Introduction to Emulsion Technology, Emulsifiers and Stability

By: Dr. Lisa Zychowski

1. Emulsions

An emulsion is a dispersed system consisting of at least two immiscible liquid phases. The dispersed liquid is usually in the form of droplets and is referred to as the dispersed, discontinuous, or internal phase. The liquid surrounding these droplets is the continuous or external phase (McClements, 2015). These dispersions can be of two main formats, oil-in-water (o/w) or water-in-oil (w/o); most food systems are o/w emulsions, such as milk, soup, or salad dressing, but there are some instances of w/o, such as margarine or butter (Dickinson, 1999; Widlak, Hartel, & Narine, 2001). Although not as commonly utilised, multiple emulsions can be prepared that are composed of larger droplets that contain another dispersed phase such as oil-in-water-in-oil (o/w/o), or vice versa (w/o/w; Fig 1.1). These can be used for controlled release or to protect certain ingredients, such as probiotics (Akoh & Min, 2008).

All emulsions are inherently unstable systems due to the thermodynamic incompatibility of the oil and water phases at the interface (McClements, 2015). The unfavourable interaction between oil and water drives the droplets to merge and inevitably separate into different phases. Factors such as the emulsifiers used, droplet size, type of emulsion, and fat or water concentration can all influence emulsion stability (David Julian McClements, 2004).

The type of emulsifier and/or surfactant employed is one of the most important factors to consider when developing an emulsion. Emulsifiers and surfactants are typically amphiphilic molecules, which means they have both polar and nonpolar regions. This chemical structure gives them the ability to absorb to an interface and create a protective membrane around the dispersed phase. Surfactants are typically smaller molecules such a lecithin or tween, while emulsifiers tend to be larger biopolymers such as proteins or hydrocolloids. Both compounds are surface active and are used in different applications or in tandem to create emulsion systems.

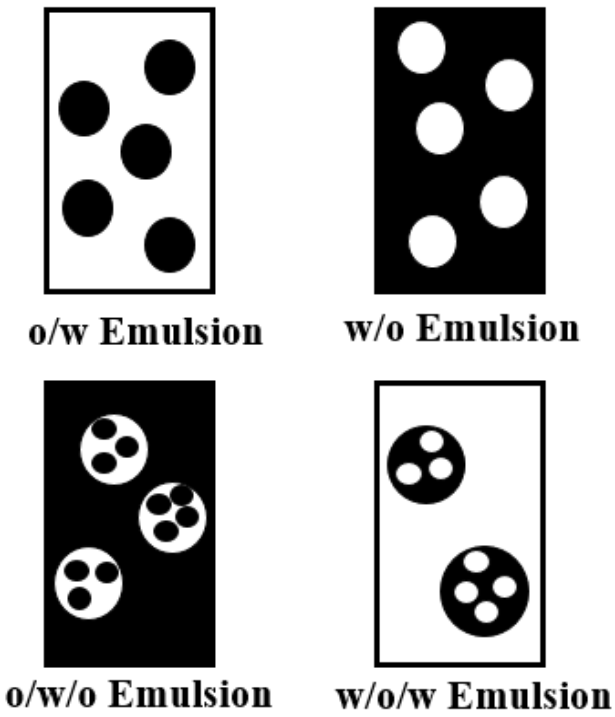

Figure 1.1 *The two-main single (top) and double emulsion varieties (bottom).*

1.1 Emulsion formation and stability

When the immiscible phases of an emulsion are mixed, they generally separate, as this is the most thermodynamically stable state. Thus, in order to mix the liquids, a mechanical force is required to combine the two phases into an emulsion. Often a two-step process, emulsion formation requires mixing by means of high shear mixing, homogenisation, membrane processing, and/or ultrasonication. In emulsion formation a course emulsion is first created to mix the two phases. Afterwards, high shear is applied to reduce the size of the dispersed droplets (Dickinson, 1999).

The quantity and time of the shear applied influences the size and stability of the dispersed droplets (Damodaran, Parkin, & Fennema, 2007). For example, in a study by Desrumaux and Marcand (2002), the average size of an emulsion droplet before final homogenisation (coarse emulsion) was 30 μm. When 50 MPa of pressure was applied to the emulsion from homogenisation, the average emulsion droplet size decreased to 0.7 μm, and with a higher homogenisation pressure (250 MPa) the droplet size decreased further to 0.25 μm. While shear is needed to create an emulsion to prevent the droplets from merging during formation, a

sufficient quantity of an emulsifier must also be present to absorb onto the droplet surface. The emulsifier will interact with both the dispersed and continuous phase to create a barrier between the two liquids. This barrier could consist of one or more emulsifiers but needs to be effective in preventing the dispersed droplets from interacting (D. J. McClements, 2004; Palanuwech & Coupland, 2003).

