Article:
Torres, O, Murray, B orcid.org/0000-0002-6493-1547 and Sarkar, A orcid.org/0000-0003-1742-2122 (2016) Emulsion microgel particles: Novel encapsulation strategy for lipophilic molecules. Trends in Food Science & Technology, 55. pp. 98-108. ISSN 0924-2244

https://doi.org/10.1016/j.tifs.2016.07.006

Emulsion microgel particles: Novel encapsulation strategy for lipophilic molecules

Ophelie Torres [1], Brent Murray [1] and Anwesha Sarkar [1]*,

[1] Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

*Corresponding author:

Dr. Anwesha Sarkar

Food Colloids and Processing Group,

School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK.

E-mail address: A.Sarkar@leeds.ac.uk (A. Sarkar).

Tel.: +44 (0) 113 3432748.

Abstract

Background

Lipophilic molecules such as flavours, essential oils, vitamins and fatty acids are difficult to deliver in food matrices owing to their limited solubility, rapid oxidation and degradation during physiological transit. Among the technologies available to deliver lipophilic molecules, emulsion microgel particles are a relatively new class of soft solid particles of discrete size, shape, and interesting release properties.

Scope and approach

Relevant literature concerning the processing of emulsion gels and emulsion microgel particles has been reviewed. Factors affecting the mechanical properties of protein-stabilized emulsion gels with key emphasis on the role of "active" and "inactive fillers" are discussed. Technologies for creation of emulsion gel particles using top-down and bottom-up approaches has been covered. Special attention was dedicated to the release mechanisms from emulsion microgel particles via swelling and erosion.

Key findings and conclusions

Emulsion gels with "active fillers" offer the potential to create emulsion microgel particles using top-down approach. Polymer extrusion, multiple emulsion templating, fluid gels are few routes for creating emulsion microgel particles using bottom-up approaches. Although whey protein has been well researched, modified starch, plant proteins need to be investigated for design of new emulsion microgel particles that can act as surfactant and bulk gelling agents in their own right through intelligent tuning of processing conditions. If designed carefully with an end goal of "controlled delivery" in mind, responsiveness to oral temperature, gastric enzymes, intestinal pH etc, can be built into emulsion microgel particles so that they may find novel applications in food, pharmaceutical and personal care industries.

Key words

Emulsion microgel particle; filler-matrix interaction; whey protein; swelling, matrix erosion

1. Introduction

Lipophilic active molecules, such as fat soluble vitamins, flavourings, fatty acids and essential oils pose challenges for their application in food matrices as they are water insoluble. They tend to oxidize rapidly in the presence of air, light and heat. Additionally, due to their hydrophobic nature, most of these compounds are difficult to deliver in human physiology and are generally partially absorbed by the body or their biological activity is partly or fully degraded during their transit. Thus, there is a huge need to protect these lipophilic compounds without environmental degradation and tailor their release at a physiological site, such as burst release of flavours or essential oils in mouth or protect the omega-3 fatty acids during gastric transit and release them in the intestine.

A wide range of technologies have been developed to encapsulate lipid molecules, such as emulsions, emulsion gels, liposomes, micelles, nanoparticles, etc. Each of these have their own specific advantages and disadvantages in terms of protection, delivery, cost, regulatory status, ease of use, biodegradability and biocompatibility (McClements & Li, 2010). Among these, emulsions gels are an alternative technique that allows stabilization and delivery of lipophilic compounds in food matrices. Emulsion gels are frequently produced in food products, such as, sausages, yogurt, dairy desserts, cheese, etc. (Mun, Kim, Shin, & McClements, 2015). Currently, there has been an upsurge in research efforts in the domain of emulsion gels resulting in engineering of novel soft solids, such as emulsion fluid gels and emulsion microgel particles. To understand different terminologies used in the literature, definitions of each of these classes of emulsion gels with their corresponding microstructures are included in Table 1.

Emulsion microgel particles are a relatively new class of soft solids, particularly in food research. Emulsion microgel particles have similar polymer chemistry to emulsion gels though their physical arrangement and scale is different. Both emulsion gels and emulsion microgel particles have oil and gel phases but microgels are much smaller discrete particles with well-defined spherical shape (Thorne, Vine, & Snowden, 2011). In emulsion gels, the emulsion droplets are stabilised by emulsifiers and heterogeneously distributed in a continuous gel matrix whereas in emulsion microgel particles, emulsion droplets are stabilised by an emulsifier and gelling agent, creating a soft solid shell around several emulsion droplets which are then incorporated into a continuous gel matrix. Therefore, in emulsion gels before gelation of the matrix, emulsion droplets are rather mobile due to Brownian motion and can be unstable due to faster flocculation, coalescence and creaming. Meanwhile, in emulsion microgel particles, several emulsion droplets are entrapped into a soft solid shell providing better control of droplet size, mobility and mechanical properties (Mun, Kim, & McClements, 2015; Ruffin, Schmit, Lafitte, Dollat, & Chambin, 2014; Zhang, Zhang, Decker, & McClements, 2015; Zhang, Zhang, Tong, Decker, & McClements, 2015). Additionally, microgel particles have been demonstrated to protect against oxidation lipophilic compound such as polyunsaturated fatty acids (Augustin & Sanguansri, 2012; Beaulieu, Savoie, Paquin, & Subirade, 2002; Chung, Degner, Decker, & McClements, 2013; Mao & Miao, 2015; Matalanis, Jones, & McClements, 2011; Velikov & Pelan, 2008).

The microgel particle encapsulation method has been described as “smart” because the size, physicochemical properties of these particles are tuneable and allow the microgel to swell or de-swell, as well as degrade in response to specific temperature, pH, ionic strength, enzymatic conditions (Ballauff & Lu, 2007; Kawaguchi, 2014; Shewan & Stokes, 2013; Wei, Li, & Ngai, 2016). Hence, emulsion microgel particles can be effective for site-dependent release of lipophilic bioactives (Ching, Bansal, & Bhandari, 2016). For instance,

93 incorporation of filled hydrogel particles in low fat dairy products have been found to retain
94 the sensory attributed of the dairy product by controlling the release of lipophilic aroma and
95 mimicking fat droplet functionality (Chung, et al., 2013; Joye & McClements, 2014; Malone
96 & Appelqvist, 2003; Malone, Appelqvist, & Norton, 2003; Oliver, Berndsen, van Aken, &
97 Scholten, 2015; Oliver, Scholten, & van Aken, 2015; Pizzoni, Compagnone, Di Natale,
98 D'Alessandro, & Pittia, 2015; Zhang, Zhang, Chen, Tong, & McClements, 2015). Hydrogel
99 particles encapsulating hydrophilic compounds have been well studied and reviewed by Joye
100 and McClements (2014) and McClements (2015) as well as protein-based microgels has been
101 investigated by Dickinson (2015). Nevertheless to our knowledge, no review on emulsion
102 microgel particles encapsulating lipophilic compounds is available. Hence, this review aims
103 to detail the formation of emulsion microgel particles and their application for controlled
104 release of lipophilic compounds.
105 We begin by covering the basic processing steps of emulsion gels since this sets the scene for
106 the top-down approach of making emulsion microgel particles from the parent emulsion gel.
107 In the second section, we discuss the role of oil droplet, "filler" or gel "matrix", and
108 interactions that govern the mechanical properties of emulsion gels. We have focussed
109 mainly on whey protein (from bovine milk) and also covered the few available publications
110 on modified starch-based systems, since both these biopolymers have potential to act as
111 surfactants and gelling agent when subjected to suitable processing conditions. The third
112 section deals with the bottom-up approach of preparation of emulsion microgel particles
113 using polymer extrusion, multiple emulsion-based templating or the fluid gel route. Finally,
114 we discuss the different release mechanisms of these emulsion microgel particles.
115

2. Formation of Emulsion Gels

The formation of emulsion gels is generally a two-step process as shown in Figure 1. The first step involves the formation of an oil-in-water emulsion. During high shear mixing, such as high pressure homogenization, colloid milling, etc., globular whey proteins unfold and adsorb onto the surface of oil droplets due to their surface active properties, decreasing the interfacial tension between the oil and aqueous phase and stabilizing the oil droplets via the electrostatic stabilization (Dickinson, 2012; Kakran & Antipina, 2014; Sala, de Wijk, van de Velde, & van Aken, 2008; Sarkar & Singh, 2016). The second step involves the formation of a three-dimensional protein network entrapping the emulsified oil droplets by gelling the continuous phase (Figure 1) by heat, salt and/or acid treatment.

In the same way, modified starch, which has been modified by attaching hydrophobic octenyl succinic acid moieties has been well reported in literature as both an emulsifier and starch is well known as a thickening agent. Because of the free carboxylic acid side chain present in OSA, OSA-starch could be considered as a weakly negatively surface active charged polyelectrolyte (Shogren, Viswanathan, Felker, & Gross, 2000). Tesch, Gerhards, and Schubert (2002) investigated the use of OSA starches as a surfactant. They reported that OSA starch has similar surface activity and surface tension to whey protein due to its amphiphilic nature (Wang, et al., 2011). The stabilisation mechanism imparted to emulsions is primarily steric due to the adsorbed branched amylopectin chains (Chivero, Gohtani, Yoshii, & Nakamura, 2016; Domian, Brynda-Kopytowska, & Oleksza, 2015; Ettelaie, Holmes, Chen, & Farshchi, 2016; Tesch, et al., 2002). Many authors have been studying the gelatinization properties of OSA starches since compared to native starches which swell and melt at high temperature, OSA-starches exhibit lower gelatinization temperatures (Bao, Xing, Phillips, & Corke, 2003; Bhosale & Singhal, 2007; Ortega-Ojeda, Larsson, & Eliasson, 2005; Sweedman, Tizzotti, Schäfer, & Gilbert, 2013; Thirathumthavorn & Charoenrein, 2006).

