Edible Pickering Emulsion Technology:

Fabrication of Edible Particle Stabilised Double Emulsions

By

Laudina Jeneise Duffus

A thesis submitted to

The University of Birmingham

for the degree of

DOCTOR OF ENGINEERING

School of Chemical Engineering

College of Physical and Engineering Sciences

The University of Birmingham

December 2016

Abstract

Water-in-oil-in-water (W/O/W) double emulsion systems provide an innovative approach for the development of low-fat healthier foods. By replacing a proportion of the oil phase of a simple oil-in-water (O/W) emulsion with an internal water phase, the overall oil volume within the emulsion system can be decreased, with potentially negligible changes to its organoleptic properties. However, double emulsions are notoriously unstable for adequate periods of time, largely due to the existence of two oppositely curved water-oil (W/O) and oil-water (O/W) interfaces in close proximity. The present study investigates the use of Pickering stabilisation in order to enhance the stability of double emulsions. Pickering stabilisation mechanisms are reputed for superior, longer term stabilisation capacities when compared to conventional surfactant stabilised emulsions, but edible particles with Pickering functionality are scarce.

The work in this thesis explores the impact of introducing Pickering stabilisation to a double emulsion structure, initially at only one of the two water/oil interfaces (either W/O or O/W) and ultimately across the entire interfacial areas. Initial work conducted centred on investigating the role of a range of edible particulates as potential Pickering stabilisers in simple emulsions (both W/O and O/W emulsion types). Based on the knowledge gained from these studies, a range of Pickering-Surfactant stabilised double emulsions (with particles or surfactant stabilising alternate interfaces), using a range of surfactants, and Pickering only stabilised double emulsion systems were prepared and analysed in terms of their microstructure, stability and encapsulation efficiencies.

Acknowledgements

I would like to thank:

- My academic supervisors, Dr. Fotis Spyropoulos and Professor Ian Norton in Birmingham for providing support and also giving me the opportunity to pursue an EngD.
- My industrial supervisor Paul Smith at Cargill.
- The EngD Programme Manager: Dr. Richard Greenwood.
- Everyone who has been involved in the wider ePET project over the years as well as the Microstructure Research Group.
- The EPSRC, Cargill and Innovate UK for generous financial support over the four years.
- My friends and family in Birmingham and beyond who always encouraged me to keep going. I got here in the end Grandma, Grandad, rest in peace.
- Mom, Dad, and Chaa-zaq, thanks for being there, and for all your support and advice and for pushing me forwards and always knowing the right things to say.
- Jermaine, thanks for all your love, patience, late nights and salted caramel lattes and for putting up with me during the writing process.

Table of Contents

List of Figures

List of Tables

Chapter One

Thesis Introduction

1.1. Introduction

Chapter Overview

The goal of this chapter is to:

i. Present an overview of the background and motivation for this thesis in terms of the novelty and value to research.
ii. Provide an introduction to Pickering stabilisation and emulsion science and its applicability in fat reduction and encapsulation with food systems; emphasis is placed upon providing the reader with the general principles, definitions, and terminology necessary to follow chapters 2, 3, 4 and 5.
iii. Outline the industrial relevance/implications of the work to the sponsoring company (Cargill).
iv. State the specific thesis aims and objectives.
v. Explain how the thesis is organised.

1.2. Research Motivation

Obesity is the medical condition where a person's body fat has accumulated to the extent that it could have a negative impact on their health. Not only does obesity increase the burden on the National Health Services (NHS) across the UK today but it also lowers the quality of life for the proportion of the population that suffer from it. At present, the direct cost of obesity related health problems to the NHS is £4.2bn; indirectly the cost is £16bn in the UK. Unfortunately, projections from Foresight expect this direct cost to double in the next 40 years and expectations are that indirect costs of obesity will rise to £50bn [1]. Recent reports from the

NHS suggest that half of the UK's population will be considered obese by 2030 as this problem becomes more widespread [1]. Commonly, obesity is mainly associated with the excessive consumption of fats in food. Fats are present in food formulations, such as chocolate, fried foods, whipped cream, processed meats and butter. In particular, fats are integral in common food emulsions such as margarine, mayonnaise and salad dressings as they are largely constituted of a water and an oil phase blended together to form the emulsion. The main challenges for formulation engineers with respect to the obesity problem are twofold. Not only must the reduced fat emulsion be as stable as a conventional full fat oil-water emulsion, it must also be of an acceptable taste quality and appearance. This is because although healthier, consumers will still expect the engineered emulsion formulations to deliver a pleasurable eating experience.

Formulation engineering can go some way to tackle the obesity issue by engineering food emulsions with a reduced fat content and hence producing healthier foods. This can be achieved via a variety of stabilisation mechanisms and complex microstructural arrangements such as multiple emulsions and specifically double emulsions. In particular and further in support of the project goals, the W/O/W emulsion system provides a solution for healthier food emulsions. By replacing a proportion of the oil phase with an internal water phase, the overall volume of oil within the emulsion system can be lowered. Hence, an emulsion is consequently produced possessing an overall fat content that is reduced compared to a simple O/W emulsion. Edible double emulsions are a fairly recent phenomenon, however pace is being gained within industry with respect to their development as their benefits and uses become ever more recognised.

However, although beneficial to food industries, multiple emulsions are notoriously unstable for sufficient periods of time (i.e. 1 week), largely due to the additional, oppositely curved, oil-water interfaces. Efforts have been made to enhance their stability and particle stabilisation has been proven to be successful for this purpose [2-4]. Pickering stabilisation has long been recognised as a very useful and suitable method for producing emulsions with superior stability when compared to conventional surfactant stabilised emulsions. Pickering particles accumulate at the oil-water interface in the form of a densely packed layer; the mechanism by which this occurs depends largely upon the particle's physical properties and chemical structure. This layer then protects the emulsion system against droplet flocculation and coalescence via a steric mechanism. In comparison, surfactants stabilise emulsions via an electrostatic or steric mechanism where surfactants, in constant dynamic equilibrium, lower the interfacial tension between the oil and water phases by adsorbing to the interface.

Consequently, the issue still remains to understand and produce double emulsions using Pickering particles as the sole stabilisation species. Additionally, another challenge exists to ensure that these novel double emulsions are made with edible Pickering particles which are of a recognised food-grade standard. Further to this, the edible Pickering particles must also be incorporated at an emulsion formulation weight percentage that is in alignment with governmental food safety standards and legislation. The aim of this project and the subsequent project plan is to discover whether such emulsions can be successfully formulated. At the outset of this work, it was scientifically uncertain as to whether double emulsions could be fabricated using the edible particulate materials described in this thesis in the absence of any surfactant. It was also uncertain whether any subsequent double emulsions of this nature would possess stability against coalescence or complete phase separation comparable to or better than conventional surfactant stabilised double emulsions. The identification of edible Pickering particles, development of formulation design rules, formulation processing routes and stability criteria described within this thesis, aim to lead to the discovery of these new emulsion systems.

1.3. Industrial Relevance

Industrial sponsorship of this project was provided by Cargill Ltd which is a multinational company specialising in the provision of food, agriculture, financial and industrial products and services on a global scale. Their operations span a wide range of markets and geographies and this project was related to the Food Ingredients and Products sector, providing services to food and beverage manufacturers, foodservice companies and retailers with food and beverage ingredients and new food applications. Specifically working with the European Research & Development centre based in Belgium, where Cargill customers look for scientific expertise when the challenge is enhancing an existing product, improving process efficiencies, or uncovering a solution that helps them launch a first-to-market innovation. This project is in alignment with Cargill’s goal to leverage their research and development capabilities in order to generate distinctive value through new, improved products and innovative ways to reduce costs or both [5].

The application of edible particle stabilised double emulsions in the food industry has the potential to positively impact the aforementioned obesity issue. Consequently, the development and understanding of such formulations and their processing routes is the motivating force of this research. This is in-line with Cargill’s Health and Nutrition product portfolio which is primarily concerned with the provision of solutions regarding today's most pressing health concerns, including heart health, bone health, digestive health, obesity, weight management, tooth protection, energy management and general wellness [5].

Double emulsions stabilised using only edible particles have the potential for inclusion within such a category. Therefore, through the exploration of edible particles, development of double emulsions and understanding processing effects on emulsion microstructure and stability is of paramount importance may lead to the commercialisation of healthy double emulsions stabilised solely by edible particles, not only through Cargill and its commercial stakeholders, but the food industry in general.

1.4. Aims and Objectives

The overall purpose of this work was to deliver the understanding of formulation and processing requirements necessary for the development of edible surfactant-free W/O/W double emulsions using particles to stabilise the emulsion interfaces. The objectives of this study are as follows:

- To investigate different particle types spanning a range of chemistries and material compositions with respect to their Pickering functionality for both O/W and W/O emulsions/interfaces. Particular emphasis is placed on using as-obtained particulate material requiring no further modification.
- To design double W/O/W emulsions using a combination of Pickering and surfactant emulsifiers by firstly substituting surfactant for particles initially at one interface only to understand any additional benefits gained through the replacement of surfactant by particles at either interface.
- To develop characterisation approaches to reliably interrogate the double emulsion microstructure, specifically to characterise inner water phase and determine inner droplet encapsulation efficiency as a function of formulation and processing route used.
- To design and fabricate double W/O/W emulsions using only edible Pickering particles to stabilise emulsion droplets and assess their stability over time.
- To study and develop an understanding of the effect of primary and secondary processing routes and internal water phase concentration on Pickering-only double emulsion stability and microstructure.

1.5. Thesis Layout

Arranged into five chapters, this thesis contains three results chapters written in the style of peer-reviewed publications (Chapters 3, 4 & 5). Each results chapter consists of an abstract, an introduction (including the analysis of relevant literature), explanation of materials and methods, results and discussion, and conclusions and future work. The start of each results chapter states where and how the work has been disseminated.

A synopsis of each chapter is given below:

Chapter 2 – *Literature Review*

This chapter gives a background of the research area depicted in this thesis. Additionally, relevant work conducted so far in the area of double emulsions and Pickering stabilisation has been critically reviewed, emphasising the main ideologies behind the applied research directions in this work.

Chapter 3 – *A comparative study on the capacity of a range of food-grade particles to form stable O/W and W/O Pickering emulsions*

This chapter details the investigation into as-obtained edible Pickering particles. The aim of this work was to identify and analyse particulate structures that could potentially act as Pickering particles with minimal or no alteration to their chemical structure. A wide range of particles were sourced however, only a select few were carried forward and discussion around these particles forms the bulk of the written work in Chapter 2.

Chapter 4 – *Double W/O/W emulsions stabilised using various Pickering particle systems*

Work in this chapter focuses on the combination of Pickering stabilisation and double emulsions in an attempt to formulate W/O/W emulsions. Experiments were designed to investigate any benefits arising from the use of edible Pickering particles (identified in the Chapter 2) at one of or both interfaces of a double emulsion in conjunction with known conventional emulsifiers. In particular, focus was placed upon understanding differences in droplet size, emulsion stability and internal water droplet retention.

Chapter 5 – *Pickering W/O/W double emulsions stabilised solely by ethylcellulose and rutin hydrate particles*

The principal intention of the study described in Chapter 4 was to utilise edible particulate materials for the stabilisation of W/O/W double emulsions in the absence of any additional surfactants, stabilisers or emulsifiers. The influence of the emulsification process on microstructure, droplet size and stability of these novel systems was also investigated. Focus was also placed upon understanding the extent to which the formulation can serve as tool for fat content reduction through phase volume manipulation. The encapsulation efficiency of double emulsions was calculated and the way in which this is affected by processing route and phase mass fraction was evaluated.

Chapter 6 – *Overall conclusions*

This chapter includes the summary and conclusions arising from the work done in this thesis.

Chapter 7 – *Future work*

This chapter details recommendations for future work.

References

[1] Butland, B., Jebb, S., Kopelman, P., McPherson, K., Thomas, S., Mardell, J., and Parry, V., Tackling obesities: Future choices. *UK Government Office for Science,* **2007**,

[2] Oza, K.P. and Frank, S.G., Multiple emulsions stabilized by colloidal micrograstalline cellulose. *Journal of Dispersion Science and Technology,* **1989**, *10*, p. 163-185.

[3] Garti, N., Aserin, A., Tiunova, I., and Binyamin, H., Double emulsions of water-in-oil-in-water stabilized by alpha-form fat microcrystals. Part 1: Selection of emulsifiers and fat microcrystalline particles. *Journal of the American Oil Chemists Society,* **1999**, *76*, p. 383-389.

[4] Spyropoulos, F., Frasch-Melnik, S., and Norton, I.T., W/o/w emulsions stabilized by fat crystals - their formulation, stability and ability to retain salt, in *11th international congress on engineering and food* **2011**. *1*, G. Saravacos, P. Taoukis, M. Krokida, V. Karathanos, H. Lazarides, N. Stoforos*, et al.*, Eds., Procedia food science: Elsevier Science Bv, p. 1700-1708.

[5] Cargill. (**2016**, 3rd July 2016). *Health promoting ingredients* Available at: http://www.cargillfoods.com/emea/en/products/health-promoting-ingredients/index.jsp

Chapter 2

Literature Review

A proportion of this work has been published as follows:

Pichot, R., Duffus, L., Zafeiri, I., Spyropoulos, F., and Norton, I.T., (2015) Particle-stabilized food emulsions, in *Particle-stabilized emulsions and colloids: Formation* *and applications*, Eds., T. Ngai and S. Bon, RSC Soft Matter: The Royal Society of Chemistry, p. 247-282.

Chapter Overview

In order to add further context to the results chapters, it is first necessary to provide an overview of the concepts which form the basis of the research.

The goal of this chapter is to outline the following:

i. Provide an introduction to Pickering stabilisation and double emulsion science and its applicability to fat reduction and encapsulation with food systems; emphasis is placed upon providing the reader with the general principles, definitions, and terminology necessary to follow result chapters 3, 4 and 5.

ii. Present a literature review of these two research areas and describe current understanding in Pickering emulsion science and double emulsion technology.

Literature review

The work in this thesis is concerned with the design, development, and characterisation of double emulsions stabilised solely by edible particles, principally for fat reduction purposes in food emulsions. Therefore, it is necessary to broadly introduce the area of double emulsion science and Pickering emulsion technology in terms of droplet formation, interactions between droplets, the physical modes of double emulsion break-down, and types of emulsifiers and particles employed along with their role in double emulsion formation and stabilisation. This review also details how these two scientific research areas have been combined and current understanding of such systems.

2.1 Emulsions

Emulsions are liquid-liquid dispersions and are typically formed through the combination of two immiscible liquids, via the addition of an appropriate emulsifier and the application of shear. In the context of this work, the two liquids used to produce emulsions are oil and water. Oil-in-water (O/W) emulsions consist of an oil phase dispersed within a continuous aqueous phase usually containing a hydrophilic emulsifier. Conversely, water-in-oil (W/O) emulsions encompass a water phase dispersed within a continuous lipid phase within which a lipophilic emulsifier is included [1].

2.2 Double emulsions

Increasing the microstructural complexity of simple emulsions gives rise to multiple emulsions, often also referred to as ‘emulsions of emulsions’ [2]. Essentially, they are comprised of a continuous phase containing a dispersed phase. Within this dispersed phase lies a second emulsion phase. This is the simplest of multiple emulsions and is deemed to be a ternary double emulsion. Emulsions containing quaternary or further complex structures are further examples of multiple emulsions and alongside double emulsions, these types of chemical arrangements have been continually studied since their initial depiction in the early 1920s [3]. Of multiple emulsions, the most commonly used and studied are double emulsions. Within this category of emulsion, emerges two main types: water-in-oil-in-water (W/O/W) and oil-in-water-in oil (O/W/O). Both structure types are depicted in the schematic representation in Figure 2-1 and a microscopic image of a W/O/W emulsion is portrayed in Figure 2-2.

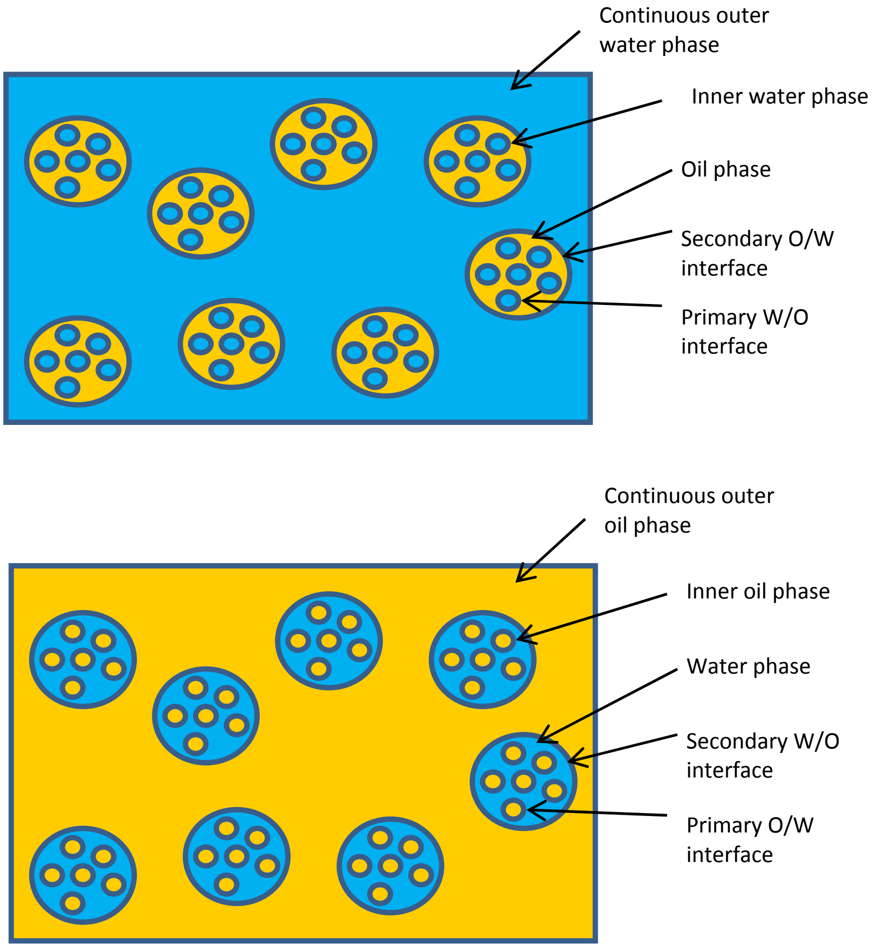

Figure 2-1 Schematic representation of W/O/W emulsion (upper diagram) and O/W/O emulsion (lower diagram).

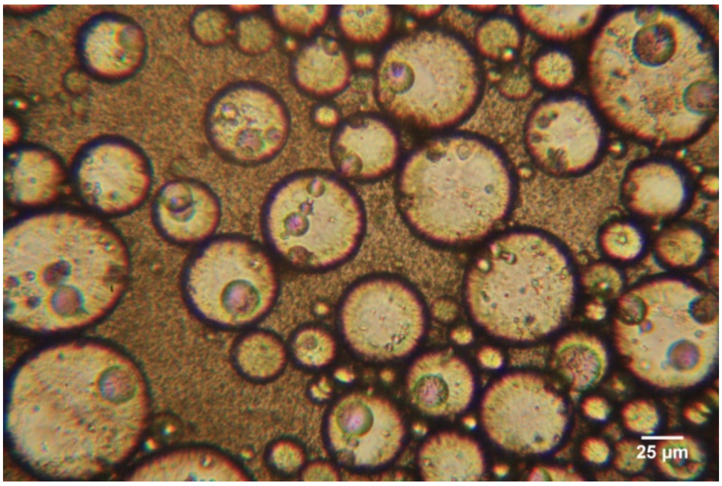

Figure 2-2 A micrograph image of a W/O/W emulsion

2.2.1 Industrial applications

The application of such emulsions in industry is far reaching and to date the potential of these types of structures have not yet been fully realised, only being more widely appreciated in the past 20 years. They have found uses in the pharmaceutical industry for example; drug targeting mechanisms [4], red blood cell substitution [5] and encapsulation of active ingredients to allow controlled release of actives [6]. In the cosmetic industry, they have been used to control release and stability of moisturising agents for the skin [7] and patents have been granted relating to both the controlled release of active ingredients to the skin [8] and delivery of desired aesthetics, i.e. pigments, in topical creams and lotions in line with consumer acceptance [9].

In the food industry, double emulsions can be utilised to improve encapsulation capabilities of active ingredients such as vitamins and minerals. Studies found in the literature show the successful employment of W/O/W emulsions in the protection of probiotics in food emulsions from degradation via gastric juice once the food had been consumed [10]. Similarly, W/O/W double emulsions were synthesised that contained sufficiently stable interfaces that protected against the release of Vitamin B1 encapsulated within the primary W/O emulsion droplets [11]. A more recent study reported that W/O/W emulsions could be used to encapsulate magnesium ions for food applications. and that the release of magnesium was primarily due to diffusion mechanisms rather than droplet coalescence within the relatively stable double emulsions produced [12]. Double emulsions have also been investigated for use in the release of aroma and flavour compounds. Orange oil was successfully encapsulated in double emulsion droplets by first creating a tertiary O/W/O/W emulsion and then evaporating the outer aqueous phase to produce a remaining O/W/O double emulsion [13].

In particular, and further in support of the project goals, W/O/W emulsions provide a solution for healthier food emulsions. By replacing a proportion of the oil phase with an internal water phase, the overall volume of oil within the emulsion system can be lowered. Hence, an emulsion is consequently produced possessing an overall fat content reduction compared with a simple O/W emulsion. A patent was granted concerning the use of double emulsions as a mechanism for fat and calorie reduction in foods such as reduced-fat dressings utilising W/O/W double emulsions [14] and studies have been carried out surrounding cheese-like products which were produced via the substitution of milk-fat with W/O/W emulsions [15]. These reduced-fat cheese-like products created were comparable to traditional full-fat white fresh cheese in terms of rheological response and image texture. Likewise, it has also been reported in literature that when comparing of the rheological properties of simple O/W emulsions against olive oil based reduced calorie food W/O/W emulsions, simple emulsions were similar to the double emulsion containing the same fraction of dispersed phase, but possessing lower oil content overall [16]. Indeed, the commercialisation of such emulsions has begun to slowly gain pace in the food industry and patents exist for the processes pertaining to the production of reduced-fat food double emulsions and low-fat double emulsion spreads [17, 18].

2.2.2 Mechanisms of instability in double emulsions

However, despite these positive findings on double emulsions for food use, they have not been used widely in the food industry to date. A major drawback for double emulsions is that they are unstable, even more so than simple emulsions as there are now two unstable interfaces present in the emulsion system instead of one. Another major drawback is the difficulty in fabricating double emulsion microstructures on a large scale (as described in more detail later in section 2.2.3). Instability of double emulsion structures can occur for a number of different reasons in addition to those typically associated with simple emulsion instability (such as Ostwald ripening, coalescence, flocculation, sedimentation and creaming). Specific to double emulsions, four possible routes that lead to a breakdown of a typical W/O/W emulsion have been described [19] and are shown in Figure 2-3.

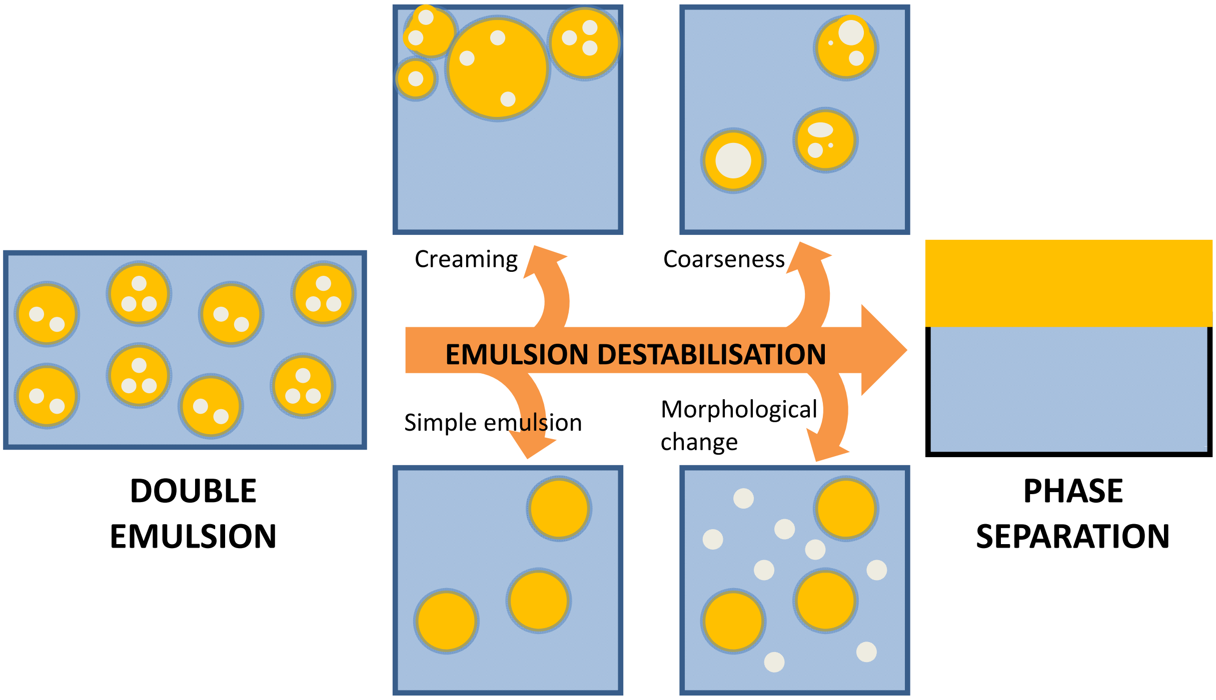

Figure 2-3 Physical destabilisation mechanisms in double emulsions

They are broadly comprised of: coalescence of the larger oil droplets (leading to creaming), coalescence of the primary internal water droplets (leading to coarser double emulsions), rupture of the oil layer separating the two distinct aqueous phases (leading to simple emulsion structures and/or phase separation) and water and water-soluble additives transporting through the oil layer (leading to evident changes in morphologies). These are the main causes for the failure of double emulsion structure stability and subsequently, factors that can influence or bring about such destabilisation occurrences include: processing routes, emulsifier selection, osmotic pressure balance and water transport (specific to W/O/W emulsion systems).

2.2.2.1 Emulsifiers

In order to deter the rapid instability of emulsions, emulsifiers are introduced to the system to enable an interfacial layer configuration around the dispersed droplets that form during processing, with the intention of prolonging complete phase separation of the two liquids. Commonly, surfactant molecules are used specifically for this purpose and adsorb at the liquid-liquid interface. The stabilising ability of such emulsifiers depends upon a variety of factors including their hydrophilic-lipophilic balance and their concentration within the system.

2.2.2.2 Type of emulsifiers and emulsifier ratios

As with simple W/O or O/W emulsions, the same types of hydrophilic and lipophilic surfactants are typically used at the liquid interfaces to form the W/O/W or O/W/O emulsions, and as a result issues have occurred concerning the monolayers that these surfactants usually form at the liquid-liquid interface. As a general rule, all surfactants possess a lipophilic and hydrophilic part in order to be surface active and typically they are formed of a hydrophilic head group and a lipophilic chain [20]. For this reason, in an emulsion, the surfactant will preferentially adsorb to the oil-water interface reducing the free energy associated with the production of a high surface area interface (during processing) and hence interfacial tension of the system is lowered [21]. For ‘lipophilic’ surfactants, the area per chain group is greater than the head group of the molecule and the surfactant monolayers curve around and form water droplets leading to W/O emulsions. As a general rule, in the case of so-called ‘hydrophilic’ surfactants, the area per head group of the molecule is larger than that of the chain section and monolayers of the surfactant will consequently curve around oil, forming oil droplets and resulting in O/W emulsions [20].

In double emulsions, where both types of emulsifier are required to stabilise the two distinct oil-water interfaces present, surfactants have been found to migrate from one interface to another, thus altering interfacial emulsifier configuration [22]. When this type of ‘disorganisation’ occurs, interfacial layers can result in a net curvature of zero and new aggregates can form, such as lamellar phases and bicontinuous emulsions, leading to double emulsion instability. Mixed reversed micelles have been also been detected in the oil phase in these types of double emulsion as surfactants molecules have migrated from their respective interfaces. This leads to solubilisation of the water soluble active ingredients and their transport to the outer water phase [20, 22]. These observations can be related to the ratio and concentration of emulsifiers used. Previous work has found that knowledge of the ratio of hydrophilic and lipophilic surfactant is important in double emulsion stability. It was reported that a ratio of 10:1 lipophilic surfactant to hydrophilic surfactant was required to form the smallest possible primary droplets and hence to produce a stable W/O/W emulsion. Any surfactant ratio using less lipophilic surfactant than this resulted in migration of hydrophilic surfactant to the primary W/O interface and eventually dispersion of the primary water droplets to the continuous aqueous phase [23].

2.2.2.3 Osmotic pressure balance

Many emulsion based food products contain salt, sugars, proteins and other water soluble additives and in W/O/W double emulsions, the salt (for instance NaCl or KCl) is often encapsulated in the internal primary aqueous phase in order to facilitate a controlled release so that less salt can be used in the formulation but the same taste properties are imparted as a full salt product [24]. However, entropy laws dictate that an electrolyte equilibrium concentration must be observed within the emulsion and so there exists a pressure pertaining to the inner water droplets, which in turn creates a pressure gradient between the inner water droplets and the outer continuous aqueous phase. This gradient leads to water transport and diffusion mechanisms occurring between the two aqueous phases in W/O/W emulsions across the separating oil phase, and this effect is magnified by a difference in electrolyte concentration between the two separate aqueous phases. Inner water droplet swelling or shrinkage depends upon the direction of the gradient and can eventually lead to double emulsion structure failure. It has been found that a small amount of electrolyte in the inner primary water droplets is required to balance the differences in pressure by osmotic opposition. However, if this electrolyte concentration is too high, osmotic pressure will cause inner water droplet deformation [25]. Therefore, in order to balance the effects of these two mechanisms, electrolyte concentration needs to be sufficiently high enough to counteract the pressure but low enough to avoid osmotic effects and water transport [26]. Additionally, in order to further limit both phenomena, both the primary and secondary interfaces should be sufficiently stabilised by the emulsifiers employed.

2.2.3 Fabrication of double emulsions

Historically, double emulsions have been prepared via one of two models: 1-step emulsification or 2-step emulsification [2]. 1-step emulsification involves forming the primary emulsion with a large excess of the emulsifier required for the primary interface and a small amount of the emulsifier needed for the secondary interface. This is then heat treated until inversion occurs and the double emulsion forms. This method, however, is difficult to reproduce consistently and can be costly, and as a consequence 2-step emulsification is more commonly employed and preferred when producing double emulsions as it is easier to control. This process involves the initial formation of the primary emulsion under high shear via homogenisation, ultrasonication or otherwise. This simple emulsion is then dispersed within a separate continuous phase and emulsification occurs in the absence of severe mixing in order to avoid disruption of the primary emulsion droplets.

The production of the double emulsion is also a determining factor for the droplet size, and with double emulsions, droplet size is a key parameter. Emulsion droplets should be smaller than 20 μm in any food emulsions in order to prevent any negative effects on sensorial properties of the food [27]. Consumer acceptance of emulsion based foods dictates that droplet sizes in excess of this lends to an ‘oily’ mouth-feel of the emulsion when consumed. Due to this, it has been deduced that in order for the primary emulsion droplets to be small enough to be integrated into secondary emulsion droplets of that size, they should be <5μm in diameter [28]. This produces problems in processing as a significant amount of energy is required to produce primary droplets of such small sizes [29] and there is great difficulty in preserving such small droplet sizes during the secondary emulsification process, where they must be able to withstand the required shear to create the secondary emulsion droplets (which need to be <20μm in size).

Conventional emulsification processes for double emulsions are based upon mixing and homogenisation. For the production of the primary emulsion, shear stress and droplet break-up is not as critical as it is for the second emulsification step and so processes such as high pressure jet homogenisation can be employed [30]. This is why only mixing is employed for this secondary step as too much shear will cause the double emulsion to not form and may result in reversion to a simple emulsion or even complete phase separation. However, if the emulsifying technique is too mild in this second step, this will result in highly polydispersed secondary W/O/W droplets [31]. Based on this, researchers have sought to develop other methods for emulsification for this second step to reduce the risk of double emulsion destabilisation. A relatively new process, membrane emulsification (Figure 2-4) and apparatus utilising T-shaped channels, can, and have often, been employed to produce double emulsions [32].

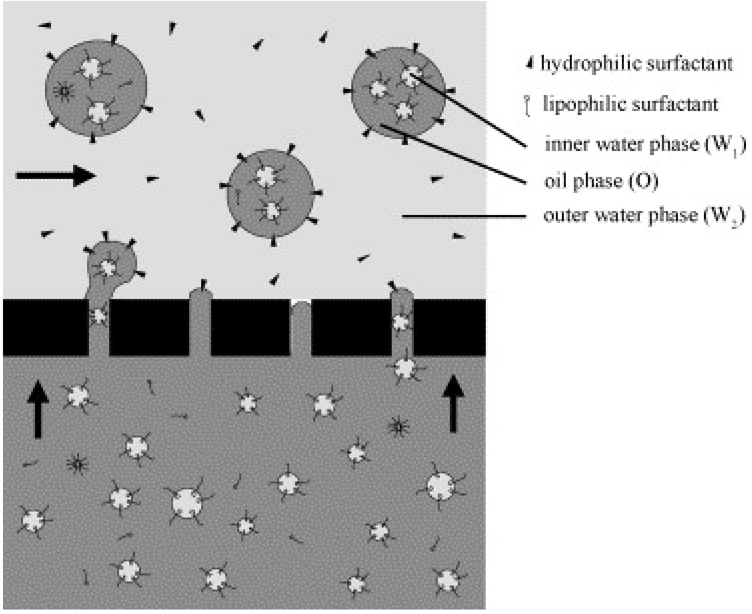

Figure 2-4 Schematic representation of a W/O/W emulsion produced by membrane emulsification where arrows represent the direction of fluid flow [32].

When producing W/OW emulsions using membrane emulsification, the W/O pre-emulsion is passed through a porous membrane and into the secondary aqueous phase. This continuous phase contains a hydrophilic surfactant and flows perpendicular to the flow of the W/O emulsion passing through the membrane, promoting formation of double emulsion droplets at the surface of the membrane. By using such a mild method for the second step of the emulsification process, prevention of primary droplet rupture can be achieved. In addition to this, membrane emulsification requires low energy densities when compared with high pressure jet homogenisation. This process can also produce smaller and more monodispersed droplets, depending on the size of the pores used within the apparatus, without causing droplet break-up that would occur when using high-shear processes for this effect [33]. However, membrane emulsification and other similar processes are not without pitfalls. They operate using low fluxes and if the flux were to be increased, droplet size would also increase which could in itself lead to emulsion destability [34]. There is currently no large scale processing equipment for membrane emulsification and thus this is not an established technology. It is quite difficult to scale-up this process which can be time consuming compared to conventional emulsification processes and added to the fact that the fluxes are quite low; it is often not cost effective for companies to employ this method in industry. However, the successful fabrication of robust Pickering stabilised double emulsions could potentially open the door to the commercialisation of these new types of emulsification routes.

2.2.4 Characterisation of double emulsion microstructure

The main difficulty in double emulsion characterisation lies in the analysis of the internal water droplets. W/O/W double emulsion droplet sizes are typically measured in a continuous aqueous medium via static multi-angle light scattering (SMLS). However due to their complex microstructure and the internal water phase, double emulsions are optically non-uniform. It has been commonly assumed in reported literature that the internal phase droplets behave as simple droplets with the same refractive index as the oil phase [35, 36]. Therefore, W/O/W droplet sizes determined via SMLS analysis can be only related to the overall oil droplets within which the primary internal water phase is contained. Combined with SMLS measurement data of the primary W/O emulsions (prior to inclusion into the secondary emulsification step), internal water droplet size can only be assumed. Therefore, micrographs of double emulsion samples are typically captured in conjunction with SMLS measurements. Techniques such as optical light and confocal microscopy as well as scanning electron microscopy are usually applied, depending upon expected droplet size range.

Recently, differential scanning calorimetry (DSC) has been suggested as a more direct approach to internal water droplet characterisation [37-39]. Classically, the thermoanalytical profile of a W/O/W double emulsion is, upon cooling, comprised of two distinct peaks each representing the two different water phases present within the system; where there is only one aqueous phase (as is the case in a simple emulsion), only one peak will exist [40] as per Figure 2-5. For the double emulsion curve within Figure 2-5, the peak labelled W_1 represents the internal water droplets and W_2 represents the outer aqueous phase.

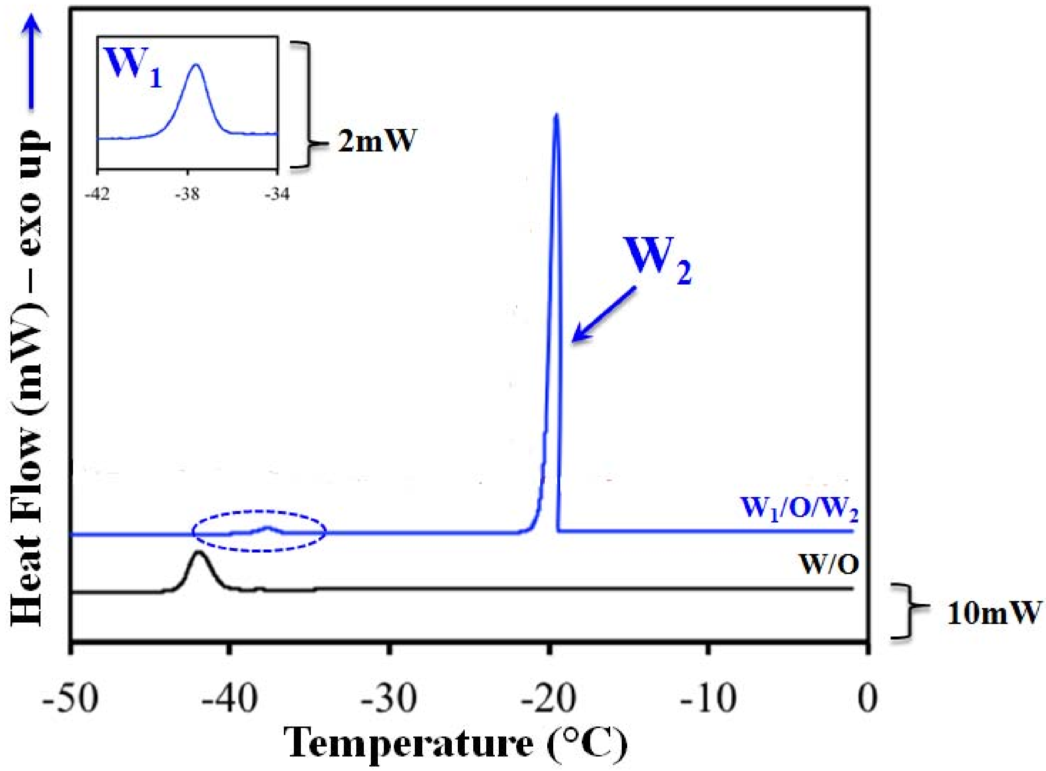

Figure 2-5 The thermoanalytical profiles of some W/O and W/O/W emulsions

It has been reported in literature that with double emulsions subjected to a constant cooling rate, solidification of the external water phase normally occurs at a much higher temperature in comparison to the inner water droplets [39]. This is due to the difference in the mechanism of nucleation between the two water phases. Freezing of the outer aqueous phase commences heterogeneously, once an ice germ (an impurity initiating ice crystal formation) forms at a nuclei, freezing rapidly occurs across the whole phase and due to the larger size of the continuous phase, the probability of the presence of an ice nuclei is significant [41]. Conversely, freezing of the internal water phase is homogenous due to the fact that the internal water phase consists of small individual water droplets. The volume of water within these droplets encapsulated in the oil phase is considerably smaller than the external aqueous phase hence the possibility of the presence of ice nuclei is vastly reduced in comparison and that ice germ only allows freezing of that individual droplet within which it is contained [42]. Consequently, the freezing point of the internal water droplets occurs at much lower temperatures than for the outer continuous aqueous phase allowing for differentiation between the two phases. Measurement of the thermoanalytical curve of the corresponding primary W/O emulsion combined with the calculation of the area under the peak relating to internal water droplets within the thermogram of the double emulsion enables determination of the encapsulation efficiency of the double emulsion structures [41, 43]. It should also be noted that an additional method for characterising the encapsulation efficiency of double emulsions is by using the Bruggeman equation [Equation 2-1].

$$\kappa = \kappa_w f_w^{3/2}$$

where κ is the emulsion conductivity, κ_w is the aqueous phase conductivity, and f_w is the volume fraction of water in the system. If the experimental value of conductivity is lower than the one expected from Bruggeman's law, this means that some water is not available to conduct electricity; and therefore indicates the occurrence of a W/O/W emulsion [44]. However, this model is limited to determining the amount of internal water phase encapsulated and provides no data or indication of internal droplet size or morphology.

2.3 Pickering stabilisation

All these factors considered, although beneficial to food industries, multiple emulsions are notoriously unstable for sufficient periods of time, largely due to the additional oil-water interface. Efforts have been made to enhance their stability and particle stabilisation has been shown to be successful for this purpose. Pickering stabilisation has long been recognised as a very useful and suitable method for producing emulsions with superior stability, since their inception at the beginning of the 20th century [45, 46]. Pickering particles accumulate at the oil-water interface in the form of a densely packed layer. This layer then protects against droplet flocculation and coalescence largely via steric and electrostatic mechanisms rather than by significantly lowering the oil-water interfacial tension as surfactants do [47]. Figure 2-6 depicts a simple schematic diagram of such an emulsion. Where solid particles are used to stabilise the emulsion droplets, many of the causes of emulsion instability associated with conventional emulsions have been eradicated or their effects greatly reduced, leading to more stable emulsions.

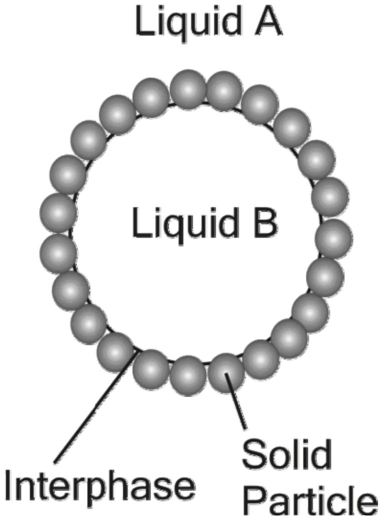

Figure 2-6 Schematic representation of a simple Pickering emulsion.

Stabilisation can be achieved via several additional mechanisms, as shown in Figure 2-7, which may be present in an emulsion [48].