Droplet interactions can result in several different types of emulsion destabilisation, such as coalescence, Oswald ripening, flocculation, phase inversion, sedimentation, and/or creaming (Fig. 1.2). Coalescence is the process by which two droplets merge during contact to form a larger droplet (Damodaran, Parkin, & Fennema, 2007). Partial coalescence can also occur and is normally seen in food emulsions when a crystallised fat crystal from one droplet pierces the lipid phase of another droplet (Dickinson, 1999). Flocculation is the process in which droplets make contact but do not merge. Oswald ripening can occur when both phases are not completely immiscible and there are different droplet sizes present in the system; here, larger droplets will form at the expense of smaller droplets (Damodaran, Parkin, & Fennema, 2007). Creaming, sedimentation, and phase inversion also result in noticeable physical changes in the emulsion structure and, in food emulsions can result in the end of shelf-life. Food emulsion instability can also come in the form of chemical degradation of the dispersed phase, such as lipid oxidation or microbial processes (David Julian McClements, 2004).

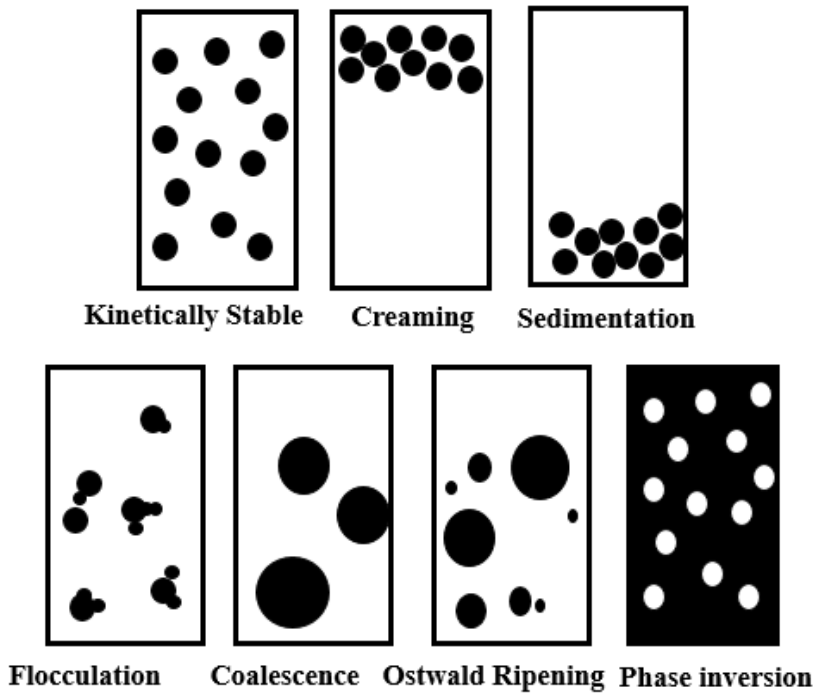

Figure 1.2 *Physical mechanisms of emulsion destabilisation adapted from McClements, 2007.*

1.2 Emulsifiers and surfactants

During emulsion formation, emulsifiers and/or surfactants present in the formulation will move to the oil/water interface and align. These surface-active molecules are typically amphiphiles, with polar and non-polar portions of the molecule and are able to interact with both emulsion phases (Hill, 1998).

The smaller compounds, known as surfactants, have a greater mobility than emulsifiers and tend to dominate the emulsion interface initially. Surfactants can help produce small droplets during emulsion formation, but are not normally used to provide long-term stability to an emulsion. Examples of surfactants utilised in the food industry are tweens, lecithin (phospholipids), and mono-and di-glycerides (Akoh & Min, 2008). Emulsifiers, on the other hand, impart long-term stability to an emulsion system, although they typically take longer to reach an emulsion interface, as they tend to be larger molecules than surfactants. Commonly utilised emulsifiers in the food industry include various proteins and hydrocolloids, such as whey, caseinates, or modified starches (Foegeding & Davis, 2011).