OSA-starches cold gelatinization properties have been attributed to the weakening of the interactions between amylopectin and amylose, caused by the improved steric repulsion disrupting starch crystalline structure after OSA modification, increasing the solubility of the modified starch and allowing OSA starch to entrap higher amounts of water (Ettelaie, et al., 2016; Sweedman, et al., 2013). Additionally, not all hydrophobic groups on the backbone of the polymer adsorb at the oil-water interface thus, hydrophobic interaction between OSA chains on neighbouring amylopectin branches can enhance the viscosity of the solution and form a polymer network (Ettelaie, et al., 2016; Ortega-Ojeda, et al., 2005; Sweedman, et al., 2013; Thirathumthavorn & Charoenrein, 2006). Interestingly, no literature was found on formation of an emulsion gel using OSA starch alone without any added surfactant or gelling agent: studies focused either on the stabilisation properties of OSA starch or on its thermal and pasting properties.

In general, different kinds of processing methods can be employed to gel the continuous phase. The key ones are heat, acid or salt treatment. Acid milk gels have deliberately been excluded here as they have been covered extensively in other reviews (Loveday, Sarkar, & Singh, 2013; Lucey & Singh, 1997).

2.1 Thermal treatment of protein stabilised emulsions

Heat treatment induces denaturation and/or thermal gelation of several biopolymers. The sol-gel transition of biopolymers can either be irreversible (whey protein) or partly reversible (starch) depending on the physical or chemical interactions involved.

On heating above the denaturation temperature (65 °C) of the key globular protein of whey – *β*-lactoglobulin, the molecule unfolds and the gelation process happens in three connected steps: denaturation, aggregation and three-dimensional network formation (Alting, Hamer, de Kruif, & Visschers, 2003; Dang, Loisel, Desrumaux, & Doublier, 2009; Nicolai, Britten, &

166 Schmitt, 2011). Structural, physical and chemical changes are induced on heating between 70
167 and 90 °C for between 5 to 60 min. When, *β*-lactoglobulin unfolds it retains its dimeric form,
168 exposing its sulfhydryl and hydrophobic groups causing the protein molecule to become
169 reactive (Moakes, Sullo, & Norton, 2015a; Wolz & Kulozik, 2015). Further rearrangement of
170 *β*-lactoglobulin secondary structure provides association points via intermolecular *β*-sheets
171 forming high molecular mass oligomers. Simultaneously, aggregation of these activated
172 molecules occurs when two unfolded molecules collide, forming the primary polymers,
173 resulting in higher molecular weight aggregates and increasing the viscosity of the system
174 (Moakes, et al., 2015a; Wijayanti, Bansal, & Deeth, 2014; Wolz & Kulozik, 2015). These
175 clusters arise from physical non-covalent interactions, such as hydrophobic, electrostatic and
176 hydrogen bond interactions between unfolded protein molecules (Boutin, Giroux, Paquin, &
177 Britten, 2007; Fitzsimons, Mulvihill, & Morris, 2008; Livney, 2010; Monahan, McClements,
178 & German, 1996). Different protein aggregation degrees can be obtained by varying
179 environmental conditions, such as protein concentration, temperature, time, pH and addition
180 of ions (Chen & Dickinson, 1998; Dang, et al., 2009; Nicolai, et al., 2011; Ruffin, et al.,
181 2014). For instance, protein concentration strongly influences whey protein aggregation
182 kinetics. Increasing the concentration of protein speeds up the denaturation process since at
183 higher protein concentration, the collision probability between molecules is increased.
184 Therefore, the aggregation rate increases and the overall protein denaturation process
185 accelerates (Dissanayake, Ramchandran, Donkor, & Vasiljevic, 2013; Wolz & Kulozik,
186 2015).

187 During further heat treatment, the aggregation process continues through chemical
188 covalent cross links such as intermolecular disulphide bonds and sulfhydryl-disulphide
189 interchange that reinforces the network permanently (Monahan, et al., 1996; Nicolai, et al.,
190 2011). In the case of whey protein-stabilized emulsions, both intra- and inter- droplet

interactions occur. As explained by Monahan, et al. (1996), denatured whey protein molecules adsorb at the oil-water interface during emulsification with hydrophobic residues located at the interface and hydrophilic residues located in the continuous phase. With time, proteins located in the continuous phase denature enabling their interaction with unfolded protein adsorbed onto oil droplets, forming an emulsion gel. These interactions can generate even thicker interfacial layers (Sarkar, Arfsten, Golay, Acquistapace, & Heinrich, 2016) and inter-droplet aggregation via disulphide interchange reactions which contributes to forming the protein gel network (Monahan, et al., 1996).

2.2 Cationic treatment

Addition of salts such as monovalent or divalent salts (NaCl, $CaCl_2$, $ZnCl_2$, $MgCl_2$) to an emulsion is another technique inducing gelation, so called cold gelation. The higher valency of multivalent ions means that they are much more effective at screening electrostatic repulsion between droplets. Furthermore, multivalent ions such as Ca^{2+} ions can specifically bind to adsorbed protein carboxylate groups on different droplet surfaces forming ion bridges (Sarkar, Kamaruddin, Bentley, & Wang, 2016).

With regard to whey protein stabilised emulsions and cold gelation induced by the addition of salts, the system must first be heat-treated to allow proteins to unfold and expose their hydrophobic patches (Dickinson, 2012). Hydrophobic patches from protein adsorbed to oil droplets can combine with hydrophobic patches located on other protein moieties leading to oil droplet aggregation. These aggregates constitute the building blocks leading to the cationic gel 3D network (Sok, Remondetto, & Subirade, 2005). Addition of calcium ions on cooling leads to further aggregation and gelation through calcium ion-mediated interactions (Bryant & McClements, 1998; Hongsprabhas & Barbut, 1997; Kuhn, Cavallieri, & Da Cunha, 2010).

3. Filler-Matrix Interactions

The rheological behaviour of emulsion filled gels has been extensively studied due to their importance in pharmaceuticals, cosmetics and foods. In 1956, Kerner established a model for gels filled with strongly bound particles, which predicts that these particles increase the storage modulus of a gel (Kerner, 1956; Oliver, Scholten, et al., 2015). Oil droplets have been reported to behave in a similar manner (Dickinson, 2012; Sala, Van Aken, Stuart, & Van De Velde, 2007). The rheological properties of an emulsion gel depend on (Dickinson & Chen, 1999; Sala, et al., 2008):

i. the properties of the background gel matrix (biopolymer composition, crosslinking density, biopolymer concentration, etc) and the properties of the emulsified oil droplets, i.e., the filler (fatty acid composition, droplet size);
ii. the filler volume fraction;
iii. the filler - matrix interactions;
iv. the state of aggregation of the filler.

In general, the final rigidity of emulsion gels is often greater than the rigidity of the corresponding protein gels without the filler due to denatured protein adsorbed on the oil droplets forming crosslinks with protein unfolded in the matrix (Dickinson, 2012).

3.1 Theoretical models

In emulsion gels, oil droplets are often hypothesized to behave like solid particles. In this case, both Van der Poel theory (1958) and Kerner theory (1956) of the shear modulus, G´, of a composite gelled material can be applied (Oliver, Scholten, et al., 2015), which are based on three assumptions (Sala, et al., 2007):

i. The filler particles are entirely adherent to the matrix,

ii. The filler particles remain as independent particles and do not interact with each other, i.e., emulsion droplets are not flocculated,

iii. The filler particles are homogeneously distributed throughout the matrix.

These theories predict three different regimes of mechanical behaviour during small deformation depending upon the filler volume fraction for given moduli of the matrix (G'_m) and filler particles (G'_f):

i. $G'_f < G'_m$: Filler particles deform more than the matrix,

ii. $G'_f = G'_m$: Filler particles deform equally to the matrix,

iii. $G'_f > G'_m$: Filler particles deform less than the matrix.

The shear modulus of liquid filler particles G'_f was later estimated by Van Vliet (1988) according to the Laplace pressure $G'_f = \frac{2\gamma}{r}$, where r is the radius of monodispersed oil droplet and γ is the oil-water interfacial tension (Sala, et al., 2007; van Vliet, 1988). In this study, Van Vliet included the aspect of non-interacting filler particles, where the storage moduli of non-interacting filled gels approached the theoretical behaviour of unfilled gel with increasing filler volume fraction (i.e., the filled gel modulus decreases with increasing filler volume fraction under small deformation).

Filler-matrix interactions are theoretically dependent on the composition of the adsorbed layer at the oil interface. Some layers can chemically interact with the polymer matrix (e.g., protein adsorbed onto oil droplets can interact with protein gel network) whereas other layers may weakly interact with the matrix (e.g., surfactant coated oil droplets weakly interact with the protein gel network) (Dickinson, 2012). The extent and strength of filler-matrix interactions are difficult to quantify since different thermal processing and distribution of surface active components between bulk and interface lead to different filler-matrix interactions. To our knowledge, no adequate method exists to directly quantify the extent and

strength of the filler-matrix interactions. Rheological measurement can, however, be an indirect method in understanding the type of interactions taking place.

In summary (Table 2), fillers can be classed as bound ("active") or unbound ("inactive") and have different effects with regards to the rheological behaviour of the emulsion gel (Dickinson, 2012; Dickinson & Chen, 1999).