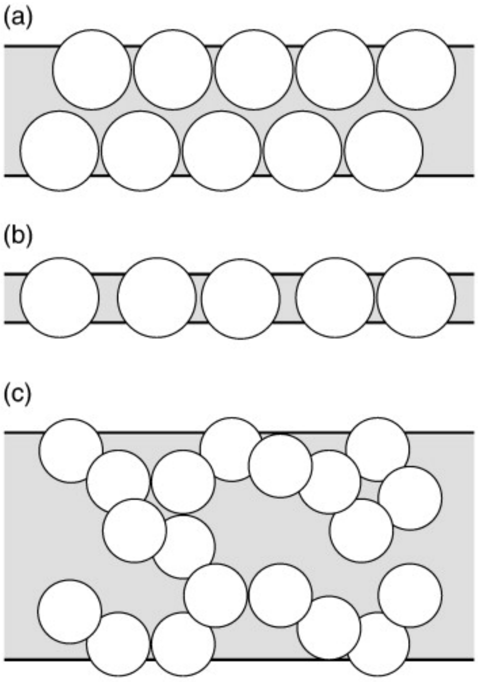

Figure 2-7 Schematic diagram showing various particle arrangements at the liquid interfaces between emulsion droplets in near proximity to one another [48].

Figure 2-7a represents the classic particle bi-layer arrangement where there are two densely packed particle monolayers close to each other that separate adjacent emulsion droplets. These thick layers then prevent droplet coalescence and flocculation by a steric mechanism and stabilises the thin film of continuous phase between the droplets. However, this ideal arrangement is not always observed with Pickering emulsions. Figure 2-7b and Figure 2-7c are also common arrangements of particles at the oil-water interface. Like Figure 2-7a, Figure 2-7b also provides a steric hindrance effect. This arrangement involves the existence of a particle monolayer that physical connects two droplets via a single dense layer of bridging particles and prevents significant particle displacement away from or within the bridging layer [49]. Figure 2-7c represents a rigid, somewhat disordered low-density network layer of aggregated particles that are held together by attractive inter-particle forces. Further studies found that by employing particles rather than traditional surfactants to stabilise oil-water interfaces, emulsions could remain stable for time periods in excess of six months [50, 51]. This was deemed to be due to the large free energy of desorption of Pickering particles from oil-water interfaces, signifying that the particles can form a very rigid interfacial film around the emulsion droplets [52]. These high energies, normally in excess of 3000kT, allow the particle adsorption to the interface to be effectively irreversible leading to exceptionally stable emulsions compared to conventional surfactant stabilised emulsions. With surfactant stabilised emulsions, the surfactant molecules are usually in rapid dynamic equilibrium between the liquid interfaces and the continuous phase (detachment energy is usually less than 10kT). Therefore, Pickering emulsions are generally considered to be far more stable than surfactant stabilised emulsions in this respect and as a consequence can also be subjected to harsher processing conditions as well as higher shear forces [51]. Pickering emulsions are also more adaptable than surfactant stabilised emulsions and can easily be inverted from W/O to O/W by simply increasing the volume fraction of water. In addition to this, these emulsions are most stable when they are close to this point, which is where the particle contact angle at the interface is equal to 90˚, and which is in complete contrast to surfactant stabilised emulsions which are least stable close to the point of balanced wettability [47].

In spite of the enhanced stability of these particle stabilised emulsions in contrast to surfactant stabilised emulsions, the stability of Pickering emulsions is dependent upon several factors. These parameters include particle type, particle wettability, particle size and concentration of particles within the formulation.

2.3.1 Wettability

In order to understand the extent to which a particle can stabilise an oil-water interface, and indeed what emulsion type will result, the wettability of the particle must be understood [53]. The morphology of Pickering emulsions do not depend on the HLB (hydrophilic-lipophilic balance) system as surfactant stabilised emulsions primarily do, but on the contact angles of the particles at the liquid-liquid interface. Essentially, the contact angle of the particle at the oil-water interface indicates which phase it is preferentially wetted by. This important parameter can determine whether water-in-oil or oil-in-water emulsions result [51].

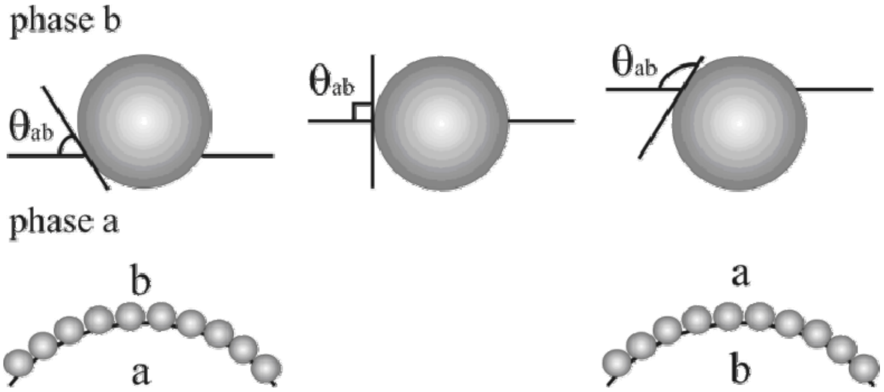

Figure 2-8 Contact angle schematic diagram at the liquid-liquid interface with regard to spherical Pickering particles [53].

When considering the schematic representation in Figure 2-8, if phase a is taken as the oil phase and phase (b) as the water phase in terms of a simple emulsion, the contact angle and wettability can be further explained in context and the contact angle is measured through the aqueous phase. Where the contact angle, θ_{ab}, is < 90˚, (as shown in the upper left hand diagram in Figure 2-8) the majority of the Pickering particle surface resides mainly in the water phase and only partially in the non-polar oil phase, at the interface between the two immiscible liquid phases. In this case, the Pickering particle can be classified as being hydrophilic, and if used solely to stabilise an emulsion, it will produce an oil-in-water emulsion. This is because the particles will create a curved monolayer in order for a larger fraction of the particle surface to remain in the water phase and hence oil droplets will form. Adversely, if $\theta_{ab} > 90^{\circ}$, a larger fraction of the particle will remain in the oil phase (upper right hand diagram in Figure 2-8) and the monolayer of particles at the interface will curve to form water droplets in a continuous oil phase, and thus result in water-in-oil emulsions. These Pickering particles are said to be lipophilic. Where θ_{ab} is 90˚, there is no curvature of the interface and there is no preference for one type of emulsion formation [47].

An equation was developed for the energy required the remove a Pickering particle from an oil-water interface, $-\Delta_{int}G$ [Equation 2-2] [54], where γ_{ow} represents the interfacial tension and r, the particle radius. This equation is not only dependent on the interfacial tension between the two immiscible phases, but also on the contact angle of the particle through the water phase, θ_W:

$$-\Delta_{int}G = \pi r^2 \gamma_{ow}(1 \pm \cos\theta_w)^2$$ **Equation 2-2**

In this equation, the terms within the bracket are positive for removal of the particle into the oil phase and negative for removal into the water phase and gravity is negligible where the particle is less than 1 μm in diameter. When contact angle, θ_W is close to 90˚, and close to the point of balanced wettability as mentioned previously, the particles possess the highest energy of attachment to the interface

[50]. However, when the contact angle approaches 0 – 20 ˚ or 160 – 180 ˚, the –$\Delta_{int}G$ rapidly decreases and becomes comparable to that of surfactants. The particles can then easily become detached from the interface and stabilisation is severely reduced [55]. The area occupied by the particle at the oil-water interface, A_e is also significant as it gives an indication to the theoretical particle saturation at the droplet interface and can demonstrate the particle concentration required to stabilise an emulsion droplet, assuming a monolayer particle arrangement at the interface (Figure 2-7a). This relationship is given by Equation 2-3 [53]:

$$A_e = \pi r^2(1 - cos^2\theta_w)$$

If emulsion stability is taken, in the broader sense, to be the ability of an emulsion to resist changes to its microstructure, then Pickering stabilisation has proven to be extremely kinetically stable compared to traditional emulsions stabilised by surfactants. However, due to the arrangement of the particles at the interface, they cannot yet be classified as stable. It was suggested that this is due to the free areas of interface between the adsorbed particles on the droplet surfaces that remain due to the geometry and homogenous wettability of the particles [54]. The positive free energy of producing this naked interface is always higher than the negative free energy contribution from the adsorbed spherical Pickering particles. Figure 2-8, and the associated description of the contact angles, is relevant only to spherical solid particles of homogenous wettability. Particles with heterogeneous wettability or ‘Janus’ particles, where the colloidal solid particles have well-defined surface areas of differing wettability, have also been explored and have been found to provide more stable Pickering emulsions as they increase the negative free energy contribution of the adsorbed particles [51]. With the application of these Janus particles as emulsifiers, it became apparent that the surface activity of these stabilising particles increased 3-fold when compared to particle of homogenous wettability [56]. They also found that these particles maintained strong surface activity for mean contact angles approaching 0˚ or 180˚.

2.3.1.1 Determination of wettability

Calculation of the wettability of Pickering particles can be extremely difficult to measure due to the powder form of the particles and their colloidal size (typically submicron), complexity in calculations and sophistication of apparatus is required [57, 58]. In spite of this, several methods have emerged for the purpose of measuring the wettability of colloidal particles. However, prior to selection, suitability to the particle types being investigated, exact measurement required as well as the purpose of the measurement must be considered and determined.

The Colloidal Probe technique uses Atomic Force Microscopy (AFM) to measure the interaction between single micrometer particles and air/water interfaces [59, 60]. Despite the accuracy of this technique, it is restricted to micron sized particles as the particle must be glued to the cantilever of the instrument for measurement to be possible. This factor also means that the technique does not yield significant amounts of data. Optical microscopy has previously been utilised via direct measurement of the side image of particles attached to liquid interfaces [61]. Although one of the simplest methods of measuring contact angles, it has a spatial resolution restricted to optical microscopy and so it is not suitable for particles smaller than a few microns. Particle trapping techniques have also been employed to this end. The Film Trapping technique traps particles in a liquid film of which the thickness is smaller than the diameter of the particle [62, 63]. The deformed film surfaces around the particle are reconstructed from the interference patterns obtained in reflected light via solving the Laplace equation of capillarity. However, this technique requires that the particles are micron sized and spherical. It is also very sensitive to errors in the film thickness used to solve the Laplace equation. The more recent Gel Trapping technique works by spreading particles at a water/air or water/oil interface at 50 ˚C, with a gelling agent incorporated in the water phase [64, 65]. The system is then cooled to form a gel and the particle monolayer is then replicated by polymethylsiloxane (PDMS) elastomer. The particles position on the PDMS surface and are then imaged via Scanning Electron Microscopy (SEM) as a way of providing data on contact angles. This method gives images with a much higher resolution than the aforementioned Optical Microscopy techniques and therefore it can be used to analyse submicron particles. The disadvantages to this technique are that there are many procedures within this method, such as the cooling and gelling steps, and they can affect the contact angle measurements. Another method was described within the literature whereby a modified Wilhelmy-plate was used to analyse the wettability of different surface modified titanium dioxide nanoparticles [57]. Using double-sided tape fully covered with the nanoparticles, they replaced the platinum plate used in the standard Kruss K100 tensiometer apparatus with it. Contact angle, θ, was calculated using Equation 2-4 where σ is the surface tension, L is the length of the plate and F is the resulting force.

$$\cos\theta = \frac{\sigma L}{F} \qquad \textbf{Equation 2-4}$$

The main advantage to this measurement technique is that it can provide contact angles for nanoparticles, however, these contact angles are considered apparent and not absolute values. A low value of the apparent contact angle indicates a more hydrophobic surface characteristic of the particle surface. Similarly, using the compressed disk method via the goniometer apparatus can provide apparent contact angle values for nanoparticles [66]. Powders are compressed into a circular disk and then placed into an open transparent glass vessel. A drop of water or oil is then placed directly onto the disk and contact angle is visualised and the Laplace-young model can be fitted via specialised software. This method is most suited towards the determination of particle hydrophilicity/hydrophobicity and lipophilic character rather than the representation of particle configuration at the liquid-liquid interface and for the purposes of the work in this thesis, this method was deemed appropriate.

2.3.2 Particle size

The relatively large size of Pickering particles, typically between 100 nm and 5 µm, when compared to surfactant molecules (approximately 0.4 nm to 1 nm) and protein molecules (normally between 1 nm and 5 nm) plays an important factor in determining the stability of the resulting emulsions [67]. The larger size of the solid particles leads to slower kinetics of adsorption at the interface and higher energy barriers to particle adsorption, both of which can strongly affect the emulsification process. The rate of droplet coalescence can exceed the rate of droplet break up during emulsification due to the time taken for the particles to adsorb to the liquid interface [68]. The slower kinetics of particle adsorption compared to surfactant adsorption means that as droplets are broken up during emulsification, there is much droplet surface area left uncovered [69]. As the particles are slow to adsorb to these newly created areas of liquid interface, they are left exposed to approaching droplets and coalescence is imminent. This in turn can lead to larger emulsion droplet sizes.

Adsorption time of particles to the liquid-liquid interface tends to increase linearly with the particle radius, and thus emulsification time was found to be longer for Pickering emulsions compared to surfactant and protein stabilised emulsions [67]. Based on the following equations, characteristic adsorption times, t_A, can be calculated depending on flow type (turbulent flow – Equation 2-5, laminar flow – Equation 2-6) [70];

$$t_A \sim \frac{\Gamma_M}{C\,(\varepsilon d)^{\frac{1}{3}}}$$ **Equation 2-5**

$$t_A \sim \frac{\Gamma_M}{C d \gamma}$$ **Equation 2-6**

With respect to t_A, in these equations dependency on the emulsifier is reflected through the ratio of the emulsifier adsorption in the complete monolayer, Γ_M, and the bulk emulsifier concentration, C. Typically for surfactants and proteins, values of Γ_M are 1.5 – 2 mg m^{-2} have been derived experimentally [71]. However, the value Γ_M for particles is represented by Equation 2-7 where ρ_P is particle mass density, r is particle radius and ϕ_{CP} is the fraction of area covered by adsorbed particles in a complete monolayer (for spherical particles it is approximately 0.907) [67]:

$$\Gamma_M \sim \rho_P \varphi_{CP} (4\pi r^3/3)/(\pi r^2)$$ **Equation 2-7**

Therefore, Γ_M is proportional to the particle radius, and t_A is seen to increase linearly as the particle sizes increase. This means that as the particle sizes increase, it takes longer for the particles to adsorb to the oil-water interface. Not only is the adsorption time of particles to the interface dependant on the particle radius, but also the stabilising capability of the particle. Considering Equation 2-2, detailed previously, the $-\Delta_{int}G$ is also dependent on the particle radius squared, and for particles of very small sizes (i.e. < 1nm) it becomes much easier for the particles to detach from the oil-water interface, whereas larger particles will adsorb to the interface more strongly. Often detachment energy is similar to conventional surfactants (< 10 kT) and hence so is the stabilising effect of these particles. Therefore, a balance in terms of the particle diameter must be struck.

2.3.3 Concentration of particles

It has previously been suggested that due to the larger demand of particle material required to be adsorbed in order to sufficiently cover the liquid interface, higher %w/w of particles were required to be present in formulations when compared to conventional emulsions [67]. Further studies concur that Pickering emulsion stability is increased by increasing particle concentration [20]. It was found that this led to an increase in the emulsion viscosity and a decrease in droplet size formed. An excess of the particle in the formulation led to a gelation of the continuous phase and a subsequent retardation of coalescence of the final emulsion and it was also found that the coalescence behaviour of particle stabilised droplets was significantly altered depending on the coverage of droplets by particles [20, 72]. Binks found that when emulsion droplets had complete particle coverage, they were stable to coalescence, as the particles formed a closely packed layer around the droplet that was able to resist the pressure gradient that drives droplets together. With low particle coverage, droplets coalesced, but with intermediate particle coverage: droplets underwent arrested coalescence where the droplets partially joined and resulted in irregularly shaped larger droplets.

Commonly, Pickering particles show a high degree of polydispersity, and thus the particle arrangement at the oil-water interface (as represented by Figure 2-7a) is rarely recognised in practice. Even when Pickering particle size distributions are fairly monodispersed, complete dispersion of the particles in the liquid medium is difficult to realise experimentally, even with high intensity homogenisation treatment. This is particularly evident with sub-micron sized particles as the large surface area of the particles makes complete dispersion from the typical powder state of the particles a thermodynamically unfavourable process. It can reduce the amount of particles adsorbing at the interface, leading to longer particle adsorption kinetics and consequently larger droplets sizes and coarser emulsions. Adversely, it also leads to aggregation of the particles within the continuous aqueous phase in O/W emulsions which can then be adsorbed to the interface and hence create particle flocculating conditions which can then improve the stability of the emulsion. Food-based O/W Pickering emulsions stabilised using chitin nanocrystals were developed and it was concluded that increasing the particle concentration from 0.01 to 0.05 % led to a reduction in average droplet size by a factor of 10 [73]. Tzoumaki *et al.*, also discovered that in terms of the rheology of the O/W emulsion, there was an additional stabilising mechanism emanating from the development of a gel-like microstructure made up of flocculated oil droplets and a viscoelastic network consisting of aggregated chitin nanocrystals in the aqueous medium amongst droplets.

2.3.4 Edible particulate materials

Renewed interest for Pickering emulsions since the 1980s mainly arose with the emergence of nanotechnologies. The development of solid particles with a broader range of properties (size, hydrophobicity, shape etc.), has allowed for researchers to get a better understanding of the mechanisms involved in the stabilisation of Pickering emulsions. Designing Pickering particles for topical applications has become a challenge since the last decade. This section reviews some of the knowledge regarding the main edible particles with potential food applications and delves into more detail on the particles used within this thesis.

Until recent years, studies conducted on Pickering stabilised emulsions focussed mainly upon inedible materials. Studies have been conducted showing the ability of hydrophilic and hydrophobic silica particles in stabilising oil-water interfaces [50, 51, 74]. In addition to silica, water-in-cyclohexane emulsions have been stabilised using polystyrene particles which formed a dense film layer around the water droplets, hindering coalescence [53], and very stable oil-in-water emulsions using clay particles as stabilisers [75]. The clay particle interactions resulted in threedimensional networks forming in the continuous phase within which oil droplets were effectively trapped. Colloidal titanium dioxide particles have also been shown to stabilise both oil-in-water and water-in-oil emulsions [58].

More recently, however, edible Pickering particles have been used to stabilise simple emulsions such as hydrocolloids, like colloidal hydrophobically modified starch particles [76-78], colloidal celluloses and various cellulose derivatives [79-81]. Such polysaccharide emulsifiers have been used to stabilise simple oil-water emulsions that were extremely stable to coalescence when compared to surfactant stabilised emulsions. Additionally, other colloidal particles, also of a biological nature, have been investigated for their potential to act as Pickering stabilisers. These include chitin nanocrystals [73], protein particles [82, 83], as well as a class of antioxidants known as flavonoids [84, 85] which have been synthesised and successfully used to emulsify oil-water emulsions. Specific to water-in-oil emulsions, wax microparticles [86, 87] and fat crystal systems [28, 87] have been exploited for the purpose of providing stabilisation to emulsions. The hydrophobic nature of these types of particles allows for their migration to the oil-water interface and in the case of the fat crystals; a sintering of the crystals was observed which enabled the formation of stabilising solid fat shells surrounding the water droplets [28].

It is important to note here that it has been well documented that protein and hydrocolloid particles such as skim milk powder, egg-yolk protein and whey protein have been historically used in the food industry as stabilisers/emulsifiers for common food emulsions [15, 88, 89]. However, it has been argued that these particles lack the ability to behave as Pickering emulsifiers as Pickering particles should remain insoluble and intact over the lifetime of the emulsion [90]. These particles, although edible, contain various types of protein aggregates and proteinaceous particles and when these self-assembled proteins adsorb to the oil-water interface, they start to breakdown into smaller fragments and rearrange themselves at the interface to form a final adsorbed protein layer [91].

A comprehensive review on edible particles for use in emulsion stabilisation has been published [92], providing further detail on many edible particle types mentioned in this section.

2.3.4.1 Polysaccharides

Polysaccharides are essentially polymeric carbohydrates and are a class of naturally occurring polymers, most abundant in agricultural feedstock and crustacean shells. Polysaccharides such as cellulose and chitin are found in nature as structural building blocks and others such as starch provide fuel for biological cells by storing solar energy in the form of sugars.

Starch is most abundant in plants, specifically in its granular form within plant cells. The starch granules consist mainly of two polymeric components; linearly structured amylose (with primary α-1,4 linkages) and branched chain amylopectin (containing approximately 5% α-1,6 linked branch points) as shown in Figure 2-9.

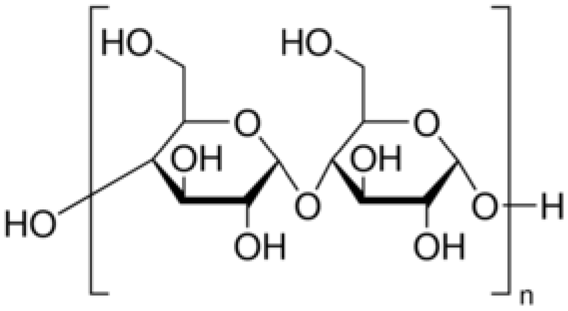

Figure 2-9 Basic chemical structure of starch

The proportions of each component differ depending upon which plant species they are derived from. Similarly, cellulose is prevalent in plant systems; it is the main structural polymer in wood and is constituted of chains of many glucose molecules as shown in Figure 2-10.

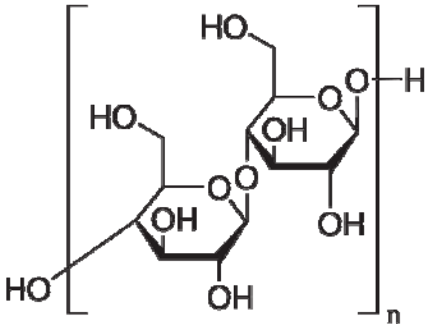

Figure 2-10 Basic chemical structure of cellulose

It supplies more than 50% of the carbon within vegetation and is the structural component of the cell walls within plants such as ramie (Boehmeria Nivea) and cotton (Gossypium) [93]. Typical sizes of native starch and cellulose particles lie within the range of several microns down to diameters in the nano range with some of the smallest native starches being rice starch (< 5 μm) and quinoa starch (500 nm – 3 μm) [77, 94]. Similarly, microcrystalline cellulose has individual particle diameters ranging from several hundred nanometres to several microns.

However prior studies suggest that by carrying out strong acid hydrolysis, rod shaped cellulose nanocrystals could be produced, measuring approximately 3 – 15 nm in width and 50 – 250 nm in length [95].

These polysaccharides are not water soluble and this insolubility in water arises from the preference of these polysaccharide molecules for partial crystallisation [96]. Nonetheless, depending upon their size, the cellulose particles can be dispersed into an aqueous solution when the water has been heated above a certain temperature, as are starch particles. Polysaccharides are used as thickeners and gelling agents in industry since they are able to form gel networks throughout the bulk to inhibit movement of both dispersed and continuous phases [97]. Besides their gelling properties, some polysaccharide structures can be tailored to adsorb at oil-water interfaces, by addition of hydrophobic chemical groups to the main hydrophilic backbone of the polysaccharide molecule. Such modification increases particle amphiphilicity and hence allows adsorption to an oil-water interface. This is the case with hydrophobically modified starches and celluloses [97]. Modification of hydrophobicity is achieved by either physical or chemical method, or combinations of these two methods [98, 99].

Dry heat can be used to alter surface character and chemical modification by either cross-linking, conversion or substitution reactions [100]. Most commonly, cellulose and starch modification occurs via chemical substitution reaction. Traditionally, substitution of the hydroxyl groups on starch particles with different alkenyl succinyl anhydrides produces modified starch with amphiphilic properties (most commonly OSA-starch) [100, 101]. In the case of cellulose, modified cellulose particles are produced via the substitution of hydroxyl groups within cellulose molecules with ether groups.

2.3.4.2 Flavonoids

Flavonoids have generated considerable interest in recent years because of the significant association between their dietary consumption and protection against disease. Flavonoids are a broad class of low molecular weight secondary plant polyphenolics, which are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings, consisting of over 4000 different compounds. Flavonoids are usually divided according to their substituents into subcategories: flavanols, flavones, flavanones, chalcones and anthocyanidines [102]. They can be further identified via different substitution patterns and groups of the C6-C3-C6 basic backbone, including the presence of an OXO-group, and a C2-C3 double bond.

Flavonoids are particularly useful as Pickering O/W emulsifiers as they are generally insoluble in water and lipid environments. Within this work, two particular flavonoids were selected for investigation; rutin hydrate and naringin.

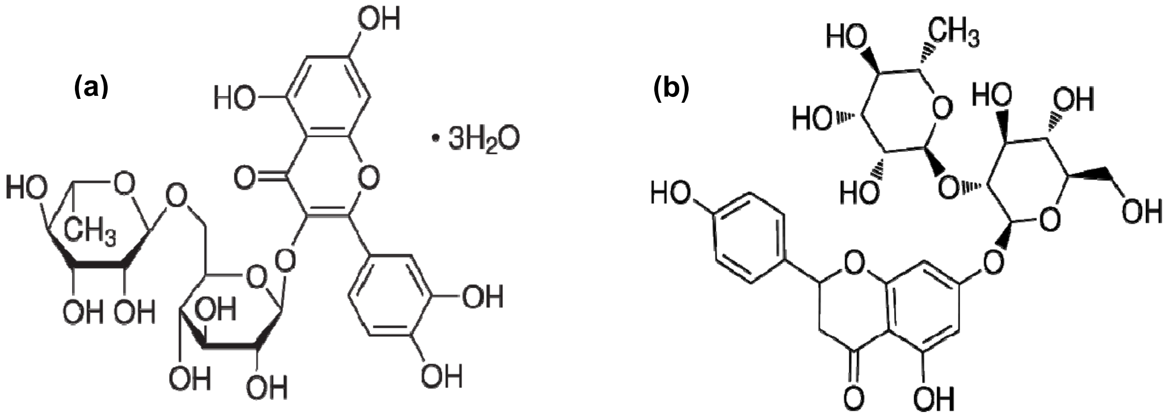

Figure 2-11 Chemical structures of (a) rutin hydrate and (b) naringin

Rutin is the rhamnoglucoside of the flavonoid Quercetin, a polyphenolic moiety, which contains the C_7-OH group and behaves as a polyprotic acid that undergoes significant dissociation between pH 4 and pH 8 by imparting a negative charge to two H^+ surface molecules [85]. The chemical structure of Naringin differs in the fact that the C_7-OH group on the naringin molecule is instead glycosylated to the disaccharide moiety. Both materials are commonly derived from grapefruit peel and juice and exhibiting antioxidant behaviour, such particles have been found to suppress oxidative stress and inflammation, curtail bioactivation of carcinogens and affect cell signalling [103, 104]. With respect to emulsion systems, very little has been reported regarding such particles for use in the stabilisation of emulsions. Studies have found naringin and rutin particles to be good O/W emulsifiers with excellent long term stabilisation capabilities and the addition of rutin to whey protein stabilised emulsions allowed for greater control over structural and oxidative stability of O/W emulsions [84, 105].

2.4 Double W/O/W Pickering emulsions

Specifically related to the project aims, only a few experiments have been successfully conducted whereby stable double emulsions have been produced via the use of particle emulsification, and these studies detail the utilisation of a combined surfactant and particulate stabilisation mechanism. The primary thinking behind this was to create very stable long term particle-stabilised emulsions by using surfactants to lower the interfacial tension between the oil and water phases in order to aid droplet break-up during emulsification. As surfactants have a faster adsorption rate to the oil-water interface than the Pickering particles, they should be able to stabilise the newly formed interfaces during the emulsification process and allow enough time for the particles to sufficiently adsorb to the interface. In turn, it was thought that this would increase the stability of double particle stabilised emulsions further by the short term guarding against droplet coalescence by surfactants during emulsification, and therefore allowing for smaller droplet sizes to be formed.

Some examples of particle and surfactant co-stabilisation of double emulsion interfaces have been described in the literature. Through the use of hydrophilic colloidal microcrystalline cellulose particles and lipophilic polysorbate (Span) surfactants, the production of W/O/W emulsions was possible which were sufficiently stable for up to 1 month [106]. Similarly, it was reported that the inclusion of cellulose nanofibrils within surfactant stabilised W/O/W emulsions significantly increased the stability of emulsion droplets against coalescence [107]. Further investigation of both of these emulsion systems found that they contained a network of the microcrystalline cellulose particles adsorbed at the secondary oil/water interface which enhanced emulsion droplet stability. Likewise, particles have been used to provide enhanced stabilisation of the secondary emulsion interface in double emulsions, but instead focus lay on O/W/O emulsions [108]. By partially coating the W/O interface with modified lipophilic clay particles, rigidity of the interface ensued. These particles also caused gelation of the continuous oil phase which acted as a barrier to sedimentation of the water globules. A combination of polymers, surfactant and colloidal silica particles was found to produce highly stable W/O/W and O/W/O emulsions that exhibited no change in secondary emulsion droplet sizes over a period of 6 months due to the elimination of surfactant diffusion which caused emulsion destabilisation [109]. Using lipophilic sub-micron crystalline fat particles as an additional stabiliser in W/O/W emulsions at the primary O/W interface, it was discovered that an enhanced stability to coalescence of the resulting emulsion was observed [110].

These experiments all provide evidence of increased stability of double emulsions via the addition of particles as stabilisers. However, they serve only to provide examples of double emulsion co-stabilisation using surfactants and particles. Even fewer studies detail the use of particles being used solely to stabilise double emulsions. One such example originates from a study conducted where a mixture of silica particles differing only in 25% SiOH content, allowed researchers to alter the wettability of the particles and synthesise two distinct types of hydrophilic and lipophilic silica particles [111]. These particles were then used in tandem in oil-water mixtures to form stable double emulsions of either O/W/O or W/O/W. Figure 2-12 shows a micrograph of the O/W/O emulsion that was produced.

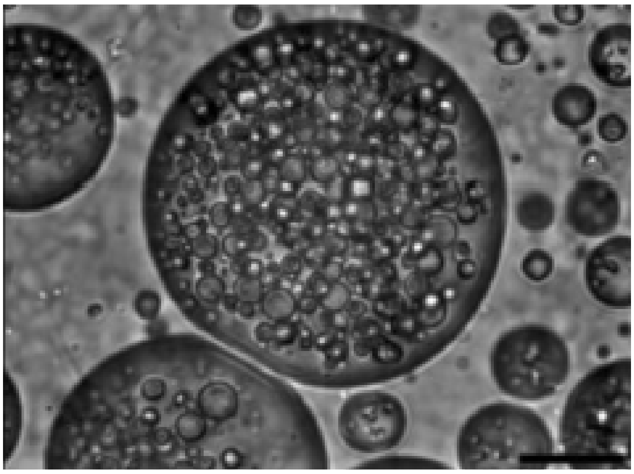

Figure 2-12 Microscopy image of O/W/O emulsion stabilised solely by silica particles (Scale bar represents 20 µm) [112].

As is evident in the microscopic image (Figure 2-12), the inner oil droplets are in close proximity to one another and despite this due to adsorbed particles at the internal interface, droplets (both inner and outer) were stable to coalescence. The inner oil droplets were also approximately 4 µm in diameter, within the acceptable range for food inner double emulsion droplets [27]. These researchers also investigated the effects of particle concentrations, volume fractions and oil types and found the double emulsions to be comparable to simple O/W and W/O particle stabilised emulsions [111]. They also reported that following particle adsorption to the respective oil-water interfaces, particle migration is minimal compared to surfactant stabilised emulsions and they found that these emulsions could be stable for in excess of 1 year.

Following on from this work, researchers developed silica-only stabilised double emulsions containing droplets and globules that were stable to coalescence for over 1 year [51]. Using toluene or triglyceride oil, lipophilic silica particles were used to stabilise the primary internal interface and hydrophilic silica particles at the outer oil-water interface. The resultant double emulsion contained oil globules of approximately 40μm in diameter which in turn contained inner water droplets of approximately 2μm in diameter. They also prepared O/W/O double emulsions by emulsifying oil into a continuous aqueous phase containing the hydrophilic silica particles and then re-emulsifying the O/W emulsion at low shear into a continuous oil phase containing lipophilic silica particles. Again they found emulsions that were stable against coalescence and sedimentation.

Key findings from these pivotal studies indicate that minimum concentrations of both types of particles are required in order to form both W/O/W and O/W/O double emulsions and below this critical concentration only simple O/W or W/O emulsions will exist. They also found that, for the different W/O/W emulsions investigated in these studies, typically 2 %w/w of each type of particle is required to produce emulsions stable to coalescence and creaming over time. By increasing the concentration of the lipophilic silica particles in the primary emulsion, an increased number of primary water droplets were formed and O/W droplets with larger diameters were formed in the secondary emulsion. In comparison, an increase in the concentration of the hydrophilic silica particles in the outer aqueous phase led to a reduction of the secondary O/W droplet diameters and an increase in the viscosity of the outer aqueous phase. As Pickering particles historically tend to exhibit very high desorption energies from the interface at which they are adsorbed, the migration of emulsifier from inner to outer surfaces is anticipated to be minimal when compared with surfactant emulsification [52].

2.5 Concluding Remarks

Despite the significant conclusions drawn from all of the experiments detailed in this review, the silica-only stabilised double emulsions are the most closely related to the challenge of producing stable and edible Pickering double emulsions [51, 111, 112]. Such findings provide evidence that stable double emulsions can be produced solely utilising Pickering stabilisation. However, these studies involve the use of silica particles as the Pickering stabilising particles and thus resultant emulsions are not widely classed as food-grade. Consequently, the challenge still remains to understand and produce double emulsions using Pickering particles as the sole stabilisation mechanism. In addition to this, another challenge exists to ensure that these novel double emulsions are solely stabilised with edible Pickering particles which are of a recognised food-grade standard. The aim of this project, and the subsequent research and experimental work conducted, was to determine whether such emulsions can be successfully synthesised. The work described in the proceeding results chapters here details development of formulation design rules, formulation processing routes and stability criteria establishment, with the aim of leading to the development and understanding of these novel emulsions.

References

[1] Aguilera, J.M. and Stanley, D.W., *Microstructural principles of food processing and engineering***1999**: Springer.

[2] Garti, N., Double emulsions — scope, limitations and new achievements. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **1997**, *123–124*, p. 233-246.

[3] Seifriz, W., Studies in emulsions. Iii-v. *The Journal of Physical Chemistry,* **1924**, *29*, p. 738-749.

[4] Omotosho, J.A., Whateley, T.L., and Florence, A.T., Release of 5-fluorouracil from intramuscular w/o/w multiple emulsions. *Biopharm Drug Dispos,* **1989**, *10*, p. 257-68.

[5] Zheng, S., Zheng, Y., Beissinger, R.L., Wasan, D.T., and McCormick, D.L., Hemoglobin multiple emulsion as an oxygen delivery system. *Biochim Biophys Acta,* **1993**, *1158*, p. 65-74.

[6] Ficheux, M.F., Bonakdar, L., Leal-Calderon, F., and Bibette, J., Some stability criteria for double emulsions. *Langmuir,* **1998**, *14*, p. 2702-2706.

[7] Laugel, C., Chaminade, P., Baillet, A., Seiller, M., and Ferrier, D., Moisturizing substances entrapped in w/o/w emulsions: Analytical methodology for formulation, stability and release studies. *Journal of Controlled Release,* **1996**, *38*, p. 59-67.

[8] Lin, Z., Schulz, W.J., and Zhang, S., Water-in-oil-in water emulsion. ed**1999**. Google Patents.

[9] Stephens, A.F., Jones, N.J., Sunkel, J.M., and Vatter, M.L., Silicone elastomer emulsion cosmetic composition comprising colorant inclusive internal phase. ed**2003**. Google Patents.

[10] Shima, M., Morita, Y., Yamashita, M., and Adachi, S., Protection of lactobacillus acidophilus from the low ph of a model gastric juice by incorporation in a w/o/w emulsion. *Food Hydrocolloids,* **2006**, *20*, p. 1164-1169.

[11] Benichou, A., Aserin, A., and Garti, N., W/o/w double emulsions stabilized with wpi–polysaccharide complexes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2007**, *294*, p. 20-32.

[12] Bonnet, M., Cansell, M., Berkaoui, A., Ropers, M.H., Anton, M., and Leal-Calderon, F., Release rate profiles of magnesium from multiple w/o/w emulsions. *Food Hydrocolloids,* **2009**, *23*, p. 92-101.

[13] Edris, A. and Bergnstahl, B., Encapsulation of orange oil in a spray dried double emulsion. *Nahrung,* **2001**, *45*, p. 133-7.

[14] Taki, J., Isomura, T., Kanda, K., Kawahara, A., Maruyama, K., and Kusumoto, S., W1/o/w2-type double emulsion dressing and method for production thereof. ed**2008**. Google Patents.

[15] Lobato-Calleros, C., Rodriguez, E., Sandoval-Castilla, O., Vernon-Carter, E.J., and Alvarez-Ramirez, J., Reduced-fat white fresh cheese-like products obtained from w1/o/w2 multiple emulsions: Viscoelastic and high-resolution image analyses. *Food Research International,* **2006**, *39*, p. 678-685.

[16] De Cindio, B. and Cacace, D., Formulation and rheological characterization of reduced-calorie food emulsions. *International Journal of Food Science & Technology,* **1995**, *30*, p. 505-514.

[17] Takahashi, Y., Yoshida, T., and Takahashi, T., Process for producing a w/o/w type multiple emulsion for medicines, cosmetics, etc. ed**1990**. Google Patents.

[18] Okonogi, S., Kato, R., Asano, Y., Yuguchi, H., Kumazawa, R., Sotoyama, K., Takahashi, K., and Fujimoto, M., Methods for producing emulsions, low-fat spread and oil-in-water-in-oil type spread. ed**1991**. Google Patents.

[19] Florence, A.T. and Whitehill, D., Some features of breakdown in water-in-oil-in-water multiple emulsions. *Journal of Colloid and Interface Science,* **1981**, *79*, p. 243-256.

[20] Binks, B.P., Particles as surfactants—similarities and differences. *Current Opinion in Colloid & Interface Science,* **2002**, *7*, p. 21-41.

[21] Nielloud, F., *Pharmaceutical emulsions and suspensions: Second edition, revised and expanded***2000**: Taylor & Francis.

[22] Garti, N. and Aserin, A., Double emulsions stabilized by macromolecular surfactants. *Advances in Colloid and Interface Science,* **1996**, *65*, p. 37-69.

[23] Matsumoto, S., Kita, Y., and Yonezawa, D., An attempt at preparing water-in-oil-in-water multiple-phase emulsions. *Journal of Colloid and Interface Science,* **1976**, *57*, p. 353-361.

[24] Pays, K., Giermanska-Kahn, J., Pouligny, B., Bibette, J., and Leal-Calderon, F., Double emulsions: How does release occur? *J Control Release,* **2002**, *79*, p. 193-205.

[25] Rosano, H.L., Gandolfo, F.G., and Hidrot, J.-D.P., Stability of w1/o/w2 multiple emulsions: Influence of ripening and interfacial interactions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **1998**, *138*, p. 109-121.

[26] Jiao, J., Rhodes, D.G., and Burgess, D.J., Multiple emulsion stability: Pressure balance and interfacial film strength. *Journal of Colloid and Interface Science,* **2002**, *250*, p. 444-450.

[27] Norton, I., Fryer, P., and Moore, S., Product/process integration in food manufacture: Engineering sustained health. *AIChE Journal,* **2006**, *52*, p. 1632-1640.

[28] Frasch-Melnik, S., Norton, I.T., and Spyropoulos, F., Fat-crystal stabilised w/o emulsions for controlled salt release. *Journal of Food Engineering,* **2010**, *98*, p. 437-442.

[29] Kentish, S., Wooster, T.J., Ashokkumar, M., Balachandran, S., Mawson, R., and Simons, L., The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science & Emerging Technologies,* **2008**, *9*, p. 170-175.

[30] Li, X., Xu, Y., Chen, G., Wei, P., and Ping, Q., Plga nanoparticles for the oral delivery of 5-fluorouracil using high pressure homogenization-emulsification as the preparation method and in vitro/in vivo studies. *Drug Dev Ind Pharm,* **2008**, *34*, p. 107-15.

[31] Kumar, R., Kumar, M.S., and Mahadevan, N., Multiple emulsions: A review. *International Journal of Recent Advances in Pharmaceutical Research,* **2012**, *2*, p. 9-19.

[32] van der Graaf, S., Schroën, C.G.P.H., and Boom, R.M., Preparation of double emulsions by membrane emulsification—a review. *Journal of Membrane Science,* **2005**, *251*, p. 7-15.

[33] Charcosset, C., Limayem, I., and Fessi, H., The membrane emulsification process—a review. *Journal of chemical technology and biotechnology,* **2004**, *79*, p. 209-218.

[34] Frasch-Melnik, S., Fat crystal-stabilised double emulsions, PhD,**2011**, University of Birmingham, UK.

[35] Pays, K., Giermanska-Kahn, J., Pouligny, B., Bibette, J., and Leal-Calderon, F., Coalescence in surfactant-stabilized double emulsions. *Langmuir,* **2001**, *17*, p. 7758-7769.

[36] Pawlik, A., Cox, P.W., and Norton, I.T., Food grade duplex emulsions designed and stabilised with different osmotic pressures. *Journal of Colloid and Interface Science,* **2010**, *352*, p. 59-67.

[37] Clausse, D., Gomez, F., Pezron, I., Komunjer, L., and Dalmazzone, C., Morphology characterization of emulsions by differential scanning calorimetry. *Advances in Colloid and Interface Science,* **2005**, *117*, p. 59-74.

[38] Schuch, A., Tonay, A.N., Köhler, K., and Schuchmann, H.P., Influence of the second emulsification step during production of w/o/w multiple emulsions: Comparison of different methods to determine encapsulation efficiency in w/o/w emulsions. *The Canadian Journal of Chemical Engineering,* **2014**, *92*, p. 203-209.

[39] Potier, L., Raynal, S., Seiller, M., Grossiord, J.L., and Clausse, D., Study of state transitions within multiple w/o/w emulsions using calorimetry (dsc). *Thermochimica Acta,* **1992**, *204*, p. 145-155.

[40] Kovács, A., Csóka, I., Kónya, M., Csányi, E., Fehér, A., and Erős, I., Structural analysis of w/o/w multiple emulsions by means of dsc. *Journal of Thermal Analysis and Calorimetry*, *82*, p. 491-497.

[41] Schuch, A., Deiters, P., Henne, J., Köhler, K., and Schuchmann, H.P., Production of w/o/w (water-in-oil-in-water) multiple emulsions: Droplet breakup and release of water. *Journal of Colloid and Interface Science,* **2013**, *402*, p. 157-164.

[42] Koop, T., Homogeneous ice nucleation in water and aqueous solutions. in *Zeitschrift für Physikalische Chemie/International journal of research in physical chemistry and chemical physics* vol. 218, ed**2004**. p. 1231.

[43] Oppermann, A.K.L., Renssen, M., Schuch, A., Stieger, M., and Scholten, E., Effect of gelation of inner dispersed phase on stability of (w1/o/w2) multiple emulsions. *Food Hydrocolloids,* **2015**, *48*, p. 17-26.