Non-ionic surface-active compounds are categorised by their hydrophilic-lipophilic balance (HLB). The calculation of HLB is as follows:

$$\mathrm{HLB} = 20\left(\frac{Mh}{M}\right) \tag{1.1}$$

where M_h = molar mass of the hydrophilic portion of the molecules and M = molecular mass of the whole molecule (Griffin, 1954; O' Dwyer, 2012). HLB values can be used to predict the surface activity of a compound and are useful when formulating an emulsion. For example in an o/w emulsion it would be advantageous to select a surface-active compound that has a higher HLB value (8-18; Table 1.1), as the compound needs to be dispersible within the continuous phase but still be able to interact with the oil droplets (Bos & van Vliet, 2001).

Table 1.1 Ranges of applications of surfactants with different hydrophilic-lipophilic balance (HLB) values

HLB value	Application
3-6	Emulsifiers of w/o emulsions
7-9	Wetting agents
8-18	Emulsifiers of o/w emulsions
13-15	Detergents
15-18	Solubilisers

Table adapted from Bos & van Vliet, 2001.

1.2.1 Food hydrocolloids as emulsifiers and stabilisers

Many hydrocolloids and protein-hydrocolloids complexes can also be employed within food emulsions. Often, food hydrocolloids act as stabilisers within the continuous phases of a food emulsion. By structuring and thickening the aqueous phase of an emulsion system, destabilisation processes, such as creaming, are often slowed. However, some hydrocolloids can stabilise the oil and water interface of an emulsion system, such as gum arabic, modified celluloses and starches, pectins and galactomanns (Dickinson, 2009; Garti & Reichman, 1993).

One of the most commonly utilised hydrocolloids is xanthan gum due to its extremely high low-shear viscosity. Oil droplets within an o/w emulsion can become trapped and sterically immobilised in the hydrated xanthan network. The yield stress created by this system tends to be larger than the buoyancy forces acting on the individual droplets, and thus, creaming is dramatically slow (Dickinson, 2003). For example, xanthan or pectin have been employed to improve the stability of wheat protein-based emulsions. Without the added hydrocolloid, the emulsion system rapidly creamed around pH values of 5 (the isoelectric point of wheat protein) and/or with the addition of salt ($CaCl_2$ ($\geq$10 mM, pH 7). Xanthan gum was found to dramatically improve emulsion stability in the CaCl2 system, even at lower pHs of 3.5 or 5, as xanthan gum was found to increase the stearic and electrostatic repulsion between the droplets. Pectin interestingly could improve the stability of wheat protein emulsion at acidic pHs but not emulsions that also had a higher level of salt. It was hypothesised that pectin, typically an anionic polysaccharide, was only weakly attached to the emulsion interface and could only provide limited stabilisation (Qiu, Zhao, & McClements, 2015).

In order to stabilise the interface of an emulsion, a hydrocolloid needs to possess both hydrophobic and hydrophilic regions. This is why proteins are often employed, as many are flexible molecules with an amphiphilic structure (D. J. McClements, 2004). However, hydrocolloids can be utilised and are often useful when the conditions of the emulsion system are unfavourable (i.e. extreme temperatures, pHs, ionic strength, calcium ion concentration ect.). For instance, casein-proteins tend to be highly susceptible to destabilisation by acidification of the aqueous medium, hence the cheese making process. Whey proteins, on the other hand, are sensitive to changes in heat. In a study performed by Chanami and McClements (2002), emulsions were stabilised by either gum arabic, modified starch or whey protein and were studied under different pHs (3 to 9), $CaCl_2$ salt concentrations (0 to 25 mM), or temperatures (30°C to 90°C). The stability of whey protein emulsions was significantly influenced by all of the chosen variables, as the main stabilising mechanism of whey protein is electrostatic repulsion. The hydrocolloid-based emulsions, however, were stabilised via stearic repulsion, and thus, were not greatly affected by the changes in pH, salt or temperature. As hydrocolloids can add a thick stabilising layer to an oil and water interface; they can in doing so, prevent aggregation over a large range of unfavourable conditions (Dickinson, 2009).

