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Duffus, Laudina J.; Norton, Jennifer E.; Smith, Paul; Norton, Ian T.; Spyropoulos, Fotios

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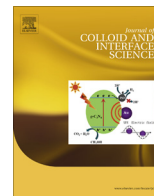
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A comparative study on the capacity of a range of food-grade particles to form stable O/W and W/O Pickering emulsions



Laudina J. Duffus^{a,*}, Jennifer E. Norton^a, Paul Smith^b, Ian T. Norton^a, Fotios Spyropoulos^a

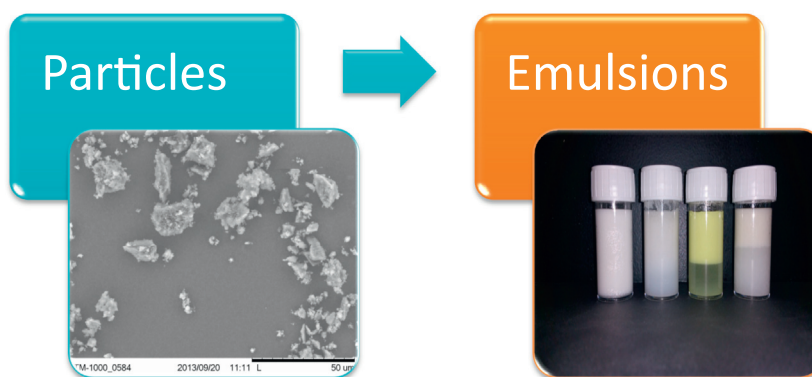
^a School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

^b Cargill, R&D Centre Europe, Havenstraat 84, B-1800 Vilvoorde, Belgium

HIGHLIGHTS

- Comparison of food-grade, as-obtained particles and subsequent emulsions.
- Development of design rules for Pickering particles for food applications.
- Production of stable food-grade Pickering nanoemulsions.

GRAPHICAL ABSTRACT



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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 μ m); 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.

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* Corresponding author.

E-mail address: lx279@bham.ac.uk (L.J. Duffus).

1. 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 largely via an electrostatic 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} ; Eq. (1)) of particles from the liquid–liquid interface [10,11]:

$$E_{det} = \pi r^2 \gamma_{ow} (1 \pm \cos \theta)^2 \quad (1)$$

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 is 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 TiO₂ [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).

2. Materials and methods

2.1. 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).

2.2. Methods

2.2.1. 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 min whilst being agitated with a magnetic stirrer. 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.

2.2.2. Preparation of particle-stabilised emulsions

Following the particle dispersion, 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 min 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, where stated.

2.2.3. Particle and emulsion droplet size measurements

Particle and emulsion droplet size distributions ($D_{3,2}$ and $D_{4,3}$) 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. 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 dusted with gold particles *in situ*, to prevent damage from the electron beam. The double emulsions were next transferred to the measurement chamber (-180 °C), and analysed using a 2 keV beam to prevent excessive damage to the sample.

2.2.4. Stability measurements

2.2.4.1. Droplet size stability. Droplet size stability was assessed over a period of 14 days by droplet sizing techniques described in Section 2.2.3. Measurements were carried out in triplicate with the mean values, unless otherwise stated, given to ± 1 standard deviation.

2.2.4.2. Creaming stability. Creaming stability was assessed in accordance to the Keowmaneechi and McClements' method [33]. 20 g 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:

$$CI = \frac{H_s}{H_e} \times 100\% \quad (2)$$

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

2.2.5. ζ -potential measurements

Particle ζ -potential measurements were 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 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.

2.2.6. 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.

2.2.7. 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 μ L) 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 θ_o (°) 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 s. All measurements were carried out in triplicate and error bars were calculated as ± 1 standard deviation.

3. Results and discussion

3.1. 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.