[44] Allouche, J., Tyrode, E., Sadtler, V., Choplin, L., and Salager, J.-L., Single-and two-step emulsification to prepare a persistent multiple emulsion with a surfactant− polymer mixture. *Industrial & engineering chemistry research,* **2003**, *42*, p. 3982-3988.

[45] Ramsden, W., Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation). -- preliminary account. *Proceedings of the Royal Society of London,* **1903**, *72*, p. 156-164.

[46] Pickering, S.U., Emulsions. *Journal of the Chemical Society,* **1907**, *91*, p. 2001 - 2021.

[47] Hunter, T.N., Pugh, R.J., Franks, G.V., and Jameson, G.J., The role of particles in stabilising foams and emulsions. *Advances in Colloid and Interface Science,* **2008**, *137*, p. 57-81.

[48] Dickinson, E., Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science,* **2010**, *15*, p. 40-49.

[49] French, D.J., Taylor, P., Fowler, J., Clegg, P.S., Liu, X., Dedinaite, A., Nylander, T., Dabkowska, A., Skoda, M., and Makuska, R., Making and breaking bridges in a pickering emulsion. *Journal of colloid and interface science,* **2015**, *441*, p. 30-38.

[50] Binks, B.P. and Lumsdon, S.O., Stability of oil-in-water emulsions stabilised by silica particles. *Physical Chemistry Chemical Physics,* **1999**, *1*, p. 3007-3016.

[51] Aveyard, R., Binks, B.P., and Clint, J.H., Emulsions stabilised solely by colloidal particles. *Advances in Colloid and Interface Science,* **2003**, *100–102*, p. 503-546.

[52] Tambe, D.E. and Sharma, M.M., The effect of colloidal particles on fluid-fluid interfacial properties and emulsion stability. *Advances in Colloid and Interface Science,* **1994**, *52*, p. 1-63.

[53] Binks, B.P. and Lumsdon, S.O., Influence of particle wettability on the type and stability of surfactant-free emulsions†. *Langmuir,* **2000**, *16*, p. 8622-8631.

[54] Levine, S., Bowen, B.D., and Partridge, S.J., Stabilization of emulsions by fine particles i. Partitioning of particles between continuous phase and oil/water interface. *Colloids and Surfaces,* **1989**, *38*, p. 325-343.

[55] Schmitt, V., Destribats, M., and Backov, R., Colloidal particles as liquid dispersion stabilizer: Pickering emulsions and materials thereof. *Comptes Rendus Physique,* **2014**, *15*, p. 761-774.

[56] Binks, B.P. and Fletcher, P.D.I., Particles adsorbed at the oil–water interface: A theoretical comparison between spheres of uniform wettability and “janus” particles. *Langmuir,* **2001**, *17*, p. 4708-4710.

[57] Stiller, S., Gers-Barlag, H., Lergenmueller, M., Pflücker, F., Schulz, J., Wittern, K., and Daniels, R., Investigation of the stability in emulsions stabilized with different surface modified titanium dioxides. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2004**, *232*, p. 261-267.

[58] Horozov, T.S., Binks, B.P., Aveyard, R., and Clint, J.H., Effect of particle hydrophobicity on the formation and collapse of fumed silica particle monolayers at the oil–water interface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2006**, *282–283*, p. 377-386.

[59] Preuss, M. and Butt, H.-J., Measuring the contact angle of individual colloidal particles. *Journal of colloid and interface science,* **1998**, *208*, p. 468-477.

[60] Gillies, G., Kappl, M., and Butt, H.-J., Direct measurements of particle–bubble interactions. *Advances in Colloid and Interface Science,* **2005**, *114–115*, p. 165-172.

[61] Hórvölgyi, Z., Nemeth, S., and Fendler, J.H., Spreading of hydrophobic silica beads at water—air interfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **1993**, *71*, p. 327-335.

[62] Hadjiiski, A., Dimova, R., Denkov, N.D., Ivanov, I.B., and Borwankar, R., Film trapping technique: Precise method for three-phase contact angle determination of solid and fluid particles of micrometer size. *Langmuir,* **1996**, *12*, p. 6665-6675.

[63] Hadjiiski, A., Tcholakova, S., Ivanov, I.B., Gurkov, T.D., and Leonard, E.F., Gentle film trapping technique with application to drop entry measurements. *Langmuir,* **2002**, *18*, p. 127-138.

[64] Paunov, V.N., Novel method for determining the three-phase contact angle of colloid particles adsorbed at air-water and oil-water interfaces. *Langmuir,* **2003**, *19*, p. 7970-7976.

[65] Cayre, O.J. and Paunov, V.N., Contact angles of colloid silica and gold particles at air-water and oil-water interfaces determined with the gel trapping technique. *Langmuir,* **2004**, *20*, p. 9594-9599.

[66] Yang, F., Liu, S., Xu, J., Lan, Q., Wei, F., and Sun, D., Pickering emulsions stabilized solely by layered double hydroxides particles: The effect of salt on emulsion formation and stability. *Journal of Colloid and Interface Science,* **2006**, *302*, p. 159-169.

[67] Tcholakova, S., Denkov, N., and Lips, A., Comparison of solid particles, globular proteins and surfactants as emulsifiers. *Physical Chemistry Chemical Physics,* **2008**, *10*, p. 1608-1627.

[68] Niknafs, N., Spyropoulos, F., and Norton, I.T., Development of a new reflectance technique to investigate the mechanism of emulsification. *Journal of Food Engineering,* **2011**, *104*, p. 603-611.

[69] Pichot, R., Spyropoulos, F., and Norton, I.T., Mixed-emulsifier stabilised emulsions: Investigation of the effect of monoolein and hydrophilic silica particle mixtures on the stability against coalescence. *Journal of Colloid and Interface Science,* **2009**, *329*, p. 284-291.

[70] Bergeron, V. and Walstra, P., Foams (chapter 7), in *Fundamentals of interface and colloid science, volume v soft colloids* **2005**. Elsevier Academic press, p.

[71] Gurkov, T.D., Russev, S.C., Danov, K.D., Ivanov, I.B., and Campbell, B., Monolayers of globular proteins on the air/water interface: Applicability of the volmer equation of state. *Langmuir,* **2003**, *19*, p. 7362-7369.

[72] Pawar, A.B., Caggioni, M., Ergun, R., Hartel, R.W., and Spicer, P.T., Arrested coalescence in pickering emulsions. *Soft Matter,* **2011**, *7*, p. 7710-7716.

[73] Tzoumaki, M.V., Moschakis, T., Kiosseoglou, V., and Biliaderis, C.G., Oil-in-water emulsions stabilized by chitin nanocrystal particles. *Food hydrocolloids,* **2011**, *25*, p. 1521-1529.

[74] Paunov, V.N., Binks, B.P., and Ashby, N.P., Adsorption of charged colloid particles to charged liquid surfaces. *Langmuir,* **2002**, *18*, p. 6946-6955.

[75] Ashby, N. and Binks, B., Pickering emulsions stabilised by laponite clay particles. *Physical Chemistry Chemical Physics,* **2000**, *2*, p. 5640-5646.

[76] Timgren, A., Rayner, M., Sjöö, M., and Dejmek, P., Starch particles for food based pickering emulsions. *Procedia Food Science,* **2011**, *1*, p. 95-103.

[77] Rayner, M., Timgren, A., Sjöö, M., and Dejmek, P., Quinoa starch granules: A candidate for stabilising food-grade pickering emulsions. *Journal of the Science of Food and Agriculture,* **2012**, *92*, p. 1841-1847.

[78] Yusoff, A. and Murray, B.S., Modified starch granules as particle-stabilizers of oil-in-water emulsions. *Food Hydrocolloids,* **2011**, *25*, p. 42-55.

[79] Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., and Norton, I.T., Investigation into the potential ability of pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. *Journal of Colloid and Interface Science,* **2012**, *366*, p. 209-215.

[80] Kalashnikova, I., Bizot, H., Cathala, B., and Capron, I., New pickering emulsions stabilized by bacterial cellulose nanocrystals. *Langmuir,* **2011**, *27*, p. 7471-7479.

[81] Paximada, P., Tsouko, E., Kopsahelis, N., Koutinas, A.A., and Mandala, I., Bacterial cellulose as stabilizer of o/w emulsions. *Food Hydrocolloids,* **2016**, *53*, p. 225-232.

[82] Paunov, V.N., Cayre, O.J., Noble, P.F., Stoyanov, S.D., Velikov, K.P., and Golding, M., Emulsions stabilised by food colloid particles: Role of particle adsorption and wettability at the liquid interface. *Journal of colloid and interface science,* **2007**, *312*, p. 381-389.

[83] de Folter, J.W., van Ruijven, M.W., and Velikov, K.P., Oil-in-water pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein. *Soft Matter,* **2012**, *8*, p. 6807-6815.

[84] Luo, Z.J., Murray, B.S., Yusoff, A., Morgan, M.R.A., Povey, M.J.W., and Day, A.J., Particle-stabilizing effects of flavonoids at the oil-water interface. *Journal of Agricultural and Food Chemistry,* **2011**, *59*, p. 2636-2645.

[85] Luo, Z.J., Murray, B.S., Ross, A.L., Povey, M.J.W., Morgan, M.R.A., and Day, A.J., Effects of ph on the ability of flavonoids to act as pickering emulsion stabilizers. *Colloids and Surfaces B-Biointerfaces,* **2012**, *92*, p. 84-90.

[86] Binks, B.P. and Rocher, A., Effects of temperature on water-in-oil emulsions stabilised solely by wax microparticles. *Journal of Colloid and Interface Science,* **2009**, *335*, p. 94-104.

[87] Hodge, S. and Rousseau, D., Continuous-phase fat crystals strongly influence water-in-oil emulsion stability. *Journal of the American Oil Chemists' Society,* **2005**, *82*, p. 159-164.

[88] Ferragut, V. and Chiralt, A., Microstructure of oil-in-water low-fat emulsions containing skim milk powder and locust bean gum. *LWT-Food Science and Technology,* **1996**, *29*, p. 648-653.

[89] Laca, A., Sáenz, M., Paredes, B., and Díaz, M., Rheological properties, stability and sensory evaluation of low-cholesterol mayonnaises prepared using egg yolk granules as emulsifying agent. *Journal of food engineering,* **2010**, *97*, p. 243-252.

[90] Dickinson, E., Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. *Trends in Food Science & Technology,* **2012**, *24*, p. 4-12.

[91] Dickinson, E., Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces B: Biointerfaces,* **2001**, *20*, p. 197-210.

[92] Tavernier, I., Wijaya, W., Van der Meeren, P., Dewettinck, K., and Patel, A.R., Food-grade particles for emulsion stabilization. *Trends in Food Science & Technology,* **2016**, *50*, p. 159-174.

[93] Araki, J., Wada, M., and Kuga, S., Steric stabilization of a cellulose microcrystal suspension by poly (ethylene glycol) grafting. *Langmuir,* **2001**, *17*, p. 21-27.

[94] Song, X., He, G., Ruan, H., and Chen, Q., Preparation and properties of octenyl succinic anhydride modified early indica rice starch. *Starch□Stärke,* **2006**, *58*, p. 109-117.

[95] Zoppe, J.O., Venditti, R.A., and Rojas, O.J., Pickering emulsions stabilized by cellulose nanocrystals grafted with thermo-responsive polymer brushes. *Journal of colloid and interface science,* **2012**, *369*, p. 202-209.

[96] Roy L, W., Solubility of polysaccharides and their behavior in solution, in *Carbohydrates in solution* **1973**. *117*, Advances in chemistry: AMERICAN CHEMICAL SOCIETY, p. 242-255.

[97] Dickinson, E., Stabilising emulsion-based colloidal structures with mixed food ingredients. *Journal of the Science of Food and Agriculture,* **2013**, *93*, p. 710-721.

[98] Tharanathan, R.N., Starch--value addition by modification. *Crit Rev Food Sci Nutr,* **2005**, *45*, p. 371-84.

[99] Magnus, G. and Ann-Charlotte, E., Starch, in *Carbohydrates in food, second edition* **2006**. CRC Press, p. 391-469.

[100] Rayner, M., Sjoo, M., Timgren, A., and Dejmek, P., Quinoa starch granules as stabilizing particles for production of pickering emulsions. *Faraday Discussions,* **2012**, *158*, p. 139-155.

[101] Caldwell, C.G. and Wurzburg, O.B., Polysaccharide derivatives of substituted dicarboxylic acids. ed**1953**. Google Patents.

[102] Uyama, H., Artificial polymeric flavonoids: Synthesis and applications. *Macromol Biosci,* **2007**, *7*, p. 410-22.

[103] Hertog, M.G.L., Feskens, E.J.M., Kromhout, D., Hollman, P.C.H., and Katan, M.B., Dietary antioxidant flavonoids and risk of coronary heart disease: The zutphen elderly study. *The Lancet,* **1993**, *342*, p. 1007-1011.

[104] Amaretti, A., Raimondi, S., Leonardi, A., Quartieri, A., and Rossi, M., Hydrolysis of the rutinose-conjugates flavonoids rutin and hesperidin by the gut microbiota and bifidobacteria. *Nutrients,* **2015**, *7*, p. 2788.

[105] Atares, L., Marshall, L.J., Akhtar, M., and Murray, B.S., Structure and oxidative stability of oil in water emulsions as affected by rutin and homogenization procedure. *Food Chemistry,* **2012**, *134*, p. 1418-1424.

[106] Oza, K.P. and Frank, S.G., Multiple emulsions stabilized by colloidal microgrystalline cellulose. *Journal of Dispersion Science and Technology,* **1989**, *10*, p. 163-185.

[107] Carrillo, C.A., Nypelö, T.E., and Rojas, O.J., Cellulose nanofibrils for one-step stabilization of multiple emulsions (w/o/w) based on soybean oil. *Journal of Colloid and Interface Science,* **2015**, *445*, p. 166-173.

[108] Sekine, T., Yoshida, K., Matsuzaki, F., Yanaki, T., and Yamaguchi, M., A novel method for preparing oil-in-water-in-oil type multiple emulsions using organophilic montmorillonite clay mineral. *Journal of Surfactants and Detergents,* **1999**, *2*, p. 309-315.

[109] Midmore, B.R. and Herrington, T.M., Silica-stabilised multiple emulsions, in *Trends in colloid and interface science xiii* **1999**. *112*, D. Težak and M. Martinis, Eds., Progress in colloid and polymer science: Springer Berlin Heidelberg, p. 115-120.

[110] Garti, N., Aserin, A., Tiunova, I., and Binyamin, H., Double emulsions of water-in-oil-in-water stabilized by alpha-form fat microcrystals. Part 1: Selection of emulsifiers and fat microcrystalline particles. *Journal of the American Oil Chemists Society,* **1999**, *76*, p. 383-389.

[111] Binks, B.P., Dyab, A.K.F., and Fletcher, P.D.I., Multiple emulsions stabilised solely by nanoparticles, in *Proceedings of 3rd World Congress on Emulsions*,**2002**, Lyon, France.

[112] Binks, B.P., Dyab, A.K.F., Fletcher, P.D.I., and Barthel, H., Multiple emulsions, Germany Patent, **2002**.

Chapter 3

A comparative study on the capacity of a range of food-grade particles to form stable O/W and W/O Pickering emulsions

A proportion of this work has been published as follows:

- L. Duffus, J.E. Norton, P. Smith, I.T. Norton, F. Spyropoulos (2016). A comparative study on the capacity of a range of food-grade particles to form stable O/W and W/O Pickering emulsions. *Journal of Colloid and Interface Science*, **473**, p. 9-21.

- L. Duffus, J.E. Norton, P. Smith, I.T. Norton, F. Spyropoulos, Characterisation & assessment of edible particles for use in Pickering emulsion systems. *Particulate Systems Analysis 2014,* Manchester, UK, September, 2014.
- L. Duffus, J.E. Norton, P. Smith, I.T. Norton, F. Spyropoulos, Edible Pickering emulsion technology in foods*. International Food Congress Novel Approaches in Food Industry*, Kusadasi, Turkey, May 2014.
- L. Duffus, J.E. Norton, P. Smith, F. Spyropoulos, I.T. Norton. Edible Pickering emulsions for healthier foods. *11th Annual Formulation Engineering Conference,* University of Birmingham, UK, April 2014
- L. Duffus, J.E. Norton, P. Smith, F. Spyropoulos, I.T. Norton. Investigation of Pickering emulsions using food-grade particles, *10th Annual Formulation Engineering Conference,* University of Birmingham, UK, April 2013

3.1 Abstract

Whilst literature describing edible Pickering emulsions is becoming increasingly available, current understanding of these systems still suffers from a lack of consistency in terms of the (processing and formulation) conditions within which these structures have been studied. The current study aims to provide a comparative analysis of the behaviour of different edible Pickering candidates and their ability to stabilise emulsion droplets, under well-controlled and uniform experimental conditions, in order to clearly identify the particle properties necessary for successful Pickering functionality.

More specifically, an extensive investigation into the suitability of various food-grade material to act as Pickering particles and provide stable oil-in-water (O/W) and water-in-oil (W/O) emulsions was carried out. Polysaccharide and flavonoid particles were characterised in terms of their size, ζ-potential, interfacial activity and wettability, under equivalent conditions. Particles were subsequently used to stabilise 20 %w/w O/W and W/O emulsions, in the absence of added surfactant or other known emulsifying agents, through different processing routes.

All formed Pickering emulsions were shown to resist significant droplet size variation and remain stable at particle concentrations between 2 and 3 %w/w. The main particle prerequisites for successful Pickering stabilisation were: particle size (200 nm – 1 μm); an affinity for the emulsion continuous phase and a sufficient particle charge to extend stability. Depending upon the employed emulsification process, the resulting emulsion formation and stability behaviour can be reasonably predicted a priori from the evaluation of specific particle characteristics.

Keywords: Pickering emulsions; wettability; Pickering particles; cellulose; flavonoids

3.2 Introduction

Pickering stabilisation [1, 2] has long been recognised as a very useful method for producing emulsions with superior stability to conventional surfactant-stabilised emulsions. Pickering particles accumulate at the oil-water interface in the form of a densely-packed layer that protects against droplet flocculation and coalescence via a steric mechanism. In comparison, surfactants stabilise emulsions via an electrostatic and steric mechanism: surfactants, comprised of a hydrophilic head group and a hydrophobic tail component, preferentially adsorb at the oil-water interface, reducing the free energy present when producing a high surface area during emulsification, and hence interfacial tension is reduced, allowing emulsions to form [3, 4].

Several factors influencing Pickering emulsion stability have been identified. These include parameters such as oil phase composition, particle characteristics, emulsification technique, and storage conditions. However, arguably the most important factors are concerned with the particle characteristics, in particular particle size and wettability [5]. Recently, it was suggested that three main steps exist for convectional (i.e. non-diffusion controlled) adsorption to the liquid-liquid interface [6, 7]. The first step involves particle collisions with free newly created interfacial areas during droplet formation. The second step is concerned with the initial adhesion of particles to this interface, where interparticle electrostatic interactions, as well as particle size properties, are extremely important. Finally, the third step involves water displacement from the particle surface by oil which is dependent upon the particle's contact angle, θ_{ow}, at the interface and hence the hydrophilic/hydrophobic character of the particle [8]. Where $\theta_{ow} < 90^\circ$ (measured through the water phase), the Pickering particle surface mainly resides in the water phase and can be classified as being predominantly hydrophilic; such particles will tend to stabilise an O/W emulsions. Adversely, if $\theta_{ow} > 90^\circ$, the (largely lipophilic) particle will predominantly remain within the oil phase and thus facilitate formation of W/O emulsions. Finally, in those cases where $\theta_{ow} = 90^\circ$, there is no net curvature of the interface and hence no preference for forming a specific emulsion type [9].

Understanding and/or modifying particle characteristics, such as wettability, surface charge, particle size and even surface activity properties, may be then used to control parameters relating to adsorption kinetics, such as the free energy of detachment (E_{det} ; Equation 3-1) of particles from the liquid-liquid interface [10, 11]:

$$E_{det} = \pi r^2 \gamma_{ow} (1 \pm \cos\theta)^2$$ **Equation 3-1**

where γ_{ow} is the interfacial tension between the oil and water phases; θ is the particle contact angle (positive or negative depending on which phase contact angle in measured through); and r is the particle radius.

Recently, research has focussed on using edible Pickering particles to stabilise simple emulsions such as hydrocolloids, rather than traditional Pickering particles such as clays [12], polystyrene [5], silica [13] and TiO_2 [14]. These edible hydrocolloid particles include colloidal hydrophobically modified starch particles [15-17], colloidal celluloses and various cellulose derivatives [18-20]. Such polysaccharide structures have been used to stabilise simple oil-water emulsions that were extremely stable to coalescence when compared to surfactant-stabilised systems. Additionally, particle stabilised emulsions were shown to possess an enhanced stability against lipid oxidation as well as an increased tolerance to shear [20, 21]. Specifically fabricated colloidal particles have also been shown to successfully stabilise simple emulsions. These include chitosan nanocrystals [22], protein particles [23, 24], and certain flavonoids [25, 26], in addition to fat crystals and wax microparticles [27, 28] and various other crystalline species [29-31].

However, despite the currently increasing knowledge on the functionality and performance of edible Pickering particles and the emulsions that these structures can subsequently stabilise, a clear disconnect amongst the available literature persists. This is mainly as a result of reported studies differing greatly with respect to a number of important emulsion parameters, such as pH conditions, particle concentration, emulsification process and processing parameters, oil type and particle dispersion methods.

The aim of the present study is to assess the potential of a range of edible particulate structures to function as Pickering particles for the stabilisation of both O/W and W/O emulsions, under well-controlled and uniform (processing and formulation) experimental conditions. The specific particulate species studied were: three modified celluloses (colloidal microcrystalline cellulose, hydroxypropyl methylcellulose and ethylcellulose) and two flavonoids (rutin hydrate and naringin), as they are edible, commercially attractive and, in the case of flavonoids, also linked to particular health benefits [32]. Particles were characterised with regards to their size, ζ-potential, particle surface activity and wettability, while the emulsions that these structures formed were evaluated in terms of their droplet size and stability. Finally, this study assessed potential advantages that these particles would impart on emulsions produced through different processing routes (i.e. high pressure homogenisation or rotor-stator mixer).

3.3 Materials & Methods

3.3.1 Materials

Distilled water and commercially available sunflower oil (purchased from a local supermarket) were used for the preparation of all emulsions. 100 g samples of emulsions were prepared at 20 %w/w dispersed phase volume and materials were used without any further purification or modification. Particles used as emulsifying agents were rutin hydrate, naringin, colloidal microcrystalline cellulose (CMCC), ethylcellulose (EC) and (hydroxypropyl)methyl cellulose (HPMC) and all were obtained from Sigma, UK. Particle concentration and water and oil phase fractions, unless stated otherwise, are given as percentages of the weight of the individual constituent over the total weight of the final emulsion (%w/w).

3.3.2 Methods

3.3.2.1 Preparation of Pickering particle dispersions

All particles were introduced to and treated in the continuous phase (between 1.5 – 3 %w/w) prior to combination with the dispersed phase to form the emulsions. 80 g of these particle dispersions were prepared and then heated with a hot plate to 45 – 50 ˚C for 40 minutes whilst being agitated with a magnetic stirrer to encourage particle dispersion. Following this, the particle dispersions were further treated by a high intensity ultrasonic vibracell processor (Sonics & Materials, Inc., CT, USA) operating at 750 W and 20 kHz.

3.3.3 Preparation of particle-stabilised emulsions

Following the particle dispersion (in water for O/W emulsion preparation and in oil for W/O emulsion preparation), 20 g of the dispersed phase was added to the particle dispersion and the mixture was emulsified using a Silverson L4RT, with an emulsion screen of 19 mm diameter, for 2 minutes at 10,000 rpm. Following mixing in the rotor-stator mixer (RSM), emulsions were then passed through a high-pressure jet homogeniser (HPH) at 900 bar.

3.3.4 Particle and emulsion droplet size measurements

Particle and emulsion droplet size distributions ($D_{3,2}$) were measured using static multi-angle light scattering (SMLS) via a Mastersizer Hydro 2000 (Malvern Instruments, UK). For smaller particles (≤ 200 nm), a Zetasizer (Malvern

Instruments, UK), employing the dynamic light scattering technique (DLS), was used which measures Brownian motion of smaller droplets. Span, defined as the width at half the height of the peak of the size distribution curve, was calculated in order to give an indication of sample polydispersity. Where the size distributions had several peaks, due to suitability, a Polydispersity Index (PDI) was calculated instead. This value is a measurement of the width of the particle size distribution and is calculated from the square of the standard deviation of the measurement divided by the mean particle diameter. All size measurements were carried out in triplicate with the mean values, unless otherwise stated, and error bars shown represent ±1 standard deviation. Visualisation of emulsions was captured via light microscope (Olympus CH2, Japan, with CCD video camera). Emulsion microstructure was also imaged using Cryo-SEM (Philips XL-30 FEG ESEM). Samples were placed in a four well holder consisting of perforated holes within a brass stage mounted on a steel rod. Samples were subsequently shock-frozen in nitrogen slush, and rapidly transferred to the preparation chamber of the SEM instrument (-140 °C). Frozen sample fracturing was achieved via a metal knife and then a gold layer was evaporated onto the sample *in situ*, to prevent damage from the electron beam. The emulsions were next transferred to the measurement chamber (-180 °C), and analysed using a 2 keV beam to prevent excessive damage to the sample.

3.3.5 Stability measurements

3.3.5.1 Droplet size stability

Droplet size stability was assessed over a period of 14 days by droplet sizing techniques described in section 3.3.4. Measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ±1 standard deviation

3.3.5.2 Creaming stability

Creaming stability was assessed in accordance to the Keowmaneechi and McClements' method [33]. 20g samples were enclosed in sealed tubes and monitored for a period of 14 days. Separation of the emulsion phases gradually occurred, resulting in an upper cream layer and a lower serum layer. Calculation of the creaming index, *CI* (%) was achieved by:

$$CI = \frac{Hs}{He} \times 100\% \qquad \textbf{Equation 3-2}$$

where, *Hs*, is the height of the lower serum layer and He is the total height of the emulsion. Measurements were carried out in triplicate with the mean values, and error bars represent ±1 standard deviation.

3.3.6 ζ-potential measurements

Particle ζ-potential measurements where performed using a Zetasizer (Malvern Instruments, UK) via pH titration (MPT-2) which combined dynamic light scattering (DLS) and electrophoresis mobility. The ζ-potential and Z-Average size (intensity weighted harmonic mean size) measurements of the aqueous particle dispersions were measured (in triplicate) as the pH of an aqueous dispersion of each of the studied particles was progressively adjusted from native pH to pH 2 and a freshly prepared sample was then adjusted from native pH to pH 8, using various concentrations of HCl and NaOH as titrants. ζ-potential data, unless otherwise stated, are given as mean values of the triplicate measurements; error bars shown represent ± 1 standard deviation also calculated from the triplicate size data.

3.3.7 Interfacial tension measurements

Interfacial tension (IFT) measurements were determined using the Wilhelmy plate method via a K100 Tensiometer (Kruss, Germany), operated at room temperature. The platinum plate was used to measure the interfacial tension between the oil phase which was pipetted onto the aqueous phase. Particles were dispersed in either the oil or aqueous phase and measurements were taken until an equilibrium interfacial tension was achieved. All interfacial tension measurements were carried out in triplicate with the mean values given to ± 1 standard deviation.

3.3.8 Wettability measurements

The hydrophobic/hydrophilic character of the particles was assessed in terms of their wettability. Measurements were recorded at room temperature on an EasyDrop goniometer (Kruss, Germany) fitted with a micro-syringe and high speed camera. Static contact angles were measured using the sessile drop method. Water or oil droplets (7.5 μL) were spotted onto compressed particle disk/pellet surfaces via the micro-syringe. The video camera was used to video-record droplet formation. The initial droplet contour was mathematically described by the Young-Laplace equation using the EasyDrop software and as such θ_W and θo (˚) that were calculated refers to the contact angle between the particle substrate and a water droplet and oil droplet, respectively. The compressed disks were prepared by placing 1 g of the pure powdered particles between the plates of a hydraulic pellet press (Graseby Specac, UK) using a 13 mm diameter die under a weight of 3 tonnes for 30 seconds. All measurements were carried out in triplicate and error bars were calculated as ±1 standard deviation.

3.4 Results & Discussion

3.4.1 Assessment of the Pickering functionality of particles

A range of particulate species were characterised in terms of their size, as dispersions within an aqueous or oil phase, charge, wettability and interfacial behaviour, in an attempt to assess their potential Pickering functionality. Specifically, the selected particles were CMCC (colloidal microcrystalline cellulose), HPMC (hydroxypropyl methylcellulose) and EC (ethylcellulose) as well as two flavonoids (rutin hydrate and naringin), due to their availability and commercial viability.

3.4.2 Effect of particle size

Particle size is an extremely important parameter when considering Pickering functionality, as it will, amongst many other phenomena occurring during emulsification, mainly govern the timescales over which particle adsorption at the oil-water interface will take place. Based on the following equations proposed by Walstra [34], t_A, time taken for particle to adsorb at the liquid-liquid interface, can be calculated depending on flow characteristics (*tf*: turbulent flow – Equation 3-3; *lf*: laminar flow – Equation 3-4):

$$t_A^{tf} \sim \frac{\Gamma_M}{C\,(\varepsilon d)^{\frac{1}{3}}}$$ **Equation 3-3**

$$t_A^{lf} \sim \frac{\Gamma_M}{C d \dot{\gamma}}$$ **Equation 3-4**

where ε is the average rate of energy dissipation per unit mass of the emulsion, d is the droplet diameter, and $\dot{\gamma}$ is the shear rate of the regular flow. With respect to t_A, emulsifier effects are introduced in these equations through the ratio of the emulsifier adsorption in the complete monolayer, Γ_M, and the bulk emulsifier concentration, C. Typically for surfactants and proteins, values of Γ_M in the range of 1.5 – 2 mg/m^2 have been measured experimentally [35]. However, Γ_M values for particles can be calculated by Equation 3-5; where ρ_P is particle mass density, r is particle radius and ϕ_{cp} is the fraction of area covered by adsorbed particles in a complete monolayer (for spherical particles it is approximately 0.907) [36]:

Equation 3-5

$$\Gamma_M \sim \rho_P \phi_{CP} (4\pi r^3/3)/(\pi r^2)$$

Therefore, Γ_M is directly proportional to the particle radius, r, and hence t_A increases as the particle size increases.

Particles investigated here were characterised in terms of their size, initially following dispersion within an aqueous medium, in order to gain insight into their potential ability to stabilise O/W emulsions. Native particle size (following mild mixing) and particle size following sonication data collected for all particle types are provided in Table 3-1; particles were sonicated at 30s time intervals until no further change to their size was recorded.

Table 3-1 Native particle size (µm), reduced particle size following sonication (µm) and the corresponding span or polydispersity indices (PDI), and Edet values (calculated from Eq. 1 where θ is individual particle contact angles with water and γ_{ow} is 25 mN/m) for cellulose (CMCC, HPMC and EC) and flavonoid (rutin hydrate and naringin) particles dispersed in an aqueous medium (measured in triplicate and ±1 standard deviation).

PARTICLE TYPE	Native Particles (aq.)			Sonicated Particles (aq.)			
	$D_{3,2}$ (µm)	Span	PDI	$D_{3,2}$ (µm)	Span	PDI	E_{det} (kT)
CMCC	1.67 ± 0.17	2.82 ± 0.02	-	1.53 ± 0.06	2.49 ± 0.04	-	1.61×10^{6}
HPMC	0.23 ± 0.07	-	0.65 ± 0.04	0.14 ± 0.03	-	0.67 ± 0.19	3.32×10^{5}
EC	109.6 ± 2.38	1.88 ± 0.01	-	63.08 ± 1.39	2.25 ± 0.01	-	3.02×10^{10}
RUTIN HYDRATE	4.69 ± 0.43	3.12 ± 0.18	-	0.18 ± 0.01	13.26 ± 0.91	-	6.04×10^{5}
NARINGIN	8.53 ± 0.39	2.41 ± 0.08	-	6.41 ± 0.41	1.85 ± 0.15	-	7.76×10^{8}

It was shown that sonolysis had either no significant effect on particle size, as was the case for CMCC and HPMC, or particle size reduction was indeed achieved, e.g. rutin hydrate (Table 3-1). CMCC, naringin, rutin hydrate and EC particle sizes were measured using the SMLS technique and rutin hydrate systems exhibited the highest level of polydispersity. Due to their smaller size, HPMC particle sizes were measured using a DLS method. Upon sonication, observation of the polydispersity indices of these samples highlights that polydispersity of HPMC particles as being unaffected. Rutin hydrate (following sonication) and HPMC (even without sonication) possess the smallest particle sizes with a significant volume (%) of particle sizes in the nano-size range. These smaller particles (rutin hydrate and HPMC) were expected to be best suited for the stabilisation of small O/W emulsion droplets. Additionally, the overall stability of an emulsion is inversely proportional to particle size, with smaller particles giving a higher packing efficiency, and therefore providing a more homogenous layer at the interface preventing coalescence [3]. On the other hand, if adsorption does occur, particle size has a direct effect on E_{det} (Eq. 1), with smaller particles (e.g. HPMC) resulting in lower E_{det} values and therefore expected to detach from the oil-water interface more easily than larger particles, especially if further processing occurs following initial droplet formation, and potentially to the detriment of emulsion stability. The paradox in this conflicting rationale serves to further stress that neither emulsion droplet size nor emulsion stability are controlled by particle size alone and that additional characteristics such as particle charge, wettability and interfacial behaviour (evaluated in later parts of the present study) must also be considered in equal terms.

Understanding of particle behaviour in a lipid environment is equally important and particularly relevant when considering the stabilisation of W/O emulsions. For this reason the size of the same particles was also measured for their dispersions within an oil medium (sunflower oil), prior to (native particles) and following ultrasound (sonicated particles) treatment (Table 3-2). The data obtained reveal that particle sizes in this case are much larger than when the same structures were dispersed in an aqueous environment, even following sonication, with the exception of EC and CMCC. EC particle size is significantly smaller when dispersed in the oil phase compared to when dispersed in an aqueous medium, suggesting that EC may be predominantly hydrophobic and more suited for the stabilisation of W/O emulsions. This is assumed to be the case as hydrophobic particles are not as easily dispersed and are more likely to form particle aggregates in an aqueous environment [9]. CMCC size is relatively small and similar in both dispersion media indicating an almost similar affinity for the particle to be placed in either phase. Ultrasound treatment appears to have little effect on particle size; only HPMC particles decrease in size and in this case the span of the particle size distribution is significantly increased as a consequence. Additionally, with the exception of EC, the span of the remaining particles increased.

Table 3-2 Native particle size (µm), reduced particle size following sonication (µm) and the corresponding span values for cellulose (CMCC and HPMC) and flavonoid (rutin hydrate and naringin) particles dispersed in a lipid medium (measured in triplicate and ± 1 standard deviation).

Particle Type	Native Particles (oil)		Sonicated Particles (oil)	
	$D_{3,2}$ (µm)	Span	$D_{3,2}$ (µm)	Span
CMCC	2.48 ± 0.71	1.77 ± 0.03	2.06 ± 0.01	23.72 ± 0.64
HPMC	22.46 ± 0.96	1.64 ± 0.18	11.77 ± 0.30	3.21 ± 1.13
EC	0.66 ± 0.01	0.38 ± 0.01	0.65 ± 0.01	0.38 ± 0.01
RUTIN HYDRATE	11.22 ± 5.44	1.85 ± 0.96	10.52 ± 0.57	23.18 ± 18.01
NARINGIN	12.49 ± 1.53	1.77 ± 0.57	18.64 ± 0.82	5.19 ± 0.57

As stressed previously, particle size is not the only determining factor and many other considerations must be accounted for when assessing the potential of particles to function as Pickering stabilisers. For example, particle size and charge in particular can be significantly affected by changes to pH conditions.

3.4.3 Effect of pH environment

Depending on the final formulation and end applications of edible Pickering emulsions, the pH of the system can vary significantly. Therefore, particle behaviour within different pH environments must be understood as changes in particle ζ-potential can potentially affect particle dispersion/aggregation. The effect of pH on the ζ-potential and size (in aqueous dispersions) of the particles studied here was determined using a titration method and the obtained data are presented in Figure 3-1 and native pH values of aqueous particle dispersions are given within the Figure 3-1 caption. It should be noted that discussion here will focus on the overall behaviour of these particles as a function of pH in qualitative rather than quantitative terms; this is mainly relevant to the collected DLS particle size data. Since the used DLS method is best suited for the characterisation of nanomaterials, accurately measuring the sizes of larger particles and/or particle aggregates (i.e. >5 μm) in the studied dispersions can become problematic. In the case of the EC particles, due to their large size, the DLS method was not able to successfully measure the larger particle aggregates (which were visible to the naked eye) accurately.

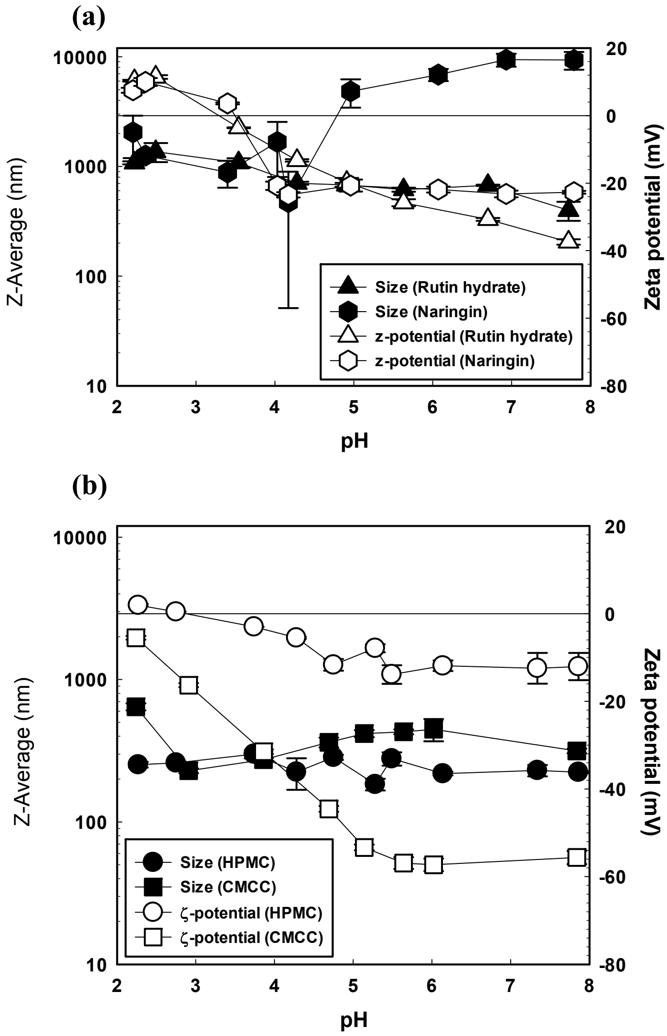

Figure 3-1 Z-Average and ζ-potential as a function of pH for: (a) flavonoid particles [rutin hydrate and naringin] and (b) cellulose particles [HPMC and CMCC]. Data points are averages of data collected in triplicate and error bars represent 1 standard deviation; where not visible, error bars are smaller than the symbols used [native pH: rutin hydrate (pH 4.6); naringin (pH 5.54); HPMC (pH 5.25); CMCC (pH 6.18)].

Analysis of the data in Figure 3-1a shows that as the pH conditions change from acidic to alkaline, the Z-Average size of the rutin hydrate particles decreases to ~400 nm with a wide but yet unimodal distribution. After the isoelectric point (IEP), at approximately pH 3.5, the ζ-potential for rutin hydrate decreases progressively to -40 mV (at pH 8). This behaviour is due to the presence of quercetin within the rutin hydrate molecular structure. This polyphenolic moiety has two C_7-$(OH)_2$ groups and behaves as a polyprotic acid undergoing significant dissociation between pH 4 and pH 8 by imparting a negative charge to two H^+ surface molecules [26]. Naringin had a similar effect with respect to the effect of pH on particle charge. When comparing the two flavonoids, a higher magnitude of negative ζ-potential was attained with rutin hydrate as the dispersion became more basic. This may be the result of the corresponding C_7-OH group on the naringin molecule being glycosylated to the disaccharide moiety, inhibiting ionisation and thus reducing negative ζ-potential magnitude.

In relation to the cellulose type particles (Figure 3-1b), a pH increase particle size decrease effect was observed with the CMCC particles where particle size decreased in acidic conditions and size reduction halted at around pH 3. Average particle size remained low and stable within a range of 200 – 400 nm as the dispersion alkalinity increased. However, the particle size distribution becomes wider and more non-uniform as alkalinity increased suggesting an increase in particle polydispersity. Negative ζ-potential increased in acidic conditions and this halted at approximately native pH at -55 mV. As pH increased from 4 to 8, negative ζ-potential remained constant at this value. Responsibility for the high ζ-potential lies potentially with the oxidation of the several –OH groups attached to the α-gluco-pyranose ring which acts as a monobasic acid [37, 38]. HPMC particles displayed a similar effect as the flavonoid samples in terms of the charge on the particles. As alkalinity of the particle aqueous dispersion increased, the particle negative charge increased past the IEP in acidic conditions and continued to increase towards a maximum and then remained constant as the pH continued to increase (see Figure 3-1b). In HPMC particles, some of the hydrogen ions present in the -OH groups in CMCC have already been substituted by methyl or hydroxypropyl radicals to obtain HPMC compounds. This reduces their ionisation potential and hence the ζ-potential of these particles is much lower compared to other particle types.

All particles possess a negative charge at their native pH with CMCC possessing the highest at -53.70 mV and this indicates that once particles adsorbed at the oil-water interface, interparticle repulsive forces amongst particles at the interface could aid droplet stability against coalescence. Therefore, prospective particle placement at the interface must also be investigated, via analysis of particle wettability and particle affectation of the oil-water interface.

3.4.4 Effect of particle surface character

Particle behaviour at the oil-water interface is highly important in understanding potential emulsion droplet formation and stability. Despite this, particle arrangement at the interface is notoriously difficult to assess and in this study two methods have been chosen in order to further understand particle behaviour; determination of particle wettability and particle effect on the interfacial tension between sunflower oil and water.

3.4.4.1 Particle wettability

The wettability (hydrophilic/lipophilic) character of the studied particles, as reflected by the individual contact angle measurements with water (θ_W) and then with oil (θ_O), can be used as an indication of the type of emulsion that these solid species would favour stabilising [5]. Accordingly, where the value for θ_W significantly exceeds θ_O for particles, they can be categorised as relatively hydrophobic, with the converse being true for hydrophilic particles.