Hydrocolloids can also be used with different proteins to create complexes at an oil and water interface. Typically, the two compounds are bound by either electrostatic or covalent forces. Covalent linkages can be formed by chemically binding the two compounds directly, i.e. amine bond between gelatine and high-methoxyl pectin (Diftis, Pirzas, & Kiosseoglou, 2005). Conjugation of the two compounds can also occur enzymatically or by directly heating the protein and hydrocolloid together. Once bound, the complex is more surface-active than the hydrocolloid alone. Thus, less hydrocolloid is required and the stearic layer from the hydrocolloid provides stability for the protein under extreme environments (Dickinson, 2009). While effective, care must be taken as an excessive quantity of the hydrocolloid can cause gelation in the aqueous phase of the emulsion, while too little can cause depletion flocculation among the oil droplets (Dickinson, 2003).

In general, it is extremely important to consider the physiochemical properties of any given emulsion system, as these factors determine the type of emulsifier that should be employed. As food products vary dramatically in processing conditions, formulation, employed pHs and salt

content; often hydrocolloids can be utilised to stabilise these systems, either in the aqueous phase or directly at the interface.

1.3 Methods to measure emulsion stability

The perceivable quality attributes of an emulsion system are strongly influenced by their physicochemical characteristics such as droplet size, charge, droplet-droplet interactions and pH. If destabilisation occurs, obvious sensory and bulk physicochemical defects can be detected. In a food emulsion system, emulsion destabilisation often results in the end of shelf-life and, thus, it is crucial to quantify how and why changes are occurring within the food system (Dickinson, 1999).

To characterise a colloidal food system multiple different analytical instruments are used to quantify emulsion characteristics, i.e., droplet size, morphology, and charge. It can also be equally important to quantify changes in the continuous phase, such as crystallisation or changes in viscosity. Often, combinations of techniques are used to elucidate the main mechanisms behind emulsion destabilisation, which in the future could aid in the creation of emulsions with improved physio-chemical properties.

1.2.1 Light Scattering

The size of the dispersed droplets is a major factor in quantifying the stability of an emulsion system. Droplet size is often evaluated using light-scattering techniques, which are related to the intensity of light from a laser. Larger particles will scatter light at narrow angles, as opposed to the smaller particles that scatter light at wider angles (Fig. 1.3b). This information is processed by a laser diffraction instrument such as a Mastersizer (Malvern Instruments Ltd., Malvern, UK; Fig. 1.3a) and the range of particle sizes present in the sample is calculated (Sprow, 1967). The most commonly utilized information from the Malvern is the $D_{(4,3)}$ value, which is the volume-based mean diameter, and the particle size distribution. This average is based on the volume of the dispersed phase instead of the number of particles. The effect of this is to increase the sensitivity of larger particles (McClements, 2007).

Light scattering is often used to measure emulsion droplet sizes and can be used when evaluating the response of emulsions to different treatments. In a study by Drapala et al. (2016), sunflower oil emulsions with whey protein and maltodextrins were evaluated for heat stability. Light scattering data showed changes in the fat globule size distribution (FGSD) after heat treatments and demonstrated that whey proteins conjugated with maltodextrins had the best

heat stability, as minimal changes in the FGSD were observed. Light scattering can also be used in a similar way to measure the size of powder particles or protein aggregates. For example, laser light scattering is often used to quantify the sizes of different dairy powders, as results obtained give a better representation of the different powder sizes than other techniques, such as microscopy (Silva & O'Mahony, 2017).