3.1.1. 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 (t_f : turbulent flow – Eq. (3); l_f : laminar flow – Eq. (4)):

$$t_A^{t_f} \sim \frac{\Gamma_M}{C(\varepsilon d)^{\frac{1}{3}}} \quad (3)$$

$$t_A^{l_f} \sim \frac{\Gamma_M}{C d \dot{\gamma}} \quad (4)$$

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/m² have been measured experimentally [35]. However, Γ_M values for particles can be calculated by

Eq. (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]:

$$\Gamma_M \sim \rho_p \phi_{cp} (4\pi r^3 / 3) / (\pi r^2) \quad (5)$$

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 1; particles were sonicated at 30 s time intervals until no further change to their size was recorded.

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 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 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

Table 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

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. 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.

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.

3.1.2. 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 Fig. 1 and native pH values of aqueous particle dispersions are given within Fig. 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. $>5 \mu\text{m}$) 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.

Table 1

Native particle size (μm), reduced particle size following sonication (μm) and the corresponding span or polydispersity indices (PDI), and E_{det} 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} (kJ)
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

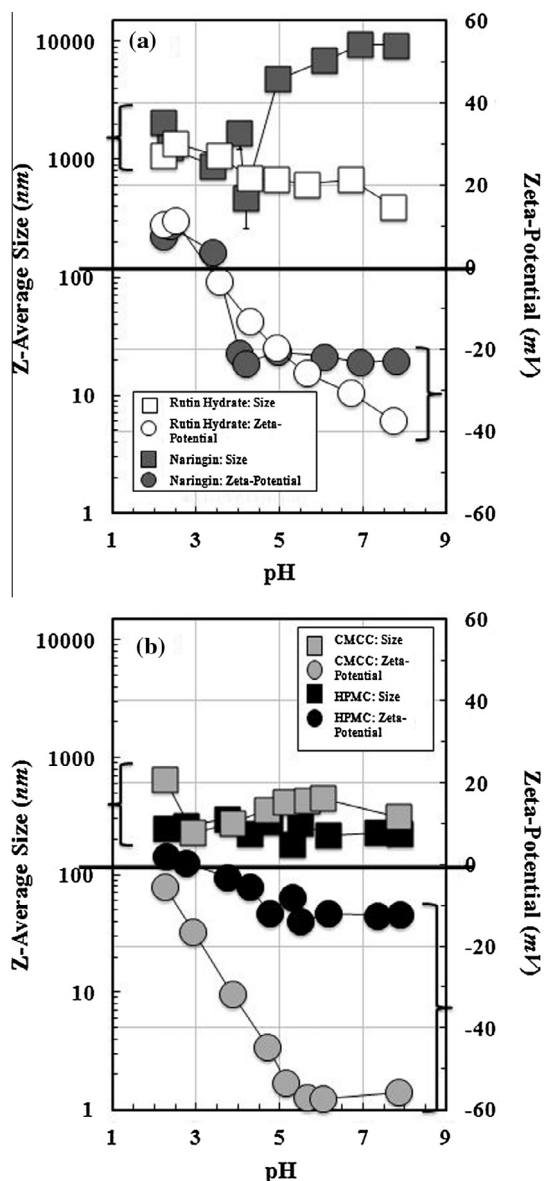


Fig. 1. Particle size and ζ -potential as a function of pH for: (a) flavonoid particles [rutin hydrate and naringin] and (b) cellulose particles [HPMC and CMCC]. Data points are averages of data collected in triplicate and error bars represent 1 standard deviation; where not visible, error bars are smaller than the used symbols. Brackets refer to the y-axis that specific data points should be read against [native pH: rutin hydrate (pH 4.6); naringin (pH 5.54); HPMC (pH 5.25); CMCC (pH 6.18)].

Analysis of the data in Fig. 1a shows that as the pH conditions change from acidic to alkaline, the 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 (Fig. 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 fairly 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 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 Fig. 1b). With 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.

3.1.3. 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 affectation of the sunflower oil–water interfacial tension.

3.1.3.1. 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.

With the exception of CMCC, all cellulose particles had θ_w values that exceeded 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 (Fig. 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 Fig. 2, rutin hydrate can be classed as relatively hydrophilic as the value of θ_o for this particle is 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.