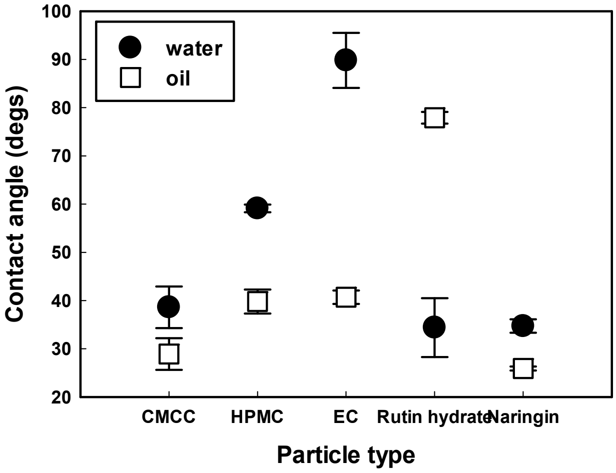

Figure 3-2 Contact angles with oil, θoil (˚) (open square symbols) and with water θwater (˚) (filled circle symbols) (measured in triplicate and error bars represent two standard deviations), for cellulose (CMCC, HPMC and EC) and flavonoid (rutin hydrate and naringin) particles. [Error bars represent ± 1 standard deviation and where not visible are smaller than symbols].

With the exception of CMCC, all cellulose particles had θ_W values that greatlyexceeded their θ_O value, and as a consequence it can be said they possess an overall hydrophobic character. In the case of CMCC, the difference between the contact angle values (Figure 3-2) is much smaller than other systems (accounting for the error in the values) indicating that there is no significant preference for either phase in comparison to the other systems. The contact angles are also fairly low for both phases with CMCC particles (similar to the particle size trend observed) indicating an affinity for both phases and as such, there can be no prediction of preferential emulsion type formation based on the wettability character of this particle type. In the case of the flavonoid particles, via analysis of Figure 3-2, rutin hydrate can be classed as relatively hydrophilic as the value of θ_O for this particle is significantly higher than its value of θ_W, highlighting that it is preferentially wetted by the water phase. Naringin particles contact angle values are similar to CMCC, in that they are low with respect to both phases, suggesting no significant preference for either phase.

The wettability of the particles can be related, in part, to the chemical structure of the materials. In the case of the celluloses, chemical alteration of the CMCC particles allows for the derivation of both HPMC and EC materials via the partial substitution of the hydrophilic –OH groups for non-polar hydrocarbons such as methyl (for HPMC) or ethyl structures (for EC) both of which are hydrophobic functional groups. Due to this, strong hydrophobic zones form on these materials. For CMCC, the many hydrophilic –OH groups present on the lipophilic cellulose backbone means it would be anticipated that they should have an affinity for both oil and water phases. Where the flavonoid particles are concerned, the rutin compound has been hydrated and therefore, when introduced to an aqueous phase, dissolution will always occur to some extent. Additionally, the added water molecules that have formed hydrogen bonds in the hydrated structure will repel oil droplets, allowing for an explanation for the hydrophilicity of the particle. With respect to the naringin particles, the presence of both –OH and –CH_3 groups imparts a degree of amphiphilicity to the particles, however, there are typically less hydrocarbon groups than the modified cellulose particles and so the hydrophobicity is reduced in comparison.

3.4.4.2 Interfacial tension

Although IFT measurement data is generally regarded as not as significant in Pickering systems as it is in surfactant stabilisation mechanisms, it is still important. However it is a static method and not equivalent to what occurs during the actual emulsification process. Subsequently, the effect of particles on the IFT of an immobile sunflower oil-water system was investigated (see Table 3-3). IFT was measured for particles initially dispersed in the water phase and then also measured with particles dispersed in the oil phase. Interfacial tension was also measured between water and sunflower oil in the absence of particles as a baseline for comparison purposes. Equilibrium IFT measured for such a system was 24.61±0.89 mN/m.

Table 3-3 Particle density (kg/m3) and interfacial tension (mN/m) data for: CMCC, HPMC, EC, naringin and rutin hydrate. Interfacial tension values are provided for particles placed either within the aqueous or sunflower oil phase during measurement. All particles were measured at their native pH in unbuffered MilliQ water, in triplicate and ± 1 standard deviation. [* provided from MSDS documentation]

PARTICLE TYPE	Particle density, $\rho_{particle}$ (kg/m^3)*	Equilibrium Interfacial Tension (mN/m)	
		Aqueous phase	Oil phase
CMCC	600	25.11 ± 0.12	14.38 ± 0.16
HPMC	1390	14.20 ± 0.24	4.51 ± 0.24
EC	1140	21.41 ± 0.63	6.93 ± 0.10
RUTIN HYDRATE	1820	24.46 ± 0.22	15.40 ± 0.31
NARINGIN	1660	19.13 ± 0.01	1.03 ± 0.31

Here a relationship between the hydrophobicity of the particle (see Table 3-3) and interfacial tension can be realised. The hydrophobic character of a particle can, to an extent, dictate which phase it prefers to remain in. Therefore, hydrophobic particles in an aqueous dispersion will tend to migrate towards the oil phase, and as a consequence the oil-water interface, effecting a reduction in the interfacial tension. In the case of IFT where particles are dispersed in the oil phase, sedimentation affects the oil/water IFT more strongly as a result of the Wilhelmy plate method. Hence particle size and density affect IFT, further resulting in an increased IFT reduction in all cases.

Consequently it can be seen that the predominantly hydrophobic cellulose particles, HPMC and EC, when dispersed in the aqueous phase, lowered IFT the most. Dispersion in the oil phase resulted in HPMC and EC considerably lowering IFT once again. These particle types individually have a higher density than the oil phase but possess a primarily hydrophobic character (see Figure 3-2) suggesting that they would have an affinity for the liquid-liquid interface and subsequently lower IFT for this reason, in this case. The third cellulose particle type, CMCC had a minor effect on IFT when dispersed in either oil or water as it has a similar preference for both phases (see Figure 3-2), and its density is lower than both liquid phases. It has a slight effect on IFT when dispersed in oil due to its comparatively large size and hence sedimentation effects prevail.

Comparison between the naringin IFT values when these particles are dispersed in the water phase and when they are dispersed in the oil phase indicate a discrepancy in particle ability to lower interfacial tension. Naringin appears to reduce IFT the most of all particles when dispersed in the oil phase however this is most likely due to sedimentation effects, and not a true reduction of a lowering of the oil-water interfacial tension. This is believed to be the case as it possess the largest particle size (when dispersed in oil), and a density higher than both liquid phases (densities of oil and water phases are taken as 915 and 998 kg/m^3 respectively) (see Table 3-3). Rutin hydrate appears to have the same effect on IFT as CMCC despite the high particle density; however, rutin hydrate particles are mainly hydrophilic in character and are significantly smaller than CMCC particles. Its hydrophilicity can explain why, when dispersed in the aqueous phase, the IFT remains unaffected as it has an affinity for the water phase and therefore particles are not driven to migrate across the interface towards the oil phase. Conversely, when rutin hydrate is dispersed in oil, the high particle density and hydrophilic nature promotes perturbation of the interface and hence the IFT is lowered.

3.5 Pickering emulsion behaviour

Both oil-in-water and water-in-oil emulsions were produced using the different particle types to stabilise droplets at various particle concentrations. Two processing methods were also investigated; rotor stator mixer and high pressure jet homogenisation, in order to assess the effect on emulsion microstructure.

3.5.1 Oil-in-water Pickering emulsions

Preliminary experiments regarding particle concentration and its effect on emulsion droplet size and stability, in addition to data in literature [20], suggested that for the modified polysaccharide particles, a particle concentration of > 1.5 %w/w was sufficient to adequately stabilise a 20 %w/w sunflower oil-in-water emulsion. Figure 3 shows the mean droplet size for these emulsions using the high shear mixer process described earlier as a function of storage time and hence gives an indication of emulsion stability. In terms of the flavonoid particles, it was shown that a higher particle concentration was required to produce smaller droplets, however stability of the droplets was unaffected by particle concentration. It was apparent that, with a minimum flavonoid particle concentration of 1.5 %w/w, once the particles had adsorbed to the oil-water interface, irrespective of initial droplet size or emulsion creaming, they were stable to coalescence for the duration of the stability study (i.e. 14 days) (see Figure 3-3).

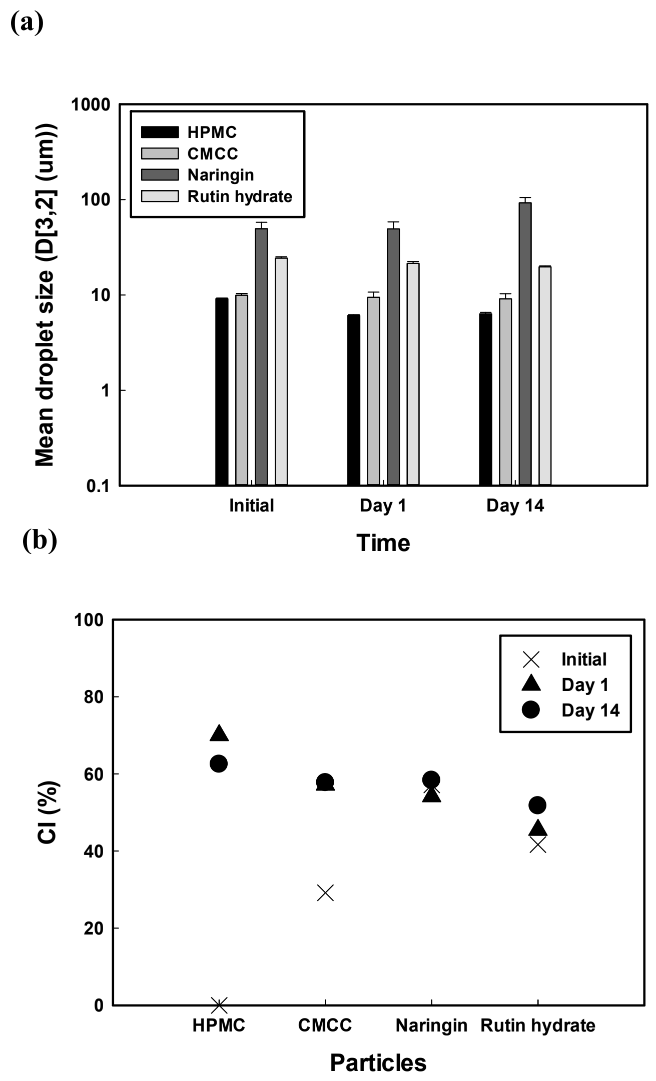

Figure 3-3 (a) Mean droplet sizes (D[3,2]) of O/W emulsions (20:80) produced using particles alone (at 1.5%w/w) to stabilise droplets initially, at day 1 and day 14 (b) Creaming index for all emulsions shown, initially, at day 1 and day 14. Measurements were recorded in triplicate and error bars represent 2 standard deviations.

Table 3-4 Particle size, initial droplet size, particle/droplet size ratio, droplet density, creaming rates, number of droplets possible given oil volume and number of droplets possible in creamed layer (Figure 3-3b) for all particle stabilised 20% O/W emulsion systems containing 1.5 %w/w particle concentration (Figure 3-3a). Measurements were recorded in triplicate and values are given to ± 1 standard deviation.

Particle Type	HPMC	CMCC	Rutin hydrate	Naringin
Particle size (µm)	0.14 ± 0.03	1.53 ± 0.06	0.18 ± 0.01	6.41 ± 0.41
Initial droplet size (µm)	9.13 ± 0.17	9.93 ± 0.43	24.30 ± 0.88	49.51 ± 8.27
Droplet/particle size ratio	63.4	6.5	135.8	7.7
Droplet density, $\rho_{droplet}$ (kg/m^3)	958.5	704.3	954.4	1356.7
Creaming rate, υ (m/s)	-1.80×10^{-6}	-1.58×10^{-5}	-3.41×10^{-5}	4.78×10^{-4}
Number of dispersed phase droplets possible (oil volume)	5.44×10^{10}	1.62×10^{11}	5.38×10^{9}	5.19×10^{7}
Number of dispersed phase droplets possible (creamed layer)	9.48×10^{10}	3.18×10^{11}	1.21×10^{10}	1.01×10^{8}

In order to drive droplet stabilisation, particle concentrations used exceeded the minimum particle concentrations required for maximum particle coverage of droplets given the oil volume used, and consequently it was expected that unadsorbed particles would remain in the continuous phase. Not only was there evidence of particle sedimentation within emulsion systems as a result but preliminary rheology measurements showed that viscosity was unaffected and emulsions behaved as Newtonian liquids. As expected based on particle size, the smaller HPMC particles produced emulsions containing the smallest oil droplets and the larger Naringin particles produced the largest droplets (see Figure 3-3). However, there is no definitive trend with respect to the particle size: droplet size ratio (Table 3-4), which again suggests that there are factors other than particle size that influence emulsion behaviour. All emulsions prepared creamed after 14 days, and creaming rates, u, (m/s) were calculated via the Stokes equation (see Equation 3-6):

$$v = \frac{2\,r^2\,(\rho_{droplet} - \rho_o)\,g}{9\,\eta}$$ **Equation 3-6**

Where r is droplet radius (µm), $\rho_{droplet}$, is the droplet density (kg/m^3), ρ_o, is the bulk phase density (kg/m^3), g, is the local acceleration due to gravity (m/s^2), and η, is the bulk phase viscosity (kg/ms). $\rho_{droplet}$, was calculated via:

$$\rho_{droplet} = \frac{m_{adp} + m_{oil}}{V_{droplet}}$$ **Equation 3-7**

Where adsorbed particle mass (kg), m_{adp}, mass of oil in the droplet (kg), m_{oil}, and volume of droplet (m^3), $V_{droplet}$, are given by:

$$m_{adp} = \rho_{particle}\,\frac{4}{3}\pi\left(\frac{D_{droplet}}{2}\right)^3 - \left(\frac{D_{oil}}{2}\right)^3$$ **Equation 3-8**

$$m_{oil} = \rho_{oil}\,\frac{4}{3}\pi\left(\frac{D_{oil}}{2}\right)^3$$ **Equation 3-9**

$$V_{droplet} = \frac{4}{3}\pi\left(\frac{D_{droplet}}{2}\right)^3$$ **Equation**

As oil droplet diameter (µm), D_{oil}, is derived from:

$$D_{oil} = D_{droplet} - 2D_{adp}$$ **Equation 3-11**

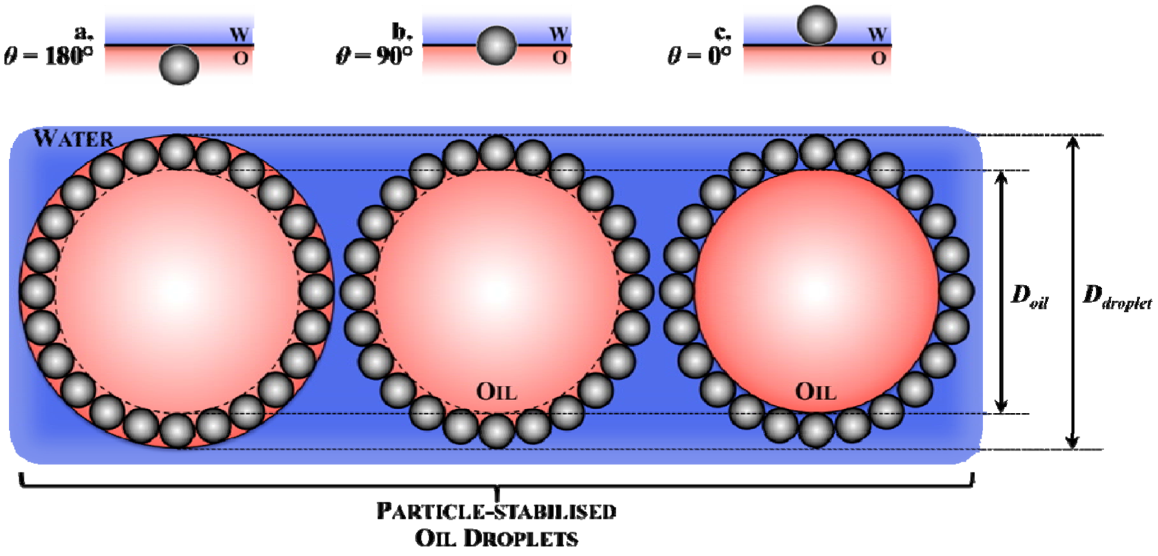

Figure 3-4 Schematic representation of the cross section of an oil droplet (O/W emulsion) stabilised by particles with interfacial arrangements corresponding to contact angles of: a. θ = 180°, b. θ = 90° and c. θ = 0°.

Assuming that particles form a uniform monolayer at the droplet surface, (as shown in schematic diagram, Figure 3-4) irrespective of particle contact angle, $\rho_{particle}$ is particle density (kg/m^3) (Table 3-3), D_{adp} is the adsorbed particle diameter, and density of oil phase, ρ_{oil} is taken as 915 kg/m^3. The three particle arrangements detailed in Figure 3-4Figure 3-4 Schematic representation of the cross section of an oil droplet (O/W emulsion) stabilised by particles with interfacial arrangements corresponding to contact angles of: a. θ = 180°, b. θ = 90° and c. θ = 0°. represent possible contact angles at the interface (measured through the water phase) as > 90˚ (Figure 4, left droplet), ~ 90˚ (Figure 3-4, centre droplet) and < 90˚ (Figure 3-4, right droplet). It can be seen that in spite of variance in contact angle, differences in oil volume amongst the three different systems is negligible where droplet sizes are equivalent.

It can be observed from Table 3-4 that as all values for droplet density are less than the density of the bulk phase (998 kg/m^3) and the values for creaming rate, (υ), are all negative, that creaming ensues as opposed to sedimentation [39] with the exception of Naringin systems. Naringin stabilised emulsions creamed at the highest rate, as can be seen in Figure 3-3b (creamed layer is largest initially) and Table 3-4 (highest υ value), however it has a positive υ value indicating that sedimentation of droplets is most likely to occur. Experimentally, this was not found to be the case as droplets creamed and this disparity may be due to incomplete droplet surface coverage by particles and not the uniform particle monolayer at the interface as is assumed when calculating creaming rates here. Incomplete droplet coverage promotes droplet coalescence and this is consistent with the fact that Naringin stabilised droplets experienced the most growth across the period of storage in comparison with the other systems.

Indeed the calculated υ values correlate well with the *C.I.* observed (Figure 3b) for all systems; those with the lowest creaming rates (i.e. HPMC) exhibited a slower increase in *C.I.* over time, whereas the naringin (highest creaming rate calculated) creamed immediately. The number of droplets possible given the volume of the creamed layer for emulsion systems appeared to be significantly larger than the number of droplets possible from the oil volume input into the emulsions in all cases, following 14 days (see Table 3-4). Essentially, this indicates that the creamed layer allows for more droplets to be produced than is possible with the volume of the oil in the system, even with maximum droplet coverage by particles assumed. This may be the result of random loose packing of particles at the interface and/or free particle aggregates present in the creamed layer.

Additionally, all particles were shown to be negatively charged at native pH (see Figure 3-1) and present in excess concentration in order to drive droplet stabilisation and so it is probable that there are significant interparticle repulsive forces within the continuous phase, leading to a larger creamed layer.

Interestingly, the CMCC stabilised emulsions creamed at an intermediate rate, but had the lowest $\rho_{droplet}$ value, which would predict the highest creaming rate. In addition, the rutin stabilised emulsions showed very little difference in droplet size over time, despite the large initial droplet size compared to the other particle stabilised systems. It can be reasoned that these anomalies are due to the wettability of these particle types (Figure 3-2). Rutin hydrate has a predominantly hydrophilic character and CMCC has no significant difference in preference for either the water or oil phase, therefore, they are more likely to provide a higher stability to droplets in an O/W emulsion system compared with the more hydrophobic particles. Additionally, CMCC particles possess a surface charge of approximately -55 mV at native pH. These particles covering the droplet surfaces are therefore more likely to repel each other more strongly than the other more weakly charged particle types (i.e. HPMC), which in turn decreases the probability of droplet coalescence, as well as hindering the occurrence of creaming.

Naringin stabilised emulsions consisted of large oil droplets, as predicted, and were unstable. This was expected due to the large size of the particles and hence the slower adsorption rates to the interface during processing. The slower kinetics of naringin particle adsorption compared to smaller particles (e.g. nano-sized) or conventional surfactant adsorption means that as droplets are broken up during emulsification, newly created areas of liquid interface are left exposed to approaching droplets encouraging coalescence. By comparison, smaller particles such as the HPMC by proxy generally produce smaller emulsion droplets. They have faster kinetics of adsorption to the interface, and this can be seen experimentally in the fact that they produced the smallest droplet sizes initially (see Figure 3-3a). However, as the magnitude of free energy of detachment of a particle, E_{det}, from the interface is inversely proportional to the particle size (see Equation 3-1), HPMC particles will also have a lower magnitude of E_{det} (although still significantly higher than typical low molecular weight surfactants) and will detach from the interface more readily than the other particles. In turn, this allows the emulsion system to become more susceptible to droplet flocculation and coalescence. As a consequence of these various factors, it was evidenced that the HPMC and naringin particles appeared to yield the least stable emulsions as the difference between the initial droplet sizes and those measured after 14 days were the highest (see Figure 3-3).

3.5.2 W/O emulsions

W/O emulsions were prepared using the particles to stabilise the interface, and emulsions were produced using the Rotor Stator Mixer (RSM) process as in the previous section with O/W emulsions. Particles were dispersed in the oil phase initially (see Table 3-2) and then 20% W/O emulsions were formed. Emulsion droplet sizes for all particles prepared via rotor-stator mixer were initially significantly larger than those found in the corresponding O/W systems and subsequently, all emulsions creamed immediately and had all phase separated within 24 hours. The exceptions were the EC particle-stabilised emulsion systems which were stable for in excess of 2 weeks and HPMC stabilised emulsions which were stable for approx 24 hours (see Figure 3-5). Both particle types underwent ultrasound treatment prior to emulsification.

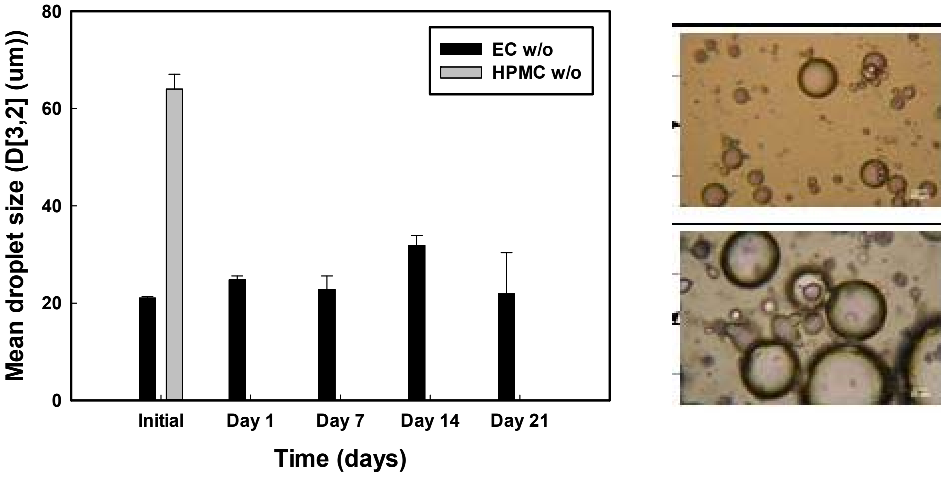

Figure 3-5 Mean droplet size (μm) as a function of time for 20% w/w water-in-oil emulsions stabilised by different particles and prepared via rotor-stator mixer at 10,000rpm for 2 minutes. Measurements were performed in triplicate and error bars represent ± 1 standard deviation. [Error bars where not visible are smaller than symbols]. Micrograph inserts indicate initial emulsion droplets formed using HPMC (top) and EC (bottom) particles.

These two particle types were expected to stabilise W/O emulsions, largely due to their predominantly hydrophobic nature, which also meant that they dispersed more easily in the oil phase compared to the other particles assessed. The lack of HPMC-stabilised droplet stability in comparison to the EC stabilised emulsions indicates rapid droplet coalescence. This is potentially due to incomplete particle coverage leaving exposed droplet surface area left susceptible to droplet-droplet coalescence, which is expected due to the large and polydispersed HPMC particles when dispersed in oil. Indeed, EC stabilised emulsions were more stable than emulsions using HPMC to stabilise droplets due to the smaller initial droplet size as EC particles were found to be smaller in size (Table 3-2) when dispersed in the continuous oil phase promoting smaller droplet formation during processing in comparison.

3.5.3 Effect of processing conditions

Pickering particles were used to produce simple O/W emulsions in order to assess and compare the effects of low (Silverson mixer) and high (HPH) shear emulsification processes on resulting emulsion microstructure, specifically in terms of droplet size and stability. Figure 3-6 depicts 20% w/w sunflower oil emulsions prepared using various particles at a pre-determined minimum particle concentration, individual to that particle. Based on preliminary work conducted surrounding particle concentration effects, 2% w/w modified polysaccharide particles was seen to be adequate to produce stable emulsions and so this was used for HPMC and CMCC particle stabilised emulsions. For the flavonoid particles, based upon the same premise, 2.5% w/w naringin and 3% w/w rutin hydrate were used, and again EC particles were unable to produce stable O/W emulsions, and so processing type comparisons were not completed for this particle type.

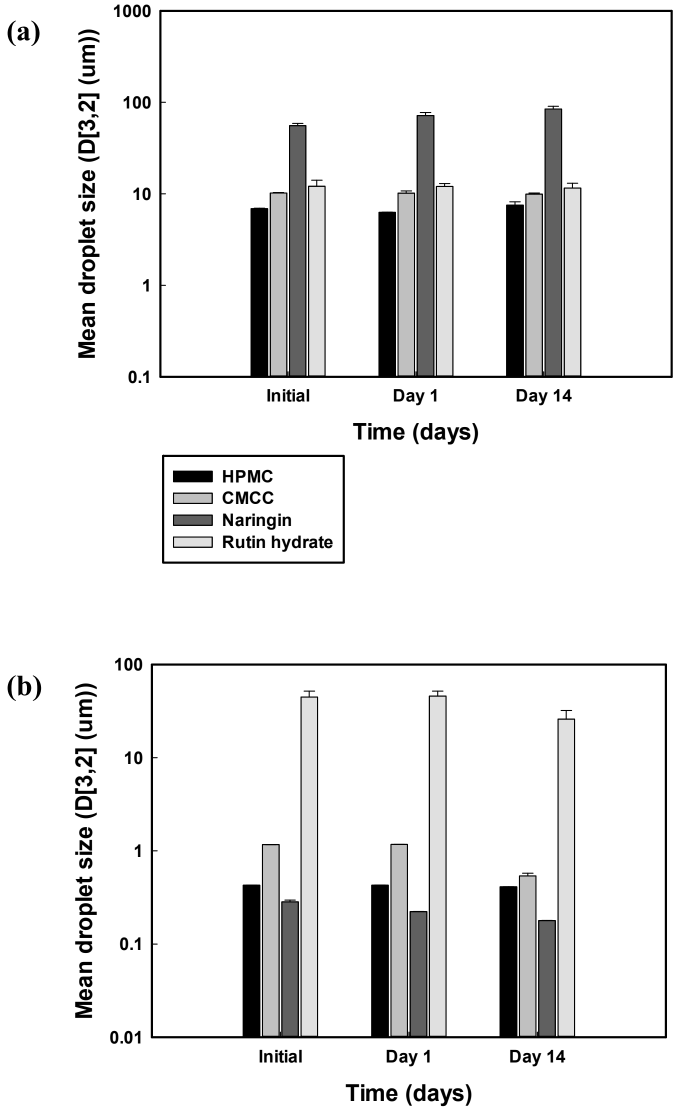

Figure 3-6 Mean droplet size (µm) as a function of time for 20% w/w sunflower oil-in-water emulsions stabilised by different particles and prepared via (a) RSM at 10,000rpm for 2 minutes and (b) 5 passes via HPH at 900bar. Measurements were performed in triplicate and error bars represent ± 1 standard deviations.

Most particles produced smaller, more stable droplets with the increased particle concentration (see Figure 3-3a and Figure 3-6a) and there again with the higher energy process, HPH (see Figure 3-6a and Figure 3-6b), as would be expected [40, 41]. Although sonolysis treatment was previously shown to not significantly reduce the particle size of both Naringin and CMCC particles, emulsions produced were nano-sized. Both particles did not significantly reduce IFT when dispersed in the aqueous phase (Table 3-2) therefore this significant reduction in emulsion droplet size appears to be caused by a particle size reduction. This reduction was more than likely due to the higher pressure breaking down particles in the HPH pressure chamber during processing. These smaller particles were then able to diffuse to the interface more quickly as smaller droplets were produced during homogenisation.

Conversely, the data suggests that rutin hydrate particles produced smaller droplets with the lower energy process, RSM, than with the HPH process (see Figure 3-7).

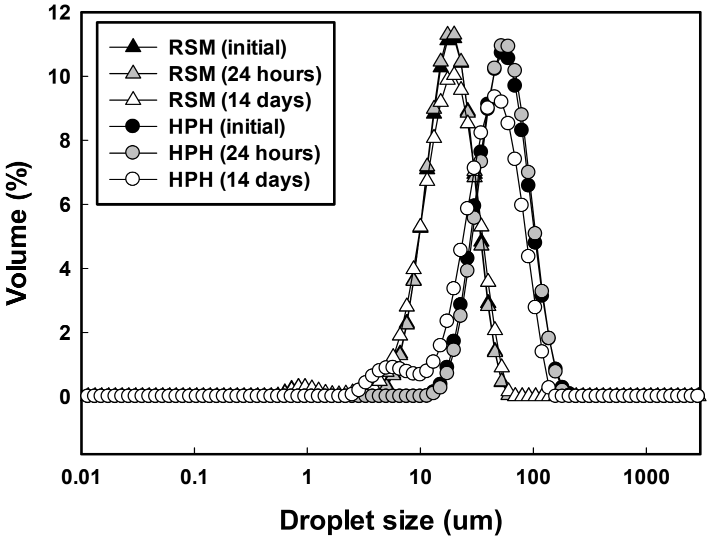

Figure 3-7 Droplet size distribution curves for 20 %w/w sunflower oil emulsions stabilised with 3 %w/w rutin hydrate particles produced via RSM and HPH processing at various time points.

For this, there are two possible, separate but plausible, explanations. Firstly, it could be reasoned that this effect occurred due to the fact that the emulsions were processed using the RSM initially; they were then passed through the HPH where the previously formed droplets were broken up further into smaller droplets. The larger size and wide distribution of rutin hydrate particles means that they cannot adsorb at the interface as rapidly as conventional surfactants and so as the high energy process causes the previously formed droplets to break into smaller ones, the newly created interface is not sufficiently covered by particles and so droplets coalesced. Equally, it could be reasoned that the measurements given by the Mastersizer apparatus incorrectly observed flocculated oil droplets (and not particle aggregates as the refractive indices of rutin hydrate (~1.77) and sunflower oil (~1.47) are too dissimilar) as individual droplets. Micrographs were collected of all emulsion samples in order to corroborate droplet size measurements and all samples correlated well (as shown in Figure 3-8c, Figure 3-8d and Figure A2) with the exception of the emulsions formed using rutin hydrate via HPH processing. Based on optical imaging analysis subsequently conducted (see Figure 3-8), and the fact that all emulsions creamed but remained stable to coalescence for the duration of the study, it appears that the latter may be the case.

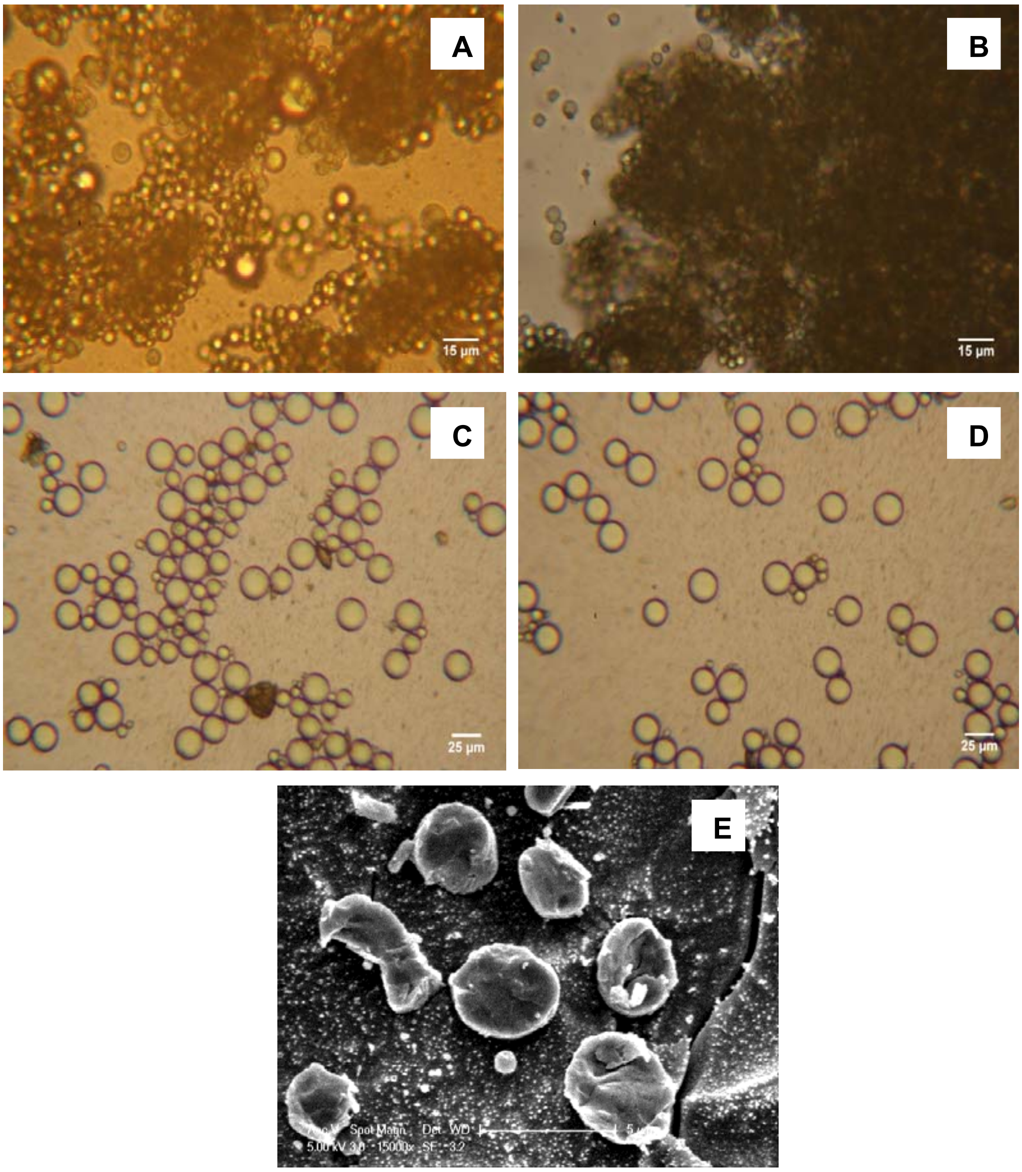

Figure 3-8 Micrographs of rutin hydrate stabilised 20%w/w sunflower oil emulsions (a) produced via 3 passes through HPH after initial preparation and (b) emulsion at t = 14 days (c) produced by RSM processing following initial preparation (d) emulsion at t = 14 days and (e) SEM micrograph of emulsion produced via 3 passes through HPH after initial preparation

The micrographs appear to show droplets and droplet floccs of ~1 – 5 µm in diameter which is not significantly represented in the corresponding distribution curves. Additionally, the stability of these rutin hydrate stabilised emulsions produced via HPH processing was relatively high (see Figure 3-7). After 14 days droplet size changed slightly and the main peak that was observed previously (~100 µm) decreased in size along with the appearance of a small peak in the 1 – 10 µm range. Micrographs show the existence of aggregated droplets irrespective of processing method, however this effect is exaggerated in the rutin stabilised HPH processed emulsions (Figure 3-8). SEM micrographs (Figure 3-8e) show no evidence of bridging flocculation within these emulsions and this suggests that network stabilisation or particle-particle electrostatic interactions occur within the continuous phase, which began to break down over time allowing for the detection of the individual droplets. Rutin hydrate particles were also shown earlier to be weakly charged at native pH in comparison to the other particles studied here (~10 mV), which decreases the potential for droplet-droplet repulsion to occur. Hence, rutin hydrate stabilised droplets are more likely to flocculate than the other more highly charged particle systems. The pronounced flocculation of the HPH droplets compared to the RSM systems is likely due to the higher energy input from the HPH process. Previous studies conducted found that the introduction of superfluous energy to solid lipid nanoparticles, via exposure to light and elevated temperatures, resulted in a decrease in particle zeta potential [42]. In this study, the additional energy and pressure imparted via HPH has potentially led to changes in the crystalline structure of the rutin particles and a decrease in particle surface charge. Hence stabilisation of the emulsion through electrostatic repulsion may be further reduced and droplet flocculation is more prominent.

Following processing by RSM, as only EC and HPMC produced W/O emulsions that were stable enough to be measured these emulsions were passed through the HPH. The other emulsions separated immediately and so could not be correctly passed through the HPH. Consideration of Figure 3-9 suggests that HPH pass number had little effect on EC stabilised W/O emulsion droplet size: droplet sizes remain comparable to those found when the RSM method was utilised.

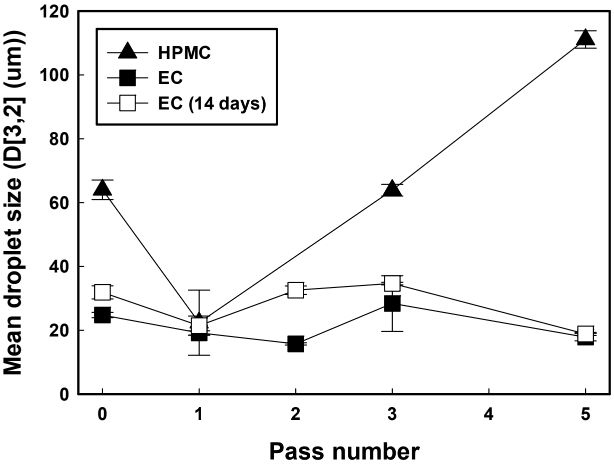

Figure 3-9 Mean droplet sizes of 20%w/w W/O emulsion (2.5%w/w particles) for HPMC and EC stabilised systems. (0 pass number value on x-axis relates to RSM method prior to HPH processing). Measurements were performed in triplicate and error bars represent ± 1 standard deviations. [Error bars where not visible are smaller than symbols].

Not only does this indicate the minimum droplet size of this formulation, but it also highlights the shear tolerance of such emulsions. In the case of the HPMC systems, there was an initial decrease in droplet size following the first pass. Subsequent passes however resulted in a gradual increase in droplet size. This may be a consequence of over processing whereby as droplet breakup continued to occur during processing, droplet coalescence increased. HPMC has a lesser degree of hydrophobicity in comparison to EC meaning particles are less likely to hold a fixed position at the interface during processing, which can go some way to explaining this phenomenon.

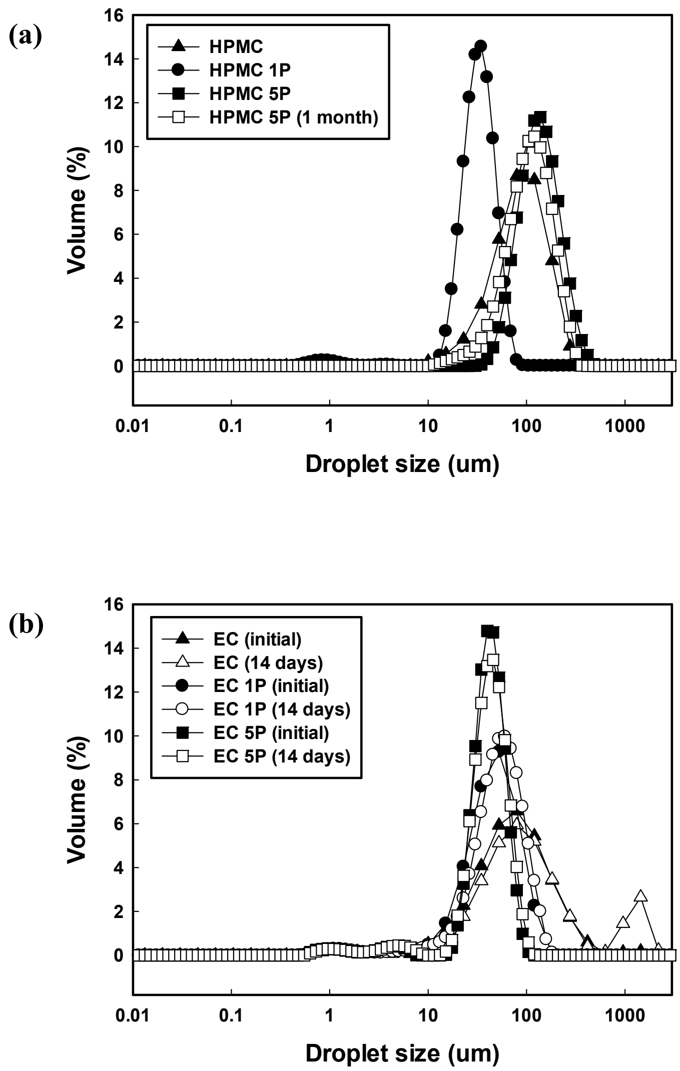

Figure 3-10 Droplet size distribution curves for 20%w/w aqueous emulsions stabilised with 2.5%w/w (a) HPMC particles after preparation via RSM only, HPH 1 pass and HPH 5 passes and (b) EC particles via RSM only, HPH 1 pass and HPH 5 passes.

Of all emulsions produced, all EC emulsions and the HPMC emulsion following 5 passes via HPH, remained stable (see Figure 3-10). All other emulsions (produced via either emulsification method) creamed immediately after preparation and after

24 hours had phase separated. The data regarding the HPMC emulsions shows that following 1 pass through the HPH, droplet sizes become more stable and then with each subsequent pass, droplet size increased. However, HPMC emulsion following 5 passes via HPH (see Figure 3-10a) represents the largest average droplet size of all the HPMC W/O emulsions was, however, the most stable system and when visualised this showed the smallest droplet size The slight shift of the distribution curve causing a decrease in droplet size after 1 month (see Figure 3-10a) in addition to the fact that there was no creaming of the emulsion, once again suggests that a network stabilisation mechanism occurring in the bulk may be breaking down over time and smaller droplets or smaller droplet flocs are being identified by the characterisation technique. This is similar to what was observed for the rutin hydrate-stabilised O/W emulsions prepared via HPH processing, and this may be occurring via a similar mechanism. Hence, these HPMC stabilised droplets are more likely to flocculate than the other more highly charged particle systems. EC stabilised emulsions appeared to be the most stable over time and droplet size reduction was observed upon HPH processing and increasing pass number (Figure 3-10b). Over time, there was very little change in the droplet size distributions for all pass numbers, although the emulsion made via Silverson only did show an increase in droplet size, and the emulsion produced following 5 passes via HPH was the most stable. It could be said that the superior stability of these emulsions is a result of electrostatic repulsions occurring between droplets suppressing droplet coalescence, due to the high charge carried by the EC particles at native pH, in a similar fashion to the CMCC stabilised O/W emulsions. However, they are also nanosized when dispersed in oil (especially

when compared to the large aggregates they formed in water; see Table 3-2), and possess a significant hydrophobic character, both of which are also contributing factors to the increased stability of these emulsions.