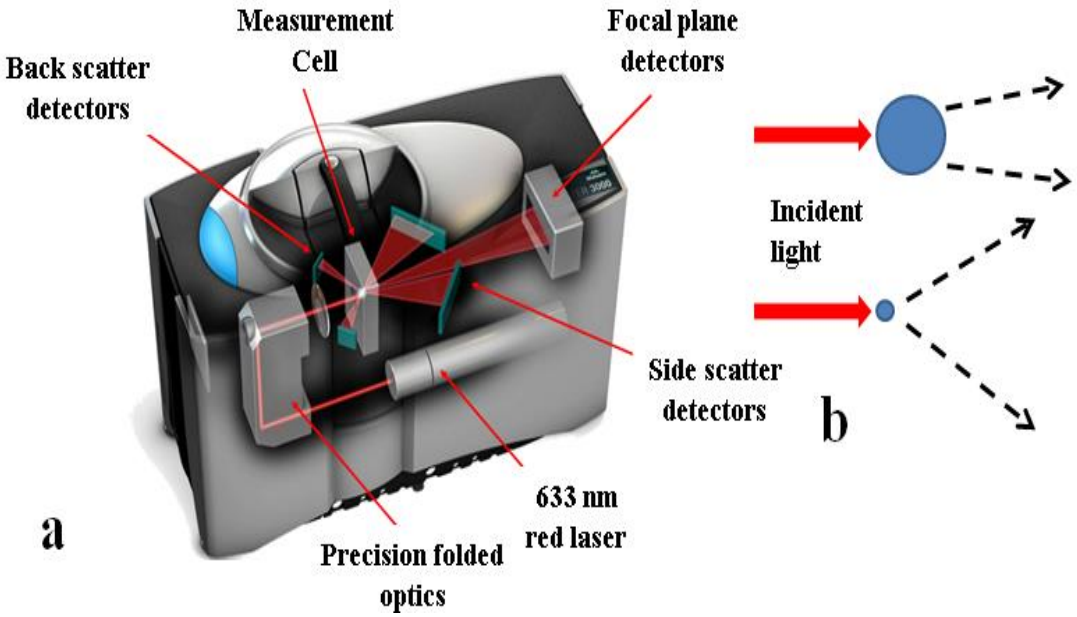

Figure 1.3 *(a) Diagram of light scattering device (Malvern Mastersizer) utilised to measure particle size. Red laser light is first bent by precision folded optics and is then passed through the measurement cell. Here the laser light is scattered by (b) different particles, which is then interrupted by the different detectors. The focal plane detector is mainly used for small angle scattering (larger particles), while the other detectors (side and back scatter) capture larger angle scattering (smaller particles; Malvern 2017)*

Light scattering can also be used to measure emulsion creaming or sedimentation, with systems, such as the Turbiscan (Turbiscan MA2000, Formulaction, France) or Lumifuge (Adaptive Instruments, West Sussex, UK). During creaming in o/w emulsions, the lower density droplets will move upwards, which also results in a clearing of droplets from the bottom of the tube (McClements, 2007). The Turbiscan can measure this change in the stability o/w emulsion by backscattering photons along the length of a tube containing the emulsion. This is then picked up and interrupted by an infrared diode and compared against the emulsion before

storage. A LUMiFuge functions in a similar way but uses centrifugation to accelerate the aging process (McClements, 2015).

1.2.2 Zeta potential

Depending on the system, electrostatic charge (zeta potential, ζ) can also give an indication of colloid stability. The ζ-potential is the most stable when the pH of the system is far from the isoelectric point of the compounds present at the emulsion interface. Other factors such a concentration of salts can also influence the ζ-potential and, thus, it important to establish the electrostatic charge of the emulsifier (Drapala, 2016). ζ-potential information is also useful in understanding the general behaviour of proteins and other dispersible compounds at various pHs and conditions (Hunter, 2013).

To measure ζ-potential, an instrument such as the Zetasizer (Malvern Instruments Ltd., Malvern, UK) is used, which sends an electrical current through a diluted colloidal sample. The electrical potential of the samples at the "shear plane" can give an indication of the attraction/repulsion forces present on the dispersed droplet (McClements, 2007). Often this is used, along with other techniques such as light scattering and microscopy, to gauge the mechanism of emulsion destabilisation in a system.

For example, ζ-potential was utilised in a study evaluating the emulsion stability in a high salt ($CaCl_2$) system with lactoferrin and/or β-casein emulsions. ζ-potential decreased in emulsions with a mixture of lactoferrin and β-casein or β-casein alone at the interface but no change was observed with $CaCl_2$ addition in emulsions with just lactoferrin. This information was combined with light scattering data, which showed that emulsions made with lactoferrin or both β-casein and lactoferrin had no significant change in particle size, while emulsions with β-casein alone had a large increase in emulsion droplet size. From this information the authors were able to conclude that lactoferrin can protect β-casein-stabilised emulsions from calcium-induced flocculation, as it electrostatically interacts with the negative phosphates groups on β-casein and can block calcium phosphate interactions (McCarthy, Kelly, O'Mahony, & Fenelon, 2014).

1.2.3 Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) offers a unique opportunity to visualise different emulsion components, such as proteins, surfactants, and lipids. This is achieved through specific fluorescent probes emitting different wavelengths of light from pre-labelled samples.

Separate channels then process the intensity emitted from the sample and images are created and overlapped to give insight into the multi-component microstructure of the sample (Fig. 1.4). While CLSM can be a powerful tool, care must be taken to minimise sample disturbance, i.e., emulsion destabilisation from fluorescent dye addition (Auty, Twomey, Guinee, & Mulvihill, 2001).