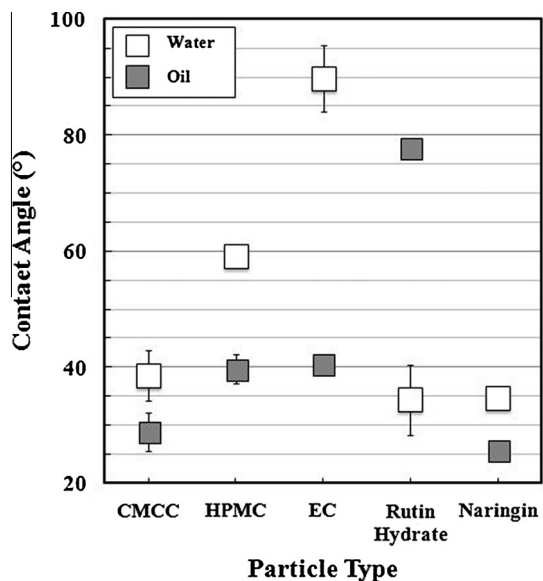


Fig. 2. Contact angles with oil, θ_{oil} (°) (open square symbols) and with water θ_{water} (°) (filled circle symbols) (measured in triplicate and error bars represent two standard deviations), for cellulose (CMCC, HPMC and EC) and flavonoid (rutin hydrate and naringin) particles. [Error bars represent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

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.

3.1.3.2. 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). 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.

Here a relationship between the hydrophobicity of the particle (see Fig. 2) 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

Table 3

Particle density (kg/m^3) 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.

Particle type	Particle density, $\rho_{particle}$ (kg/m^3) ^a	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

^a Provided from MSDS documentation.

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 Fig. 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 Fig. 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). 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.

3.2. 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.

3.2.1. 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. Fig. 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 Fig. 3).

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,

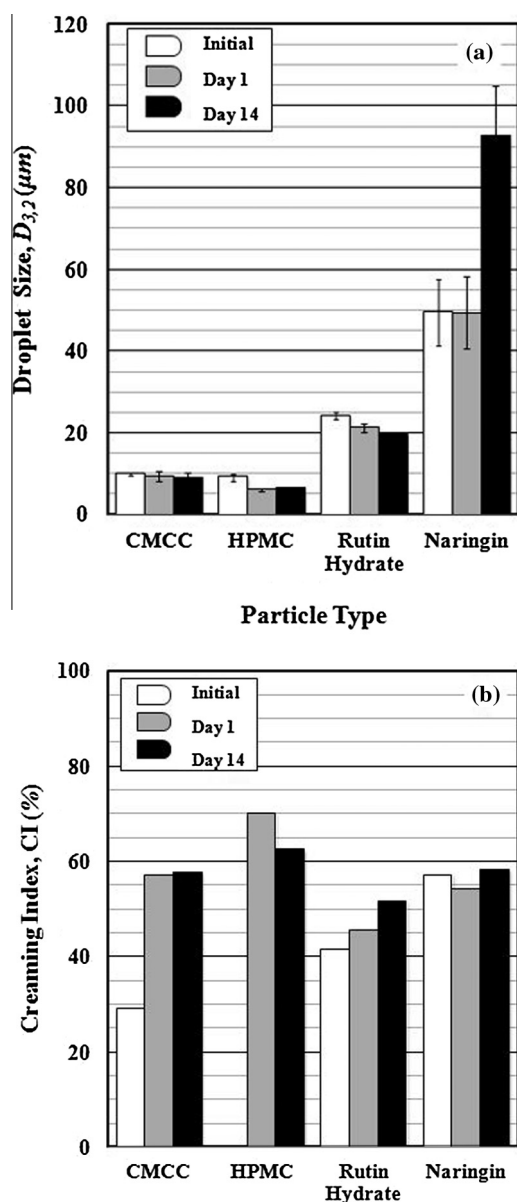


Fig. 3. (a) Mean droplet sizes ($D_{3,2}$) of O/W emulsions (20:80) produced using particles alone (at 1.5% w/w) to stabilise droplets initially, at day 1 and day 14 (b) Creaming index for all emulsions shown, initially, at day 1 and day 14. Measurements were recorded in triplicate and error bars represent 2 standard deviations. [Error bars where not visible are smaller than symbols].