3.6 Conclusions

A range of food grade materials, and the O/W and W/O emulsions stabilised by such particles, were investigated (see Appendix, Figure A1). Particle characteristics were analysed in an attempt to predict the droplet size, microstructure and stability of the simple emulsions that they would produce. Based on this experimental data, and theory found in the literature [5, 9], it can be said that particle characterisation can be used to predict emulsion type produced, emulsion droplet sizes and stability of such emulsions. Extensive investigation of particle properties under equivalent conditions within this study allows for cohesion in Pickering particle studies as reported work thus far does not attempt to analyse such a diverse range of particles comparatively. Previous emulsion studies regarding flavonoid particles involve the use of such particles to stabilise *n*-tetradecane-in-water systems [25]; EC particles were used in water-in-2-octyldodecanol emulsions [18]; work involving CMCC describe sunflower oil-in-water emulsion production at longer processing timeframes than shown here [20]. By analysing these different particles under the same formulation and processing parameters, a more specific and accurate set of design rules for Pickering particles can be developed and be applicable to a wider range of Pickering candidate materials for use in food emulsions. Consequently, such rules can be used to predict particle stabilised emulsion characteristics prior to emulsion formulation, thus facilitating particle selection in food emulsion research and development efforts. It was found that the main prerequisites for particles to be able to stabilise small emulsion droplets are small particle size ranging between 200 nm and 1 µm, an affinity for the bulk phase and particle charge to aid stability; particle surface activity appears to be a less significant factor. These rules are applicable to the 20 %w/w O/W emulsions, where this oil volume was chosen as it is commonly used in food emulsions. Further investigation is required surrounding the effects on these parameters where higher oil mass fractions are used.

Furthermore, it was shown that lower particle concentrations compared to surfactants and particles (typically c. 10 %w/w) could be used to stabilise emulsions. It was also shown that network stabilisation plays a large role in the stability of a proportion of these types of emulsions, both O/W and W/O, and that this is not easily represented in traditional emulsion characterisation procedures, to this end, further investigation is required. Larger particles, Naringin and CMCC, were able to form food-grade Pickering nanoemulsions within this work that were stable against droplet-droplet coalescence for extended periods of time, whereas flavonoid stabilised O/W emulsions were previously reported within the micron size range [25]. These findings show that the HPH emulsification process breaks down particle sizes further and in turn this paves the way for the preparation of food emulsion systems with more complex microstructures such as double or tertiary emulsions.

From a commercial perspective, these food grade particles are relatively cheap, abundant in nature and in the case of the flavonoids have been shown to provide health benefits upon human consumption. It has also been shown here that only relatively small particle concentrations are required to successfully stabilise emulsions that, depending on the emulsification process, contain droplet sizes comparable to conventional surfactant stabilised emulsions, giving rise to the production of clean label food-grade emulsions for use in many applications in the food industry, in particular the health and dietary sectors.

References

[1] Ramsden, W., Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation). -- preliminary account. *Proceedings of the Royal Society of London,* **1903**, *72*, p. 156-164.

[2] Pickering, S.U., Emulsions. *Journal of the Chemical Society,* **1907**, *91*, p. 2001 - 2021.

[3] Hunter, T.N., Pugh, R.J., Franks, G.V., and Jameson, G.J., The role of particles in stabilising foams and emulsions. *Advances in Colloid and Interface Science,* **2008**, *137*, p. 57-81.

[4] Niknafs, N., Spyropoulos, F., and Norton, I.T., Development of a new reflectance technique to investigate the mechanism of emulsification. *Journal of Food Engineering,* **2011**, *104*, p. 603-611.

[5] Binks, B.P. and Lumsdon, S.O., Influence of particle wettability on the type and stability of surfactant-free emulsions†. *Langmuir,* **2000**, *16*, p. 8622-8631.

[6] Yuan, Q., Cayre, O.J., Manga, M., Williams, R.A., and Biggs, S., Preparation of particle-stabilized emulsions using membrane emulsification. *Soft matter,* **2010**, *6*, p. 1580-1588.

[7] Manga, M.S., Cayre, O.J., Williams, R.A., Biggs, S., and York, D.W., Production of solid-stabilised emulsions through rotational membrane emulsification: Influence of particle adsorption kinetics. *Soft Matter,* **2012**, *8*, p. 1532-1538.

[8] Pichot, R., Spyropoulos, F., and Norton, I.T., O/w emulsions stabilised by both low molecular weight surfactants and colloidal particles: The effect of surfactant type and concentration. *Journal of Colloid and Interface Science,* **2010**, *352*, p. 128-135.

[9] Aveyard, R., Binks, B.P., and Clint, J.H., Emulsions stabilised solely by colloidal particles. *Advances in Colloid and Interface Science,* **2003**, *100–102*, p. 503-546.

[10] Frelichowska, J., Bolzinger, M.-A., and Chevalier, Y., Pickering emulsions with bare silica. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2009**, *343*, p. 70-74.

[11] Pichot, R., Spyropoulos, F., and Norton, I.T., Mixed-emulsifier stabilised emulsions: Investigation of the effect of monoolein and hydrophilic silica particle mixtures on the stability against coalescence. *Journal of Colloid and Interface Science,* **2009**, *329*, p. 284-291.

[12] Ashby, N. and Binks, B., Pickering emulsions stabilised by laponite clay particles. *Physical Chemistry Chemical Physics,* **2000**, *2*, p. 5640-5646.

[13] Paunov, V.N., Binks, B.P., and Ashby, N.P., Adsorption of charged colloid particles to charged liquid surfaces. *Langmuir,* **2002**, *18*, p. 6946-6955.

[14] Stiller, S., Gers-Barlag, H., Lergenmueller, M., Pflücker, F., Schulz, J., Wittern, K., and Daniels, R., Investigation of the stability in emulsions stabilized with different surface modified titanium dioxides. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2004**, *232*, p. 261-267.

[15] Timgren, A., Rayner, M., Sjöö, M., and Dejmek, P., Starch particles for food based pickering emulsions. *Procedia Food Science,* **2011**, *1*, p. 95-103.

[16] Yusoff, A. and Murray, B.S., Modified starch granules as particle-stabilizers of oil-in-water emulsions. *Food Hydrocolloids,* **2011**, *25*, p. 42-55.

[17] Rayner, M., Timgren, A., Sjöö, M., and Dejmek, P., Quinoa starch granules: A candidate for stabilising food□grade pickering emulsions. *Journal of the Science of Food and Agriculture,* **2012**, *92*, p. 1841-1847.

[18] Melzer, E., Kreuter, J., and Daniels, R., Ethylcellulose: A new type of emulsion stabilizer. *European Journal of Pharmaceutics and Biopharmaceutics,* **2003**, *56*, p. 23-27.

[19] Kalashnikova, I., Bizot, H., Cathala, B., and Capron, I., New pickering emulsions stabilized by bacterial cellulose nanocrystals. *Langmuir,* **2011**, *27*, p. 7471-7479.

[20] Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., and Norton, I.T., Investigation into the potential ability of pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. *Journal of Colloid and Interface Science,* **2012**, *366*, p. 209-215.

[21] Lee, L.L., Niknafs, N., Hancocks, R.D., and Norton, I.T., Emulsification: Mechanistic understanding. *Trends in Food Science & Technology,* **2013**, *31*, p. 72-78.

[22] Shi, W.-J., Tang, C.-H., Yin, S.-W., Yin, Y., Yang, X.-Q., Wu, L.-Y., and Zhao, Z.-G., Development and characterization of novel chitosan emulsion films via pickering emulsions incorporation approach. *Food Hydrocolloids,* **2016**, *52*, p. 253-264.

[23] Paunov, V.N., Cayre, O.J., Noble, P.F., Stoyanov, S.D., Velikov, K.P., and Golding, M., Emulsions stabilised by food colloid particles: Role of particle adsorption and wettability at the liquid interface. *Journal of colloid and interface science,* **2007**, *312*, p. 381-389.

[24] de Folter, J.W., van Ruijven, M.W., and Velikov, K.P., Oil-in-water pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein. *Soft Matter,* **2012**, *8*, p. 6807-6815.

[25] Luo, Z.J., Murray, B.S., Yusoff, A., Morgan, M.R.A., Povey, M.J.W., and Day, A.J., Particle-stabilizing effects of flavonoids at the oil-water interface. *Journal of Agricultural and Food Chemistry,* **2011**, *59*, p. 2636-2645.

[26] Luo, Z.J., Murray, B.S., Ross, A.L., Povey, M.J.W., Morgan, M.R.A., and Day, A.J., Effects of ph on the ability of flavonoids to act as pickering emulsion stabilizers. *Colloids and Surfaces B-Biointerfaces,* **2012**, *92*, p. 84-90.

[27] Hodge, S. and Rousseau, D., Continuous-phase fat crystals strongly influence water-in-oil emulsion stability. *Journal of the American Oil Chemists' Society,* **2005**, *82*, p. 159-164.

[28] Binks, B.P. and Rocher, A., Effects of temperature on water-in-oil emulsions stabilised solely by wax microparticles. *Journal of Colloid and Interface Science,* **2009**, *335*, p. 94-104.

[29] Hodge, S. and Rousseau, D., Flocculation and coalescence in water-in-oil emulsions stabilized by paraffin wax crystals. *Food research international,* **2003**, *36*, p. 695-702.

[30] Frasch-Melnik, S., Norton, I.T., and Spyropoulos, F., Fat-crystal stabilised w/o emulsions for controlled salt release. *Journal of Food Engineering,* **2010**, *98*, p. 437-442.

[31] Kurukji, D., Pichot, R., Spyropoulos, F., and Norton, I.T., Interfacial behaviour of sodium stearoyllactylate (ssl) as an oil-in-water pickering emulsion stabiliser. *Journal of Colloid and Interface Science,* **2013**, *409*, p. 88-97.

[32] Hertog, M.G.L., Feskens, E.J.M., Kromhout, D., Hollman, P.C.H., and Katan, M.B., Dietary antioxidant flavonoids and risk of coronary heart disease: The zutphen elderly study. *The Lancet,* **1993**, *342*, p. 1007-1011.

[33] Keowmaneechai, E. and McClements, D.J., Influence of edta and citrate on physicochemical properties of whey protein-stabilized oil-in-water emulsions containing cacl2. *J Agric Food Chem,* **2002**, *50*, p. 7145-53.

[34] Lyklema, J., *Fundamentals of interface and colloid science: Soft colloids***2005**, *5*: Academic press.

[35] Gurkov, T.D., Russev, S.C., Danov, K.D., Ivanov, I.B., and Campbell, B., Monolayers of globular proteins on the air/water interface: Applicability of the volmer equation of state. *Langmuir,* **2003**, *19*, p. 7362-7369.

[36] Tcholakova, S., Denkov, N., and Lips, A., Comparison of solid particles, globular proteins and surfactants as emulsifiers. *Physical Chemistry Chemical Physics,* **2008**, *10*, p. 1608-1627.

[37] Saric, S.P. and Schofield, R.K., The dissociation constants of the carboxyl and hydroxyl groups in some insoluble and sol-forming polysaccharides. *Proc R Soc Lond A Math Phys Sci,* **1946**, *185*, p. 431-47.

[38] de Graaf, R.A., Broekroelofs, G.A., Janssen, L.P.B.M., and Beenackers, A.A.C.M., The kinetics of the acetylation of gelatinised potato starch. *Carbohydrate Polymers,* **1995**, *28*, p. 137-144.

[39] McClements, D.J., *Food emulsions: Principles, practices, and techniques, third edition***2015**: CRC Press.

[40] Chevalier, Y. and Bolzinger, M.-A., Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2013**, *439*, p. 23-34.

[41] Timgren, A., Rayner, M., Dejmek, P., Marku, D., and Sjöö, M., Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride. *Food Science & Nutrition,* **2013**, *1*, p. 157-171.

[42] Freitas, C. and Müller, R.H., Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (sln™) dispersions. *International Journal of Pharmaceutics,* **1998**, *168*, p. 221-229.

Chapter 4

Double W/O/W emulsions stabilised using various Pickering particle systems

Part of the work in this chapter has been disseminated as follows:

- L Duffus, P.Smith, I.T. Norton, F.Spyropoulos. Fabrication and stability of edible Pickering W1/O/W2 double emulsions, *Journal of Colloid and Interface Science*, In preparation.
- L Duffus, P.Smith, I.T. Norton, F.Spyropoulos. Formulation and stability of edible Pickering W1/O/W2 double emulsions, *13th International Hydrocolloids Conference*, Guelph, Canada, May 2016.
- L. Duffus, J.E. Norton, P. Smith, F. Spyropoulos, I.T. Norton. Formulating double emulsions stabilised using edible Pickering particles, *Rideal AGM Meeting SCI,* London, UK, April 2015.
- L. Duffus, J.E. Norton, P. Smith, F. Spyropoulos, I.T. Norton. Formulation of double emulsions stabilised by Pickering particles. *12th Annual Formulation Engineering Conference,* University of Birmingham, UK, March 2015.
- L. Duffus, J.E. Norton, P. Smith, I.T. Norton, F. Spyropoulos. Formulation & stability of double emulsions solely stabilised by edible particles. *Advances in*

Food Processing: Challenges for the Future, Sao Paulo, Brazil, November 2014.

4.1 Abstract

Double water-in-oil-in-water (W/O/W) emulsions present an opportunity for the production of healthier food emulsions, however, their complex structure and vast interfacial area means that they are significantly more unstable than simple emulsions. Pickering stabilisation, reputed for superior, longer term stabilisation capacities when compared to surfactant stabilised emulsions, provides the opportunity to enhance double emulsion stability. It was the intention to assess whether Pickering stabilisation can provide any advantage by initially adopting it for only one of the two interfaces within a double emulsion (i.e. internal W/O interface, or the external O/W interface).

In this study, double emulsions were solely stabilised by novel natural and modified edible particles at both interfaces and in the absence of any additional surfactants or stabilisers. Pickering-Pickering and Pickering-Surfactant double emulsion systems were formulated using a range of different particles and surfactants and subsequently compared in terms of their droplet size, thermoanalytical profiles and stability.

Pickering-Pickering and Pickering-Surfactant double emulsion were formed and depending on emulsifier selection, emulsions were stable to complete loss of internal water phase for 1 month. Solely Pickering-stabilised double structures were found to possess superior stability and primary emulsion droplet retention in comparison to Pickering-Surfactant and Surfactant-only stabilised double emulsion formulations.

Keywords: Pickering emulsions; double w/o/w emulsions; polysaccharides; flavonoids

4.2 Introduction

The complex microstructure of double emulsions is comprised of a simple emulsion consisting of a dispersed phase, within which a separate emulsion is contained [1, 2]. Commonly, this takes the form of a water-in-oil (w/o) emulsion dispersed within a secondary aqueous phase in order to produce a final water-in-oil-in-water (w/o/w) double emulsion [3, 4]. In the food industry in particular, such double emulsions can be utilised to improve encapsulation capabilities of active ingredients such as vitamins and minerals [5-11]. Studies have also been carried out concerning the use of double emulsions as a mechanism for fat and calorie reduction in foods [12-20]. However, despite these encouraging findings on double emulsions for food use, they have not yet been widely used in the food industry to date. The major drawback for double emulsions is that they are extremely unstable, even more so than simple emulsions as there are now two unstable interfaces present in the formulation and an accompanying vast increase in interfacial area [21, 22].

A theory that may allow for increased stability in double emulsions is to alter the stabilisation mechanism employed. Solid stabilised emulsions, or ‘Pickering’ emulsions and have long been recognised as possessing superior stability properties when compared to conventional surfactant-stabilised emulsions. First developed at the turn of the 20th century [23, 24], they are defined as emulsion droplets coated by a layer of adsorbed colloidal (micro- or nano-) solid particles at the oil-water interface [25-33]. Although few, several experiments have been successfully conducted and completed whereby stable double emulsions have been produced via the use of combined surfactant and particle stabilisation [34-38]. The primary thinking behind this was to create very stable long term particle-stabilised emulsions by using surfactants to lower the interfacial tension between the oil and water phases in order to aid droplet break-up during emulsification. As surfactants have a faster adsorption rate to the oil-water interface than the Pickering particles, they were able to stabilise the newly formed interfaces during the emulsification process and allow enough time for the particles to sufficiently adsorb to the interface [39]. In turn it was thought that this would increase the stability of double particle stabilised emulsions further by the short term guarding against droplet coalescence by surfactants during emulsification and therefore allowing for smaller droplet sizes to be formed [40].

Previous studies provide evidence of prolonging double emulsion stability through the use of particle-surfactant combination. By replacing surfactant with particles at one of the two interfaces within a double emulsion, droplet size stability has been vastly improved. Examples of such combinations include lipophilic polysorbate and microcrystalline cellulose [34], polyglycerol polyricinoleate (PGPR) and colloidal starch [41, 42], PGPR and kafirin nanoparticles [43] and PGPR and fat crystals [38, 44, 45].

The aim of this work is to also combine these two key research areas in an attempt to formulate W/O/W emulsions. However, the primary intention is to use novel edible particulate materials at both interfaces and in the absence of silica or any additional surfactants or stabilisers; an approach that provides the opportunity for the production of stable, clean-label and surfactant-free food double emulsions. Furthermore, it was the aspiration to investigate any benefits arising from the use of these edible Pickering particles at one of or both interfaces of a double emulsion in conjunction with known conventional emulsifiers. In particular, focus was placed upon understanding differences in droplet size stability and inner water phase characterisation. The specific particles explored were: three modified celluloses (colloidal microcrystalline cellulose, hydroxypropyl methylcellulose and ethylcellulose), sodium stearoyl lactylate and a flavonoid, rutin hydrate, as they are readily commercially available, edible, are relatively cheap and often can be derived from waste products. To this end, a range of Pickering-Pickering (using different particles) and Pickering-Surfactant (using a range of surfactants) double emulsion systems were prepared and compared in terms of their microstructure and stability.

4.3 Materials and Methods

4.3.1 Materials

Distilled water and commercially available sunflower oil were used for the preparation of all emulsions. Particles used as emulsifying agents were rutin hydrate (RH), colloidal microcrystalline cellulose (CMCC), ethylcellulose (EC) and (hydroxypropyl)methyl cellulose (HPMC) and all were obtained from Sigma, UK. Grindsted® SSL P 55 Veg Kosher (sodium stearoyl lactylate) (SSL) was obtained from (Danisco, UK) Dyes Rhodamine B and perylene were obtained from Sigma, UK. Surfactants used were Tween 20 (sorbitan monolaurate) (T20) was obtained from Sigma, UK, and Grinsted® PGPR 90 (polyglycerol polyricinoleate) (PGPR) which was obtained from Danisco, Switzerland. Sodium caseinate from bovine milk (NaCas) was also obtained from Sigma, UK. All materials were used without any further purification or modification. Percentages of water and oil phases, in addition to emulsifying agents and all other emulsion formulation components, were calculated as the weight of the individual constituent per weight of the final emulsion (simple or multiple), as detailed in the relevant section.

4.3.2 Methods

4.3.2.1 Preparation of Pickering particle dispersions

All particles were introduced to and treated in the continuous phase prior to combination with the dispersed phase to form the emulsions. 80 g of these particle dispersions were prepared and then heated with a hot plate to 45 – 50 ˚C for 40 minutes whilst being agitated with a magnetic stirrer to encourage particle dispersion. Following this, particle dispersions were further treated by a high intensity ultrasonic vibracell processor (Sonics & Materials, Inc., CT, USA) operating at 750 W and 20 kHz.

4.3.2.2 Fabrication of simple emulsions

Following the particle dispersion preparation, 20 g of the dispersed phase was added to the particle dispersion (unless otherwise stated) and the mixture was emulsified using a rotor-stator mixer (RSM) (Silverson L4RT, emulsion screen diameter 19mm), for 2 minutes at 10,000 rpm.

4.3.2.3 Fabrication of double emulsions

10% W/O primary emulsions were made by dispersing 15 g of water into a dispersion consisting of 132 g of sunflower oil and 3 g of EC or PGPR. These primary W/O emulsions were prepared with the addition of a small amount of Rhodamine B dye (≤ 0.05 %w/w), premixed in an aqueous solution, and included in the dispersed phase as a microscopy marker. 20 g of this W/O emulsion was then dispersed in 80g of a secondary water phase dispersion (made up of 77 g water and 3 g of emulsifier). The final double emulsion structure was then produced via low shear RSM processing (Silverson L4RT, emulsion screen diameter 19mm), for 2 minutes at 3,500 rpm. The ratios of W_2 to W/O used were 80:20 unless stated otherwise. Double emulsions have been labelled according to the emulsifier used at each interface, for instance PGPR/T20 emulsions refers to double emulsions where PGPR was used to stabilise the internal W/O primary interface and T20 was used to stabilise the outer secondary O/W interface.

4.3.2.4 Characterisation of dispersions

Particle and micelle sizes were measured via static multi-angle light scattering using a Mastersizer Hydro 2000 (Malvern Instruments, UK) or Zetasizer. All measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ± 1 standard deviation. ζ-potential analyses were performed on the Zetasizer Nano Series (Nano ZS) equipped with MPT-2 multipurpose titration unit (Malvern Instruments, UK). Four drops of 1% w/w particle or surfactant were diluted into 25 g distilled water and gently shaken for 30 s to fully disperse droplets. This was immediately transferred to a specialised zeta cell (Malvern Instruments, UK) and measured. ζ-potential measurements were carried out in triplicate and values reported are given to ± 1 standard deviation.

4.3.2.5 Characterisation of emulsion droplet size

All emulsion droplet size sizes were measured via static multi-angle light scattering using a Mastersizer Hydro 2000 (Malvern Instruments, UK). All measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ± 1 standard deviation. Where double emulsions are concerned imaging was particularly important as, in terms of light scattering, double emulsions are optically non-uniform due to the internal droplet phase. Pays, et al. [46] assume that the internal phase droplets behave as simple droplets with the same refractive index as the oil phase. Micrographs were therefore taken in conjunction with Mastersizer measurements and generally correlated well with one another. Visualisation of both simple and double emulsions was captured via light microscopy (Olympus CH2, Japan, with CCD video camera). Double emulsion microstructure was also imaged using a Leica TCS SPE confocal microscope (Leica Microsystems, UK). A drop of emulsion was placed onto a glass microscope slide and a cover slip placed over. Separate fluorescent emission spectra were obtained for the oil phase (stained with perylene) and internal water phase (stained with Rhodamine B), enabling spatial identification of each phase within the double emulsion microstructure.

4.3.2.6 Differential Scanning Calorimetry (DSC)

DSC experiments were performed on a DSC 8000 differential scanning calorimeter (PerkinElmer Inc., USA). Analysis was performed on simple W/O and corresponding W/O/W emulsion samples at various time points, with samples stored at room temperature. 5.5 ± 1.5 mg of each sample were weighed using an analytical balance directly into a specialised stainless steel DSC crucible. All emulsions were cooled from 20 ˚C to -60 ˚C at a rate of 5 ˚C min^{-1}, held at -60 ˚C for 2 mins and then ramped back up to 20 ˚C at the same rate. The reference crucible was left empty. The cooling enthalpies reported for emulsions were obtained by calculating the area under the curve with a linear baseline using the instrument software (Pyris software). These enthalpies are $\Delta H'$ (J/g), which represents the enthalpy change during crystallisation of the internal water phase within the double emulsion, and $\Delta H'$ (J/g) which is the enthalpy change during freezing of the dispersed phase of the primary W/O emulsion. These values were calculated using the Pyris software via division of the enthalpy of the specific transition by the mass of the sample in the aluminium pan. Division of these values by the dispersed phase volume fraction of that particular emulsion allows for the amount of water present in the internal phase in double emulsions and the amount of water dispersed in the primary emulsion to be deduced. However, for the purposes of this study, primary droplet dispersed phase (0.2) and internal water phase (0.02) volume fractions remained constant, formulations differed only in terms of the emulsifier or particle type employed. Therefore, for comparison purposes, directly comparing the peak areas as obtained by the Pyris software was deemed sufficient. All DSC measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ± 1 standard deviation.

4.4 Results & Discussion

4.4.1 Simple Emulsions

Investigation of the effect of stabilisers on simple O/W and W/O emulsion interfaces serves as a route to understanding the two interfaces (primary W/O and secondary O/W interfaces) involved in the complex double emulsion microstructure. By preparing simple O/W and W/O emulsions using the emulsification processes that would be used during double emulsion production, some insight can be gained into the interfacial behaviour of the W/O/W emulsions. Suitable Pickering particles previously identified in Chapter 2 were compared against conventional emulsifiers commonly used in the stabilisation of food emulsions.

4.4.1.1 Water-in-oil emulsions

A key factor in double emulsion stability is the primary emulsion. It is formed first and must be as stable as possible to ensure that both stabiliser migration between the two interfaces and droplet breakup during the secondary processing step are minimised. PGPR was selected as a comparison for EC particles to produce W/O emulsions. Emulsions were formed via high shear; RSM was used at 10,000 rpm for 10 mins. Results are shown in Figure 4-1.

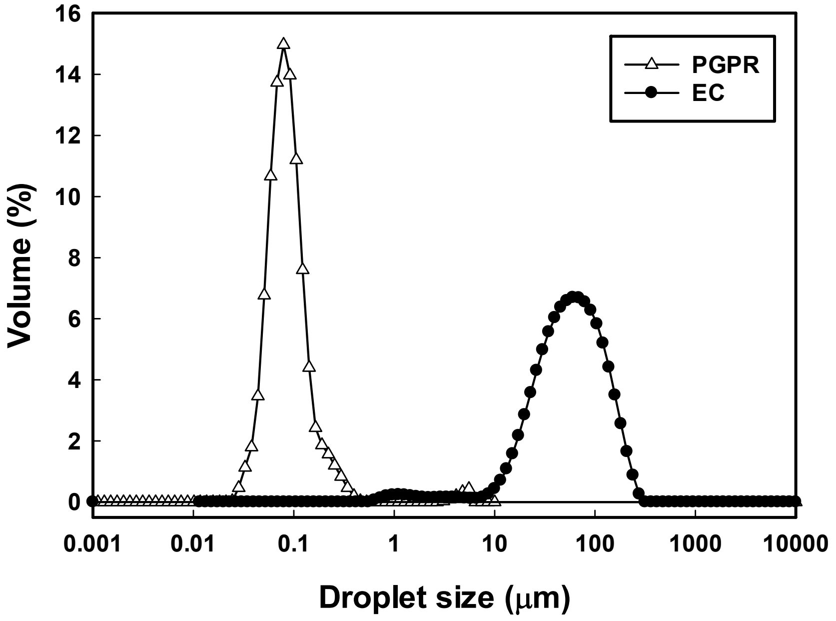

Figure 4-1 Droplet size (nm) of W/O emulsions produced using PGPR and EC in an attempt to stabilise the emulsion interface. All measurements were taken in triplicate and error bars represent ± 1 standard deviation.

Droplet sizes for the surfactant stabilised emulsions, irrespective of water phase volume, are significantly smaller than those stabilised using EC particles. This trend was also reflected in the differing material sizes, with the EC particles (see Table 3-2) being significantly larger than PGPR monomers. As the EC emulsion droplets and particles are larger than the surfactant monomers and consequently PGPR stabilised droplets, (Figure 4-1), they are more likely to sediment which consequently increases the probability of droplet coalescence and hastens complete phase separation due to the close proximity of droplets to one another [47]. However, due to the assumed Pickering mechanism of the EC particle adsorption to the interface and the known associated enhanced stability compared to conventional surfactants, this effect of droplet coalescence maybe offset.

4.4.1.2 Oil-in-water emulsions

Critical to prevention of reversion to a simple emulsion is the formation of the secondary O/W interface within a double emulsion. During the secondary emulsification step, the secondary emulsifier should form a stable interfacial layer around the oil phase (containing the primary W/O droplets), without disrupting the primary interface. Rutin hydrate, SSL, CMCC and HPMC particles were selected to stabilise the O/W emulsions, and surfactant, T20, and protein, NaCas, were used for comparison purposes. Particle and surfactant micelle size was measured as well as ζ-potential and subsequent emulsions were prepared using the different materials. In this case, simple O/W emulsions were produced using a lower shear emulsification process as would be used for the secondary emulsification step when preparing a double emulsion. The low shear technique used in this case was HSM at 3,500 rpm for 2 mins and droplet size distributions, alongside material size and ζ-potential, are depicted in Figure 4-2.

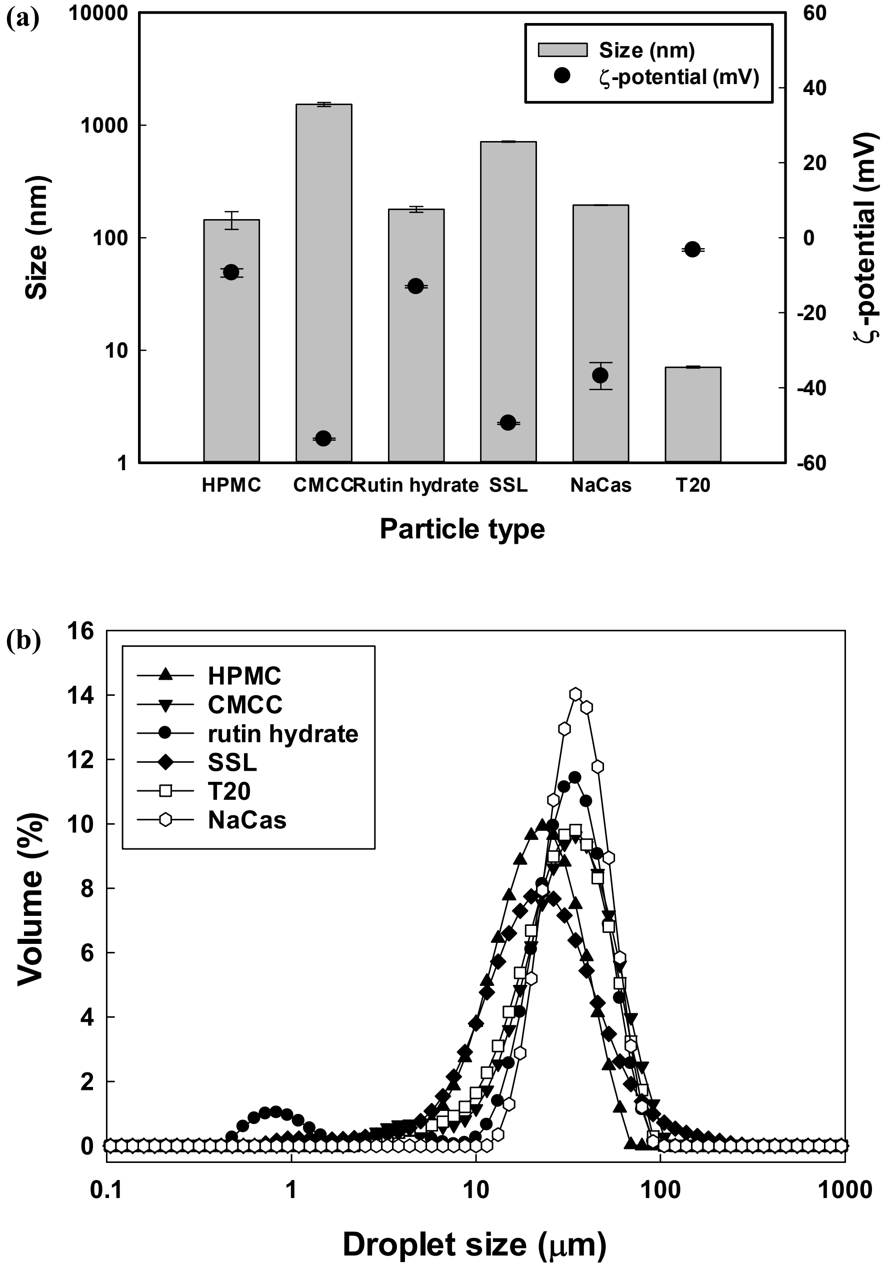

Figure 4-2(a) Bar chart indicating particle and micelle sizes (nm) and scatter plot representing ζ-potential (mV) for materials used to produce simple o/w emulsions (b) Droplet size distributions of o/w emulsions produced with 20 %w/w sunflower oil using rutin hydrate, CMCC, HPMC and Tween 20 to stabilise the interface. All measurements were taken in triplicate and error bars represent ± 1 standard deviations.

Of the Pickering systems described in Figure 4-2, HPMC particles are the smallest in terms of size and emulsion droplet size is the smallest also, albeit only slightly. Overall, the T20 surfactant micelles were the smallest in size (and it will normally be the even smaller surfactant monomers adsorbing at the O/W interface), however the droplet size distribution of the resulting emulsion droplets are comparable to that of the other formulations with larger particle sizes, i.e. CMCC. This discrepancy suggests that the processing method employed is a more dominant factor in the determination of emulsion droplet size. This is particularly noteworthy as T20 is a conventional surfactant. The surface activity of T20 enables a reduction in the interfacial tension between the two immiscible liquid phases and is pre-disposed for adsorption to the liquid-liquid interface, unlike the Pickering materials, yet irrespective of this, the droplet size distributions of the emulsions are analogous. In relation to double emulsions, it is implied here that the secondary interface formation is more dependent on the processing method used than the stabilising material properties. ζ-potential can be related to the surface charge at the interface once surfactant or particle adsorption occurs. CMCC, SSL and NaCas had the highest ζ-potential and it would be expected that these materials would form interfacial layers with higher electrostatic repulsion amongst droplets rendering them more stable against droplet coalescence and emulsion failure.

4.4.2 Double emulsion microstructure and stability

Double emulsions were initially prepared using conventional surfactants, and Figure 4-3 depicts an example, specifically PGPR to stabilise the internal W/O emulsion and Tween 20 to stabilise the final (W/O)/W interface. This formulation was used as a basis for comparison against double emulsion systems using Pickering particles at one or both interfaces. All emulsions were prepared via the same processing method in order to eliminate any microstructural changes as a result of processing conditions.

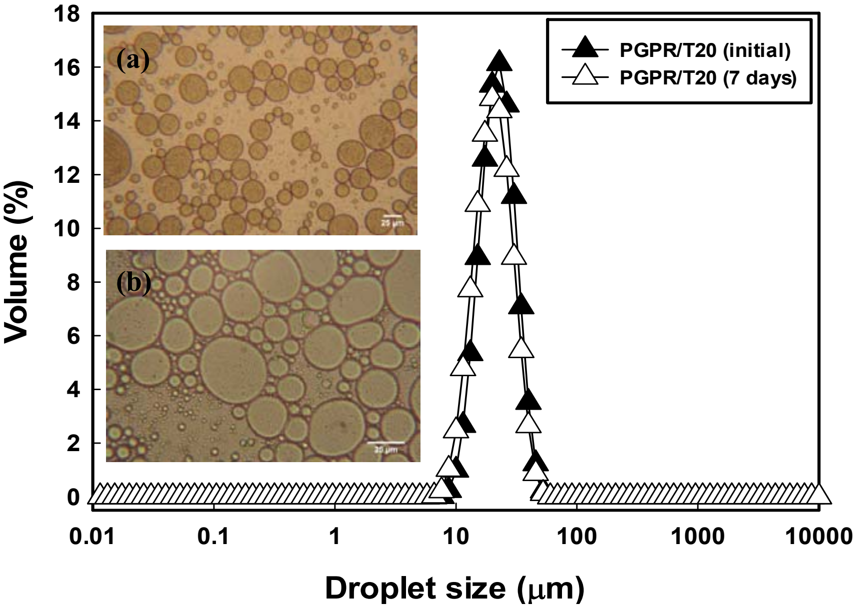

Figure 4-3 Droplet size distribution for double emulsion using PGPR at primary interface and T20 at secondary interface after preparation, and double emulsion after 7 days, and micrographs of double emulsion (a) immediately following preparation and (b) after 7 days. Scale bars represent 25 μm.

From analysis of the droplet size distributions of the relevant simple emulsions, it can be seen that theoretically, the surfactant-only stabilised PGPR/T20 double emulsion system should work (Figure 4-3). The PGPR stabilised W/O droplets are small enough to be encapsulated into the larger T20 stabilised O/W droplets. Immediately following processing, micrographs showed the existence of double emulsion structures (see inset (a) in Figure 4-3), and following 7 days storage, emulsion droplet size change was minimal (Figure 4-3). However, micrographs of the 7 day aged samples exhibited mainly oil droplets and a drastically reduced number of double emulsion structures. This correlates well with previous studies; Dragosavac *et al.,* (2012) found that double emulsions stabilised using such a surfactant combination, whilst retaining an overall similar droplet size over time, were devoid of the finely dispersed inner water droplets. Of the four destabilisation mechanisms described by Florence and Whitehill [21], these findings suggest that rupture of the oil film separating to two distinct water phases is the chief cause of instability in this instance. Subsequently, replacement of surfactant by particles at one of the two interfaces was investigated in an effort to enhance stability and maintain double emulsion integrity over time.

4.4.3 Surfactant at the internal interface

Double emulsions were produced using surfactant to stabilise the primary W/O interface and particles to stabilise the secondary O/W interface, and vice versa. A schematic diagram (Figure 4-4) depicts the difference between the two stabilisation arrangements at the interfaces within the double emulsions.

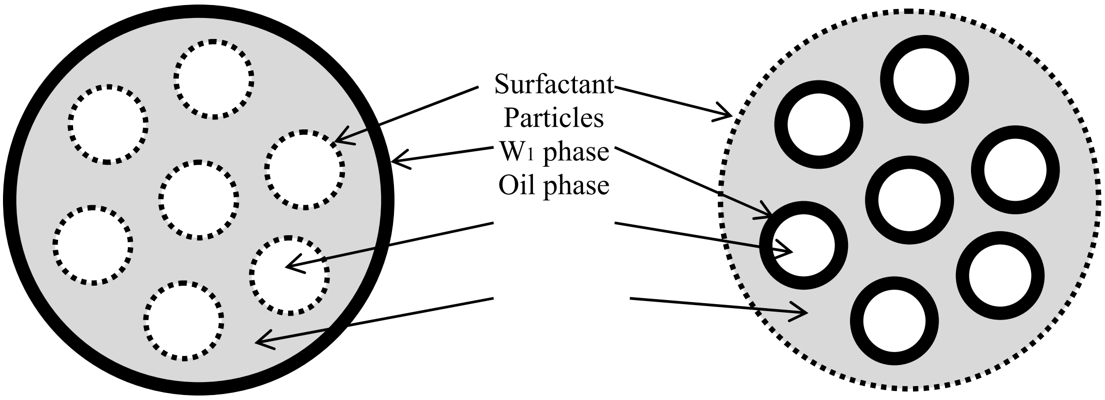

Figure 4-4. Schematic diagram representing the 2 different types of Pickering-Surfactant stabilised double emulsion systems

W/O/W emulsions were initially produced using surfactant, PGPR, at the primary interface and particles at the secondary interface.

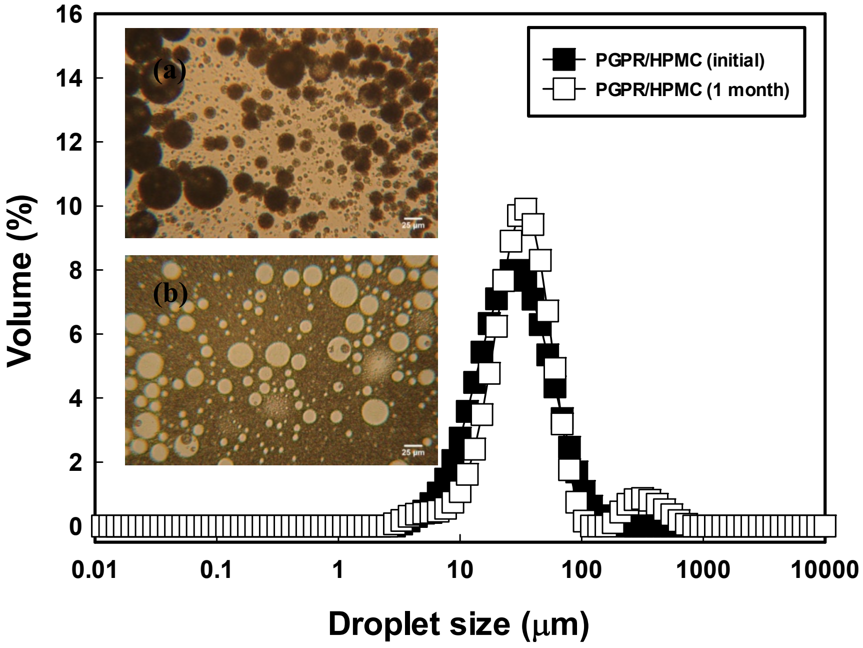

Figure 4-5. Droplet size distribution for PGPR/HPMC double emulsion after preparation, and double emulsion after 1 month, and micrographs of double emulsion (a) immediately following preparation and (b) after 1 month. Scale bars represent 25 µm.

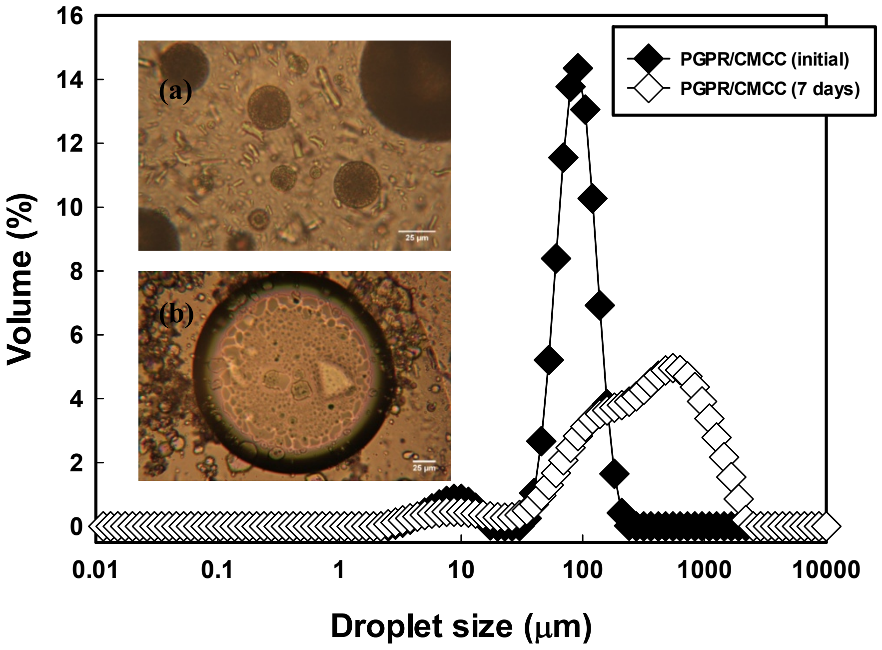

Figure 4-6. Droplet size distribution for PGPR/CMCC double emulsion after preparation, and double emulsion after 7 days, and micrographs of double emulsion (a) immediately following preparation and (b) after 7 days. Scale bars represent 25 µm.