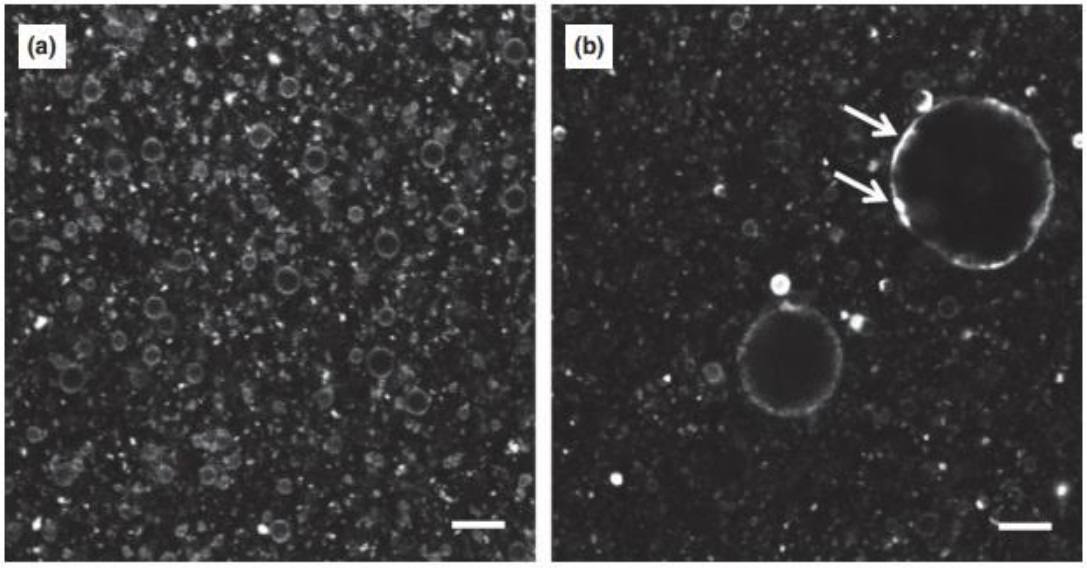

Figure 1.4 *High-magnification confocal micrographs of emulsions showing the uneven protein coverage in emulsions with (a) 0% lecithin and (b) 1% lecithin. Scale bar = 5 µm. Arrows highlight the variable thickness of protein at the oil droplet interface (Drapala, Auty, Mulvihill, & O'Mahony, 2015).*

Emulsion characteristics, such as droplet morphology and size, can be elucidated from CLSM images, along with protein distribution and interfacial thickness. Often, CLSM visualisation makes it easier to identify the causes of emulsion destabilisation, and it is often employed along with other techniques in fundamental emulsion studies.

For example, in a study by Drapala et al. (2015), model infant formula emulsions were produced with protein hydrolysate and varying levels of lecithin. The addition of lecithin into the system was found to decrease interfacial tension and to decrease the fat globule size. However, at lower levels of lecithin (1-3%), coalescence occurred and a bimodal fat globule distribution was detected. Images of coalesced droplets captured using CLSM showed an uneven distribution of protein at the droplet interfaces of the emulsion suggesting possible partial displacement of the whey protein by lecithin (Fig. 1.4).

1.2.4 Cryogenic scanning electron microscopy

Cryogenic scanning electron microscopy (Cryo-SEM) is another useful technique for directly visualising the microstructure of emulsions. As emulsions are typically liquid systems, the sample is first rapidly frozen using liquid nitrogen. After freeze-fracturing, the sample is placed under a vacuum, sublimated, and coated with a conductive metal such as platinum. The sample can be imaged in the frozen hydrated state using a focused electron beam. During imaging, primary electrons penetrate the sample and incident electron scattered from the sample are interrupted by detectors as images.

Similar to CLSM, Cyro-SEM can be a helpful tool in visualising structure but expertise is needed to minimise ice crystal development in high-moisture systems, such as o/w emulsions (Auty, Twomey, Guinee, & Mulvihill, 2001; Kelly, 2015). Cryo-SEM is often useful for visualising crystal development in emulsion systems, as this is difficult to see using CLSM. In a study by Norton et al. (2009), cocoa-butter emulsion systems were successfully imaged using Cyro-SEM, showed that in tempered emulsions a smooth layer of crystalline fat on the surface aided in emulsion stability. Fat-based Pickering emulsions, that exploit crystalline fat for interfacial stability, are often also characterised by Cryo-SEM (Frasch-Melnik, Norton, & Spyropoulos, 2010; Frasch-Melnik, Spyropoulos, & Norton, 2010).