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 flavonoid particles produced the largest droplets (see Fig. 3). However, there is no definitive trend with respect to the particle size: droplet size ratio (Table 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, v , (m/s) were calculated via the Stokes equation (see Table 4):

$$v = \frac{2r^2(\rho_{\text{droplet}} - \rho_o)g}{9\eta} \quad (6)$$

where r is droplet radius (μm), ρ_{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). ρ_{droplet} , was calculated via:

$$\rho_{\text{droplet}} = \frac{m_{\text{adp}} + m_{\text{oil}}}{V_{\text{droplet}}} \quad (7)$$

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:

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

$$m_{\text{oil}} = \rho_{\text{oil}} \frac{4}{3} \pi \left(\frac{D_{\text{oil}}}{2} \right)^3 \quad (9)$$

$$V_{\text{droplet}} = \frac{4}{3} \pi \left(\frac{D_{\text{droplet}}}{2} \right)^3 \quad (10)$$

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

$$D_{\text{oil}} = D_{\text{droplet}} - 2D_{\text{adp}} \quad (11)$$

Assuming that particles form a uniform monolayer at the droplet surface, (as shown in schematic diagram, Fig. 4) irrespective of particle contact angle, ρ_{particle} is particle density (kg/m^3) (Table 3), D_{adp} is the adsorbed particle diameter, and density of oil phase, ρ_{oil} is taken as $915 \text{ kg}/\text{m}^3$. The three particle arrangements detailed in Fig. 4 represent possible contact angles at the interface (measured through the water phase) as $>90^\circ$ (Fig. 4, left droplet), $\sim 90^\circ$ (Fig. 4, centre droplet) and $<90^\circ$ (Fig. 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 4 that as all values for droplet density are less than the density of the bulk phase ($998 \text{ kg}/\text{m}^3$) and the values for v 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 Fig. 3b (creamed layer is largest initially) and Table 4 (highest v value), however it has a positive v 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 v values correlate well with the C.I. observed (Fig. 3b) for all systems; those with the lowest creaming

Table 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 (Fig. 3b) for all particle stabilised 20% O/W emulsion systems containing 1.5% w/w particle concentration (Fig. 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, ρ_{droplet} (kg/m^3)	958.5	704.3	954.4	1356.7
Creaming rate, v (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 (creamied layer)	9.48×10^{10}	3.18×10^{11}	1.21×10^{10}	1.01×10^8

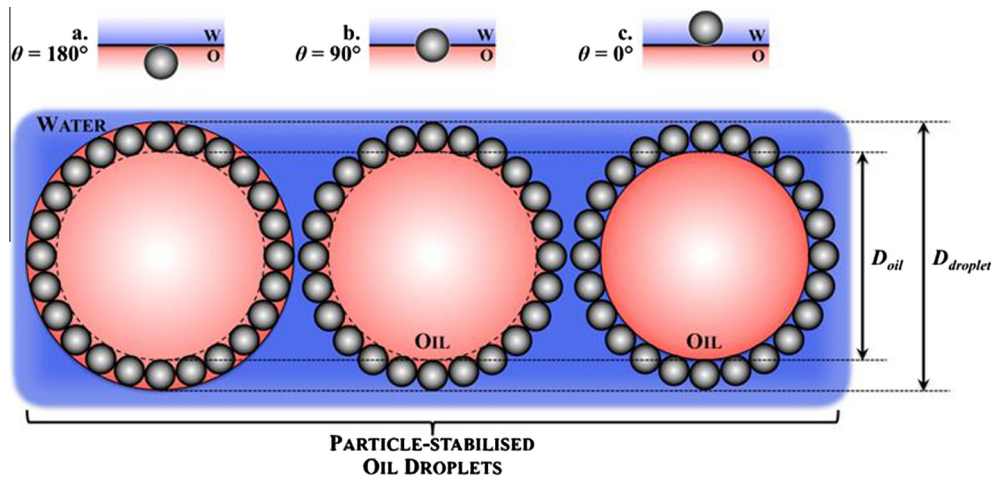


Fig. 4. Schematic representation of the cross section of an oil droplet (O/W emulsion) stabilised by particles with interfacial arrangements corresponding to contact angles of: (a) $\theta = 180^\circ$, (b) $\theta = 90^\circ$ and (c) $\theta = 0^\circ$.