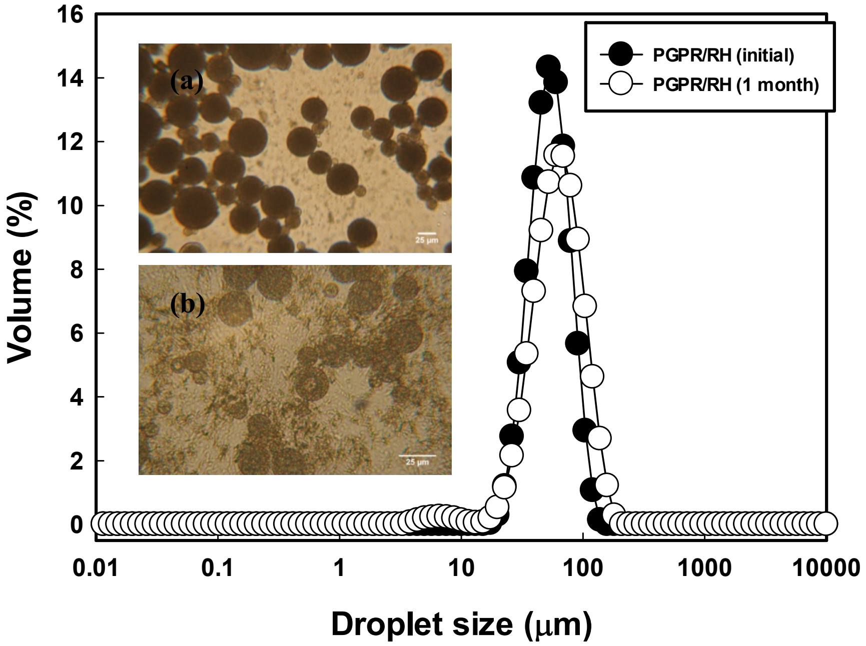

Figure 4-7. Droplet size distribution for PGPR/RH double emulsion after preparation, and double emulsion after 1 month, and micrographs of double emulsion (a) immediately following preparation and (b) after 1 month. Scale bars represent 25 μm.

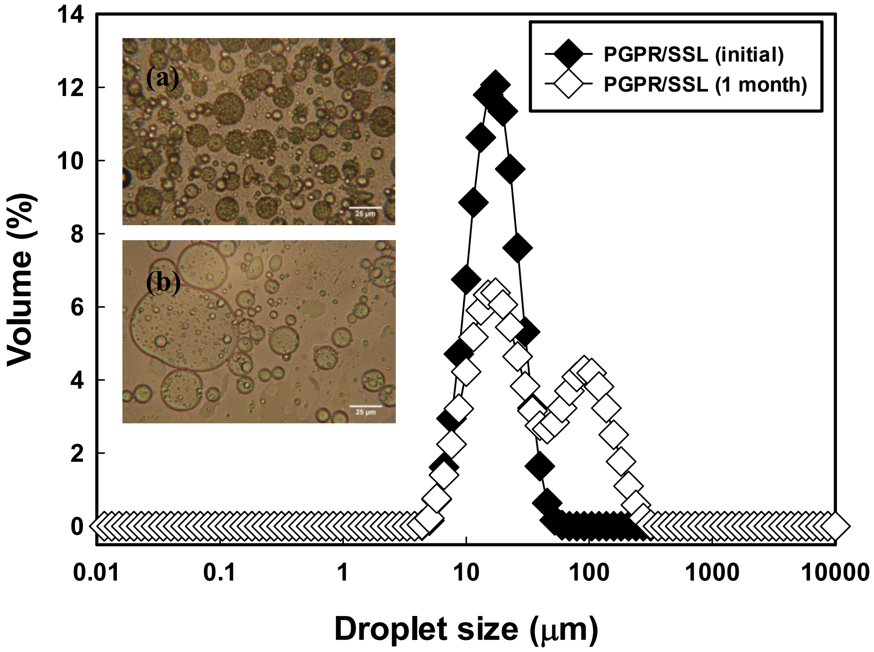

Figure 4-8.Droplet size distributions of PGPR/SSL double emulsion after preparation, and double emulsion after 1 month, and micrographs of double emulsions (a) immediately following preparation and (b) after 1 month. Scale bars represent 25 µm.

Analysis of micrographs of PGPR/HPMC, PGPR/CMCC, PGPR/RH and PGPR/SSL double emulsion systems immediately following preparation reveals the existence of double emulsion structures. In all instances it is evident that the primary emulsion droplets are significantly smaller than the double emulsion droplets (Figure 4-1 & Figure 4-2b) therefore encapsulation of the primary W/O droplets should be straightforward ensuring more stable double emulsion droplets. Despite this, the three systems vary in stability, and three different phenomena are evidenced.

The PGPR/CMCC double emulsion system phase separated at day 7 and PGPR/HPMC and PGPR/RH emulsion droplets were stable for 1 month, however double emulsion structure was reduced with emulsions where HPMC was used to stabilise the secondary interface. In the case of these PGPR/HPMC double emulsions (Figure 4-5), a similar effect to PGPR/T20 emulsions (Figure 4-3) was observed. Overall external droplet size remained similar over time with the development of a small peak between 100 – 1000 µm promoting droplet-droplet coalescence. Despite this, analysis of micrographs shows a transition from an initial W/O/W structure to a simple O/W emulsion, thus highlighting a failure in the stabilisation of the primary W/O interface. Differently, PGPR/CMCC double emulsions (Figure 4-6), suffered a total loss of double emulsion structure, a large increase in droplet size and phase separated upon 1 week of storage. PGPR/SSL and PGPR/RH emulsions (Figure 4-7 and Figure 4-8) maintained both a double emulsion structure and micrographs indicate an increase in internal water droplet size, manifested in an increase in double emulsion droplet size as a function of time. This is in agreement with results pertaining to PGPR/fat crystal stabilised double emulsions previously published in literature [45] and it is believed that the PGPR/RH oil droplets have swollen in order to accommodate this increase. Overall, this is appears to be indicative of sufficient stabilisation of both interfaces within the emulsion as no significant loss of internal water droplet phase was observed and double emulsion structure was maintained. The PGPR/SSL emulsion droplet size distribution developed bimodality and although double emulsions were still in existence, internal droplets appear to be larger after 1 month. Similar to the PGPR/RH systems, the oil droplets appear to swell in order to accommodate this increase in internal droplet size, however the bimodality is conducive to droplet growth by way of Ostwald ripening, i.e. pressure gradient. This suggests that use of RH provides a more robust interfacial layer than SSL for long term stabilisation.

The instability of double emulsion structures pertaining to PGPR/HPMC and PGPR/CMCC emulsions in comparison to PGPR/RH is potentially due to the fact that the PGPR surfactant is lipophilic and has a low HLB value whereas the rutin hydrate particles possess a strong hydrophilic character; significantly more so than both the HPMC and CMCC particles as shown in Chapter 3 (Figure 3-2). This suggests that competition for the same interface within the double emulsion is minimised within the flavonoid system (PGPR/RH emulsion), inhibiting movement of the adsorbed particles from the secondary interface and thus promoting longer term stability than the cellulose systems. Additionally, preliminary experiments and prior literature [49, 50] found that where particles and surfactant exist in the same emulsion formulation, surfactant-particle interactions can exist. These studies also show that these interactions can take the form of surfactant adsorption to particle surfaces altering original particle wetting properties. In the case of these double emulsion systems, PGPR may have adsorbed to the rutin hydrate particle surface altering wettability, potentially improving the contact angle at the oil-water interface, enhancing droplet stability.

4.4.4 Particles at the internal interface

As a starting point, attempts were made to stabilise double emulsions through the application of EC particles as the internal phase emulsifier and T20 and then NaCas, for secondary interface stabilisation. For comparative purposes, the same processing methods were employed as described in the previous section for continuity, and no stable double emulsion structures were able to form with both formulations.

EC/HPMC, EC/CMCC, EC/RH and EC/SSL double emulsions were produced in the absence of surfactant whereby EC particles were applied for the inner W/O interface stabilisation and particles (rutin hydrate, HPMC, CMCC or SSL) were used to stabilise the outer O/W interface. Figure 4-9 shows the droplet size distributions of these emulsions, both initially and following storage.

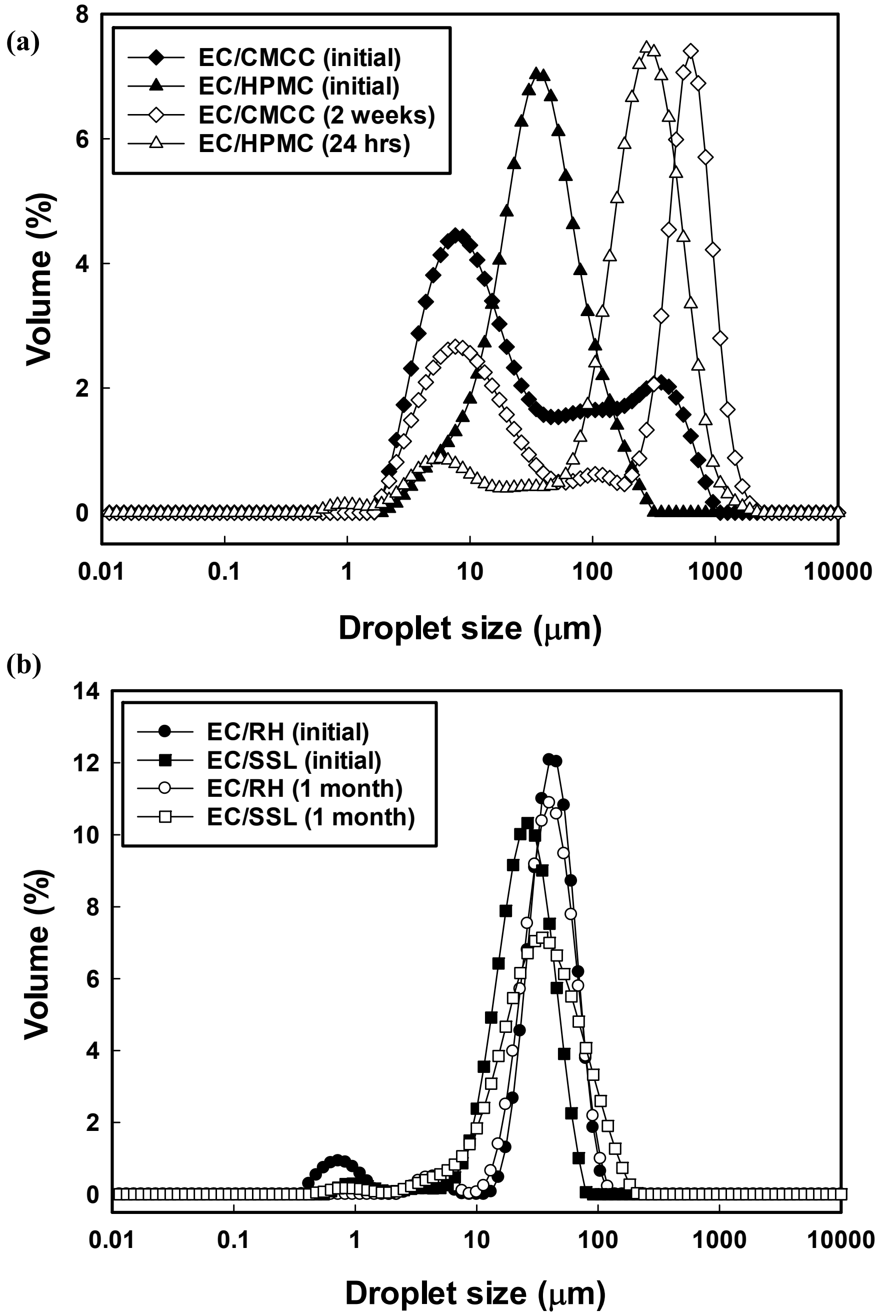

Figure 4-9 Droplet size distributions for particle-only stabilised double emulsions following preparation (closed symbols and solid line plots) and following storage (open symbols and dashed line plots).

Of these 4 different systems, only the formulations containing rutin hydrate and SSL were stable (top plot, Figure 4-9). The double emulsions, where CMCC and

HPMC were used to stabilise the secondary interfaces, were not stable, and the micrographs show that only a simple emulsion structures existed. This may be due to the similarities in their cellulosic physicochemical properties and hence adsorption competition for the same interface leading to simple emulsion formation and not a double emulsion structure; HPMC and EC particles are both predominantly hydrophobic and CMCC has no distinct preference for either the aqueous or lipid phase over the other.

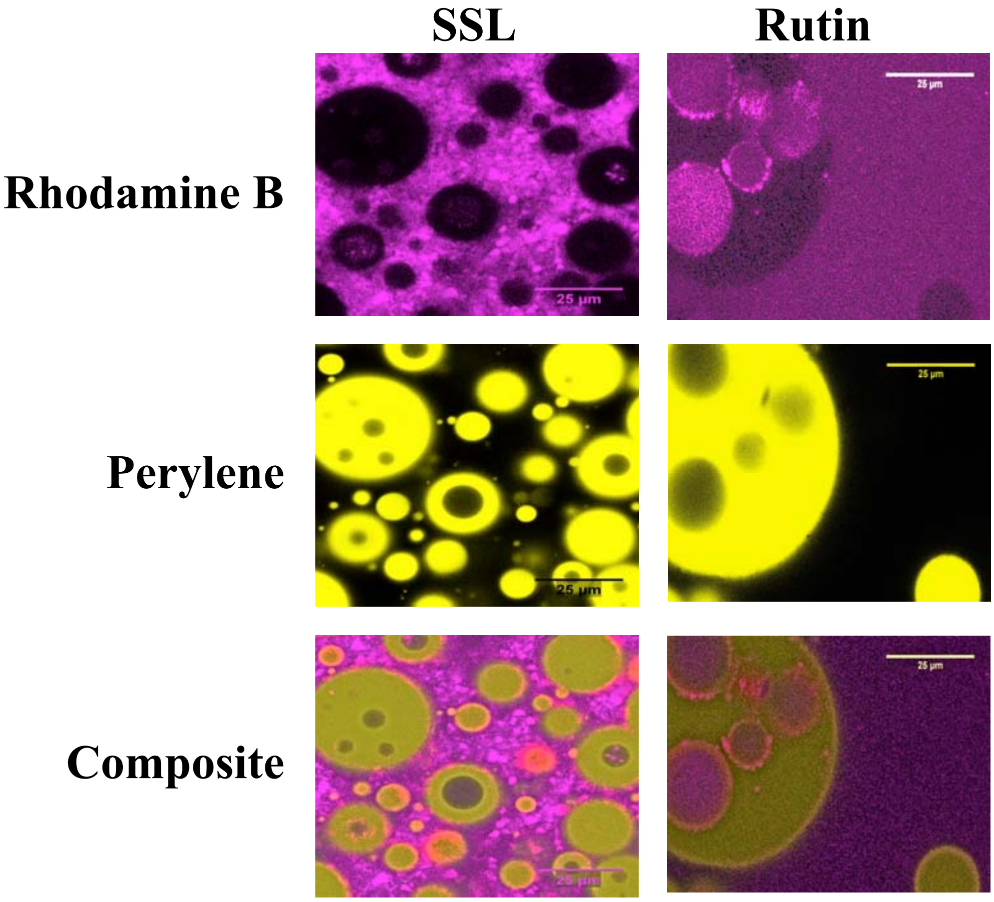

Figure 4-10.Confocal micrographs of EC/RH and EC/SSL double emulsions. Images were taken following 1 month storage.

With the EC/RH and EC/SSL systems, although in both cases the primary W/O emulsion droplet size distribution was similar (Figure 4-1) to the double emulsion droplet size distributions, W/O/W structures were formed and were stable for more than 1 month (Figure 4-9). Confocal micrographs show the presence of double emulsion structures in both systems (Figure 4-10) following 1 month of storage at room temperature. SSL emulsions exhibited an increase in droplet size upon storage whereas droplet size change was minimal with the rutin hydrate systems. The superior stability of EC/RH double emulsions may be due to rutin hydrate particles being mainly hydrophilic whereas EC particles possess a predominately hydrophobic character. Therefore the difference in the wettability of these two particle systems is more significant in comparison to the previous cellulose only systems (the EC/HPMC and EC/CMCC formulations), and consequently they are more likely to successfully adsorb at the two different interfaces present in a double emulsion system and not migrate to the opposing interface encouraging emulsion destabilisation. It was also observed from the confocal micrographs (Figure 4-10) that SSL emulsions are also less able to inhibit Rhodamine B leaching from the internal aqueous phase into the outer water phase when compared to the EC/RH emulsions. This suggests that the interfacial layer provided by SSL is much more permeable in comparison.

4.4.5 Effect of stabiliser combination on internal water phase

In order to be used as a reliable fat replacement tool in food applications, understanding and characterising the extent to which oil can be replaced is imperative. Alas, determination of the amount of internal water captured within oil droplets in a double emulsion is notoriously difficult due to the complex microstructure.

Previous literature reports describe the use of DSC measurements for the characterisation of inner water phase in double emulsions [51]. The temperature of crystallisation, T_c, and magnitude of the enthalpy change during crystallisation of the internal water phase within the double emulsion, $\Delta H'$ (J/g) (circled in Figure 4-11) can be related to inner water droplet size and how this is affected by storage time. From this data, droplet growth over time, water mass transfer and relative encapsulated droplet size can be deduced.

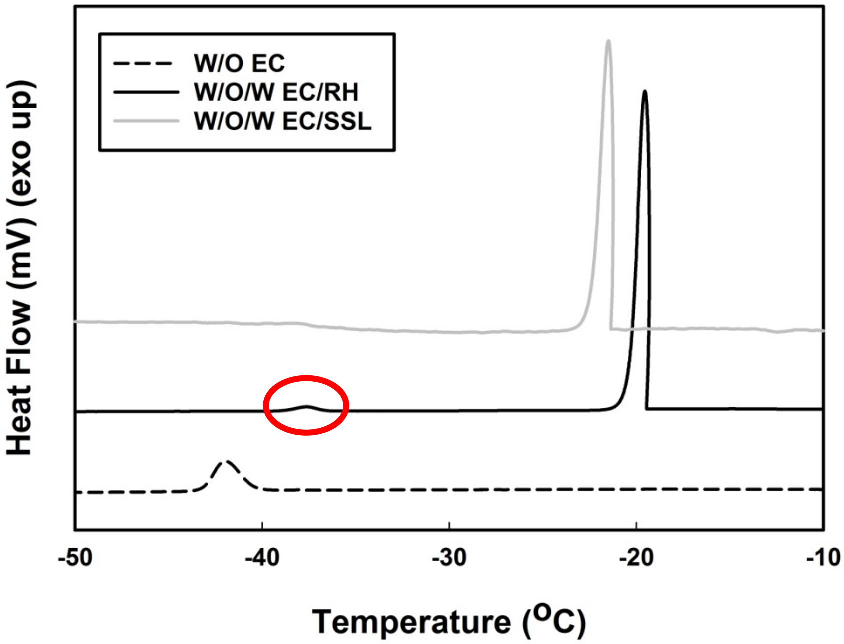

Figure 4-11.Thermoanalytical DSC profiles (upon cooling from 20ºC to -60ºC) of W/O/W emulsions stabilised using EC for the primary interface and either rutin hydrate or SSL particles for the secondary interface and profile of the EC stabilised W/O emulsion used to produce both emulsions. Measurements were taken immediately following preparation.

In this study, data derived from the thermoanalytical curves of double emulsions have been used to determine how emulsifier selection and combination influences the inner water phase within double emulsion structures. Both initial and storage $\Delta H'$ and T_c values of double emulsions and their respective W/O emulsions are shown in Table 4-1.

Table 4-1.Mean $\Delta H'$ and $\Delta H''$ (J/g) values, for different systems as a function of time (initial and 1 month), and their respective Tc (˚) values. Measurements were performed in triplicate and ± represents 1 standard deviation.

EMULSION NAME	W/O/W				W/O	
	Initial $\Delta H'$ (J/g)	Initial inner droplet T_c (˚)	1 month $\Delta H'$ (J/g)	1 month inner droplet T_c (˚)	$\Delta H''$ (J/g)	Initial w/o droplet T_c (˚)
PGPR/RH	5.83 ± 1.81	-41.89 ± 0.82	–	–	15.70 ± 3.59	-43.06 ± 1.86
PGPR/SSL	3.83 ± 1.74	-40.07 ± 2.37	–	–		
EC/RH	3.44 ± 1.52	-38.43 ± 0.98	*1.74 ± 0.99*	*-39.57 ± 0.13*	40.98 ± 4.56	-42.04 ± 0.12
EC/SSL	2.16 ± 1.73	-39.30 ± 1.45	*2.64 ± 1.60*	*-37.99 ± 2.32*		

Systems utilising PGPR (in the stabilisation of the primary w/o droplets) had marginally larger $\Delta H'$ values and experienced crystallisation of the inner water droplets at lower average temperatures than the EC double emulsions (Table 4-1). Overall, this implies a larger number of encapsulated droplets and smaller internal water droplet sizes in the PGPR systems; in accordance with w/o droplet size measurements (Figure 4-1), where PGPR stabilised w/o droplets were much smaller than EC w/o droplets. In terms of the extent of encapsulation, the ratio of the $\Delta H'$ values to the $\Delta H'$ values for the PGPR systems is smaller in comparison to the EC systems (**Error! Reference source not found.**), largely due to the large $\Delta H'$ values for the EC systems. This indicates that there are potentially more primary droplets formed or, in accordance with the droplet size data in Figure 4-1, that the droplets are larger than the PGPR stabilised primary emulsions. As a consequence, this indicates that as there are potentially a lower number of smaller PGPR W/O droplets compared to the EC emulsions; encapsulation efficiency should be higher in the PGPR double emulsion systems. Despite this, analysis of the 1 month old PGPR double emulsion thermoanalytical profiles highlights a transformation into single emulsion structures (Figure 4-12).

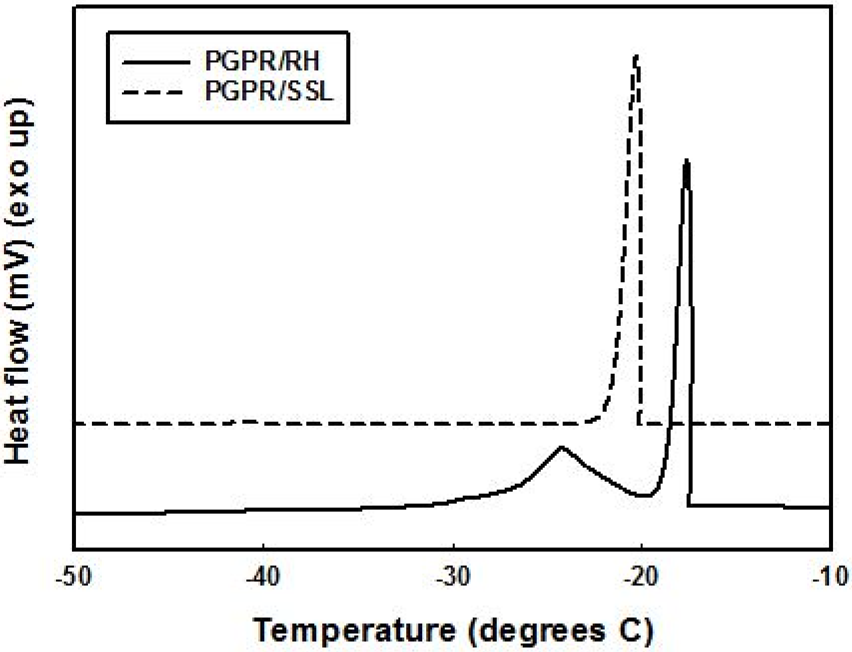

Figure 4-12. Thermoanalytical DSC profiles of a W/O/W emulsion stabilised using PGPR for the primary interface and either rutin hydrate or SSL particles for the secondary interface. Measurements were taken following 1 month storage.

In the thermoanalytical profiles of the PGPR/RH system after 1 month storage, a shoulder developed on the main peak relating to the secondary continuous water phase, as shown in Figure 4-12. Typically, when such profiles are observed for double emulsions, it is indicative of either inner droplet coalescence or diffusion of inner droplets into the continuous water phase during measurement [52]. Analysis of micrographs of both emulsion systems (micrograph inserts in Figure 4-7 and Figure 4-8) shows only a minor increase in internal water droplet size. Moreover, in both cases, there is a reduction in $\Delta H'$ of the peak relating to inner water droplets which indicates the occurrence of inner droplet diffusion into the outer aqueous phases and the loss of internal water phase volume. Therefore it can be concluded that water diffusion predominantly occurs during the measurement and these findings are in agreement with the PGPR double emulsion systems described in literature [52]. The fact that this doesn't occur during measurement of the PGPR double emulsions immediately following preparation, as described in literature, suggests that internal water droplet diffusion to the external water phase is retarded due to the secondary particle stabilised interface.

In contrast, EC double emulsion formulations were more stable against loss of internal droplets as indicated by the fact that no significant change in $\Delta H'$ occurred over time (Table 4-1). Furthermore, the Tc of inner droplets remained stable over time indicating no significant change in internal droplet size, and hence internal water droplet coalescence was minimal. Overall, this data signals the existence of a robust internal EC stabilised interface in both emulsions.

With respect to the secondary interface, the use of SSL or rutin hydrate appears to have very little effect on $\Delta H'$ or T_c values. This indicates that inner droplet integrity relies more upon the stability of the internal w/o interface than the secondary interface. Although, confocal images of SSL double emulsions showed a leaching of water soluble dye out of the inner water phase into the outer secondary water phase, DSC measurements were not in agreement. This shows that although Rhodamine B can traverse the oil layer and diffuse into the secondary aqueous phase in the SSL W/O/W emulsion systems, the SSL interfacial layer does not appear to allow the internal water molecules to do the same.

4.5 Conclusions

The aim of this work was to assess whether Pickering stabilisation could provide any additional benefits by the initial replacement of surfactant with particles at only one of the two interfaces present in double emulsions. It was observed that double emulsion stability was extended via the employment of particles at either interface in comparison to the surfactant-only stabilised double emulsions, and even further improved by the application of particles at both interfaces.

Deconstruction of these double emulsion systems via individual interface assessment highlighted the factors influencing pseudo interfacial layer formation; primary interface is more dependent upon material properties, secondary interface formation is process driven. Overall however, the compatibility of the emulsifiers used is vital in double emulsion stability. It was found that if the two materials used were too similar in terms of their hydrophilic/lipophilic character, no double emulsion structure forms or were extremely unstable.

The thermoanalytical profiles of the more stable double emulsions (those still containing encapsulated internal water droplets upon 1 month storage) highlighted the presence of a higher number of smaller internal water droplets where PGPR was used to stabilise the primary interface. Despite this, on the whole double emulsions using EC to stabilise the internal water droplets were more stable, irrespective of the emulsifier selected to stabilise the larger oil droplets. As a comparison to systems where PGPR is used to stabilise internal water droplets, DSC studies showed that the EC double emulsion formulations are much less susceptible to disruption via environmental temperature changes and storage time. This is in agreement with studies exhibiting particle stabilised double emulsions with superior stability compared with surfactant stabilised systems [26]. The results of this study also highlighted the limitations of the DSC technique for analysis of these particle stabilised double emulsion systems as there was evidence of droplet migration during DSC measurement. This advocates the need for always using a secondary analysis tool for these systems in tandem such as microscopy to substantiate findings.

No attempts were made here to balance the osmotic pressure within these systems and consequently further studies should involve the introduction of electrolytes (i.e. salts, proteins) to these formulations to deter water diffusion across the oil layer and consequently stem emulsion destabilisation. This may lead to the formation of stable W/O/W emulsions arising from some of the formulations shown here that were previously unsuccessful.

References

[1] Garti, N., Double emulsions — scope, limitations and new achievements. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **1997**, *123–124*, p. 233-246.

[2] Garti, N. and Aserin, A., Double emulsions stabilized by macromolecular surfactants. *Advances in Colloid and Interface Science,* **1996**, *65*, p. 37-69.

[3] Seifriz, W., Studies in emulsions. Iii-v. *The Journal of Physical Chemistry,* **1924**, *29*, p. 738-749.

[4] van der Graaf, S., Schroën, C.G.P.H., and Boom, R.M., Preparation of double emulsions by membrane emulsification—a review. *Journal of Membrane Science,* **2005**, *251*, p. 7-15.

[5] Benichou, A., Aserin, A., and Garti, N., W/o/w double emulsions stabilized with wpi–polysaccharide complexes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2007**, *294*, p. 20-32.

[6] Bonnet, M., Cansell, M., Berkaoui, A., Ropers, M.H., Anton, M., and Leal-Calderon, F., Release rate profiles of magnesium from multiple w/o/w emulsions. *Food Hydrocolloids,* **2009**, *23*, p. 92-101.

[7] Sagalowicz, L. and Leser, M.E., Delivery systems for liquid food products. *Current Opinion in Colloid & Interface Science,* **2010**, *15*, p. 61-72.

[8] Shima, M., Morita, Y., Yamashita, M., and Adachi, S., Protection of lactobacillus acidophilus from the low ph of a model gastric juice by incorporation in a w/o/w emulsion. *Food Hydrocolloids,* **2006**, *20*, p. 1164-1169.

[9] Flaiz, L., Freire, M., Cofrades, S., Mateos, R., Weiss, J., Jiménez-Colmenero, F., and Bou, R., Comparison of simple, double and gelled double emulsions as hydroxytyrosol and n-3 fatty acid delivery systems. *Food Chemistry,* **2016**, *213*, p. 49-57.

[10] Aditya, N.P., Aditya, S., Yang, H., Kim, H.W., Park, S.O., and Ko, S., Co-delivery of hydrophobic curcumin and hydrophilic catechin by a water-in-oil-in-water double emulsion. *Food Chemistry,* **2015**, *173*, p. 7-13.

[11] Aditya, N.P., Aditya, S., Yang, H.-J., Kim, H.W., Park, S.O., Lee, J., and Ko, S., Curcumin and catechin co-loaded water-in-oil-in-water emulsion and its beverage application. *Journal of Functional Foods,* **2015**, *15*, p. 35-43.

[12] Takahashi, Y., Yoshida, T., and Takahashi, T., Process for producing a w/o/w type multiple emulsion for medicines, cosmetics, etc. ed**1990**. Google Patents.

[13] Lobato-Calleros, C., Rodriguez, E., Sandoval-Castilla, O., Vernon-Carter, E.J., and Alvarez-Ramirez, J., Reduced-fat white fresh cheese-like products obtained from w1/o/w2 multiple emulsions: Viscoelastic and high-resolution image analyses. *Food Research International,* **2006**, *39*, p. 678-685.

[14] Taki, J., Isomura, T., Kanda, K., Kawahara, A., Maruyama, K., and Kusumoto, S., W1/o/w2-type double emulsion dressing and method for production thereof. ed**2008**. Google Patents.

[15] Jiménez-Colmenero, F., Potential applications of multiple emulsions in the development of healthy and functional foods. *Food Research International,* **2013**, *52*, p. 64-74.

[16] Jimenez-Colmenero, F., Salcedo-Sandoval, L., Bou, R., Cofrades, S., Herrero, A.M., and Ruiz-Capillas, C., Novel applications of oil-structuring methods as a strategy to improve the fat content of meat products. *Trends in Food Science & Technology,* **2015**, *44*, p. 177-188.

[17] De Cindio, B. and Cacace, D., Formulation and rheological characterization of reduced-calorie food emulsions. *International Journal of Food Science & Technology,* **1995**, *30*, p. 505-514.

[18] Muschiolik, G., Multiple emulsions for food use. *Current Opinion in Colloid & Interface Science,* **2007**, *12*, p. 213-220.

[19] Fernández-Martín, F., Freire, M., Bou, R., Cofrades, S., and Jiménez-Colmenero, F., Olive oil based edible w/o/w emulsions stability as affected by addition of some acylglycerides. *Journal of Food Engineering,* **2017**, *196*, p. 18-26.

[20] Oppermann, A.K.L., Piqueras-Fiszman, B., de Graaf, C., Scholten, E., and Stieger, M., Descriptive sensory profiling of double emulsions with gelled and non-gelled inner water phase. *Food Research International,* **2016**, *85*, p. 215-223.

[21] Florence, A.T. and Whitehill, D., Some features of breakdown in water-in-oil-in-water multiple emulsions. *Journal of Colloid and Interface Science,* **1981**, *79*, p. 243-256.

[22] Ficheux, M.F., Bonakdar, L., Leal-Calderon, F., and Bibette, J., Some stability criteria for double emulsions. *Langmuir,* **1998**, *14*, p. 2702-2706.

[23] Ramsden, W., Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation). -- preliminary account. *Proceedings of the Royal Society of London,* **1903**, *72*, p. 156-164.

[24] Pickering, S.U., Emulsions. *Journal of the Chemical Society,* **1907**, *91*, p. 2001 - 2021.

[25] Dickinson, E., Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science,* **2010**, *15*, p. 40-49.

[26] Aveyard, R., Binks, B.P., and Clint, J.H., Emulsions stabilised solely by colloidal particles. *Advances in Colloid and Interface Science,* **2003**, *100–102*, p. 503-546.

[27] Levine, S., Bowen, B.D., and Partridge, S.J., Stabilization of emulsions by fine particles i. Partitioning of particles between continuous phase and oil/water interface. *Colloids and Surfaces,* **1989**, *38*, p. 325-343.

[28] Schmitt, V., Destribats, M., and Backov, R., Colloidal particles as liquid dispersion stabilizer: Pickering emulsions and materials thereof. *Comptes Rendus Physique,* **2014**, *15*, p. 761-774.

[29] Ashby, N. and Binks, B., Pickering emulsions stabilised by laponite clay particles. *Physical Chemistry Chemical Physics,* **2000**, *2*, p. 5640-5646.

[30] Yusoff, A. and Murray, B.S., Modified starch granules as particle-stabilizers of oil-in-water emulsions. *Food Hydrocolloids,* **2011**, *25*, p. 42-55.

[31] Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., and Norton, I.T., Investigation into the potential ability of pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. *Journal of Colloid and Interface Science,* **2012**, *366*, p. 209-215.

[32] Song, X., Pei, Y., Qiao, M., Ma, F., Ren, H., and Zhao, Q., Preparation and characterizations of pickering emulsions stabilized by hydrophobic starch particles. *Food Hydrocolloids,* **2015**, *45*, p. 256-263.

[33] Rousseau, D., Trends in structuring edible emulsions with pickering fat crystals. *Current Opinion in Colloid & Interface Science,* **2013**, *18*, p. 283-291.

[34] Oza, K.P. and Frank, S.G., Multiple emulsions stabilized by colloidal microgrystalline cellulose. *Journal of Dispersion Science and Technology,* **1989**, *10*, p. 163-185.

[35] Carrillo, C.A., Nypelö, T.E., and Rojas, O.J., Cellulose nanofibrils for one-step stabilization of multiple emulsions (w/o/w) based on soybean oil. *Journal of Colloid and Interface Science,* **2015**, *445*, p. 166-173.

[36] Sekine, T., Yoshida, K., Matsuzaki, F., Yanaki, T., and Yamaguchi, M., A novel method for preparing oil-in-water-in-oil type multiple emulsions using organophilic montmorillonite clay mineral. *Journal of Surfactants and Detergents,* **1999**, *2*, p. 309-315.

[37] Midmore, B.R. and Herrington, T.M., Silica-stabilised multiple emulsions, in *Trends in colloid and interface science xiii* **1999**. *112*, D. Težak and M. Martinis, Eds., Progress in colloid and polymer science: Springer Berlin Heidelberg, p. 115-120.

[38] Garti, N., Aserin, A., Tiunova, I., and Binyamin, H., Double emulsions of water-in-oil-in-water stabilized by alpha-form fat microcrystals. Part 1: Selection of emulsifiers and fat microcrystalline particles. *Journal of the American Oil Chemists Society,* **1999**, *76*, p. 383-389.

[39] Hu, Z., Ballinger, S., Pelton, R., and Cranston, E.D., Surfactant-enhanced cellulose nanocrystal pickering emulsions. *Journal of Colloid and Interface Science,* **2015**, *439*, p. 139-148.

[40] Timgren, A., Rayner, M., Dejmek, P., Marku, D., and Sjöö, M., Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride. *Food Science & Nutrition,* **2013**, *1*, p. 157-171.

[41] Matos, M., Timgren, A., Sjoo, M., Dejmek, P., and Rayner, M., Preparation and encapsulation properties of double pickering emulsions stabilized by quinoa starch granules. *Colloids and Surfaces a-Physicochemical and Engineering Aspects,* **2013**, *423*, p. 147-153.

[42] Mun, S., Choi, Y., Park, K.-H., Shim, J.-Y., and Kim, Y.-R., Influence of environmental stresses on the stability of w/o/w emulsions containing enzymatically modified starch. *Carbohydrate Polymers,* **2013**, *92*, p. 1503-1511.

[43] Xiao, J., Lu, X., and Huang, Q., Double emulsion derived from kafirin nanoparticles stabilized pickering emulsion: Fabrication, microstructure, stability and in vitro digestion profile. *Food Hydrocolloids,* **2017**, *62*, p. 230-238.

[44] Garrec, D.A., Frasch-Melnik, S., Henry, J.V.L., Spyropoulos, F., and Norton, I.T., Designing colloidal structures for micro and macro nutrient content and release in foods. *Faraday Discussions,* **2012**, *158*, p. 37-49.

[45] Frasch-Melnik, S., Fat crystal-stabilised double emulsions, PhD,**2011**, University of Birmingham, UK.

[46] Pays, K., Giermanska-Kahn, J., Pouligny, B., Bibette, J., and Leal-Calderon, F., Coalescence in surfactant-stabilized double emulsions. *Langmuir,* **2001**, *17*, p. 7758-7769.

[47] McClements, D.J., *Food emulsions: Principles, practices, and techniques, second edition***2004**: Taylor & Francis.

[48] Dragosavac, M.M., Holdich, R.G., Vladisavljević, G.T., and Sovilj, M.N., Stirred cell membrane emulsification for multiple emulsions containing unrefined pumpkin seed oil with uniform droplet size. *Journal of Membrane Science,* **2012**, *392–393*, p. 122-129.

[49] Pichot, R., Duffus, L., Zafeiri, I., Spyropoulos, F., and Norton, I.T., Particle-stabilized food emulsions, in *Particle-stabilized emulsions and colloids: Formation and applications* **2015**. T. Ngai and S. Bon, Eds., Rsc soft matter: The Royal Society of Chemistry, p. 247-282.

[50] Tambe, D.E. and Sharma, M.M., Factors controlling the stability of colloid-stabilized emulsions: I. An experimental investigation. *Journal of Colloid and Interface Science,* **1993**, *157*, p. 244-253.

[51] Potier, L., Raynal, S., Seiller, M., Grossiord, J.L., and Clausse, D., Study of state transitions within multiple w/o/w emulsions using calorimetry (dsc). *Thermochimica Acta,* **1992**, *204*, p. 145-155.

[52] Schuch, A., Deiters, P., Henne, J., Köhler, K., and Schuchmann, H.P., Production of w/o/w (water-in-oil-in-water) multiple emulsions: Droplet breakup and release of water. *Journal of Colloid and Interface Science,* **2013**, *402*, p. 157-164.

Chapter 5

Double W/O/W emulsions stabilised solely by Pickering particles

A proportion of this work has been published as follows:

- L. Duffus, P.Smith, I.T. Norton, F.Spyropoulos. Pickering W/O/W double emulsions stabilised solely by ethylcellulose and rutin hydrate particles, *Langmuir*, In preparation.
- L. Duffus, P.Smith, I.T. Norton, F.Spyropoulos. Formulation and stability of edible Pickering W1/O/W2 double emulsions, *13th International Hydrocolloids Conference*, Guelph, Canada, May 2016.
- L. Duffus, J.E. Norton, P. Smith, F. Spyropoulos, I.T. Norton. Formulating double emulsions stabilised using edible Pickering particles, *Rideal AGM Meeting SCI,* London, UK, April 2015.
- L. Duffus, J.E. Norton, P. Smith, F. Spyropoulos, I.T. Norton. Formulation of double emulsions stabilised by Pickering particles. *12th Annual Formulation Engineering Conference,* University of Birmingham, UK, March 2015.
- L. Duffus, J.E. Norton, P. Smith, I.T. Norton, F. Spyropoulos. Formulation & stability of double emulsions solely stabilised by edible particles. *Advances in Food Processing: Challenges for the Future,* Sao Paulo, Brazil, November 2014.

5.1 Abstract

Double water-in-oil-in-water (W/O/W) emulsions present an opportunity for the production of healthier food emulsions, however, their complex structure and vast interfacial area means that they are significantly more unstable than simple emulsions. Use of Pickering stabilisation, reputed for superior, longer term stabilisation capacities when compared to surfactant stabilised emulsions, increases the possibility of double emulsion stability enhancement. Work presented in the previous chapters provided a strong indication that replacing surfactant with particulate materials at either interface provided a stability advantage to double emulsions. Therefore in this work, the aim was to fabricate double emulsions solely stabilised via ethylcellulose and rutin hydrate edible particles in the absence of any additional surfactants or stabilisers. The effect of a range of processing routes and variation of encapsulated water droplet concentration on emulsion microstructure and stability was investigated as well as the relationship of such parameters with encapsulation efficiency. Edible particle stabilised double emulsions with internal water droplet volume ranging from 1 – 16 %w/w were successfully formulated and remained stable against total loss of inner water phase for 1 month. Encapsulation efficiencies exceeding 89 % were possible depending on the processing route selected, and due to the extremely stable droplet interfaces created during primary emulsion formation; there is minimal disruption to primary water-in-oil droplets during secondary emulsification processing. For this reason, it was found that water-in-oil droplet size distribution can be a limiting factor for encapsulation.

Keywords: Pickering emulsions; double w/o/w emulsions; ethylcellulose; flavonoids

5.2 Introduction

Multiple emulsions are often also known as 'emulsions of emulsions' [1]. Essentially, these complex systems are comprised of a continuous phase containing a dispersed phase which itself has another separate dispersed phase contained within. These types of emulsion microstructures have been studied since their initial description in the early 1920s [2]. Of multiple emulsions, the most commonly used and studied are double emulsions and within this category two main types emerge; water-in-oil-in-water (W/O/W) and oil-in-water-in oil (O/W/O) [3-6], and more recently oil-in-oil-in-oil double emulsions are also being investigated [7]. Typically double emulsions are formed utilising a 2-step emulsification processing route. This process involves the initial formation of the primary emulsion under high shear via homogenisation, ultrasonication or otherwise. This simple emulsion is then dispersed within a separate secondary continuous phase containing a secondary emulsifier and emulsification occurs in the absence of severe mixing in order to avoid disruption of the primary emulsion droplets [8-10]. Control and understanding of these emulsification steps leads to better management of double emulsion droplet size and stability.