1.4 Interfacial tension

When emulsifiers or surfactants align and interact at an emulsion interface, a decrease in the free energy of the system occurs. This decrease in free energy makes emulsion formation possible and can be quantified through interfacial tension measurements (Kim & Burgess, 2001). Interfacial tension is commonly utilised to quantify the surface activity of a compound. Traditionally performed with a Langmuir tray, interfacial tension measurements have become possible with optical devices, du Noüy rings, and Wilhelmy plates. The Wilhelmy plate (Krüss GmbH, Hamburg, Germany) is useful for emulsion systems as dynamic interfacial tension can be recorded over time. Thus, it is possible to quantify how quickly a compound can move to the interface, changes in interfacial tension over time, and if the compound is stable at the interface (Drapala, 2016).

The Wilhelmy plate is a thin vertical plate, which first operates by making contact with the heaviest (higher density) immiscible phase. The second phase (typically the lighter oil phase) is then added to cover the plate and the plate begins to pull against the interface (Fig. 1.10).

The tension or force present at the interface is quantified using a microbalance (equation 1.2). If a surface-active compound is present, that force will be lower than for a clean interface (Drelich, Fang, & White, 2002).

$$\gamma = \frac{F}{L \cos \theta} \tag{1.2}$$

where γ is the interfacial tension (mN/m), *F* is the pull force (mN) acting on the plate, *L* is the plate length (i.e., 2 × plate width; mm) and θ is the contact angle between liquid meniscus and the plate (Drapala, 2016).

By measuring interfacial tension, is it also possible to observe how different compounds behave at an emulsion interface. For example, Li et al. (2016) used dynamic interfacial tension measurements to demonstrate that mixtures of whey protein isolate (WPI) and β-casein were able to lower interfacial tension to a greater extent than WPI alone; these results, along with CLSM images, which showed both proteins co-localised at the emulsion interface, suggest a potential interaction with the two proteins at the emulsion interface. Similar results have been observed in emulsion systems with proteins and other surfactants, such as lecithin (Drapala, Auty, Mulvihill, & O'Mahony, 2015; Sünder, Scherze, & Muschiolik, 2001).

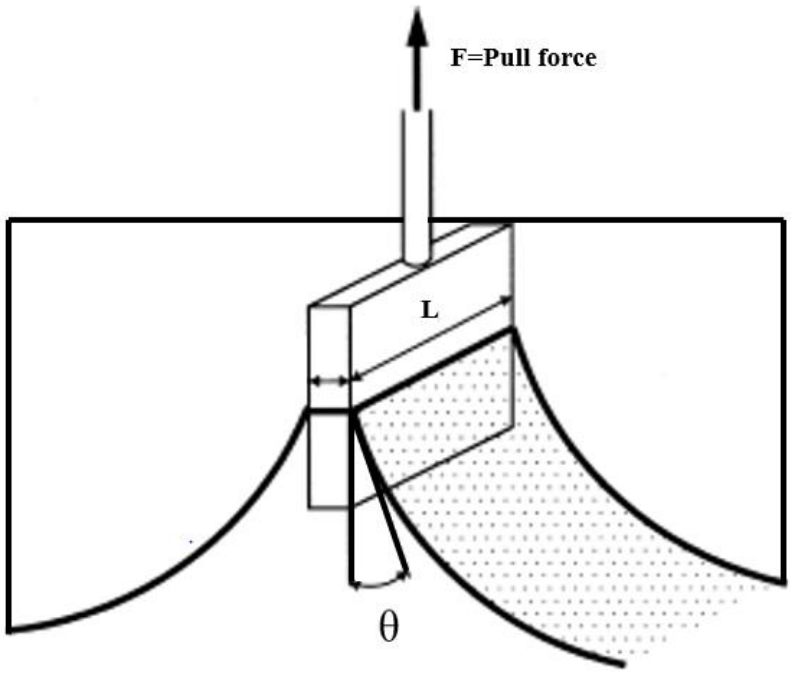

Figure 1.5 *Schematic of Wilhelmy plate geometry used for interfacial tension measurements (Drelich et al., 2002, Drapala, 2016)*

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