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 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 Fig. 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 ρ_{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 (Fig. 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 Fig. 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 Eq. (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 Fig. 3).

3.2.2. W/O emulsions

W/O emulsions were prepared using the particles to stabilise the interface, and emulsions were produced using the RSM process

as in the previous section with O/W emulsions. Particles were dispersed in the oil phase initially (see Table 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 h. 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 h (see Fig. 5). Both particle types underwent ultrasound treatment prior to emulsification.

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 2) when dispersed in the continuous oil phase promoting smaller droplet formation during processing in comparison. Additionally EC particles were more negatively charged at their native pH than HPMC particles increasing the probability of interparticle repulsive forces between EC stabilised droplets.

3.3. 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. Fig. 6 depicts 20% w/w sunflower oil emulsions prepared using

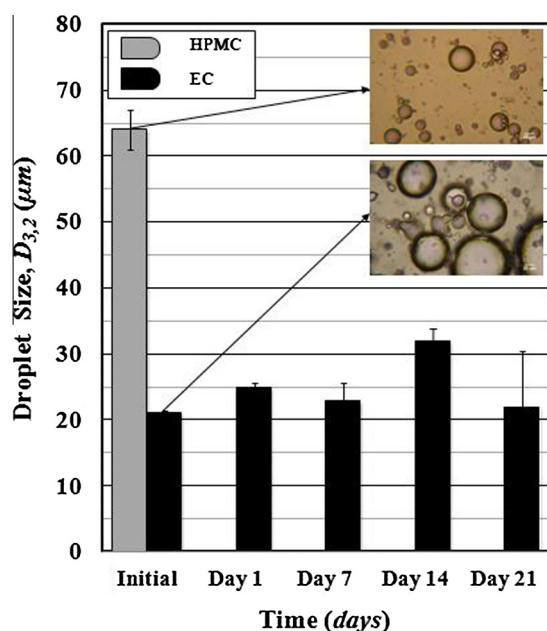


Fig. 5. Mean droplet size (μm) as a function of time for 20% w/w water-in-oil emulsions stabilised by different particles and prepared via rotor–stator mixer at 10,000 rpm for 2 min. Measurements were performed in triplicate and error bars represent ± 1 standard deviation. [Error bars where not visible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

