	[115, 116]

- k = rate of lipid digestion, φ_{max} = maximum extent of FFA release (observed or theoretical), $t_{1/2}$ = time interval whereby half of the initial concentration of lipids has been converted to FFA (observed or theoretical)

No data (-), Derived data from the graphs (*), Bypassed proteolysis (**), Indicative