3.2 Factors affecting the mechanical properties of emulsion gels

The presence of oil droplets affects the overall rheological behaviour of emulsion gels depending on several factors. Extensive studies have been carried out on filler-matrix interactions, particularly in whey protein emulsion gels (Dickinson, 1998). Table 3 shows a compilation (non-exhaustive) of various whey protein-based emulsion gels, where the whey protein-stabilized emulsion droplets act as active fillers and the factors which dictate different kinds of interactions and resultant rheological behaviour. In this section, we describe some of these systems with respect to two key variables, i.e., filler and matrix properties.

3.2.1 Effect of Filler

i. Types of emulsifier

The type of emulsifying agent dictates the nature of interactions between the droplet surface and matrix (i.e., active or inactive filler). Whey protein stabilised emulsion droplets in a whey protein gel generally acts as "active" or "bound" fillers and enhance the gel strength. On the other hand, droplets stabilised by non-ionic or ionic surfactant will interact weakly with protein gel matrix, decreasing the storage modulus (Chen, Dickinson, Lee, & Lee, 2001; Dickinson & Chen, 1999; McClements, Monahan, & Kinsella, 1993). These "inactive" or "unbound fillers" will decrease the elastic modulus - except if the droplets are small and rigid - compared to active fillers, regardless of droplet size, droplet volume fraction, etc (Dickinson

& Chen, 1999) (Figure 2). In the case of a mixed monolayer of protein and surfactants, such as Tween 20, surfactants tend to displace the proteins due to their stronger affinity for the oil droplet interfaces. Hence, oil droplets will not interact with the protein matrix, weakening chemical affinities between the filler and the matrix, resulting in the decrease of gel strength (Chen, Dickinson, Langton, & Hermansson, 2000; Dickinson, 2012; Dickinson & Chen, 1999; Sala, et al., 2008). For instance, Chen, et al. (2000) investigated the viscoelastic properties of heat set WPI stabilised emulsion gels in presence or absence of added emulsifier. The study showed that whey protein emulsion gel had a five times higher G´ (5.05 kPa) as compared to that of mixed whey protein + Tween 20-stabilized emulsion gel (0.95 kPa).

ii. Droplet volume fraction

Several authors have reported that increasing the concentration of active fillers increases G´ (Dickinson & Chen, 1999; Sok, et al., 2005; Yost & Kinsella, 1992). Chen and Dickinson (1998) studied the effect of droplet volume fraction (0-45 vol%) on 10 wt% whey protein emulsion gels and observed a significant increase in gel strength when droplet volume fraction was above 20 vol% (Table 3).

iii. Emulsion droplet size and emulsifier concentration

A balance has to be found between emulsion droplet size and emulsifying agent concentration. Small droplets have a larger surface area which needs to be covered by surfactant. Thus, a high concentration of emulsifier is required to avoid bridging flocculation and aggregation. With larger droplets, an excess of micellar emulsifier might lead to depletion flocculation which can be beneficial in increasing the gel strength as discussed in the next section (Boutin, et al., 2007; Chen & Dickinson, 1998; Yost & Kinsella, 1992).

In terms of emulsion droplet size, McClements, et al. (1993) reported that emulsion gels prepared with 10 wt% whey protein isolate (WPI) and 40 wt% oil droplets showed a 100 % increase in G´ on decreasing the mean droplet diameter (d_{32} value) from approximately 4 to 0.7 μm. Decreasing emulsion droplet size at a constant volume fraction increases the total droplet surface area. With this increase in surface area-to-volume ratio, they become more closely packed and the number of protein interactions between droplets increases (Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken, 2009). Therefore, smaller droplets reinforce the matrix and increase the Young's modulus to a greater extent. Droplets larger than the pore size of the matrix might disrupt the three-dimensional network and may also result in lowering the modulus even though they are active fillers (McClements, et al., 1993; Yost & Kinsella, 1992).

iv. Flocculation of emulsion droplets

Recently, Oliver, Berndsen, et al. (2015) showed effects of emulsion droplet clustering using emulsions (1 wt% WPI, 40 wt% oil) in a gelatin matrix (4 to 10 wt%). At a slow gelation rates, depletion interactions allowed aggregation of droplets in the absence of other attractive interactions between adjacent droplets. This led to a heterogeneous distribution of droplets distribution in a homogeneously gelled matrix (Oliver, Berndsen, et al., 2015). Clustering of emulsion droplet thus lead to an increase in the G´. Sala, et al. (2007) also showed that aggregated emulsion droplets had a greater impact on the rheological properties of the emulsion gels due to the increase in localized volume fraction. van Aken, Oliver, and Scholten (2015), explained the effect of particle clustering using a theoretical model. This model recognizes that the deformability of aggregated particles is linked to the volume fraction inside each cluster and their firmness.

v. Solid Fat content

The firmness of aggregated droplets can be increased by increasing the solid fat content of droplets, which also increases the G'. The effective modulus of liquid oil droplets is related to their Laplace pressure $\Delta P = \frac{2\gamma}{r}$ where r is the radius of monodispersed oil droplet and γ is the oil-water interfacial tension for an emulsion (Oliver, Scholten, et al., 2015; van Vliet, 1988). The modulus of solid fat droplets is related to the presence of a fat crystal network enhancing the droplets rigidity. Therefore, a higher solid fat content containing larger fat crystal network increases the firmness of the emulsion droplet which in turn increases G´ of the emulsion gel. Oliver, Scholten, et al. (2015) showed that at 4 °C, 9 % (w/w) WPI stabilised emulsion gel with low solid fat content (27%) had a 20-fold lower tangential stress (12.1 kPa), compared to that with higher solid fat content (61.6%, 251.7 kPa). Furthermore, compared to liquid oil droplets, higher solid fat droplets are more prone to partial coalescence due to fat needles from one droplet protruding to the adjacent droplets. Such partial coalescence can significantly increase the effective droplet volume fraction, which strengthens the emulsion gel further (Dickinson, 2006; Oliver, Scholten, et al., 2015; Yost & Kinsella, 1992).

vi. Interfacial ageing

In case of emulsion gels with active fillers, the extent of strengthening is also dependent on the age of the interfacial adsorbed layer if it consists of a biopolymer (Dickinson, 2012). Studies conducted by Chen and Dickinson (1999b) have indicated that aged (1 day to 1 week) protein-stabilised emulsion droplets have weaker affinities for the protein in the matrix (G´ < 1 kPa) as compared to freshly prepared emulsion gel (G´ > 3 kPa). Interactions can occur between folded and unfolded protein in the bulk and protein already adsorbed at the surface of the emulsion droplets within the solution although the aged adsorbed protein will be

unfolded in a different way. The sulfhydryl groups can lose their reactivity due to rapid conformational changes of the adsorbed protein structure during surface ageing. Therefore, the filler and matrix are less bound to each other, which decreases G´ (Chen & Dickinson, 1999b).

3.2.2 Matrix Properties

The concentration of gelling agent influences the rheology of emulsion gels as explained by the van Vliet theory of emulsion gels with either active or inactive fillers. The modulus increases or decreases depending on the ratio between the matrix modulus (affected by the gelling agent concentration, etc.) and the filler modulus (Table 2). For the matrix itself, variation of gelling agent concentration typically alters the G´ according to a power law relation, i.e., $G' \approx c^n$ (c: concentration of protein; n: power law) (Puyol, Pérez, & Horne, 2001). At high concentrations of gelling agent, the number of bonds between molecules are more important than at lower concentrations. Decreasing the amount of voids (free space) in gels leads to denser gels (Boutin, et al., 2007). Studies conducted on emulsion gels, as described in Table 3, also show similar results, i.e., higher matrix concentration leads to firmer gels (Fitzsimons, et al., 2008; Oliver, Scholten, et al., 2015; Sala, et al., 2009; Tesch, et al., 2002). For instance, Chen and Dickinson (1998) reported that increasing the concentration of WPI from 1 wt% to 8 wt% in an emulsion gel containing 20 vol% oil nearly doubled the G' of the emulsion gel. Nevertheless, a critical gelling agent concentration was noticed by Chen and Dickinson (1998) for active oil droplets, which depends on the oil volume fraction and the source of the protein. For instance, a pure protein gel formed with 14 wt% WPI had the same strength as a emulsion gel formed with 3 wt% WPI and a high filler volume fraction (45 vol.% oil). Above this critical matrix concentration, the storage modulus

388 of the matrix G'_m is so high that the effect of the filler is insignificant (Chen & Dickinson,
389 1998; van der Poel, 1958).
390 In summary, the mechanical behaviour of a gel can be controlled by tuning the properties of
391 the inner phase (emulsion droplets) and the biopolymer matrix. Interestingly, most literature
392 on emulsion gels with active fillers has focussed on whey protein based emulsion gels.
393 Literature on starch based emulsion gels where modified starch acts as both surfactant and
394 gelling agent appears largely unexplored. This might be an interesting field of research to
395 explore systematically to understand if OSA starch-stabilized droplets act as active fillers or
396 not, and whether interfacial OSA starch interacts with the starch present at the continuous
397 phase during thermal gelation.
398

399 ## 4. Emulsion microgel particle formation

400 Emulsion microgel particles can be formed using two routes – a top-down' approach or a
401 bottom-up' approach. In the top-down approach, large materials are broken down into small
402 particles with the use of specific shearing equipment (McClements, 2014). For instance,
403 emulsion gels with or without added lipophilic bioactive molecules, can be sheared in a
404 controlled manner resulting in small gel particles. It can be hypothesized that the properties
405 of filler-matrix interaction will be critical for the break-up of such microgel particles. This
406 facile processing route has been successfully used in whey protein-based microgels (Sarkar,
407 Murray, et al., 2016), and holds potential for creation of emulsion microgel particles too. In
408 theory, emulsion gels with active fillers should be better for this top-down processing so that
409 the oil droplets contained within do not coalesce and leak out of the gel particles during the
410 shearing process. This is a research question which needs exploration. In comparison the
411 bottom-up approach is based on the spontaneous formation of particles due to alteration of
412 molecular interactions forcing molecules to rearrange themselves (McClements, 2014). In

this case the starting emulsion is directly gelled into micron-sized soft emulsion particles using different techniques under appropriate conditions such as ionic strength, temperature, pH, etc.