The major drawback for double emulsions is their inherent instability, even more so than simple emulsions as there are now two unstable interfaces in close proximity present in the formulation [11]. By using Pickering stabilisation in the place of surfactants, it has been shown that it is possible to successfully prolong the stability of double emulsions [12-14]. First developed by Ramsden and Pickering [15, 16], Pickering particle stabilised emulsions are defined as emulsion droplets coated by a layer of adsorbed colloidal (micro- or nano-) solid particles at the oil-water interface [17-19]. Studies have been completed that provide evidence of increased stability of edible double emulsions via the addition of edible particles as emulsifiers using fat particles [20-23], starches [24, 25] and pectin-WPI complexes [26]. Despite this, they serve only to provide examples of double emulsion co-stabilisation using surfactants and particles. Surprisingly, even fewer studies detail the use of particles being used solely to stabilise double emulsions and these studies largely involve the use of silica based colloidal particles [27-30] or other combinations of inorganic particles [31, 32] and are not solely focussed upon food-grade Pickering particles. This study aims to build upon this knowledge as well as the knowledge gained in the prior chapters and stabilise double emulsions using edible ethylcellulose and rutin hydrate particles.

Rutin is the rhamnoglucoside of the flavonoid Quercetin. Readily sourced from common citrus fruits, it is classified as a secondary plant metabolite. Exhibiting antioxidant behaviour, it has been found to suppress oxidative stress and inflammation, curtail bioactivation of carcinogens and affect cell signalling [33, 34]. Typically insoluble in both water and oil environments, it is commonly derived from grapefruit peel and juice, and largely used as a food supplement to boost antioxidant intake. Chat et al., (2011) reported that the solubilisation and radical scavenging activity of rutin was increased in the presence of certain surfactant micelles [35]. With respect to emulsion systems, Luo et al., (2011) have found it to be a good O/W emulsifier with excellent long term stabilisation capabilities [36]. Ethylcellulose is a mainly hydrophobic, non-water soluble cellulose ether polymer, consisting of an anhydroglucose repeating backbone, where a large proportion of the hydroxyl groups on the repeating structure are etherified with ethyl groups [37]. For stabilisation of the primary interface, ethylcellulose particles were selected as not only are they food-grade, being derived primarily from wood and cotton, but ethylcellulose has been found to be an excellent emulsifier for air/water foams [38] and W/O emulsions [39].

The principal intention of this study was to utilise the aforementioned edible particulate materials for the stabilisation of W/O/W double emulsions in the absence of silica particles or any additional surfactants or stabilisers. This innovative approach provides a foundation for the production of stable, clean-label and surfactant-free food double emulsions. Furthermore, the aim was to gain insight into the influence of emulsification process on microstructure, droplet size and stability of these novel systems. Thirdly, focus was placed upon understanding the extent to which the formulation can serve as tool for fat content reduction through phase volume manipulation. Previously reported as a suitable direct technique for the characterisation of inner water droplets in double emulsions, differential scanning calorimetry (DSC) [40-43], was used to determine the encapsulation efficiency of double emulsions and to evaluate how this is affected by processing route and phase mass fraction.

5.3 Materials and Methods

5.3.1 Materials

Distilled water and sunflower oil (purchased from a local supermarket) were used for the preparation of all emulsions. Particles used as emulsifying agents were rutin hydrate (RH) and ethylcellulose (EC) and were obtained from Sigma, UK. Rhodamine B and Perylene dyes were obtained from Sigma, UK. All materials were used without any further purification or modification. Percentages of water and oil phases, in addition to emulsifying agents and all other emulsion formulation components, were calculated as the weight of the individual constituent per weight of the final emulsion (simple or multiple), as detailed in the relevant section.

5.3.2 Methods

5.3.2.1 Fabrication of Pickering particle dispersions

All particles were dispersed into and treated in the sunflower oil or water phase prior to combination with the alternate phase to form the emulsions. These particle dispersions were prepared and then heated with a hot plate at various temperatures ranging from 45 to 95 ˚C for durations between 40 and 60 minutes whilst being agitated with a magnetic stirrer to promote particle dispersion. Following this, aqueous particle dispersions were further treated by a high intensity ultrasonic vibracell processor (Sonics & Materials, Inc., CT, USA) operating at 750W and 20 kHz, for 2 minutes. Following the continuous phase/particle dispersion treatment, the corresponding dispersed phase was added and the mixture was emulsified using different techniques.

5.3.2.2 W/O emulsions

Three different emulsification methods were investigated for the preparation of the primary W/O emulsions:

Primary emulsification method 1 (PM1): Emulsions were formed via rotor stator mixing (RSM) using a Silverson L4RT (emulsion screen diameter 19mm), at a rotational speed of 10,000 rpm for 10 minutes.

Primary emulsification method 2 (PM2): The emulsion pre-mixes were subjected to high intensity ultrasonic processing using a 12 mm diameter probe for 2 min (20 kHz, 95% amplitude).

Primary emulsification method 3 (PM3): Emulsions were firstly processed using PM1 and then passed through an air-driven M1 10S Microfluidiser (Microfluidics, USA) fitted with a cooling tube for up to 5 passes at an operating pressure of 100 MPa for each pass.

In PM1 and PM2, 100 g emulsion samples were produced which consisted of a dispersed phase of 10 g of water containing a small amount of Rhodamine B dye (≤ 0.1 %w/w), which was then added to 88 g of sunflower oil containing 2 g EC particles and a small amount of lipophilic Perylene dye (≤ 0.1 %w/w). In PM3, 500 g samples were produced using 50 g of water containing the Rhodamine B dye (≤

0.1 %w/w) which was added to 440 g of sunflower oil containing 10 g of EC particles and a small amount of Perylene dye (≤ 0.1 %w/w).

5.3.2.3 W/O/W emulsions

For the investigation of secondary emulsification methods, the primary emulsification step was kept constant and PM1 was used. In each method, 20g of the primary emulsion was added to 77g of water containing 3g of rutin hydrate particles prior to emulsification. The different secondary emulsification steps investigated were:

Secondary emulsification method 1 (SM1): Double emulsion structure was produced via a Silverson L4RT rotor-stator mixer (emulsion screen diameter 19mm) using a rotational speed of 3,500 rpm for 2 minutes.

Secondary emulsification method 2 (SM2): The double emulsion pre-mixes were subjected to low intensity ultrasonic processing using a 12 mm diameter probe for 2 min (20 kHz, 20% amplitude).

Secondary emulsification method 3 (SM3): Mixing via magnetic stirrer for 2 min at a rotational speed of 1325 rpm.

Formulations used in double emulsion phase volume studies are shown in **Error! Reference source not found.** and PM1 and SM1 were used to prepare 100 g samples of these emulsions.

5.3.2.4 Droplet sizing measurements

Emulsion droplet sizes were measured in a continuous aqueous medium via static multi-angle light scattering (SMLS) using a Mastersizer Hydro 2000 (Malvern Instruments, UK). All measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ± 1 standard deviation. Where double emulsions are concerned imaging was particularly important as, in terms of light scattering, double emulsions are optically non-uniform due to the internal droplet phase. Pays, et al. [44] assume that the internal phase droplets behave as simple droplets with the same refractive index as the oil phase. Micrographs were therefore taken in conjunction with Mastersizer measurements. Visualisation of both simple and double emulsions was captured via light microscope (Olympus CH2, Japan, with CCD video camera). Double emulsion microstructure was also imaged using a Leica TCS SPE confocal microscope (Leica Microsystems, UK). A drop of emulsion was placed onto a glass microscope slide and a cover slip placed over. Separate fluorescent emission spectra were obtained for the oil phase (stained with perylene) and internal water phase (stained with Rhodamine B), enabling spatial identification of each phase within the double emulsion microstructure.

One-way ANOVA statistical tests including Tukey’s Pairwise Comparisons (Minitab 17 statistical software) were carried out in order to determine significant differences (p value < 0.05) amongst double emulsion droplet sizes of varying formulations.

5.3.2.5 Differential Scanning Calorimetry (DSC)

DSC experiments were performed on a DSC 8000 differential scanning calorimeter (PerkinElmer Inc., USA). Analysis was performed on simple W/O and the corresponding W/O/W emulsion samples at various time points, with samples stored at room temperature. 5.5 ± 1.5 mg of each sample was weighed directly into a specialised stainless steel DSC crucible. All emulsions were cooled from 20 ˚C to -60 ˚C at a rate of 5 ˚C /min, held at -60 ˚C for 2 mins and then heated back up to 20 ˚C at the same rate. The reference crucible was left empty. The cooling enthalpies reported for emulsions were derived through integration of the relative area under the respective thermoanalytical curve with an applied linear baseline using the instrument software (Pyris). By dividing this value by the mass of the sample in the aluminium pan, these ΔH values generated by the software were then used to calculate encapsulation efficiency, EE (%). EE is described as the amount of water present in the internal water phase in the final double emulsion compared to the amount of water added during the primary emulsification step and is calculated using the following equation [43]:

$$EE = \left(\left(\frac{\Delta H'}{\varphi'}\right) / \left(\frac{\Delta H''}{\varphi''}\right)\right) \times 100\% \qquad \textbf{Equation 5-1}$$

Here, $\Delta H'$ (J/g) represents the enthalpy change during crystallisation of the internal water phase within the double emulsion. $\Delta H'$ (J/g) is the enthalpy change during freezing of the dispersed phase of the primary W/O emulsion. φ' is the mass fraction of the internal water phase within the double emulsion and φ'' is the mass fraction of the aqueous phase in the primary W/O emulsion. By dividing the ΔH

values by their respective φ, the amount of water within each phase can be determined and hence EE can be calculated (as described in [43]). All DSC measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ± 1 standard deviation.

5.4 Results & Discussion

5.4.1 Formulation of Pickering only double emulsions

Double emulsion formulations constituted 2 %w/w internal water phase and 0.4 %w/w EC particles dispersed within a 17.6 %w/w oil phase, to form 10% W/O primary emulsions. This primary W/O emulsion was then dispersed into a secondary aqueous medium consisting of 3 %w/w rutin hydrate particles dispersed within an 77 %w/w external water phase to form the overall double emulsion. This base formulation was used as an initial starting point, and PM1 and SM1 emulsification methods were used to prepare these systems (Figure 5-1).

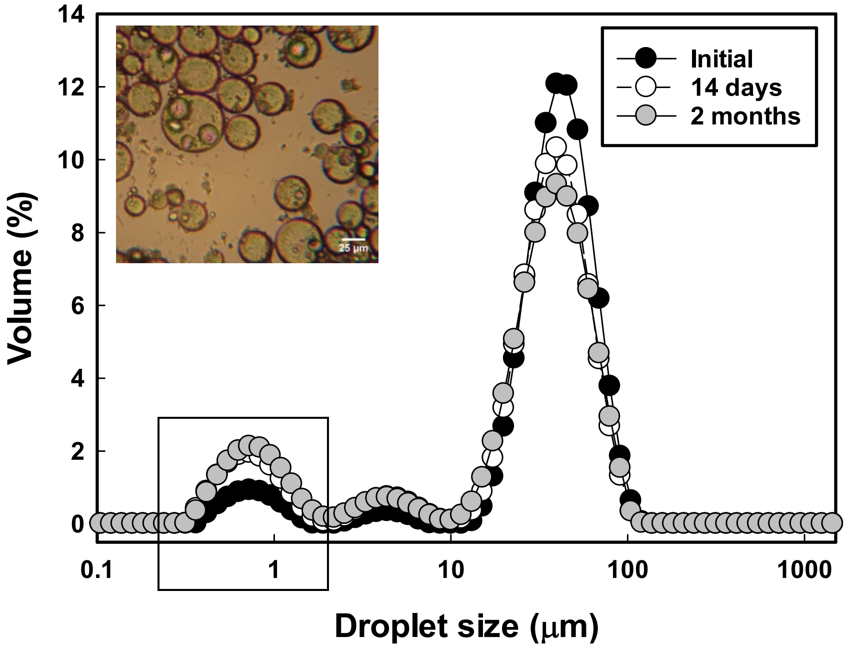

Figure 5-1.Droplet size distributions for particle-only EC/Rutin stabilised double emulsions consisting of 10% w/o primary emulsion comprising 20% of the final double emulsion and following preparation and following storage. The micrograph insert shows double emulsion droplets following 2 months storage. Scale bars represent 25 μm.

Essentially, these double emulsions were comprised of a 10% w/o emulsion which represented 20% of the overall double emulsion. Previous experimental work in Chapter 4 showed that in the production of simple emulsions via Silverson mixer, 2%w/w EC and 3% rutin hydrate particle concentrations were sufficient for the stabilisation of 10% w/o and 20% o/w emulsions with very stable droplets, respectively. The double emulsions formed were stable for in excess of 2 months (Figure 5-1). The boxed area of the droplet size distribution in Figure 5-1 relates to free rutin particle concentration present in the continuous phase, (confirmed via rutin hydrate particle size measurement and double emulsion optical light micrograph analysis). Rutin particles were deliberately included within formulations in excess of the minimum particle concentration required for the total surface area of oil droplets calculated in order to promote faster particle adsorption at the secondary oil-water interface during processing and hence smaller droplet sizes. The increase in the area of this peak over time coupled with the decrease in the height of the peak relating to the double emulsion droplets is indicative of double emulsion droplet coalescence, and consequently an increase in free rutin hydrate particle concentration within the continuous phase. Despite this, micrographs confirmed the presence of double emulsion structures formed via this formulation even after 2 months storage (micrograph insert, Figure 5-1). The stability of these double emulsions is largely due to rutin hydrate particles being mainly hydrophilic and EC particles possess a predominately hydrophobic character. Therefore the difference in the wettability of these two particle systems is significant and consequently they are more likely to successfully adsorb at the two different interfaces present in a double emulsion system and not migrate to the opposing interface encouraging droplet coalescence and emulsion destabilisation.

5.4.2 Effect of processing route on double emulsion microstructure

By assessment of several different processing routes for these Pickering-only stabilised double emulsions, the aim was to not only minimise primary emulsion droplet size in order to maximise internal water droplet inclusion into the double emulsion secondary droplets, but to also create robust primary and secondary interfaces to limit droplet coalescence. A range of different high and low shear emulsification processes were explored in order to achieve these ambitions and to further understand their influence on these double emulsion microstructures.

5.4.3 Effect of primary emulsification step

Typically, the first emulsification step within double emulsions is performed under high shear in an attempt to create small W/O droplets to maximise droplet encapsulation during the secondary emulsification step. Therefore, in this study, double emulsions were produced whereby PM1 (Silverson), PM2 (ultrasonication), and PM3 (microfluidisation) processing methods were used in the fabrication of primary emulsion droplets for use in double emulsions [45]. SM1 was employed as the secondary emulsification step. The same formulation exhibited in Figure 5-1 was used in all cases to form the overall double emulsion structure for comparative purposes. Subsequent droplet size distributions are displayed in Figure 5-2.

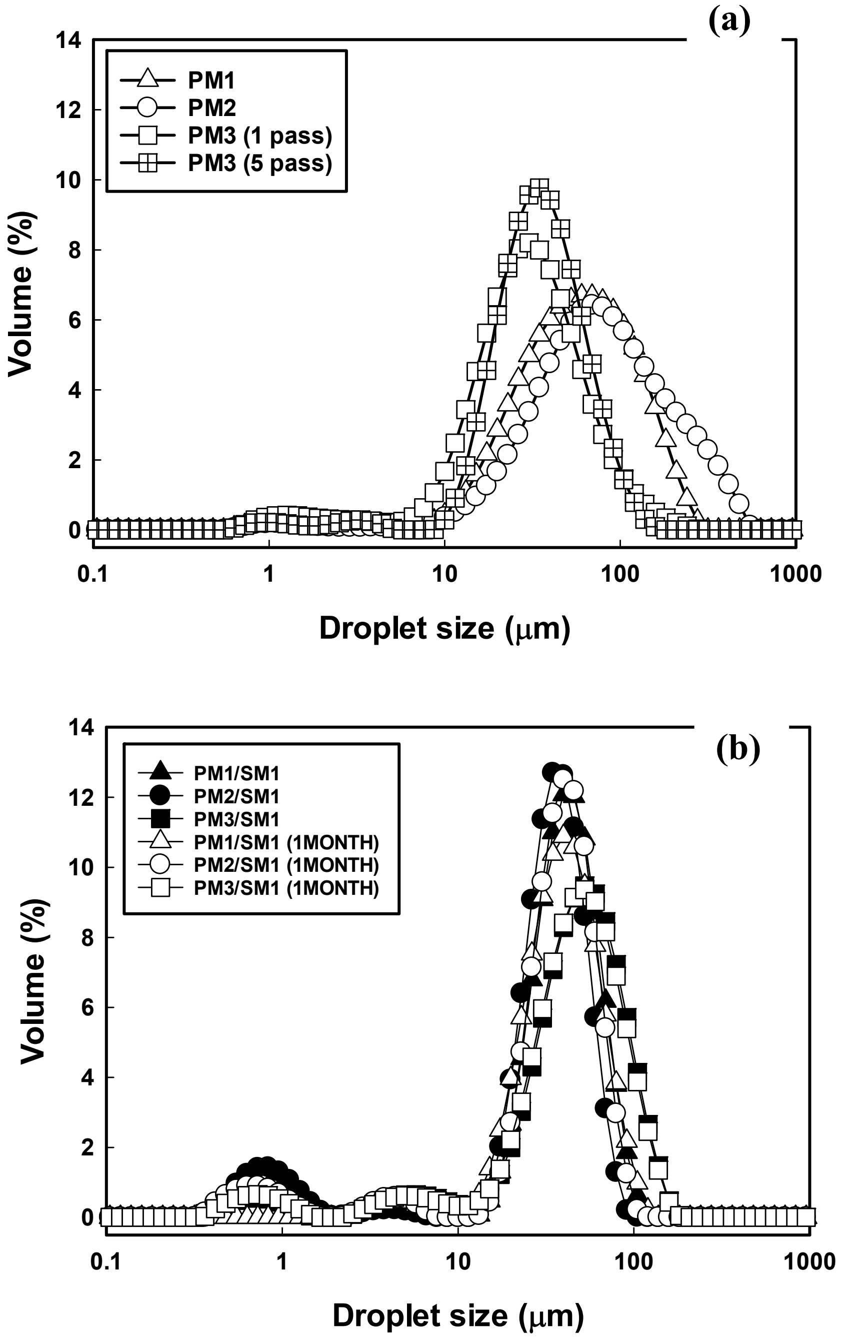

Figure 5-2. Droplet size distributions for (a) EC stabilised 10% w/o emulsions prepared via different processes following initial preparation and (b) double emulsions prepared via different primary emulsification routes, following preparation and after 1 month storage.

W/O emulsion droplets formed using the different processes displayed differences in droplet size; the higher energy process (PM3), produced the smallest droplet sizes as anticipated. However, further passes through the microfluidiser apparatus showed no further significant decrease in droplet size, indicating that a minimum droplet size for this formulation was realised via the microfluidiser processing route (Figure 5-2a) and 1 pass was sufficient. Double emulsions were consequently formulated whereby the different primary emulsification processes were used and the subsequent secondary emulsification step, SM1, remained constant across the three systems (Figure 5-2b). Despite the trend found with the W/O emulsion droplet sizes, the corresponding double emulsion droplet size distributions were largest when microfluidisation was used as the primary emulsification technique Figure 5-2b. The simplest explanation for this is that potentially more primary droplets were formed of an appropriate size to enable encapsulation within the secondary double emulsion droplets, consequently causing a slight swelling of the final double emulsion droplets.

Primary emulsion droplets appear to be similar in size to the subsequent double emulsions however; micrographs confirmed the presence of double emulsion structures, whereby the internal droplets are considerably smaller than the oil droplets within which they are contained. In addition to this, although double emulsions formed via PM2/SM1 experienced droplet growth and the PM1/SM1 W/O/W emulsion droplet distribution became wider, all three emulsions remained stable against phase separation and loss of double emulsion microstructure integrity upon 1 month of storage. There are potentially two mechanisms via which this phenomenon occurs; further reduction of primary droplet size during secondary emulsification step allowing for better encapsulation, or only a proportion of the primary droplet distribution are actually encapsulated. Further investigation around characterisation of inner water droplets and encapsulation efficiency reported later in this study can provide further insight.

5.4.4 Effect of secondary emulsification step

The influence of the secondary emulsification step on droplet size and stability of these systems was investigated through the exploration of three different lower shear processing routes. The importance of applying a low shear process for the second emulsification step lies in the need for the minimisation of primary droplet disruption when forming the overall double emulsion structure. Nevertheless, a significant shear must be imparted to the pre-emulsion so that the final double emulsion droplets are of an appropriate size and narrow distribution so as to discourage droplet-droplet coalescence. Double emulsions were produced using EC to stabilise the inner W/O interface and rutin hydrate to stabilise the outer O/W interface. Formulations utilising mass fractions of 2 %w/w W_1, 17.6 %w/w O and 77 %w/w W_2 were used again in order to maintain consistency when comparing processing methods. PM1 was used for the primary emulsification step in all subsequent emulsions (droplet size distributions shown in Figure 5-2a) and the different secondary emulsification processes described in section 5.2.2.3 were investigated. Emulsion droplet size was measured as a function of time and micrographs were captured following emulsion preparation as shown in Figure 5-3.

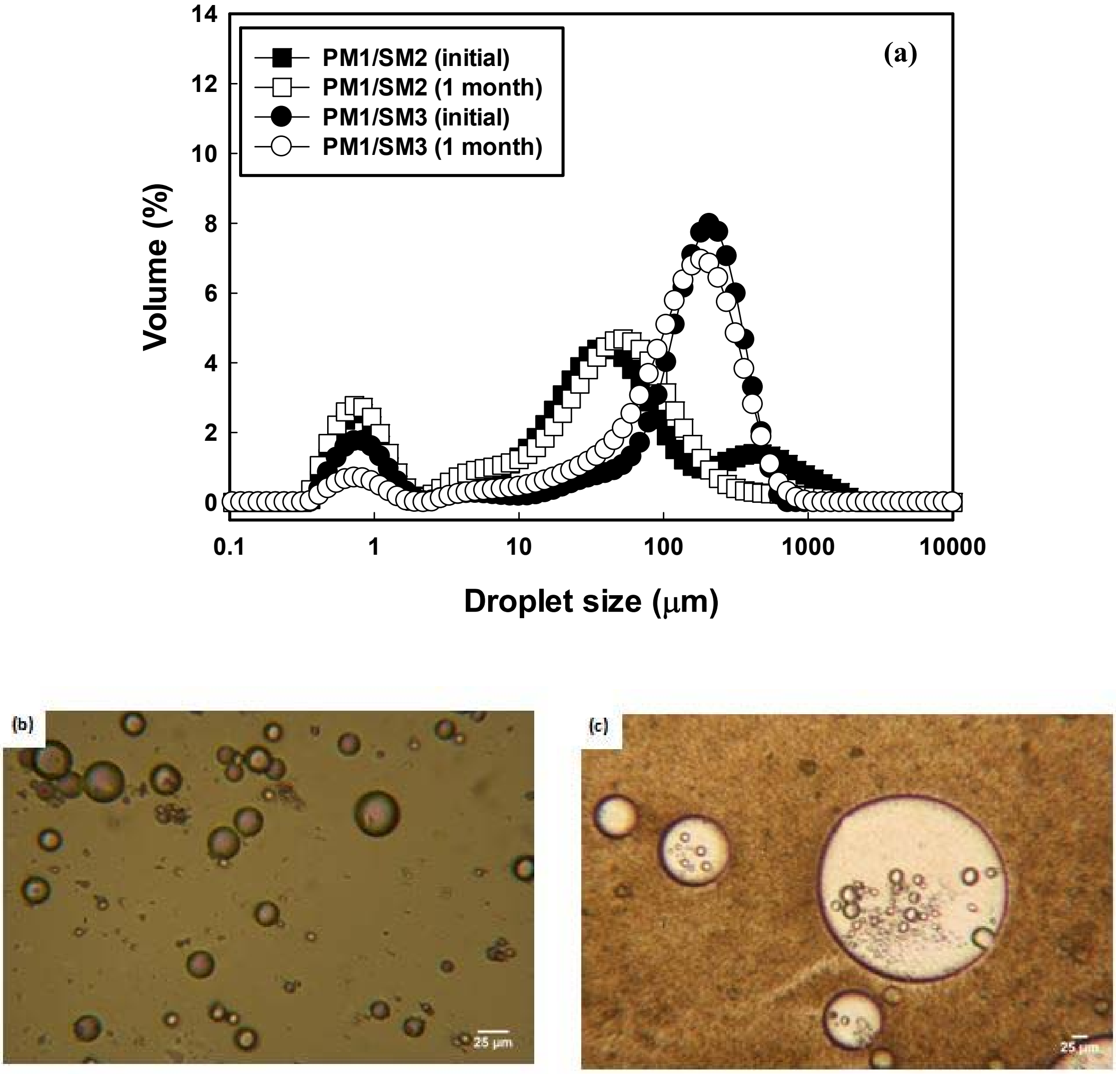

Figure 5-3 (a) Droplet size distributions (μm) for double emulsions formed using various processing routes for the primary emulsion and the secondary emulsion droplets, as function of time and micrographs of emulsions formed via: (b) SM2, and (c) SM3. Micrographs for SM1 are shown in Error! Reference source not found.**. Scale bars represent 25 μm.**

Where techniques are used for the secondary emulsification process that provides a lower shear than SM1 processing, i.e. SM3, a significant increase in droplet size is observed (Figure 5-3a). As Pickering particles are generally not regarded as being surface active, they are not predisposed to adhere to the interface in the same manner as conventional surfactants. For this reason, initial particle adsorption to the interface is significantly more random by comparison. Therefore, by applying a more intense processing method, the probability of particles occupying the interface between the two immiscible liquids is increased. This reasoning is partly supported by the fact that, although processing time was kept constant (2 mins) across all secondary emulsification stages, the formulations prepared using SM1 for the secondary emulsification step produced emulsion droplets of a smaller size (Figure 5-1). Micrographs of emulsions correlated well with droplet size distributions (Figure 5-3), however the emulsions produced via the higher shear SM2 process depicted the existence of only simple emulsion droplets signifying that where the secondary process is too intense, primary W/O droplets suffered disruption, and the double emulsion structure is compromised. In spite of this, the other two emulsions (where PM1/SM1 (Figure 5-1) and PM1/SM3 (Figure 5-3a) processing combinations were employed) appeared to be stable following 1 month storage and double emulsion droplet integrity was maintained despite negligible droplet size change over time. Although both systems were stable, double emulsions formed exclusively via rotor-stator mixing (PM1/SM1 processing) were shown to possess double emulsion microstructures of a more appropriate size (i.e. ~ 25 µm) for consumer taste acceptance within food emulsions than those formed via PM1/SM3 [46]. Subsequently, experiments were conducted in order to assess the effect of droplet size and microstructure as a function of SM1 processing time. This was carried out in order to understand how well emulsion droplets could withstand additional processing and resulting droplet sizes as a function of time are shown in Figure 5-4.

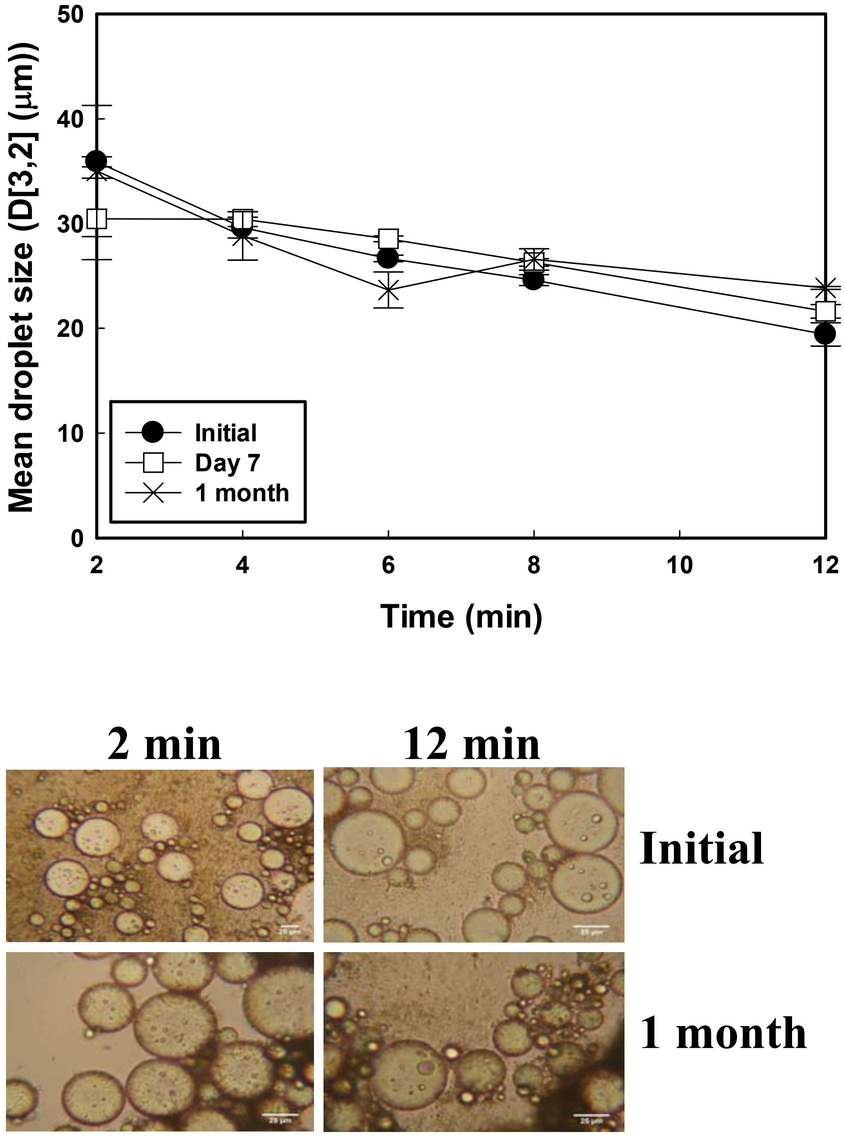

Figure 5-4.Mean droplet sizes (µm) of double emulsions following several passes through RSM process immediately following preparation, and after 7 days and 1 month storage. Measurements were performed in triplicate and error bars represent ± 1 standard deviation. [Error bars where not visible are smaller than symbols]. Micrographs represent double emulsion samples with different processing times and at different time points. Scale bars represent 25 µm.

All emulsions were stable to phase separation for up to 1 month stored at room temperature and processing time appears to effect a reduction in droplet size. However, as processing time increased, emulsion droplet size distributions became wider. Micrographs of the emulsions indicated that the W/O droplet size increased and became more polydispersed with increasing processing time (Figure 5-4). For the sample processed for 12 minutes, internal water droplets become more polydispersed in comparison to those double emulsions with shorter processing times, leading to a higher probability of internal droplet-droplet coalescence and the existence of more simple O/W droplets (Figure 5-4). This change in droplet size was expected to be due to over-processing and disruption of droplets leading to coalescence, however the secondary rutin hydrate stabilised droplets displayed very little change in droplet size upon storage; this effect was specific to the EC stabilised W/O droplets. This may be due to the predominantly hydrophobic character of the EC particles. Repeated W/O droplet breakup and thus EC particle exposure to the larger secondary aqueous phase promotes EC particle aggregation and so reformation of the initially small W/O primary emulsion droplets is less likely to occur as processing time progresses. Previous experimental work showed that continued processing of EC stabilised W/O emulsions via high pressure jet homogenisation and droplet size increase was not observed irrespective of pass number [47]. This further supports the notion that the excess aqueous environment present in double emulsions, compared to the EC W/O formulation, leads to the destabilisation of EC particles and inner water droplets.

5.4.5 Effect of mass fraction on double emulsion microstructure

Following on from the identification of the stable EC and rutin stabilised Pickering-Pickering double emulsion systems and processing route (PM1/SM1), the next objective was to investigate increasing internal water phase volume within the double emulsion structures as a means of reducing overall oil content further. Double emulsions were produced at a range of phase volumes via the formulations shown in Table 5-1.

Table 5-1Composition of double emulsions of differing phase volume

EMULSION NAME	DOUBLE EMULSION COMPOSITION (%w/w)				
	W_1 phase	**Primary interface stabiliser**	**Oil phase**	**W_2 phase**	**Secondary interface stabiliser**
10/10	1.00	0.20	8.80	87.00	3.00
10/20	2.00	0.40	17.60	77.00	3.00
10/40	4.00	0.80	35.20	57.00	3.00
20/10	2.00	0.20	7.80	87.00	3.00
20/20	4.00	0.40	15.60	77.00	3.00
20/40	8.00	0.80	31.20	57.00	3.00
40/10	4.00	0.20	5.80	87.00	3.00
40/20	8.00	0.40	11.60	77.00	3.00
40/40	16.00	0.80	23.20	57.00	3.00

Double emulsions were fabricated and mean droplet size and micrographs were recorded as a function of internal water mass as well as time. This was done in order to assess double emulsion structure integrity as well as emulsion stability. Figure 5-6 shows the droplet size distribution of the primary emulsions formed via PM1 and used to form the inner primary emulsions of the double emulsions represented in Figure 5-6.

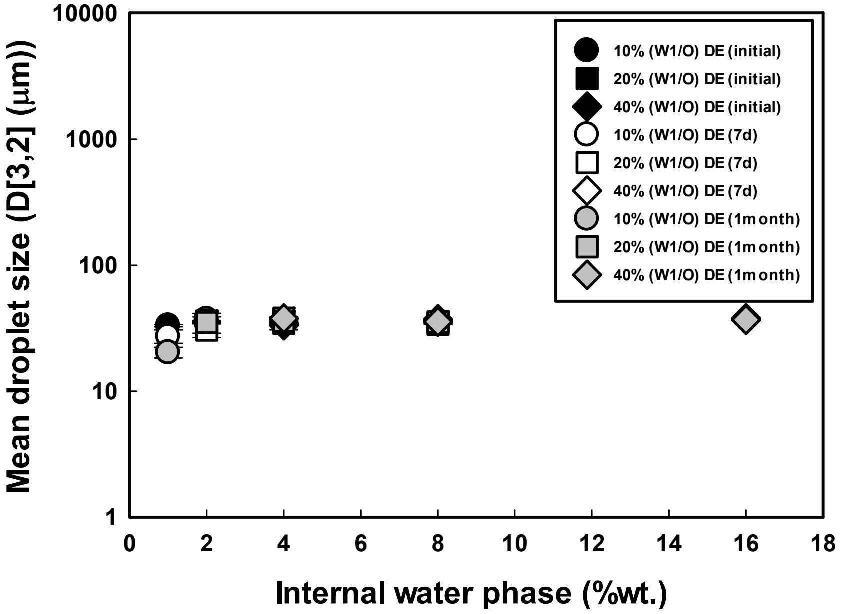

Figure 5-5 Droplet sizes (μm) for double emulsions produced at different internal water phase volumes (○ represent 0.2 %w/w EC□ represent 0.4 %w/w EC,◇represent 0.8 %w/w, black symbols represent droplet size following preparation, white symbols represent 7 day storage and grey symbols represent 1 month storage).

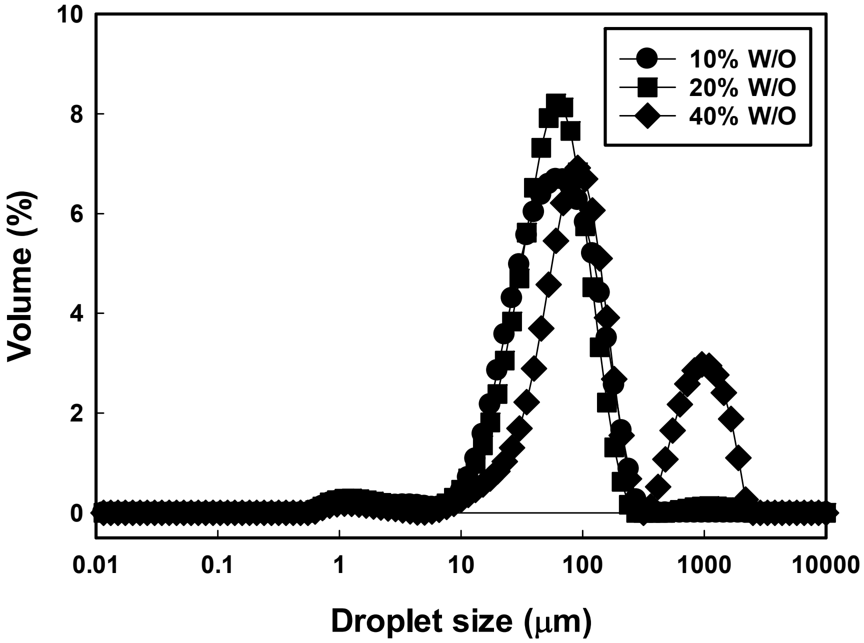

Figure 5-6.Droplet size distributions of primary emulsions used in double emulsion formation. Measurements were performed in triplicate and error bars represent ± 1 standard deviation. [Error bars where not visible are smaller than symbols].

Mean droplet size (D[3,2]) values shown in Figure 5-6 are the result of droplet size distributions that have been truncated. This was done in order to obtain a more accurate mean droplet size for double emulsions via exclusion of the proportion of the size distribution relating to free rutin hydrate particle concentration (highlighted in the box, Figure 5-1), previously confirmed via sample imaging. The data shows that increasing the internal water concentration results in a slight increase in mean droplet size. Over time however, although droplet growth was observed, there was no complete emulsion failure for any system, irrespective of phase volume. One-way ANOVA tests performed using droplet sizes for all formulations shown in

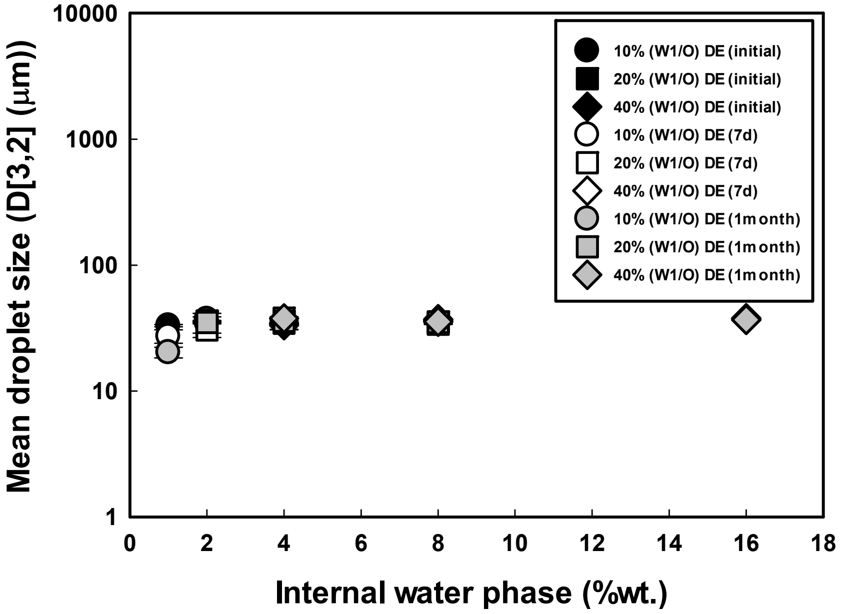

Figure 5-5 gave a p value < 0.05 highlighting no statistically significant differences amongst droplet sizes regardless of emulsion composition. In terms of stability and double emulsion droplet size variation over time, one-way ANOVA tests also showed that there was no significant difference between initial droplet size and droplet size following 1 month of storage for these double emulsion systems, irrespective of phase volume formulation.

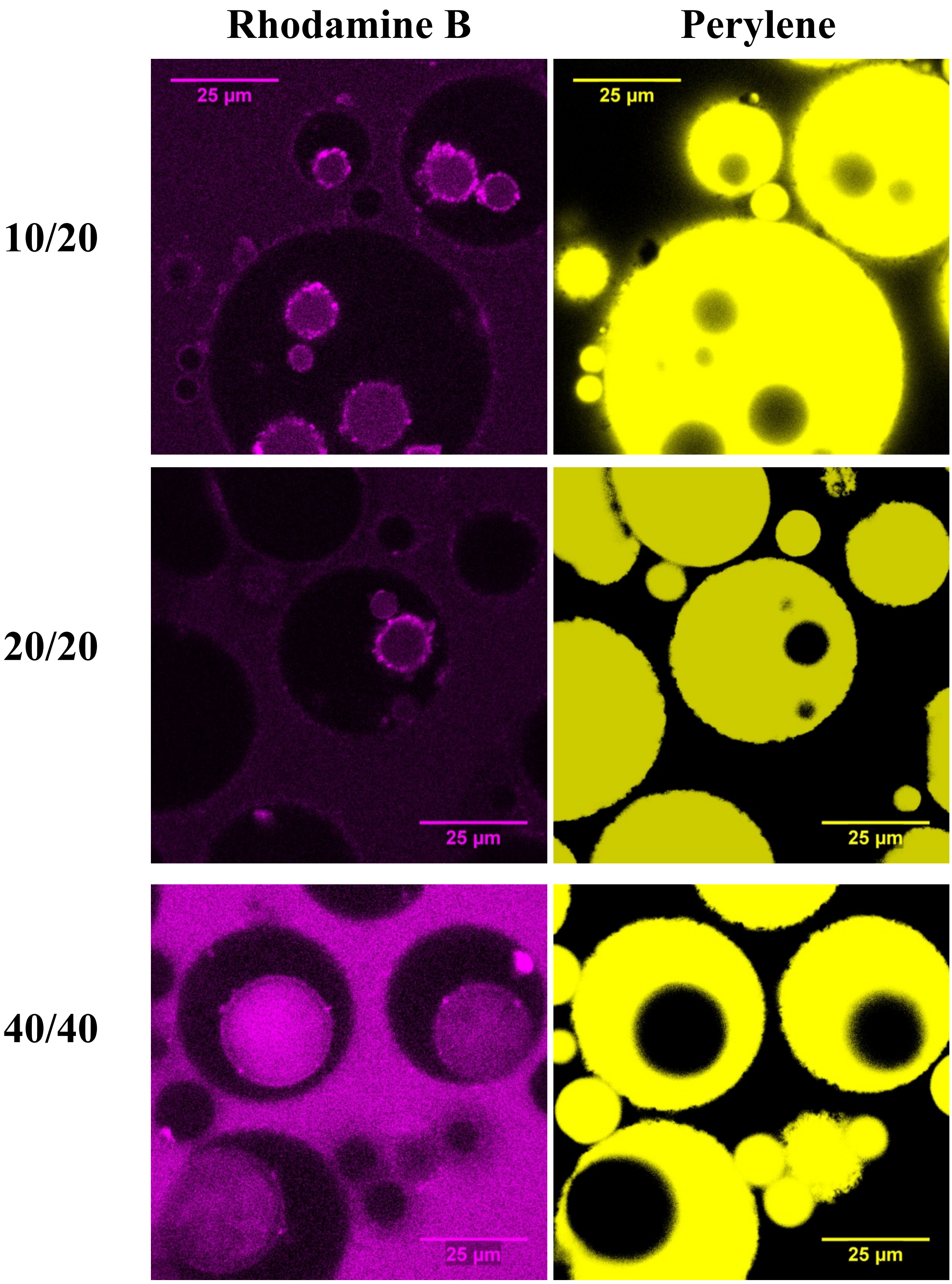

Figure 5-7. Confocal micrographs (of 10/20, 20/20 and 40/40) to compare inner water droplet sizes

Confocal micrographs (Figure 5-7) confirm the presence of double emulsion structures and support droplet sizing measurements of the overall double emulsion structure. 40/40 double emulsion droplets appear to show larger inner water droplets compared to the other two systems (10/20 and 20/20 emulsion systems), however this is in line with the associated 40 % W/O primary emulsion droplets which are larger than the 10% and 20% W/O primary emulsion droplets (Figure 5-6). Nevertheless, confocal imaging is not able to successfully provide solid quantitative data for internal water droplet encapsulation. Analysis of the thermoanalytical curves of the W/O/W and corresponding simple W/O emulsions allows for the characterisation of inner water droplet yield during the secondary emulsification step. To this end, DSC measurements were performed in an attempt to address this concern with regard to these Pickering only systems.