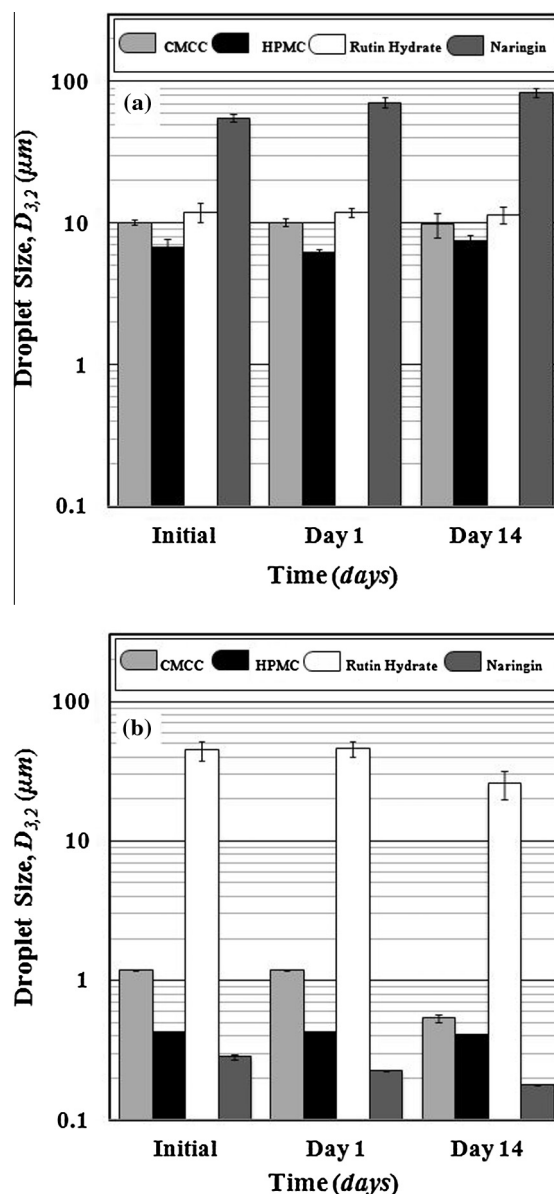


Fig. 6. Mean droplet size (μm) as a function of time for 20% w/w sunflower oil-in-water emulsions stabilised by different particles and prepared via (a) RSM at 10,000 rpm for 2 min and (b) 5 passes via HPH at 900 bar. Measurements were performed in triplicate and error bars represent ± 1 standard deviations. [Error bars where not visible are smaller than symbols].

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.

Most particles produced smaller, more stable droplets with the increased particle concentration (see Figs. 3a and 6a) and there again with the higher energy process, HPH (see Fig. 6a and b), 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 (Fig. S1). Both particles did not significantly reduce IFT when dispersed in the aqueous phase (Table 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 Fig. 7). 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 has 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 Fig. 8c and d) with the exception of the emulsions formed using rutin hydrate via HPH processing. Based on optical imaging analysis subsequently conducted (see Fig. 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.

The micrographs appear to show droplets and droplet floccs of $\sim 1\text{--}5\ \mu\text{m}$ 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 Fig. 7). After 14 days droplet size changed slightly and the main peak that was observed previously

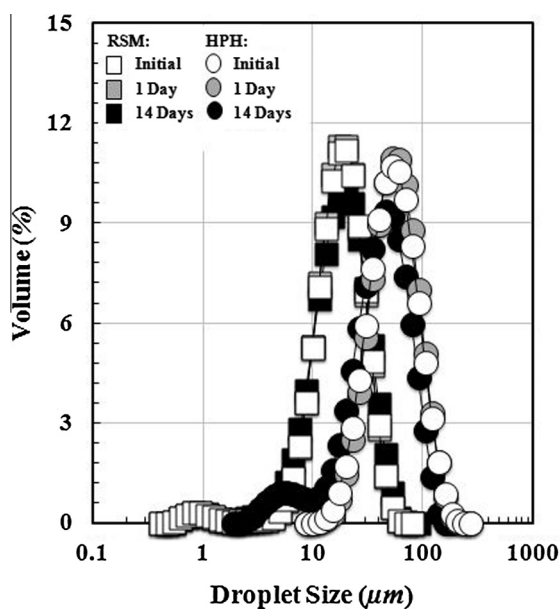


Fig. 7. Droplet size distribution curves for 20% w/w sunflower oil emulsions stabilised with 3% w/w rutin hydrate particles produced via RSM and HPH processing at various time points.

($\sim 100\ \mu\text{m}$) decreased in size along with the appearance of a small peak in the $1\text{--}10\ \mu\text{m}$ range. Micrographs show the existence of aggregated droplets irrespective of processing method, however this effect is exaggerated in the rutin stabilised HPH processed emulsions (Fig. 8). SEM micrographs (Fig. 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 ($\sim 10\ \text{mV}$), 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 Fig. 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.

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.