4.1 Formation of emulsion microgel particles using bottom-up approaches

4.1.1 Polymer extrusion route

Polymer extrusion is a process in which a polymer at relatively high concentration is forced through a nozzle at a certain pressure, flow rate and temperature. The polymer extruded through the nozzle usually changes texture due to the release of steam or reaction with ions, leading to its gelation (Harper & Clark, 1979). Whey protein microgel particles without filler emulsions have been successfully prepared using this technique by extruding denatured WPI into $CaCl_2$ solution (Egan, O'Riordan, O'Sullivan, & Jacquier, 2014). This method required a heating step during which whey proteins were denaturated and polymerized into soluble aggregates, followed by a cooling step and the subsequent addition of calcium ions, which results in the formation of a network via Ca^{2+}-mediated interactions of soluble aggregates. This Ca^{2+}-mediated cold gelation of whey protein may be compared to alginate gelation resulting from a dimeric association of guluronic and mannuronic acid regions with Ca^{2+} in the "egg box" formation.

Formation of emulsion microgel particles via extrusion can also be achieved by passing the emulsion through a nozzle where gelled emulsion particles would exit the extrusion device due to heat, salt or acid treatment or their combination. Pre-treated whey protein-stabilised emulsions have been reported to successfully gel into emulsion microgel particles or "emulsion gel beads" using such an external gelation method (Beaulieu, et al., 2002; Egan, Jacquier, Rosenberg, & Rosenberg, 2013; Ruffin, et al., 2014). The technique involved extruding emulsion droplets stabilised by denatured WPI through a syringe into a

bath containing $CaCl_2$ solution (Egan, Jacquier, Rosenberg, & Rosenberg, 2013). Calcium ions had numerous effects on the elasticity, size and morphology of the resultant particles (Beaulieu, et al., 2002; Liang, Leung Sok Line, Remondetto, & Subirade, 2010). Higher concentrations of $CaCl_2$ led to a decrease in the size of the microgel particle as well an increase in their sphericity. Beaulieu, et al. (2002) related this effect to the increase in kinetics of gelation via calcium ions. An increase in Ca^{2+} concentration increases the amount of ionic bridges formed between calcium ions and sulfhydryl groups on the protein which increases protein-protein interactions and aggregation, leading to an accelerated formation of a three dimensional network. As the gelation kinetic is accelerated droplets do not have enough time to destabilize via screening of electrostatic repulsion by Ca^{2+} and small emulsion microgel particles can be produced. In the case of the internal gelation technique, emulsification of oil containing insoluble calcium present as $CaCO_3$ with denatured WPI is the first step. The emulsion gels due to the addition of acid, releasing the calcium ions.

As compared to the top-down approach, the bottom-up approach of polymer extrusion excludes the use of high temperature on the encapsulated bioactive molecule since it is a cold gelation technique. However, the main disadvantage of the polymer extrusion technique is the large size of the microgel particles formed (> 500 μm), though internal gelation has the ability to form smaller particles (< 100 μm). Particles size formed via external gelation mainly depend on the nozzle or syringe diameter which has a restricted range of sizes. In comparison, particles size formed via internal gelation depends on the emulsion droplet size generated by the multiple emulsion which can be controlled by the concentration of emulsifier, homogenization conditions, concentration of $CaCO_3$, stirring rate and oil volume fraction allowing better control over particle size than the former. As particles over 100 μm impact the sensory perception of food, these might have some adverse sensory aspects when incorporated in food.

As well as external or internal gelation via polymer extrusion, co-extrusion techniques have also been investigated in literature. In this case, a surfactant stabilised emulsion is first prepared and then mixed with alginate solution followed by Ca^{2+}-ion mediated gelation using a spray aerosol method. Ching, Bansal, and Bhandari (2015) showed that the alginate microgel particles with filled emulsion droplets had droplet sizes in the range of 20-80 µm. During this process, there is a possibility that as the droplets are gelled, shrinkage of the microgel particles might force the droplets closer to each other and result in droplet coalescence. Furthermore, it requires an additional hydrocolloid, such as alginate, which increases the cost and this also might not be thermodynamically compatible with the biopolymer used to stabilize the emulsion (e.g., protein) or lead to depletion flocculation of the droplets before gelation can occur.

4.1.2 Multiple emulsion templating route

Sung, Xiao, Decker, and McClements (2015) described a new method of producing emulsion microgel particles using a multiple emulsion templating route. In this study, Sung, et al. (2015) gelled the aqueous phase of an oil-in-water-in-oil multiple emulsion via thermal gelation of the whey protein in the inner aqueous phase. The emulsion microgel particles were separated from the secondary oil phase using an organic solvent. The advantage of this method is that it produces small particles (mean diameter (d_{32}) ~ 12 µm). However, this method is time consuming due to the number of processing steps required and use of organic solvent which limits its applications in food. Egan, et al. (2013) prepared emulsion microgel particles using a combination of the internal gelation method and multiple emulsion templating. Compared to external gelation, this technique was mainly affected by the stirring rate, which allowed reduction of the size of the particles below 100 µm.

4.1.3 Fluid gel route

Recently, the new technique of fluid gels has been presented by Moakes, Sullo, and Norton (2015b) building on research done on multiple emulsion-based hydrogels and shear gels. This is a bottom-up approach as shear is applied to the biopolymer solution that is undergoing a sol-gel transition. This prevents the formation of a continuous gel network and instead produces discrete spherical gel particles (Garrec & Norton, 2012; Moakes, et al., 2015b). The particle size and morphology of the microgels formed are controlled by the shear rate and thermal history of the biopolymers. Research on fluid gels formed with whey protein, at a typical concentration of 10 wt% shows that the shear applied to the primary aggregates of whey protein restricts particle-particle aggregation and therefore complete whey protein gelation does not occur. This restricted sol-gel transition alters whey protein interactions forcing the molecules to rearrange themselves. In terms of thermal treatment, the rapid heating rate increases particle-particle interactions due to Brownian motion and also the strengthening of hydrophobic interactions between protein aggregates makes them resistant to shear. Therefore, large aggregated particles are formed. Low heating rates, in comparison, decreases the protein aggregation rate and do not strengthen hydrophobic interactions. Thus, aggregates formed are smaller and single non aggregated particles can also be produced (Moakes, et al., 2015a).

Using the same design principle, emulsion fluid gel particles were prepared. An oil-in-water emulsion (5 to 20 vol% oil) was first stabilised using a solution of WPI (5 to 30 wt%). The emulsion was then heat treated (0.5 $^{\circ}$C/min to 80 $^{\circ}$C), which started protein denaturation process and hydrophobic aggregation. Shear (450 rpm) was applied preventing gelation of the emulsion in entirety. As a result, WPI adsorbed onto the oil droplets was gelled forming emulsion fluid gel particles (Moakes, et al., 2015b).

5. Delivery of lipophilic molecules using emulsion microgel particles

In general, on ingestion, an emulsion microgel particle is expected to be exposed to a wide range of physical (e.g. shear and temperature) and biochemical (e.g. dilution, ionic strength, pH, pepsin, amylase, pancreatin, mucins and bile salts) conditions as it passes through the mouth into the stomach and then the intestines (Singh & Sarkar, 2011). During its physiological transit, the emulsion microgel particle can release the encapsulated active molecule by two approaches: 1. Swelling of the particle due to pH and environmental ionic strength and 2. Erosion due to enzymatic degradation or shear. Figure 3 illustrates the release of active molecules when triggered by particular physical and/or biochemical factors.

5.1 Swelling of emulsion microgel particles

The swelling of an emulsion gel particle containing ionized or ionisable groups can occur depending on the pH and ionic strength of the environment (Beaulieu, et al., 2002). As illustrated in Figure 3, when emulsion microgels with ionisable groups are exposed to a specific pH, loss of attractive electrostatic interactions drive the charged groups apart. This repulsion might lead to the swelling of emulsion microgel particle, which increases the pore size (Zhang, Zhang, Chen, et al., 2015). If the lipophilic active molecules are smaller than the stretched pores, they can more easily diffuse out or, if the active molecules are electrostatically bound they would be more easily released if there is change in ionic environment. Therefore, controlling the environment or tuning the microgel can allow control of their swelling ratio.

