

Emulsifiers of Pickering-like characteristics at fluid interfaces

Spyropoulos, Fotis; Clarke, Christopher; Kurukji, Daniel; Norton, Ian T.; Taylor, Phil

DOI:

[10.1016/j.colsurfa.2020.125413](https://doi.org/10.1016/j.colsurfa.2020.125413)

License:

Other (please provide link to licence statement)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Spyropoulos, F, Clarke, C, Kurukji, D, Norton, IT & Taylor, P 2020, 'Emulsifiers of Pickering-like characteristics at fluid interfaces: Impact on oil-in-water emulsion stability and interfacial transfer rate kinetics for the release of a hydrophobic model active', *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 607, 125413. <https://doi.org/10.1016/j.colsurfa.2020.125413>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

1 **Emulsifiers of Pickering-like characteristics at fluid interfaces:**
2 **Impact on oil-in-water emulsion stability and interfacial transfer**
3 **rate kinetics for the release of a hydrophobic model active**

4 *Fotis Spyropoulos^a, Christopher Clarke^a, Daniel Kurukji^b, Ian T. Norton^a, Phil Taylor^a*

5 ^aSchool of Chemical Engineering, University of Birmingham, UK

6 ^bFormulation Technology Group, Syngenta Ltd, Jealott's Hill International Research Centre,
7 UK

8
9 **Abstract**

10 The influence of interfacial layer composition on the release kinetics of a model hydrophobic
11 active (dimethyl phthalate, DMP) from oil-in-water (o/w) emulsions relevant to foods is
12 reported. The present study considers various food-relevant emulsifiers known to form
13 colloidal particles in aqueous solutions. These range from a low molecular weight surfactant
14 and protein polyelectrolytes, to biopolymer complexes and solid particles: sodium stearyl
15 lactylate (SSL); bovine serum albumin (BSA); sodium caseinate (NaCAS), chitosan (Ch);
16 BSA/Ch or NaCAS/Ch complexes; and silica (Pickering) nanoparticles (A200), were all
17 investigated. In all cases, DMP release from the oil droplets of the o/w emulsions was
18 controlled by the interfacial transport of the active rather than by its diffusion through the
19 globules' interior. Release data followed first-order kinetics, where emulsions stabilised by soft
20 (protein/polysaccharide complexes) colloidal structures were shown to provide similar
21 ($528 \text{ nm}^2 \text{ s}^{-1}$) or even lower interfacial rate constants ($241 \text{ nm}^2 \text{ s}^{-1}$) to harder (silica) particulate
22 entities ($625 \text{ nm}^2 \text{ s}^{-1}$). SSL (a lamellar-phase forming surfactant that has been previously
23 suggested to stabilise o/w emulsions via a mechanism closely resembling that of Pickering
24 particles) exhibited the lowest interfacial release rate ($17 \text{ nm}^2 \text{ s}^{-1}$). Overall, the present study
25 contributes to current understanding on how emulsion interfacial architecture can be controlled
26 to provide desirable molecular release performances.

27
28 **Keywords:**

29 oil-in-water emulsions; Pickering stabilisation; interfacial transfer rate; sodium stearyl
30 lactylate; protein/polysaccharide complexes; silica nanoparticles.

31

* Corresponding Author: F.Spyropoulos@bham.ac.uk

1. Introduction

The encapsulation of functional ingredients is a key part of formulation science. Such functional species encompass flavour molecules; pharmaceuticals; nutraceuticals; coatings; agrochemicals; biologicals such as DNA, RNA, and peptides; and many more. Microstructure design for encapsulation invariably involves spatial and temporal control of the active ingredient(s) [1]. This may be employed to increase active stability, taste masking (e.g. peptide bitterness) [2], control delivery rates to biological targets, and/or to co-formulate otherwise physically disparate actives within the same liquid formulation [3],[4].

Emulsions offer a convenient template for encapsulation and controlled delivery of functional molecules due to their multi-phase components (oil, water, emulsifier). Emulsion composition/physical properties are defined by parameters including dispersed phase chemistry, emulsifier composition, and droplet size distribution. The material composition and processing route together determine the final microstructure, and this ultimately dictates the equilibrium and kinetic phenomena of dissolved functional material.