5.4.6 Influence of processing and mass fraction on encapsulation efficiency

The DSC profiles of different double emulsion formulations (shown in Table 5-1) and emulsions prepared with processing methods described previously, in order to analyse their effect on primary water droplet encapsulation and retention over time. Typically, the thermoanalytical profile of a W/O/W double emulsion displays two distinct peaks each corresponding to the crystallisation transitions of the two different water phases present within the system; where there is only one aqueous phase (as is the case in a simple emulsion), only one peak will exist [48]. It has been reported in literature that with double emulsions, solidification of the external water phase normally occurs at a much higher temperature in comparison to the inner water droplets [49], and the temperature at which they crystallise, T_c, can indicate droplet size; smaller droplets freeze at lower temperatures. Literature reviews of such studies have been published and can provide further reading regarding the mechanisms by which this occurs [50, 51]. More recent literature suggests that measurement of the thermoanalytical curve of the corresponding primary W/O emulsion combined with the calculation of the area under the peak corresponding to internal water droplets within the double emulsion profile, encapsulation efficiency of the double emulsion structures can be determined [40, 43].

The thermoanalytical profile for an EC stabilised 10% W/O emulsion and the corresponding EC and rutin hydrate stabilised W/O/W emulsion (where 20 %w/w of the primary emulsion constituted the final double emulsion formulation) is shown in Figure 5-8. As an example, values for $\Delta H'$ would be derived from the peak at approximately –38˚C on the W/O/W profile, representing the internal water phase.

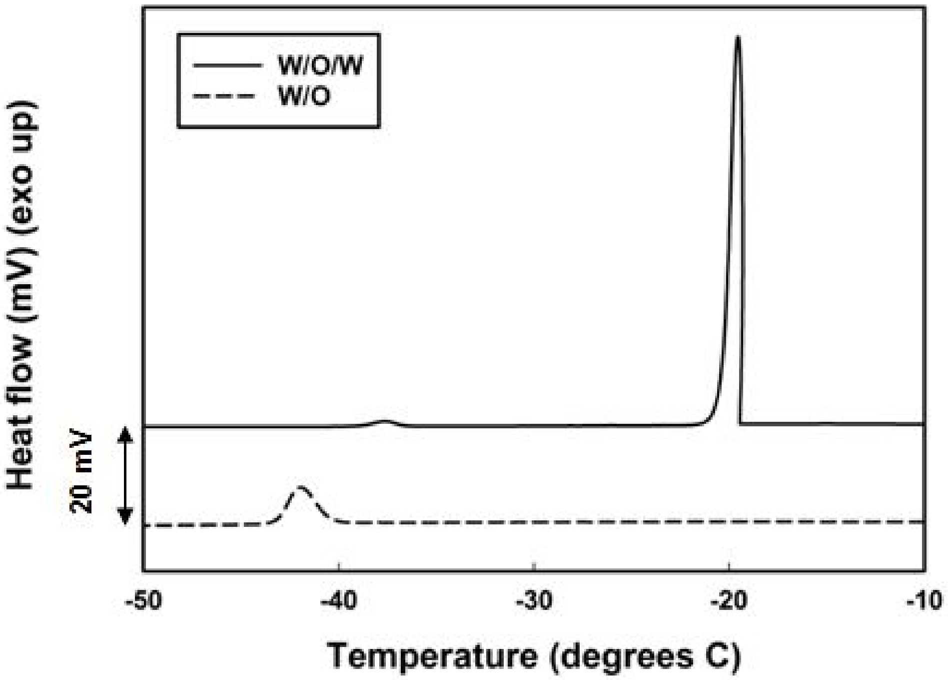

Figure 5-8. Thermoanalytical DSC profiles of a 10/20 double W/O/W emulsion stabilised using EC for the primary interface and rutin hydrate for the secondary interface and the corresponding EC stabilised 10% W/O primary emulsion used to formulate the final double emulsion structure. Measurements were taken immediately following sample preparation.

DSC analysis of the double emulsions and respective primary W/O emulsions produced using different phase volumes (Figure 5-6) and different processing routes (Figure 5-2 and Figure 5-3) not only allows for a better understanding of initial encapsulation efficiencies following the second emulsification step but also primary droplet retention over time. Thermoanalytical curves for double emulsions using the 10/20, 20/20 and 40/40 formulations, those formed via different processes, and the corresponding primary W/O emulsions were compared. This was done in order to investigate processing influence and the effect of increasing the primary emulsion concentration on encapsulation as well as further understanding the mechanism responsible for allowing the droplet size of these systems to remain constant (Figure 5-6) whilst phase volume changes.

Using the thermoanalytical curves for the different simple and double emulsions as well as those taken upon storage, it was possible to derive the initial encapsulation efficiency (EE) for the formulations that produced double emulsion structures, and how the internal droplets behave over time. Such values are exhibited in Table 5-2 and EE and T_c following preparation and after 1 month for the different systems are depicted in Figure 5-9.

Table 5-2 Mean $\Delta H'$ and $\Delta H''$ (J/g) values, for all of the different systems as a function of time (initial and 1 month), and their respective mass fractions, φ' and φ'', in the final double emulsion formulations. Measurements were performed in triplicate and ± represents 1 standard deviation.

EMULSION NAME	Emulsification methods	Initial $\Delta H'$ (J/g)	1 month $\Delta H'$ (J/g)	φ'	$\Delta H''$ (J/g)	φ''
10/20a	PM1/SM1	3.44 ± 1.52	*1.74 ± 0.99*	0.02	40.98 ± 4.56	0.20
20/20	PM1/SM1	3.45 ± 1.95	*2.21 ± 1.24*	0.04	35.26 ± 23.26	0.20
40/40	PM1/SM1	1.45 ± 0.07	*1.13 ± 0.60*	0.16	52.14 ± 31.25	0.40
10/20b	PM2/SM1	1.18 ± 1.25	*4.09 ± 0.72*	0.02	38.27 ± 21.57	0.20
10/20c	PM3/SM1	1.15 ± 0.74	*3.79 ± 1.54*	0.02	15.92 ± 14.91	0.20
10/20d	PM1/SM3	5.92 ± 1.72	*1.81 ± 0.67*	0.02	40.98 ± 4.56	0.20

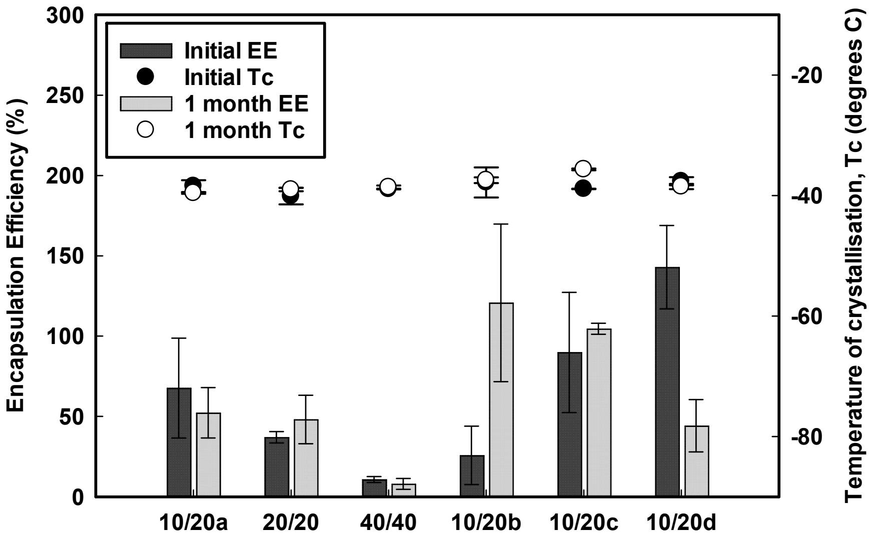

Figure 5-9. Encapsulation efficiencies, EE (%) (vertical bar plots) and temperature of crystallisation, Tc (˚C) (scatter plots) of double emulsions of differing mass fractions and different processing routes over time.

In terms of the primary processing route, the 10/20c double emulsions formed through PM3/SM1 processing methods had the highest EE values (Figure 5-9). This primary emulsification route was found to produce the largest double emulsion droplets despite having the smallest primary w/o droplets in contrast to the other primary emulsification routes (Figure 5-2); the other two double emulsions formed via PM1 and PM2 were comparable in mean droplet size. Previous literature report that double emulsions with larger oil droplets tended to have higher EE [42], and results shown here are in agreement. The higher EE of the PM3/SM1 double emulsions compared to the other SM1 systems confirms that a larger proportion of the primary droplets were encapsulated alluding to a primary droplet size dependency, more w/o droplets of an appropriate size were encapsulated.

Where the secondary emulsification process was altered and SM3 was used as an alternative, the largest emulsion droplets were formed in conjunction with the highest encapsulation efficiency overall (Figure 5-3a and Figure 5-9), once again in agreement with findings in literature. These 10/20d emulsions had initial EE values surpassing 100 % which points towards an internal water mass exceeding the input from the primary W/O emulsion droplets. In this case it seems that a proportion of the external water phase was captured within the internal phase during the secondary emulsification step. It appears that as the primary emulsion is introduced to the system during the second processing step, primary W/O droplets were disrupted and increased in size prior to encapsulation within the oil phase of the final double emulsions structure due to the low shear imparted by SM3. As a consequence, smaller W/O droplets did not reform as with other higher shear processes i.e. SM1. Not only did this lead to the creation of larger oil droplets, but also larger encapsulated W/O droplets.

With respect to phase volume, an increase in the mass fraction of the inner water phase yielded a reduction in EE and this can also be related back to primary emulsion droplet size. The droplet size distribution for 10% and 20% W/O emulsions are very similar, the main difference being the development of a peak at ~1000 µm with the 20% W/O emulsion which increased in area with 40% W/O (Figure 5-6). The bimodality and consequent high polydispersity of these droplet size distributions means that the number of primary droplets of an appropriately small size readily available for encapsulation upon commencement of the secondary emulsification step is significantly reduced. As a consequence, lower EE are observed for the related double emulsions. These findings not only suggest that larger oil droplets tend to equate to a higher EE but that small primary droplet size is also an influencing factor. Secondly, it also reinforces the fact that the primary W/O emulsion droplets undergo minimal disruption during the second processing step, SM1.

For most systems, following 1 month storage at room temperature, EE did not significantly vary indicating a high level of stability of both interfaces and minimal inner droplet diffusion to the outer aqueous phase. This correlates well with the minimal droplet size change over time (Figure 5-2, Figure 5-3 and Figure 5-6). The only exceptions were 10/20b and 10/20d samples. The 10/20b double emulsions EE increased over time. As mentioned prior, initially the droplet size of these emulsions was comparable to those of 10/20a double emulsions, although after 1 month, 10/20a droplet size distribution became slightly wider, whereas 10/20b mean droplet size increased (Figure 5-2b). This analogous increase in EE with droplet size over time is indicative of oil droplet coalescence, which in turn promotes internal water droplet coalescence and a subsequent increase in inner droplet size creating a pseudo increase in EE. With respect to the 10/20d emulsions, although droplet sizes for this formulation remained stable to complete emulsion failure, there was a significant decrease in $\Delta H'$ and EE following storage (Figure 5-9 and Table 5-2). Signifying a loss of internal water phase, this was corroborated with micrographs captured after initial preparation and after 1 month (Figure 5-10).

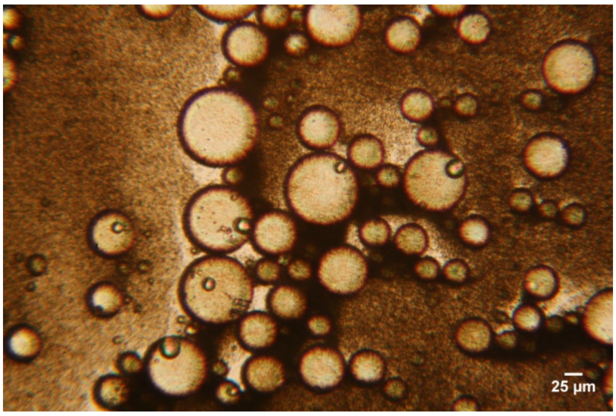

Figure 5-10.Micrograph of 10/20d double emulsion following 1 month storage. Scale bar represents 25 µm.

This loss of inner water phase was potentially due to the large double emulsion droplet size and accompanying wide droplet size distribution, promoting coalescence by way of Ostwald ripening [52].

The temperatures of crystallisation, T_c, of the peaks relating to the inner water droplets on the W/O/W thermoanalytical profiles are displayed in Figure 5-9. T_c was consistent across all double emulsions, initially and Schuch et al., 2013 state that the smaller the droplets, the lower the number of water molecules can become the centre of a critical germ and so freezing temp decreases as droplet size decreases [40]. Remaining constant across all systems initially, values for T_c indicate that there is a critical droplet size for inner water droplets; droplets above that size are not encapsulated. T_c for 10/20c emulsions increased upon 1 month of storage and this suggests an increase in internal droplet size. As it was observed that more primary droplets were encapsulated with this particular system (compared to the other SM1 formulations), inner droplet coalescence is more likely to occur due to the higher number of droplet in close proximity. Despite this, these double emulsion droplets were still stable against complete reversion to a simple emulsion or phase separation.

5.5 Conclusions

The aim of this work was to use Pickering stabilisation in an attempt to formulate food-grade W/O/W emulsions, in the absence of silica or any additional known surfactants or stabilisers. Furthermore, it was the aspiration to investigate the effects of processing route and mass fraction alterations on emulsion microstructure and encapsulation efficiency.

For the first time, Pickering w/o/w emulsions were successfully synthesised using EC and rutin hydrate particles only and possessed an excellent level of stability against emulsion failure or conversion to a simple o/w emulsion. It was also possible to increase the internal water from 1 %w/w to 16 %w/w and maintain comparable overall double emulsion droplet sizes and stability. The high level of stability of the double emulsion interfaces, also confirmed with DSC via analysis of T_c, diversifies the potential applications of these systems. Not only has the suitability of these systems for fat reduction purposes been realised, but active ingredient encapsulation and taste masking can potentially be more easily achieved.

DSC allowed for the successful measurement of encapsulation efficiency and, in terms of EE correlation to overall emulsion droplet size, results were in agreement with those reported in literature [41-43]. Although such studies found that oil droplet size is directly proportional to primary droplet yield, it is apparent here that internal water droplet size is also detrimental to encapsulation efficiency within these systems. Internal droplets must be below ~ 30 μm (the secondary oil droplet size) prior to inclusion in the formulation during the secondary emulsification step.

Rotor stator mixing for both emulsification steps was found to be the most suitable for the production of these systems with appropriately sized double emulsion droplets [46]. However, prolonged processing of emulsions resulted in a reduction of internal water droplets, due to the repeated exposure of EC particles to the external aqueous phase. Moving forward, alternative 'drop forming' methods such as membrane emulsification could be applied to these Pickering systems in order to further control internal water droplet encapsulation [53]. However, the low fluxes and membrane pore blocking issues associated with such methods still remain barriers to large scale mass production of these types of particle stabilised double emulsions. From a commercial standpoint, future work can focus on understanding the sensory properties of these formulations and efforts made to qualify the systems for consumer taste acceptance. Additionally, studies investigating the integration of food ingredients, common in downstream food products, such as salts, active ingredients, thickeners and stabilisers must also be considered.

References

[1] Garti, N., Double emulsions — scope, limitations and new achievements. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **1997**, *123–124*, p. 233-246.
[2] Seifriz, W., Studies in emulsions. Iii-v. *The Journal of Physical Chemistry,* **1924**, *29*, p. 738-749.

[3] Muschiolik, G., Multiple emulsions for food use. *Current Opinion in Colloid & Interface Science,* **2007**, *12*, p. 213-220.

[4] Takahashi, Y., Yoshida, T., and Takahashi, T., Process for producing a w/o/w type multiple emulsion for medicines, cosmetics, etc. ed**1990**. Google Patents.

[5] Alberini, F., Dapelo, D., Enjalbert, R., Van Crombrugge, Y., and Simmons, M.J.H., Influence of dc electric field upon the production of oil-in-water-in-oil double emulsions in upwards mm-scale channels at low electric field strength. *Experimental Thermal and Fluid Science,* **2017**, *81*, p. 265-276.

[6] Giroux, H.J., Robitaille, G., and Britten, M., Controlled release of casein-derived peptides in the gastrointestinal environment by encapsulation in water-in-oil-in-water double emulsions. *LWT - Food Science and Technology,* **2016**, *69*, p. 225-232.

[7] Tyowua, A.T., Yiase, S.G., and Binks, B.P., Double oil-in-oil-in-oil emulsions stabilised solely by particles. *Journal of Colloid and Interface Science,* **2017**, *488*, p. 127-134.

[8] Kumar, R., Kumar, M.S., and Mahadevan, N., Multiple emulsions: A review. *International Journal of Recent Advances in Pharmaceutical Research,* **2012**, *2*, p. 9-19.

[9] van der Graaf, S., Schroën, C.G.P.H., and Boom, R.M., Preparation of double emulsions by membrane emulsification—a review. *Journal of Membrane Science,* **2005**, *251*, p. 7-15.

[10] Frasch-Melnik, S., Fat crystal-stabilised double emulsions, PhD,**2011**, University of Birmingham, UK.

[11] Florence, A.T. and Whitehill, D., Some features of breakdown in water-in-oil-in-water multiple emulsions. *Journal of Colloid and Interface Science,* **1981**, *79*, p. 243-256.

[12] Midmore, B.R. and Herrington, T.M., Silica-stabilised multiple emulsions, in *Trends in colloid and interface science xiii* **1999**. *112*, D. Težak and M. Martinis, Eds., Progress in colloid and polymer science: Springer Berlin Heidelberg, p. 115-120.

[13] Sekine, T., Yoshida, K., Matsuzaki, F., Yanaki, T., and Yamaguchi, M., A novel method for preparing oil-in-water-in-oil type multiple emulsions using organophilic montmorillonite clay mineral. *Journal of Surfactants and Detergents,* **1999**, *2*, p. 309-315.

[14] Carrillo, C.A., Nypelö, T.E., and Rojas, O.J., Cellulose nanofibrils for one-step stabilization of multiple emulsions (w/o/w) based on soybean oil. *Journal of Colloid and Interface Science,* **2015**, *445*, p. 166-173.

[15] Ramsden, W., Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation). -- preliminary account. *Proceedings of the Royal Society of London,* **1903**, *72*, p. 156-164.

[16] Pickering, S.U., Emulsions. *Journal of the Chemical Society,* **1907**, *91*, p. 2001 - 2021.

[17] Dickinson, E., Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science,* **2010**, *15*, p. 40-49.

[18] Garti, N., Binyamin, H., and Aserin, A., Stabilization of water-in-oil emulsions by submicrocrystalline alpha-form fat particles. *Journal of the American Oil Chemists Society,* **1998**, *75*, p. 1825-1831.

[19] Binks, B.P. and Whitby, C.P., Nanoparticle silica-stabilised oil-in-water emulsions: Improving emulsion stability. *Colloids and Surfaces a-Physicochemical and Engineering Aspects,* **2005**, *253*, p. 105-115.

[20] Pawlik, A., Cox, P.W., and Norton, I.T., Food grade duplex emulsions designed and stabilised with different osmotic pressures. *Journal of Colloid and Interface Science,* **2010**, *352*, p. 59-67.

[21] Fernández-Martín, F., Freire, M., Bou, R., Cofrades, S., and Jiménez-Colmenero, F., Olive oil based edible w/o/w emulsions stability as affected by addition of some acylglycerides. *Journal of Food Engineering,* **2017**, *196*, p. 18-26.

[22] Garti, N., Aserin, A., Tiunova, I., and Binyamin, H., Double emulsions of water-in-oil-in-water stabilized by alpha-form fat microcrystals. Part 1: Selection of emulsifiers and fat microcrystalline particles. *Journal of the American Oil Chemists Society,* **1999**, *76*, p. 383-389.

[23] Binks, B.P., Dyab, A.K.F., Fletcher, P.D.I., and Barthel, H., Multiple emulsions, Germany Patent, **2002**.

[24] Matos, M., Timgren, A., Sjoo, M., Dejmek, P., and Rayner, M., Preparation and encapsulation properties of double pickering emulsions stabilized by quinoa starch granules. *Colloids and Surfaces a-Physicochemical and Engineering Aspects,* **2013**, *423*, p. 147-153.

[25] Marefati, A., Sjoo, M., Timgren, A., Dejmek, P., and Rayner, M., Fabrication of encapsulated oil powders from starch granule stabilized w/o/w pickering emulsions by freeze-drying. *Food Hydrocolloids,* **2015**, *51*, p. 261-271.

[26] Lutz, R., Aserin, A., Wicker, L., and Garti, N., Double emulsions stabilized by a charged complex of modified pectin and whey protein isolate. *Colloids and Surfaces B: Biointerfaces,* **2009**, *72*, p. 121-127.

[27] Binks, B.P., Particles as surfactants—similarities and differences. *Current Opinion in Colloid & Interface Science,* **2002**, *7*, p. 21-41.

[28] Aveyard, R., Binks, B.P., and Clint, J.H., Emulsions stabilised solely by colloidal particles. *Advances in Colloid and Interface Science,* **2003**, *100–102*, p. 503-546.

[29] Garrec, D.A., Frasch-Melnik, S., Henry, J.V.L., Spyropoulos, F., and Norton, I.T., Designing colloidal structures for micro and macro nutrient content and release in foods. *Faraday Discussions,* **2012**, *158*, p. 37-49.

[30] Williams, M., Warren, N.J., Fielding, L.A., Armes, S.P., Verstraete, P., and Smets, J., Preparation of double emulsions using hybrid polymer/silica particles: New pickering emulsifiers with adjustable surface wettability. *Acs Applied Materials & Interfaces,* **2014**, *6*, p. 20919-20927.

[31] Zou, S.W., Liu, H., Yang, Y., Wei, Z.J., and Wang, C.Y., Multihollow nanocomposite microspheres with tunable pore structures by templating pickering double emulsions. *Reactive & Functional Polymers,* **2013**, *73*, p. 1231-1241.

[32] Dyab, A.K.F., Macroporous polymer beads and monoliths from pickering simple, double, and triple emulsions. *Macromolecular Chemistry and Physics,* **2012**, *213*, p. 1815-1832.

[33] Hertog, M.G.L., Feskens, E.J.M., Kromhout, D., Hollman, P.C.H., and Katan, M.B., Dietary antioxidant flavonoids and risk of coronary heart disease: The zutphen elderly study. *The Lancet,* **1993**, *342*, p. 1007-1011.

[34] Amaretti, A., Raimondi, S., Leonardi, A., Quartieri, A., and Rossi, M., Hydrolysis of the rutinose-conjugates flavonoids rutin and hesperidin by the gut microbiota and bifidobacteria. *Nutrients,* **2015**, *7*, p. 2788.

[35] Chat, O.A., Najar, M.H., Mir, M.A., Rather, G.M., and Dar, A.A., Effects of surfactant micelles on solubilization and dpph radical scavenging activity of rutin. *Journal of Colloid and Interface Science,* **2011**, *355*, p. 140-149.

[36] Luo, Z.J., Murray, B.S., Yusoff, A., Morgan, M.R.A., Povey, M.J.W., and Day, A.J., Particle-stabilizing effects of flavonoids at the oil-water interface. *Journal of Agricultural and Food Chemistry,* **2011**, *59*, p. 2636-2645.

[37] Mallipeddi, R., The application of coarse particle ethylcellulose and high molecular weight polyethylene oxide in the production of beads by extrusion-spheronization, 3349136 Ph.D.,**2009**, University of the Sciences in Philadelphia, Ann Arbor.

[38] Jin, H., Zhou, W., Cao, J., Stoyanov, S.D., Blijdenstein, T.B.J., de Groot, P.W.N., Arnaudov, L.N., and Pelan, E.G., Super stable foams stabilized by colloidal ethyl cellulose particles. *Soft Matter,* **2012**, *8*, p. 2194-2205.

[39] Melzer, E., Kreuter, J., and Daniels, R., Ethylcellulose: A new type of emulsion stabilizer. *European Journal of Pharmaceutics and Biopharmaceutics,* **2003**, *56*, p. 23-27.

[40] Schuch, A., Deiters, P., Henne, J., Köhler, K., and Schuchmann, H.P., Production of w/o/w (water-in-oil-in-water) multiple emulsions: Droplet breakup and release of water. *Journal of Colloid and Interface Science,* **2013**, *402*, p. 157-164.

[41] Schuch, A., Tonay, A.N., Köhler, K., and Schuchmann, H.P., Influence of the second emulsification step during production of w/o/w multiple emulsions: Comparison of different methods to determine encapsulation efficiency in w/o/w emulsions. *The Canadian Journal of Chemical Engineering,* **2014**, *92*, p. 203-209.

[42] Schuch, A., Wrenger, J., and Schuchmann, H.P., Production of w/o/w double emulsions. Part ii: Influence of emulsification device on release of water by coalescence. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2014**, *461*, p. 344-351.

[43] Oppermann, A.K.L., Renssen, M., Schuch, A., Stieger, M., and Scholten, E., Effect of gelation of inner dispersed phase on stability of (w1/o/w2) multiple emulsions. *Food Hydrocolloids,* **2015**, *48*, p. 17-26.

[44] Pays, K., Giermanska-Kahn, J., Pouligny, B., Bibette, J., and Leal-Calderon, F., Coalescence in surfactant-stabilized double emulsions. *Langmuir,* **2001**, *17*, p. 7758-7769.

[45] Silva, E.K., Rosa, M.T.M.G., and Meireles, M.A.A., Ultrasound-assisted formation of emulsions stabilized by biopolymers. *Current Opinion in Food Science,* **2015**, *5*, p. 50-59.

[46] Norton, I., Fryer, P., and Moore, S., Product/process integration in food manufacture: Engineering sustained health. *AIChE Journal,* **2006**, *52*, p. 1632-1640.

[47] Duffus, L.J., Norton, J.E., Smith, P., Norton, I.T., and Spyropoulos, F., A comparative study on the capacity of a range of food-grade particles to form stable o/w and w/o pickering emulsions. *Journal of Colloid and Interface Science,* **2016**, *473*, p. 9-21.

[48] Kovács, A., Csóka, I., Kónya, M., Csányi, E., Fehér, A., and Erős, I., Structural analysis of w/o/w multiple emulsions by means of dsc. *Journal of Thermal Analysis and Calorimetry*, *82*, p. 491-497.

[49] Potier, L., Raynal, S., Seiller, M., Grossiord, J.L., and Clausse, D., Study of state transitions within multiple w/o/w emulsions using calorimetry (dsc). *Thermochimica Acta,* **1992**, *204*, p. 145-155.

[50] Clausse, D., Gomez, F., Pezron, I., Komunjer, L., and Dalmazzone, C., Morphology characterization of emulsions by differential scanning calorimetry. *Advances in Colloid and Interface Science,* **2005**, *117*, p. 59-74.

[51] Clausse, D., Differential thermal analysis, differential scanning calorimetry, and emulsions. *Journal of Thermal Analysis and Calorimetry,* **2010**, *101*, p. 1071-1077.

[52] Taylor, P., Ostwald ripening in emulsions. *Advances in Colloid and Interface Science,* **1998**, *75*, p. 107-163.

[53] Pawlik, A.K. and Norton, I.T., Encapsulation stability of duplex emulsions prepared with spg cross-flow membrane, spg rotating membrane and rotor-stator techniques—a comparison. *Journal of Membrane Science,* **2012**, *415–416*, p. 459-468.

Chapter 6

Overall Conclusions

6.1 Objectives

The work detailed within this thesis has focussed on the design and development of W/O/W double emulsions stabilised solely by edible particles. In order to achieve this, the objectives were as follows:

- To investigate different “as-obtained” edible particles spanning a range of chemistries and material compositions with respect to their Pickering functionality.
- To design double W/O/W emulsions using Pickering stabilisation at either the W/O internal interface or the external O/W interface in order to understand whether replacement of surfactant by particles produces any additional stability.
- To design double W/O/W emulsions using only edible Pickering particles to stabilise emulsion droplets.
- To study and develop an understanding of the effect of the primary and secondary emulsification steps and internal water phase concentration on Pickering-only double W/O/W emulsion stability and microstructure.
- To characterise inner water phase and determine inner droplet encapsulation efficiency as a function of the formulation and processing route used.

6.2 Conclusions

Initially, the principle aim of this work was to identify and analyse particulate structures that could potentially act as Pickering particles with minimal or no alteration to their chemical structure. A wide range of particles were sourced and filtered and consequently only a select few were carried forward. Analysis of the different particle types under the same formulation and processing parameters, allowed for the development of a more specific and accurate set of design rules for Pickering particles which were shown to be applicable to a wider range of Pickering candidate materials for use in food emulsions. It was found that the main prerequisites for particles to be able to stabilise small emulsion droplets are small particle size ranging between 200 nm and 1 μm, an affinity for the continuous phase and particle charge to aid stability. It appeared that particle surface activity appears to be a less significant factor as there was no significant correlation found to exist between particle affectation of oil / water interfacial tension and emulsion stability when compared to the surfactant systems mainly due to larger particle size (in comparison to surfactant micelles) and sedimentation effects. These design rules were used to predict particle stabilised emulsion characteristics prior to emulsion formulation, thus facilitating particle selection in food emulsion research and development efforts. In producing simple W/O and O/W emulsions using the particles identified, it was found that lower particle concentrations (1.5 – 3 %w/w) could be used to stabilise droplets compared to amounts typically used in industry (c. 10 %w/w), potentially improving cost effectiveness of systems commercially. This work on simple emulsions also included a comparative study on the use of high and low shear emulsification techniques to produce emulsions and it was found that nanoemulsions could be fabricated using the high shear process and micron sized particles, Naringin and CMCC, were able to stabilise nanosized droplets. This indicated that the high shear process was able to fragment particles during emulsification that were then stable enough to successfully stabilise the oil-water interface and create stable nanoemulsions.

Based on these findings, work then focussed on the combination of Pickering stabilisation and surfactants in an attempt to formulate W/O/W emulsions. Experiments were designed to investigate any benefits arising from the use of the edible Pickering particles identified at one of the interfaces of a double emulsion in conjunction with known conventional emulsifiers. In particular, focus was placed upon understanding differences in droplet size, emulsion stability and internal water droplet retention. As was expected, the long term stability of double emulsions was extended via the employment of particles at either interface replacing surfactant in contrast to the surfactant-only stabilised double emulsions, due to the enhanced stability of the oil / water interface provided by Pickering stabilisation. It was also found that the stability of the internal W/O interface in a double emulsion is more dependent upon the material properties whereas the formation and stability of the secondary outer O/W interface was primarily process driven. Principally, the compatibility of the emulsifiers used is vital in double emulsion stability. If the two materials used were too similar in terms of their hydrophilic / lipophilic character, no double emulsion structure forms or resulting emulsions were extremely unstable irrespective of how stable the equivalent simple emulsions were.

Where PGPR was used to stabilise the primary interface, and particles were used to stabilise the external interface, the thermoanalytical profiles (derived from the DSC measurements) of the more stable double emulsions displayed a higher number of smaller internal water droplets. However despite this, when performing the DSC measurements on the PGPR double emulsion systems there was evidence of migration of internal water droplets to the external aqueous phase which did not occur with the EC emulsion systems. This further indicated that even though PGPR formed smaller W/O droplets, those stabilised by EC were less susceptible to microstructural transformation due to environmental changes and hence were more stable. This migration occurring with the PGPR double emulsion systems during the course of the DSC measurement also signifies the need for additional analysis techniques (i.e. microscopy) to be used in conjunction with techniques such as DSC for double emulsion characterisation.

Following on from this, investigations were made into the use of these edible particulate materials for the stabilisation of W/O/W double emulsions in the absence of any additional surfactants, stabilisers, or emulsifiers. The influence of the emulsification process on microstructure, droplet size and stability of these novel systems was also explored. Focus was also placed upon understanding the extent to which the formulation can serve as tool for fat content reduction through phase volume manipulation. The encapsulation efficiency of double emulsions was also calculated and the way in which this is affected by processing route and phase mass fraction was evaluated. Consequently, Pickering W/O/W emulsions were successfully synthesised, without the use of any known emulsifiers or stabilisers, using EC and rutin hydrate particles only and the emulsions possessed a superior level of stability against emulsion failure or conversion to a simple O/W emulsion compared to surfactant stabilised double emulsions. Moreover, it was possible to increase the internal water concentration from 1 %w/w to 16 %w/w and maintain comparable overall double emulsion droplet sizes and stability. Through the use of DSC measurements, it was possible to build a thermoanalytical profile of the different double emulsion systems and better understand internal water droplet characteristics through analysis of the enthalpy change during crystallisation associated with these internal droplets. It was apparent here that internal water droplet size is detrimental to encapsulation efficiency within these systems. Internal droplets must be below ~ 30 μm (i.e. the secondary oil droplet size) prior to the secondary emulsification step to ensure encapsulation efficiency is maximised.

In terms of the emulsification process, rotor stator mixing for both emulsification steps was found to be the most suitable for the production of these systems in terms of stability, as it created enough shear to allow droplets to form and particles to accumulate at the interface without significantly disrupting the primary emulsion during the secondary emulsification step. However prolonged processing of emulsions resulted in a reduction of the number of internal water droplets, due to the repeated exposure of EC particles to the external aqueous phase. This promoted particle aggregation during droplet break up and formation during the course of emulsification, reducing the probability of complete droplet coverage of particles and hence the formation of droplets small enough for encapsulation.

.

Chapter 7

Future Work

1. *Analysis of a larger and even more diverse range of edible particulate materials in order to further define the particle design rules developed*

The ability to successfully analyse a range of different particles in the same manner for use as emulsifiers was demonstrated. These learnings could be applied to a further set of differing particle types that fit the criteria for use in food emulsions, to allow the particle design rules to be more finely tuned and applicable to an even wider set of particle types. Additionally, the simple emulsion work described in Chapter 3 showed that network stabilisation plays a large role in the stability of some of these types of emulsions, both O/W and W/O, and that this is not easily represented in traditional emulsion droplet size characterisation procedures. Future experiments could be performed to investigate alternate ways of analysing these types of Pickering emulsions.

2. *Determination of the effect of electrolytes inclusion in these double emulsion formulations on emulsion stability and microstructure*

With regard to the double emulsions, no attempts were made here to balance the osmotic pressure within these systems and consequently further studies should involve the introduction of electrolytes (i.e. salts, proteins) to these formulations to deter water diffusion across the oil layer and consequently stem emulsion destabilisation. This may lead to the formation of stable W/O/W emulsions arising from some of the formulations shown here that were previously unsuccessful and even to more complex emulsion microstructures such tertiary or quaternary emulsions.

3. *Develop a deeper understanding of the rheological profile of these double emulsions and how this compares to simple Pickering emulsions and conventional surfactant stabilised emulsions*

Some preliminary rheological experiments were conducted comparing double Pickering- only stabilised emulsions (where ethylcellulose was used to stabilise the internal interface and rutin hydrate particles were used to stabilise the external interface) and O/W rutin hydrate stabilised emulsions. Simple and double emulsions were equivalent in terms of continuous phase concentration and rutin hydrate concentration. Although results showed little difference in rheological behaviour between the two types of emulsions, further work is required to gain a complete understanding of the rheological behaviour of these types of double emulsions in comparison to other surfactant stabilised simple and double emulsions.

4. *Active ingredient encapsulation*

DSC measurements allowed for the analysis of T_c and ΔH values of double emulsions and in turn diversified the analysis tools available for the characterisation of these Pickering double emulsions studies within this work. Furthermore, the high level of stability of the Pickering only stabilised double emulsions in comparison to the surfactant only and Pickering-surfactant stabilised double emulsions was determined. Future experiments investigating active ingredient encapsulation and taste masking can be carried out to expand the industrial application repertoire of these novel double emulsion systems outside of fat reduction in food emulsions and potentially into pharmaceutical formulations.

5. *Developing further knowledge on the effect of applying Particle / Surfactant combinations at the same interface in the double emulsion formulations.*

Some preliminary experiments were performed investigating the use of particle-surfactant mixtures with varying ratios to stabilise the external interface in the double emulsions. Initial results indicated that there was no significant gain in terms of droplet size stability when compared to Pickering-only stabilised double emulsions and this may be due to the presence of some surfactant-only stabilised emulsion droplets. Further experiments are required testing different particle-surfactant combinations and concentrations, assessing the performance of any subsequent double emulsions against the highly stable Pickering only double emulsions studied here. Further exploration of such mixed surfactant-particle systems for use as double emulsion interface stabilisers would allow an opportunity to investigate whether there is any stability benefit to be gained from the employment of such combinations.

6. *Investigation of the fabrication of double emulsions via 'drop forming' emulsification methods to determine whether more unimodal droplet size distributions could be attained.*

In terms of processing, going forward, alternative 'drop forming' methods such as membrane emulsification could be applied to these Pickering systems in order to further control internal water droplet encapsulation volume and encapsulation efficiency. However, the low fluxes and membrane pore blocking issues associated with such methods still remain barriers to large scale mass production of these types of particle stabilised double emulsions.

7. *Developing sensory profiles for these Pickering-only stabilised double emulsion and conversion into commercial food products*

From a commercial standpoint, future work can focus on understanding the sensorial properties of these formulations and efforts could be made to qualify the systems for consumer taste acceptance for use in commercial food emulsions. Additionally, studies investigating the integration of food ingredients, common in downstream food products, such as salts, active ingredients, thickeners and stabilisers must also be considered.

Appendix

Additional Emulsion Images

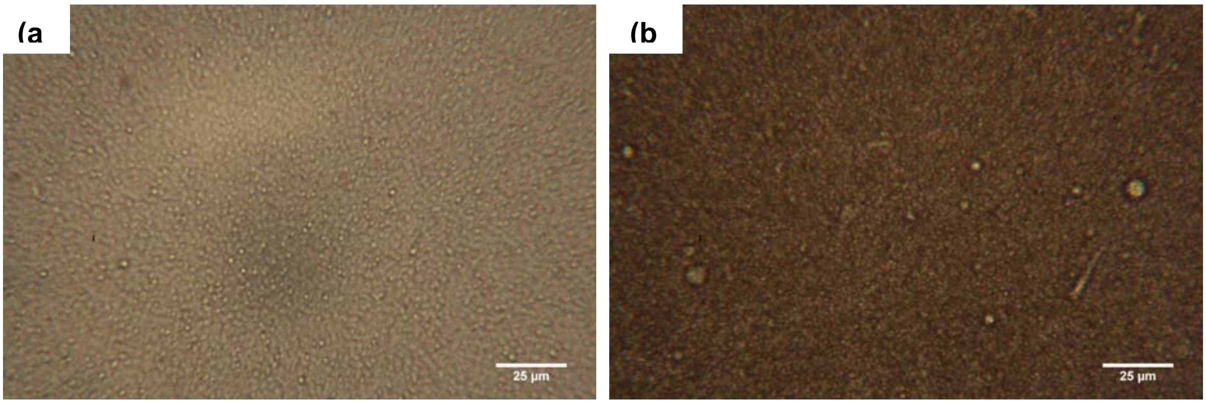

Fig. A1 Simple 20 %w/w O/W emulsions processed via HPH stabilised using (a) naringin and (b) CMCC particles, following preparation.

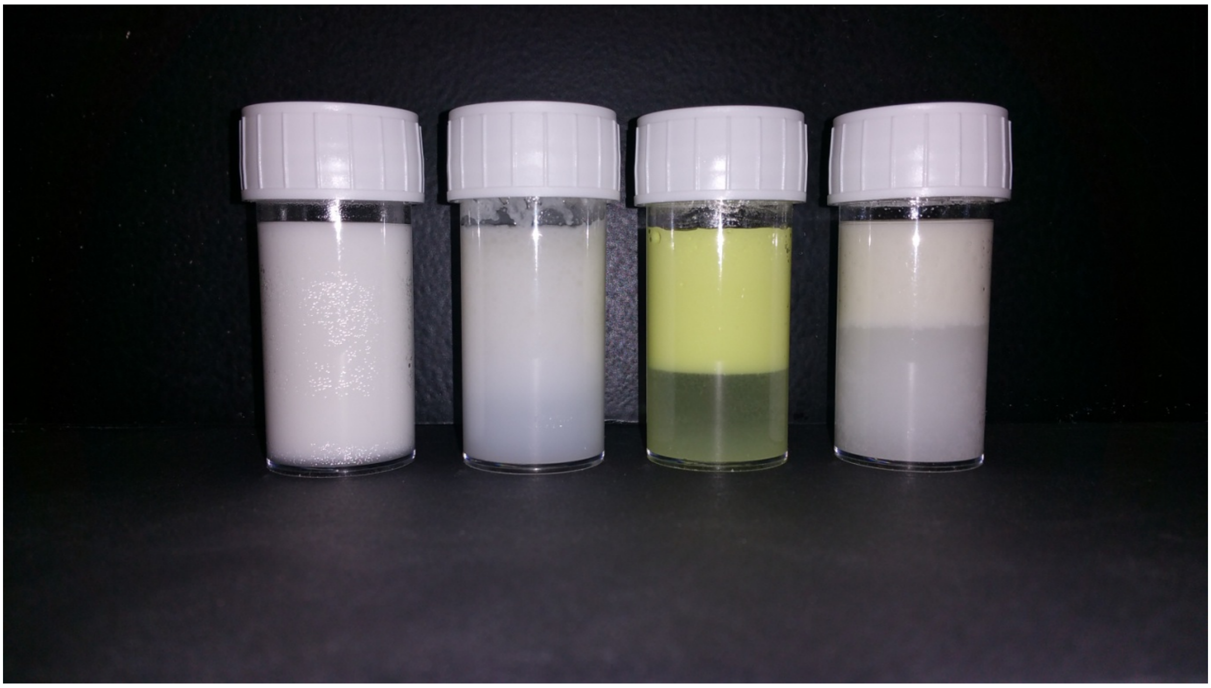

Fig. A2. Simple 20 %w/w O/W emulsions stabilised using: (left to right) HPMC, CMCC, rutin hydrate and naringin particles, following preparation.