This swelling ratio can be calculated in two different ways:

i. Fick's model of diffusion: This can be used to predict the release of the entrapped lipophilic molecules from swollen gels (Paulsson & Edsman, 2002; Ritger & Peppas, 1987);

$$Q = 2C_0 \frac{Dt^{1/2}}{\pi}$$

Q: amount of active molecule released per unit area

C_0: initial concentration of active molecule in emulsion microgel particle

t: time elapsed since release experimented started

D: diffusion coefficient of active molecule in the emulsion microgel

ii. Swelling ratio: This calculates the weight change before and after incubation of emulsion gel particles in a particular environmental condition, such as oral or gastrointestinal phase (Beaulieu, et al., 2002; Gunasekaran, Ko, & Xiao, 2007; Liang, et al., 2010)

$$Swelling\ ratio\ or\ water\ uptake\ ability\ (\%) = \frac{W_w - W_d}{W_d} \times 100$$

W_w: wet weight of microgel particles, W_d: dry weight of microgel particles

Studies conducted on whey protein emulsion microgel particles have reported that at pre-prandial acidic / gastric pH (1.9), the particles did not extensively swell (Swelling ratio = 20%). All negatively charged carboxyl groups at the surface of the microgel particle were neutralized, however the protein chain contained few positively charged amine groups, leading to low electrostatic repulsive forces. At a pH close to the protein pI (pH 5.2), the swelling ratio was lowest (Swelling ratio = 13%) as the net charge of the protein was close to zero and thus, no electrostatic repulsion. At intestinal pH (7.5), the swelling ratio was quite high (Swelling ratio = 42%) because whey protein had a high amount of negatively charged carboxyl groups at the surface of the microgel, leading to strong electrostatic repulsive forces (Beaulieu, et al., 2002; Gunasekaran, et al., 2007; Gunasekaran, Xiao, & Ould Eleya, 2006). Additionally, several authors reported that the protein concentration also had an effect on the swelling ratio of beads. At higher protein concentration, the cross-linking density of the

562 microgel network was higher, leading to a decrease in the swelling ratio. Hence, it takes
563 longer for particles to swell and liberate the encapsulated substance, which can be a strategy
564 for slow release (Egan, et al., 2013; Gunasekaran, et al., 2007). In comparison to protein,
565 OSA starch based emulsion microgel systems might not be so much affected by the
566 environmental pH or ionic strength (Tesch, et al., 2002), since it is only weakly charged. This
567 might be an interesting area for further research.
568
569 **5.2 Matrix erosion by enzyme degradation**
570 During physiological processing, enzymes degrade biopolymers enabling erosion of the
571 matrix and release of the encapsulated compounds. As compared to swelling, the matrix is
572 disrupted either partly or completely, enabling a burst release in erosion. So, it can be
573 expected that whey protein emulsion microgel particles would be digested by proteolytic
574 enzymes, such as trypsin or pepsin, whereas OSA starch based microgel particles would be
575 digested by amylase, unless their susceptible bonds were engineered to be inaccessible or
576 partly accessible to the enzymes. Studies conducted by Beaulieu, et al. (2002) reported that
577 whey protein emulsion microgels were resistant to gastric enzymes such as pepsin but were
578 attacked by intestinal enzyme mixtures such as pancreatin, so a targeted intestinal delivery
579 seems plausible. This is quite unlike the behaviour of whey protein emulsions or whey
580 protein based microgel particles which are readily hydrolysed by pepsin at gastric pH (Sarkar,
581 Goh, Singh, & Singh, 2009; Sarkar, Murray, et al., 2016; Singh & Sarkar, 2011). Resistance
582 of emulsion microgel particles to pepsin might be attributable to the reburial of hydrophobic
583 groups during the emulsification and gelation processes, with little or no accessibility to
584 pepsin. Nevertheless, during intestinal digestion emulsion microgel particles were digested by
585 trypsin and chymotrypsin. The former acts on the carboxyl end of peptide bond involving

lysine and arginine, whereas, the later attacks peptide bonds at large hydrophobic groups (Beaulieu, et al., 2002; Gunasekaran, et al., 2007; Kananen, et al., 2000).

In modified starch hydrogels, during oral and intestinal processing, α-amylase would hydrolyse the starch to some extent. The amount of amylose contained in starch affects the gel strength of the matrix (Mun, Kim, Shin, et al., 2015) – higher amounts lead to a more compressed and packed structure (Tangsrianugul, Suphantharika, & McClements, 2015). This increase in strength and/or compactness as well as the cross-linking achieved through processing might enable prevention of immediate matrix erosion due to amylase attack (Atyabi, Manoochehri, Moghadam, & Dinarvand, 2006).

6. Conclusions and future outlook

Emulsion gels containing active or inactive fillers and their rheological properties have been well characterised. Such knowledge will enable creation of emulsion microgel particles, a new class of soft solid particles, which has attracted recent research attention. Emulsion microgels might be carefully designed using top down approaches of controlled shearing of emulsion gels with active fillers, or bottom up approaches using polymer extrusion, fluid gels, or multiple emulsion templating. Such particles could be used to release the encapsulated lipophilic phase in a sustained or burst manner via erosion due to shear treatment or enzyme, such as with amylase or pepsin, or swelling of the matrix due to changes of pH and ionic strength in the physiological regime. Whey protein based emulsion microgel particles are currently being investigated. Many food proteins and polysaccharides can be used to form edible emulsion microgel particles. Use of gelatine, modified starch and plant proteins would be of great interest, since they show potential for emulsion microgel particle formation by acting as both emulsifying and bulk gelling agents. However, to our knowledge, no systematic research has been conducted using these biopolymers.

Furthermore, these biopolymers have specific responsiveness to pH, ionic strength, enzymes, etc , which can be exploited for tailored properties. Elaboration of these systems for food, pharmaceutical and personal care industries will strongly depend on the progress in designing innovative microgels that allow site-dependent controlled release.

7. Acknowledgements

OT thanks University of Leeds for awarding a 110 Anniversary Research Scholarship for her PhD studies.

8. References

Alting, A. C., Hamer, R. J., de Kruif, C. G., & Visschers, R. W. (2003). Cold-set globular protein gels: Interactions, structure and rheology as a function of protein concentration. Journal of Agricultural and Food Chemistry, 51, 3150-3156.

Anton, M., Le Denmat, M., Beaumal, V., & Pilet, P. (2001). Filler effects of oil droplets on the rheology of heat-set emulsion gels prepared with egg yolk and egg yolk fractions. Colloids and Surfaces B: Biointerfaces, 21, 137-147.

Atyabi, F., Manoochehri, S., Moghadam, S. H., & Dinarvand, R. (2006). Cross-linked starch microspheres: effect of cross-linking condition on the microsphere characteristics. Arch Pharm Res, 29, 1179-1186.

Augustin, M. A., & Sanguansri, L. (2012). 2 - Challenges in developing delivery systems for food additives, nutraceuticals and dietary supplements A2 - Garti, Nissim. In D. J. McClements (Ed.), Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals (pp. 19-48): Woodhead Publishing.

Ballauff, M., & Lu, Y. (2007). "Smart" nanoparticles: Preparation, characterization and applications. Polymer, 48, 1815-1823.

Bao, J., Xing, J., Phillips, D. L., & Corke, H. (2003). Physical Properties of Octenyl Succinic Anhydride Modified Rice, Wheat, and Potato Starches. Journal of Agricultural and Food Chemistry, 51, 2283-2287.

Beaulieu, L., Savoie, L., Paquin, P., & Subirade, M. (2002). Elaboration and characterization of whey protein beads by an emulsification/cold gelation process: application for the protection of retinol. Biomacromolecules, 3, 239-248.

Bhosale, R., & Singhal, R. (2007). Effect of octenylsuccinylation on physicochemical and functional properties of waxy maize and amaranth starches. Carbohydrate Polymers, 68, 447-456.

Boutin, C., Giroux, H. J., Paquin, P., & Britten, M. (2007). Characterization and acid-induced gelation of butter oil emulsions produced from heated whey protein dispersions. International Dairy Journal, 17, 696-703.

Briuglia, M., Urquhart, A. J., & Lamprou, D. A. (2014). Sustained and controlled release of lipophilic drugs from a self-assembling amphiphilic peptide hydrogel. International Journal of Pharmaceutics, 474, 103-111.
Bryant, C. M., & McClements, D. J. (1998). Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends in Food Science & Technology, 9, 143-151.
Chen, J. S., & Dickinson, E. (1998). Viscoelastic properties of heat-set whey protein emulsion gels. Journal of Texture Studies, 29, 285-304.
Chen, J. S., & Dickinson, E. (1999a). Effect of surface character of filler particles on rheology of heat-set whey protein emulsion gels. Colloids and Surfaces B-Biointerfaces, 12, 373-381.
Chen, J. S., & Dickinson, E. (1999b). Interfacial ageing effect on the rheology of a heat-set protein emulsion gel. Food Hydrocolloids, 13, 363-369.
Chen, J. S., Dickinson, E., Langton, M., & Hermansson, A. M. (2000). Mechanical properties and microstructure of heat-set whey protein emulsion gels: Effect of emulsifiers. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 33, 299-307.
Chen, J. S., Dickinson, E., Lee, H. S., & Lee, W. P. (2001). Protein-based emulsion gels: Effects of interfacial properties and temperature. In E. Dickinson & R. Miller (Eds.), Food Colloids: Fundamentals of Formulation (pp. 384-391). Cambridge: Royal Soc Chemistry.
Ching, S. H., Bansal, N., & Bhandari, B. (2015). Physical stability of emulsion encapsulated in alginate microgel particles by the impinging aerosol technique. Food Research International, 75, 182-193.
Ching, S. H., Bansal, N., & Bhandari, B. (2016). Rheology of emulsion-filled alginate microgel suspensions. Food Research International, 80, 50-60.
Chivero, P., Gohtani, S., Yoshii, H., & Nakamura, A. (2016). Assessment of soy soluble polysaccharide, gum arabic and OSA-Starch as emulsifiers for mayonnaise-like emulsions. LWT - Food Science and Technology, 69, 59-66.
Chung, C., Degner, B., Decker, E. A., & McClements, D. J. (2013). Oil-filled hydrogel particles for reduced-fat food applications: Fabrication, characterization, and properties. Innovative Food Science & Emerging Technologies, 20, 324-334.
Dang, H. V., Loisel, C., Desrumaux, A., & Doublier, J. L. (2009). Rheology and microstructure of cross-linked waxy maize starch/whey protein suspensions. Food Hydrocolloids, 23, 1678-1686.
Dickinson, E. (2006). Structure formation in casein-based gels, foams, and emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 288, 3-11.
Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil droplets. Food Hydrocolloids, 28, 224-241.
Dickinson, E. (2015). Microgels - An alternative colloidal ingredient for stabilization of food emulsions. Trends in Food Science & Technology, 43, 178-188.
Dickinson, E., & Chen, J. S. (1999). Heat-Set Whey Protein Emulsion Gels: Role of Active and Inactive Filler Particles. Journal of Dispersion Science and Technology, 20, 197-213.
Dissanayake, M., Ramchandran, L., Donkor, O. N., & Vasiljevic, T. (2013). Denaturation of whey proteins as a function of heat, pH and protein concentration. International Dairy Journal, 31, 93-99.
Domian, E., Brynda-Kopytowska, A., & Oleksza, K. (2015). Rheological properties and physical stability of o/w emulsions stabilized by OSA starch with trehalose. Food Hydrocolloids, 44, 49-58.