Of all emulsions produced, all EC emulsions and the HPMC emulsion following 5 passes via HPH, remained stable (see Fig. 10). All other emulsions (produced via either emulsification method) creamed immediately after preparation and after 24 h 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 Fig. 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 (see micrograph insert, Fig. 10b). The slight shift of the distribution curve causing a decrease in droplet size after 1 month (see Fig. 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 floccs 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. HPMC particles were also shown earlier to be weakly charged at native pH as rutin hydrate particles were ($\sim 10\ \text{mV}$)

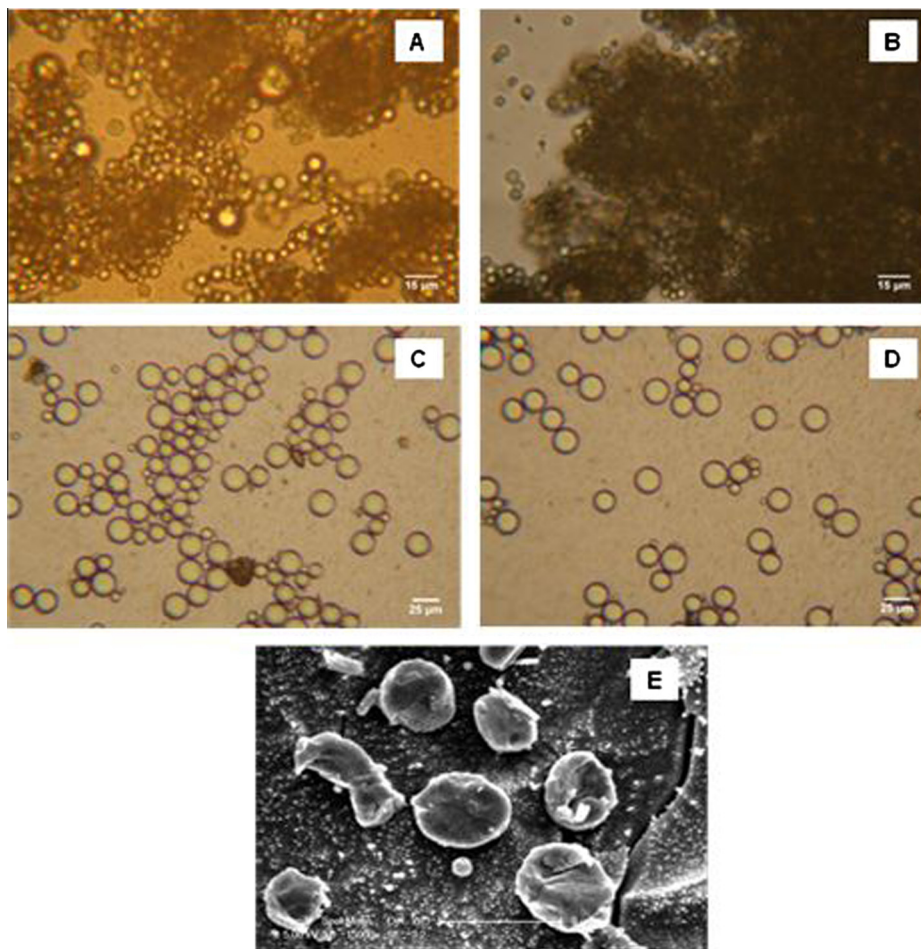


Fig. 8. Micrographs of rutin hydrate stabilised 20% w/w sunflower oil emulsions (a) produced via 3 passes through HPH after initial preparation and (b) emulsion at $t = 14$ days (c) produced by RSM processing following initial preparation (d) emulsion at $t = 14$ days and (e) SEM micrograph of emulsion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