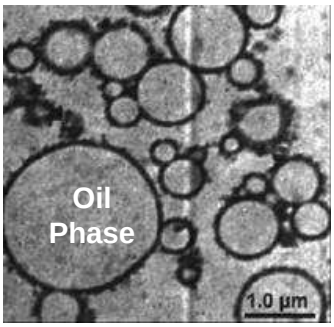
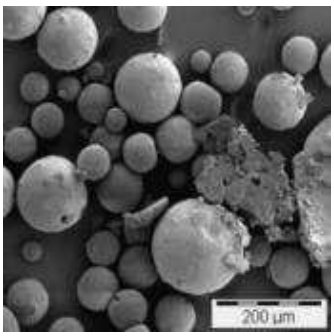
Egan, T., Jacquier, J. C., Rosenberg, Y., & Rosenberg, M. (2013). Cold-set whey protein microgels for the stable immobilization of lipids. Food Hydrocolloids, 31, 317-324.
Egan, T., O'Riordan, D., O'Sullivan, M., & Jacquier, J. C. (2014). Cold-set whey protein microgels as pH modulated immobilisation matrices for charged bioactives. Food Chemistry, 156, 197-203.
Ettelaie, R., Holmes, M., Chen, J., & Farshchi, A. (2016). Steric stabilising properties of hydrophobically modified starch: Amylose vs. amylopectin. Food Hydrocolloids, 58, 364-377.
Fitzsimons, S. M., Mulvihill, D. M., & Morris, E. R. (2008). Segregative interactions between gelatin and polymerised whey protein. Food Hydrocolloids, 22, 485-491.
Garrec, D. A., & Norton, I. T. (2012). Understanding fluid gel formation and properties. Journal of Food Engineering, 112, 175-182.
Gunasekaran, S., Ko, S., & Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. Journal of Food Engineering, 83, 31-40.
Gunasekaran, S., Xiao, L., & Ould Eleya, M. M. (2006). Whey protein concentrate hydrogels as bioactive carriers. Journal of Applied Polymer Science, 99, 2470-2476.
Harper, J. M., & Clark, J. P. (1979). Food extrusion. C R C Critical Reviews in Food Science and Nutrition, 11, 155-215.
Hongsprabhas, P., & Barbut, S. (1997). Protein and salt effects on Ca2+-induced cold gelation of whey protein isolate. Journal of Food Science, 62, 382-385.
Joye, I. J., & McClements, D. J. (2014). Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application. Current Opinion in Colloid & Interface Science, 19, 417-427.
Kakran, M., & Antipina, M. N. (2014). Emulsion-based techniques for encapsulation in biomedicine, food and personal care. Current Opinion in Pharmacology, 18, 47-55.
Kananen, A., Savolainen, J., Mäkinen, J., Perttilä, U., Myllykoski, L., & Pihlanto-Leppälä, A. (2000). Influence of chemical modification of whey protein conformation on hydrolysis with pepsin and trypsin. International Dairy Journal, 10, 691-697.
Kawaguchi, H. (2014). Thermoresponsive microhydrogels: preparation, properties and applications. Polymer International, 63, 925-932.
Kerner, E. H. (1956). The Elastic and Thermo-elastic Properties of Composite Media. Proceedings of the Physical Society. Section B, 69, 808.
Kuhn, K. R., Cavallieri, Â. L. F., & Da Cunha, R. L. (2010). Cold-set whey protein gels induced by calcium or sodium salt addition. International Journal of Food Science & Technology, 45, 348-357.
Liang, L., Leung Sok Line, V., Remondetto, G. E., & Subirade, M. (2010). In vitro release of α-tocopherol from emulsion-loaded β-lactoglobulin gels. International Dairy Journal, 20, 176-181.
Livney, Y. D. (2010). Milk proteins as vehicles for bioactives. Current Opinion in Colloid & Interface Science, 15, 73-83.
Loveday, S. M., Sarkar, A., & Singh, H. (2013). Innovative yoghurts: Novel processing technologies for improving acid milk gel texture. Trends in Food Science & Technology, 33, 5-20.
Lucey, J. A., & Singh, H. (1997). Formation and physical properties of acid milk gels: a review. Food Research International, 30, 529-542.
Malone, M. E., & Appelqvist, I. A. M. (2003). Gelled emulsion particles for the controlled release of lipophilic volatiles during eating. Journal of Controlled Release, 90, 227-241.

746 Malone, M. E., Appelqvist, I. A. M., & Norton, I. T. (2003). Oral behaviour of food
747 hydrocolloids and emulsions. Part 2. Taste and aroma release. Food Hydrocolloids,
748 17, 775-784.
749 Mao, L. K., & Miao, S. (2015). Structuring Food Emulsions to Improve Nutrient Delivery
750 During Digestion. Food Engineering Reviews, 7, 439-451.
751 Matalanis, A., Jones, O. G., & McClements, D. J. (2011). Structured biopolymer-based
752 delivery systems for encapsulation, protection, and release of lipophilic compounds.
753 Food Hydrocolloids, 25, 1865-1880.
754 McClements, D. J. (2014). Mechanical Particle Fabrication Methods. In Nanoparticle- and
755 Microparticle-based Delivery Systems (pp. 123-148): CRC Press.
756 McClements, D. J. (2015). Encapsulation, protection, and release of hydrophilic active
757 components: Potential and limitations of colloidal delivery systems. Advances in
758 Colloid and Interface Science, 219, 27-53.
759 McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: controlling
760 the digestion and release of lipophilic food components. Adv Colloid Interface Sci,
761 159, 213-228.
762 McClements, D. J., Monahan, F. J., & Kinsella, J. E. (1993). Effect of emulsion droplets on
763 the rheology of whey-protein isolate gels. Journal of Texture Studies, 24, 411-422.
764 Moakes, R. J. A., Sullo, A., & Norton, I. T. (2015a). Preparation and characterisation of whey
765 protein fluid gels: The effects of shear and thermal history. Food Hydrocolloids, 45,
766 227-235.
767 Moakes, R. J. A., Sullo, A., & Norton, I. T. (2015b). Preparation and rheological properties
768 of whey protein emulsion fluid gels. Rsc Advances, 5, 60786-60795.
769 Monahan, F. J., McClements, D. J., & German, J. B. (1996). Disulfide-mediated
770 Polymerization Reactions and Physical Properties of Heated WPI-stabilized
771 Emulsions. Journal of Food Science, 61, 504-509.
772 Mun, S., Kim, Y. R., & McClements, D. J. (2015). Control of beta-carotene bioaccessibility
773 using starch-based filled hydrogels. Food Chem, 173, 454-461.
774 Mun, S., Kim, Y. R., Shin, M., & McClements, D. J. (2015). Control of lipid digestion and
775 nutraceutical bioaccessibility using starch-based filled hydrogels: Influence of starch
776 and surfactant type. Food Hydrocolloids, 44, 380-389.
777 Nicolai, T., Britten, M., & Schmitt, C. (2011). β-Lactoglobulin and WPI aggregates:
778 Formation, structure and applications. Food Hydrocolloids, 25, 1945-1962.
779 Oliver, L., Berndsen, L., van Aken, G. A., & Scholten, E. (2015). Influence of droplet
780 clustering on the rheological properties of emulsion-filled gels. Food Hydrocolloids,
781 50, 74-83.
782 Oliver, L., Scholten, E., & van Aken, G. A. (2015). Effect of fat hardness on large
783 deformation rheology of emulsion-filled gels. Food Hydrocolloids, 43, 299-310.
784 Ortega-Ojeda, F. E., Larsson, H., & Eliasson, A. (2005). Gel formation in mixtures of
785 hydrophobically modified potato and high amylopectin potato starch. Carbohydrate
786 Polymers, 59, 313-327.
787 Paulsson, M., & Edsman, K. (2002). Controlled Drug Release from Gels Using Lipophilic
788 Interactions of Charged Substances with Surfactants and Polymers. Journal of Colloid
789 and Interface Science, 248, 194-200.
790 Pizzoni, D., Compagnone, D., Di Natale, C., D'Alessandro, N., & Pittia, P. (2015).
791 Evaluation of aroma release of gummy candies added with strawberry flavours by
792 gas-chromatography/mass-spectrometry and gas sensors arrays. Journal of Food
793 Engineering, 167, Part A, 77-86.

794 Puyol, P., Pérez, M. D., & Horne, D. S. (2001). Heat-induced gelation of whey protein
795 isolates (WPI): effect of NaCl and protein concentration. Food Hydrocolloids, 15,
796 233-237.
797 Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release II.
798 Fickian and anomalous release from swellable devices. Journal of Controlled Release,
799 5, 37-42.
800 Ruffin, E., Schmit, T., Lafitte, G., Dollat, J., & Chambin, O. (2014). The impact of whey
801 protein preheating on the properties of emulsion gel bead. Food Chemistry, 151, 324-
802 332.
803 Sala, G., de Wijk, R. A., van de Velde, F., & van Aken, G. A. (2008). Matrix properties affect
804 the sensory perception of emulsion-filled gels. Food Hydrocolloids, 22, 353-363.
805 Sala, G., Van Aken, G. A., Stuart, M. A. C., & Van De Velde, F. (2007). Effect of droplet-
806 matrix interactions on large deformation properties of emulsion-filled gels. Journal of
807 Texture Studies, 38, 511-535.
808 Sala, G., van Vliet, T., Cohen Stuart, M. A., van de Velde, F., & van Aken, G. A. (2009).
809 Deformation and fracture of emulsion-filled gels: Effect of gelling agent
810 concentration and oil droplet size. Food Hydrocolloids, 23, 1853-1863.
811 Sarkar, A., Arfsten, J., Golay, P., Acquistapace, S., & Heinrich, E. (2016). Microstructure and
812 long-term stability of spray dried emulsions with ultra-high oil content. Food
813 Hydrocolloids, 52, 857-867.
814 Sarkar, A., Goh, K. K. T., Singh, R. P., & Singh, H. (2009). Behaviour of an oil-in-water
815 emulsion stabilized by β-lactoglobulin in an in vitro gastric model. Food
816 Hydrocolloids, 23, 1563-1569.
817 Sarkar, A., Juan, J. M., Kolodziejczyk, E., Acquistapace, S., Donato-Capel, L., & Wooster, T.
818 J. (2015). Impact of protein gel porosity on the digestion of lipid emulsions. Journal
819 of Agricultural and Food Chemistry, 63, 8829-8837.
820 Sarkar, A., Kamaruddin, H., Bentley, A., & Wang, S. (2016). Emulsion stabilization by
821 tomato seed protein isolate: Influence of pH, ionic strength and thermal treatment.
822 Food Hydrocolloids, 57, 160-168.
823 Sarkar, A., Murray, B. M., Holmes, M., Ettelaie, R., Abdalla, A., & Yang, X. (2016). In vitro
824 digestion of Pickering emulsions stabilized by soft whey protein microgel particles:
825 influence of thermal treatment. Soft Matter, 12, 3558-3569.
826 Sarkar, A., & Singh, H. (2016). Emulsions and foams stabilised by milk proteins. In P. L. H.
827 McSweeney & J. A. O'Mahony (Eds.), Advanced Dairy Chemistry (pp. 133-153):
828 Springer New York.
829 Satapathy, S., Singh, V. K., Sagiri, S. S., Agarwal, T., Banerjee, I., Bhattacharya, M. K.,
830 Kumar, N., & Pal, K. (2015). Development and Characterization of Gelatin-Based
831 Hydrogels, Emulsion Hydrogels, and Bigels: A Comparative Study. Journal of
832 Applied Polymer Science, 132.
833 Shewan, H. M., & Stokes, J. R. (2013). Review of techniques to manufacture micro-hydrogel
834 particles for the food industry and their applications. Journal of Food Engineering,
835 119, 781-792.
836 Shogren, R. L., Viswanathan, A., Felker, F., & Gross, R. A. (2000). Distribution of octenyl
837 succinate groups in octenyl succinic anhydride modified waxy maize starch. Starch -
838 Stärke, 52, 196-204.
839 Singh, H., & Sarkar, A. (2011). Behaviour of protein-stabilised emulsions under various
840 physiological conditions. Adv Colloid Interface Sci, 165, 47-57.
841 Sok, L. V. L., Remondetto, G. E., & Subirade, M. (2005). Cold gelation of β-lactoglobulin
842 oil-in-water emulsions. Food Hydrocolloids, 19, 269-278.

Sung, M. R., Xiao, H., Decker, E. A., & McClements, D. J. (2015). Fabrication, characterization and properties of filled hydrogel particles formed by the emulsion-template method. Journal of Food Engineering, 155, 16-21.
Sweedman, M. C., Tizzotti, M. J., Schäfer, C., & Gilbert, R. G. (2013). Structure and physicochemical properties of octenyl succinic anhydride modified starches: A review. Carbohydrate Polymers, 92, 905-920.
Tangsrianugul, N., Suphantharika, M., & McClements, D. J. (2015). Simulated gastrointestinal fate of lipids encapsulated in starch hydrogels: Impact of normal and high amylose corn starch. Food Research International, 78, 79-87.
Tesch, S., Gerhards, C., & Schubert, H. (2002). Stabilization of emulsions by OSA starches. Journal of Food Engineering, 54, 167-174.
Thirathumthavorn, D., & Charoenrein, S. (2006). Thermal and pasting properties of native and acid-treated starches derivatized by 1-octenyl succinic anhydride. Carbohydrate Polymers, 66, 258-265.
Thorne, J. B., Vine, G. J., & Snowden, M. J. (2011). Microgel applications and commercial considerations. Colloid and Polymer Science, 289, 625-646.
van Aken, G. A., Oliver, L., & Scholten, E. (2015). Rheological effect of particle clustering in gelled dispersions. Food Hydrocolloids, 48, 102-109.
van der Poel, C. (1958). On the rheology of concentrated dispersions. Rheologica Acta, 1, 198-205.
van Vliet, T. (1988). Rheological properties of filled gels. Influence of filler matrix interaction. Colloid and Polymer Science, 266, 518-524.
Velikov, K. P., & Pelan, E. (2008). Colloidal delivery systems for micronutrients and nutraceuticals. Soft Matter, 4, 1964-1980.
Wang, X. Y., Li, X. X., Chen, L., Xie, F. W., Yu, L., & Li, B. (2011). Preparation and characterisation of octenyl succinate starch as a delivery carrier for bioactive food components. Food Chemistry, 126, 1218-1225.
Wei, J., Li, Y., & Ngai, T. (2016). Tailor-made microgel particles: Synthesis and characterization. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 489, 122-127.
Wijayanti, H. B., Bansal, N., & Deeth, H. C. (2014). Stability of Whey Proteins during Thermal Processing: A Review. Comprehensive Reviews in Food Science and Food Safety, 13, 1235-1251.
Wolz, M., & Kulozik, U. (2015). Thermal denaturation kinetics of whey proteins at high protein concentrations. International Dairy Journal, 49, 95-101.
Yost, R. A., & Kinsella, J. E. (1992). Microstructure of Whey-Protein Isolate Gels Containing Emulsified Butterfat Droplets. Journal of Food Science, 57, 892-897.
Zhang, Z., Zhang, R., Chen, L., Tong, Q., & McClements, D. J. (2015). Designing hydrogel particles for controlled or targeted release of lipophilic bioactive agents in the gastrointestinal tract. European Polymer Journal, 72, 698-716.
Zhang, Z., Zhang, R., Decker, E. A., & McClements, D. J. (2015). Development of food-grade filled hydrogels for oral delivery of lipophilic active ingredients: pH-triggered release. Food Hydrocolloids, 44, 345-352.
Zhang, Z., Zhang, R., Tong, Q., Decker, E. A., & McClements, D. J. (2015). Food-grade filled hydrogels for oral delivery of lipophilic active ingredients: Temperature-triggered release microgels. Food Research International, 69, 274-280.

890 Table 1. Definitions and microstructures (at various length scales) of different emulsion gel based
891 strategies for delivery of lipophilic molecules. (A) Transmission electron micrograph (TEM) of
892 emulsion gels (reproduced from Anton, Le Denmat, Beaumal, and Pilet (2001). (B) Scanning electron
893 micrograph (SEM) of emulsion microgel particle (reproduced from Egan, et al., 2013).

Nomenclature and Microstructure	Description	References
A) Emulsion gel		
	"Emulsion gels", also named as "emulsion hydrogel", "emulgel", "emulsion-filled gel" are defined as soft solids where emulsified lipid droplets are entrapped in a gel matrix. Generally, the emulsified lipid droplets are referred to as "fillers" and the gelled aqueous phase is referred to as the "matrix". They are formed either by suitable application of temperature, pH, ionic strength to the emulsion made with high concentration of biopolymer (especially protein in case of protein-based emulsion gel) or by addition of a gelling agent to the continuous phase forming physical cross-links between emulsion droplets. It has the advantages of both hydrogels (i.e. thermodynamic stability) and emulsions (i.e., delivery of lipid soluble molecules).	(Briuglia, Urquhart, & Lamprou, 2014; Dickinson, 2012; Oliver, Scholten, et al., 2015; Sarkar, et al., 2015; Satapathy, et al., 2015)
B) Emulsion microgel particle		
	"Emulsion microgel particles", "emulsion filled hydrogel particles", "emulsion gel beads" or "fluid emulsion gel" are a new class of particles formed by encapsulating several emulsion droplets into a soft gel-based shell either using a top-down or a bottom-up approach. Fluid emulsion gels are a specific case of emulsion microgel particles as they are formed by applying shear to the continuous phase whilst gelling the emulsion droplets.	(Beaulieu, et al., 2002; Ching, et al., 2016; Dickinson, 2015; Egan, et al., 2013; Garrec & Norton, 2012; Moakes, et al., 2015b; Sung, et al., 2015)

894

895 Table 2. Effects of active and inactive filler on the rheological behaviour of emulsion gels (G´: storage
896 modulus; ϕ: volume fraction; ↑: increase; ↓: decrease).

897

	Active / Bound filler	**Inactive / Unbound filler**
Definition	Fillers are mechanically connected to the matrix. Such interaction can occur via electrostatic, hydrogen bonding, covalent bonding and/or hydrophobic interaction	Little or no chemical or physical affinity of the fillers for the surrounding matrix; fillers behave like small holes or "voids" within the matrix
Effect on elastic modulus of the filled emulsion gel (G', Pa)	↑ or ↓ G´ depending on $\frac{G_f}{G_M}$	↓ G´
Filler volume fraction (φ, %)	↑ ϕ => ↑ G´	Little effect of ϕ on G´
Filler droplet size (μm)	↑ droplet size => ↓ G´	G´ is independent to filler droplet size

898

899

900 Table 3. Filler-matrix interactions and rheological behaviour of whey protein emulsion gels (G´:
901 storage modulus, ϕ: volume fraction; [ ]: concentration, ↑: increase; ↓: decrease; =>: leads to).