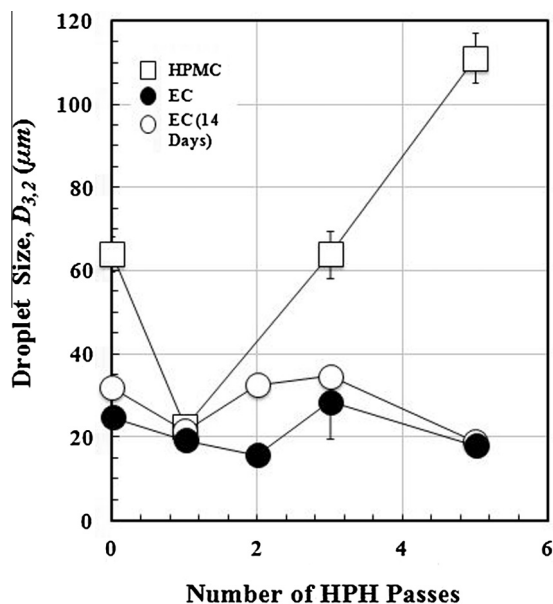


Fig. 9. Mean droplet sizes of 20% w/w W/O emulsion (2.5% w/w particles) for HPMC and EC stabilised systems. (0 pass number value on x-axis relates to RSM method prior to HPH processing). Measurements were performed in triplicate and error bars represent ± 1 standard deviations. [Error bars where not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

(see Fig. 1), which decreases the likelihood of significant droplet-droplet repulsive forces occurring. 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 (Fig. 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 2), and possess a significant hydrophobic character, both of which are also contributing factors to the increased stability of these emulsions.

4. Conclusions

A range of food grade materials, and the O/W and W/O emulsions stabilised by such particles, were investigated (see Fig. S2). Particle characteristics were analysed in an attempt to predict the droplet size, microstructure and stability of the simple

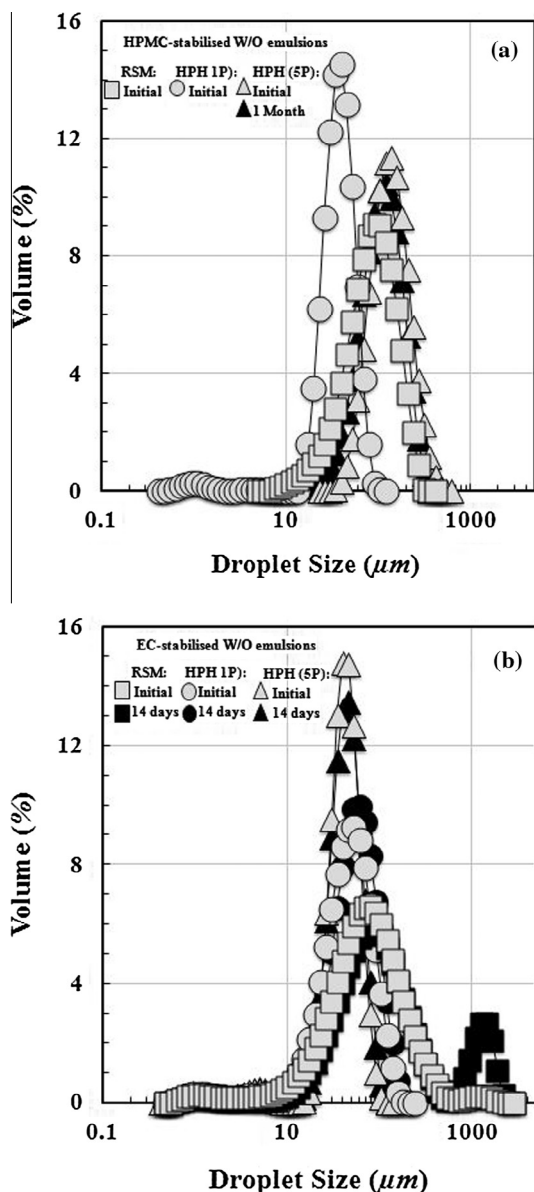


Fig. 10. Droplet size distribution curves for 20% w/w aqueous emulsions stabilised with 2.5% w/w (a) HPMC particles after preparation via RSM only, HPH 1 pass and HPH 5 passes and (b) EC particles via RSM only, HPH 1 pass and HPH 5 passes.

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-octyl-dodecanol 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 wt% 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. 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. Food-grade Pickering nanoemulsions were also produced within this work that were stable against droplet–droplet coalescence for extended periods of time, whereas flavonoid stabilised O/W emulsions were previously reported to be within the micron size range [25]. These findings in particular, pave 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.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcis.2016.03.060>.

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