Processing condition	Oil: Emulsifier ratio	Biopolymer concentration	Processing conditions	Mechanical Behaviour	References
Thermal treatment	2 : 1 10 : 1	10-15 wt%	pH ≈ 7 90 °C for 15-30 min 0- 200mM NaCl	• Active filler => ↑ G´	(McClements, et al., 1993; Sung, et al., 2015)
	5 : 1, 10 : 1	6-9 wt%	pH ≈ 7 85 °C for 30 min	• ↑ ϕ => ↑ G´ • ↑ number of crosslinks => ↑ G´ • ↓ oil droplet size => ↑ G´ Old emulsion => ↓ G´	(Chen & Dickinson, 1998, 1999a, 1999b; Chen, et al., 2000)
	1 : 4, 1 : 1, 33 : 1,	5-30 wt%	pH ≈ 4.6 – 5 50-90 °C for 15min	• ↑ [protein] =>↑ gel strength • WPI precipitates at pH close to its pI (5.2) => random aggregation and ↑ G´	(Moakes, et al., 2015b; Yost & Kinsella, 1992)
Divalent ions	1 : 1, 1.5 : 1, 2 : 1, 2.8 : 1, 4.3 : 1	8-10 wt%	pH 4-6.8 12-140 mM $CaCl_2$	• ↑ ϕ + same [Ca^{2+}] => structural changes from particulate to both fine stranded and random aggregates • Same ϕ + ↑ [Ca^{2+}] => larger gel pores + protein aggregates • ↑ [Ca^{2+}] => particulate structure of random aggregates + oil droplets flocculation (excessive calcium bridging between proteins) • ↓ [Ca^{2+}] => filamentous network	(Beaulieu, et al., 2002; Egan, et al., 2013; Sok, et al., 2005)

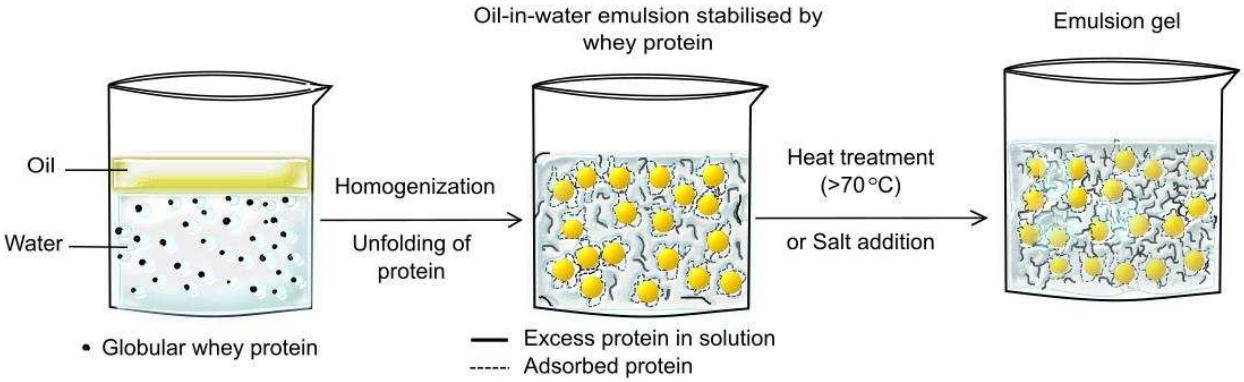
Figure captions

Figure 1. Schematic of emulsion gel formation using whey protein.

Figure 2. Schematic diagram illustrating the effect of fillers on G´. (Solid line: inactive filler; dotted line: active filler).

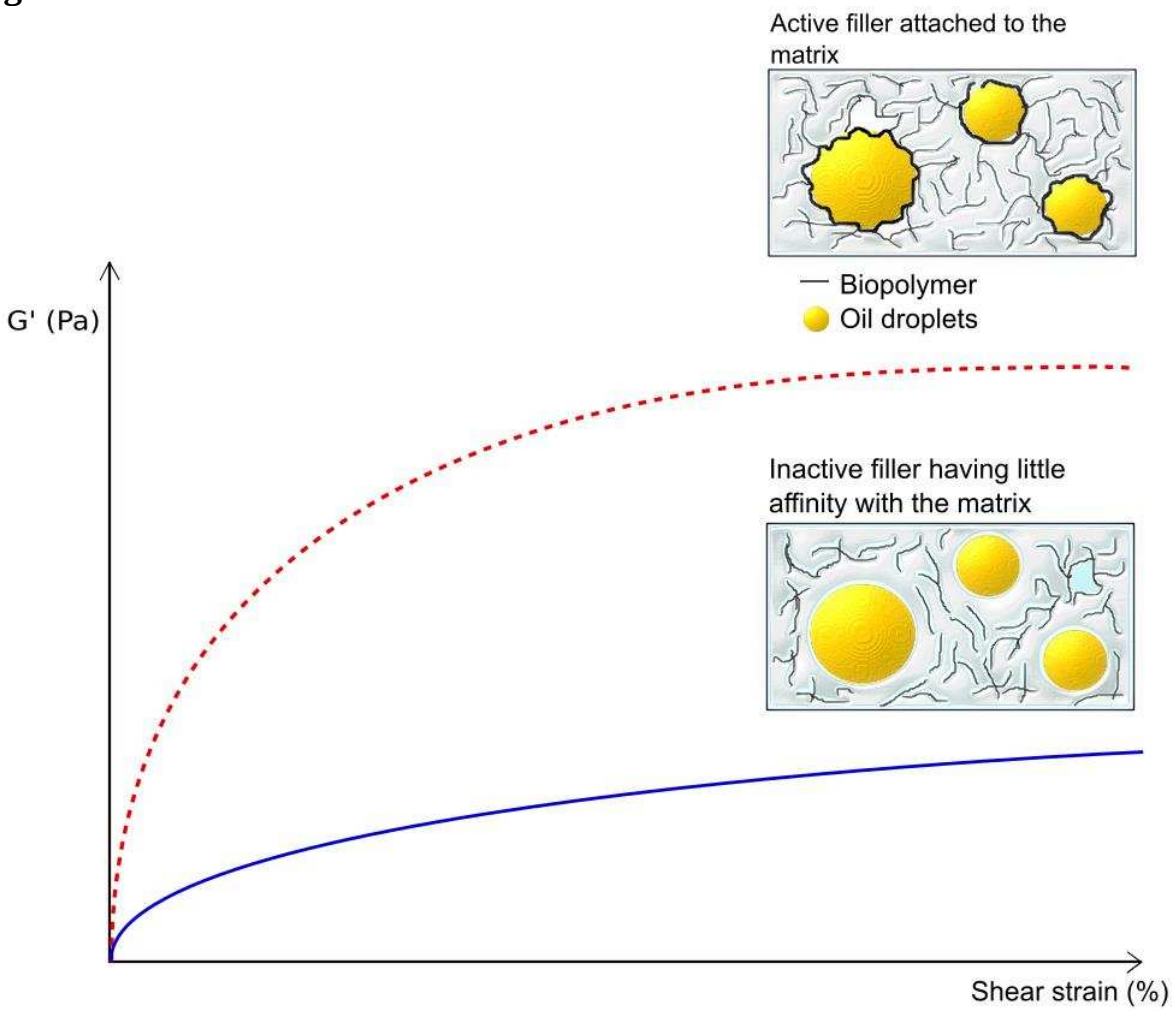
Figure 3. Schematic of controlled release of lipophilic molecules from emulsion microgel particle via swelling or matrix erosion.

912 **Figure 1.**



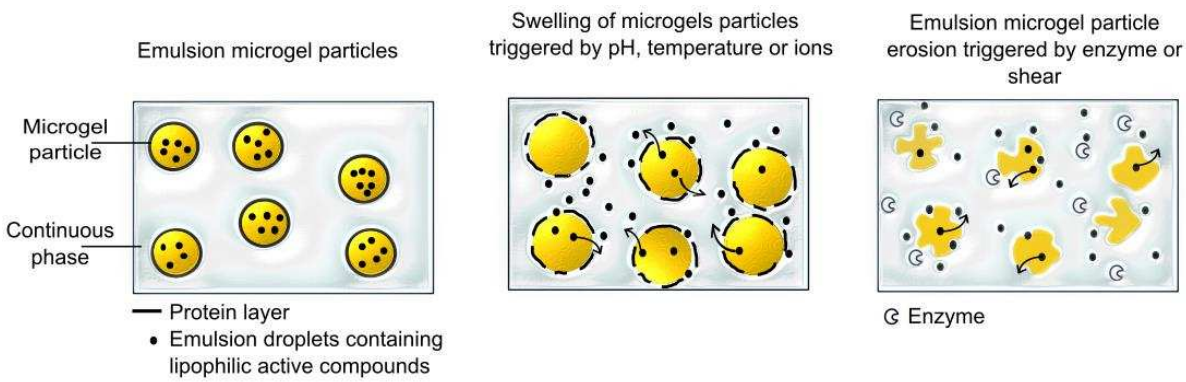
913
914

915 **Figure 2.**



916
917
918

Figure 3.


Emulsion microgel particles
Swelling of microgels particles triggered by pH, temperature or ions
Emulsion microgel particle erosion triggered by enzyme or shear
Microgel particle
Continuous phase
Protein layer
Emulsion droplets containing lipophilic active compounds
Enzyme