A typical interface occupies somewhere in the range $1-10 \text{ m}^2 \text{ g}^{-1}$ depending on average droplet size and phase density. How emulsifiers impart stability has been (and still is) extensively studied across a wide range of interfacial species [5]-[7]; it is often a key performance indicator. However, to what extent emulsifier composition controls molecular release from the emulsion disperse phase is less understood. Of particular interest is the comparative difference between a typical polyelectrolyte (e.g. protein), a hard Pickering particle (e.g. silica) and softer particles such as those formed through biopolymer complexation. Often particulate interfaces are cited as providing a greater release barrier [5], however protein interfaces can often be preferable due to their diverse functionality, biodegradability and biocompatibility [7].

A major benefit of Pickering emulsions is their ability to offer a route to capsules with well-defined interfacial thickness and permeability. Disparate chemistries including fat crystals [8] and latex particles [9] have been utilised for this purpose. Simovic *et al.* [10] demonstrate sustained release of dibutyl phthalate from nanosilica stabilised emulsions at sodium chloride concentrations capable of causing partial interfacial flocculation. Frasch-Melnik *et al.* [11] reported controlled release of sodium chloride using tripalmitin-monoglyceride water-in-oil Pickering emulsions. Crystallisation at interfaces [10],[11] is an avenue for implementation to foods [12] and pharmaceuticals, since chemical functionalisation and/or use of hazardous reagents (e.g. glutaraldehyde, isocyanate, etc.) is not required [13],[14]. For products not designed for human consumption, interfacial cross-linking has found niche applications in agrochemical formulations (e.g. capsule suspensions) to impart specific functionality (UV protection or sustained release). Interfacial crosslinking of proteins and polysaccharides using chemical (e.g. glutaraldehyde and polyphosphate crosslinking of chitosan) [15] and enzymatic methods (e.g. laccase) [16] have been reported as applicable to foods and pharmaceuticals. Since their inception, emulsions stabilised via layer-by-layer (LbL) deposition or pre-formed complexes are cited to add value for controlled release [17]. Despite this, research exploring this area in an experimentally systematic manner is limited.

Based on the aforementioned gap in knowledge, this study aims to assess the capacity of a range of emulsifier species, that are relevant to food applications, to provide an interfacial barrier that regulates the release of an encapsulated model hydrophobic active (dimethyl phthalate; DMP) from oil-in-water (o/w) emulsions. Although the chosen emulsifier species differ substantially in their fundamental structures, they have also been shown, or are considered, to produce interfaces with properties akin to those stabilised by soft or hard particles. For example, while the low molecular weight surfactant sodium stearyl lactylate has

1 been reported to possess a (liquid) crystalline structure in water [18], its action in emulsions
2 (<42°C) is claimed to provide a solid interfacial layer [19]. Other emulsifiers investigated
3 include proteins (bovine serum albumin and sodium caseinate), and their biopolymer
4 complexes formed with chitosan, which can produce soft colloidal particles that also possess
5 the capacity to stabilise emulsions. These interfaces are compared to the hard particle interfaces
6 created by hydrophilic silica (Aerosil® 200). Emulsions stabilised by all emulsifiers studied
7 here were characterised in terms of their droplet sizes. DMP release from these systems (under
8 sink conditions) was analysed using the model proposed by Washington and Evans [20] and it
9 was shown to be limited by the transfer of the active across the droplet interface created, rather
10 than by diffusion. DMP interfacial rate constants for all systems were calculated and it was
11 demonstrated that interfaces stabilised by protein/polysaccharide complexes can provide an
12 interfacial barrier of comparative or even enhanced functionality to one populated by typical
13 Pickering particles.

15 2. Experimental

16 2.1. Materials

17 Sodium caseinate (NaCAS), bovine serum albumin (BSA), dimethyl phthalate (DMP),
18 acetic acid, and low (50-190kDa) and medium (190-310kDa) molecular weight chitosan (LCh
19 and MCh, respectively), both with de-acetylation degrees >75%, were obtained from Sigma
20 Aldrich (UK). Hydrophilic nanosilica (Aerosil® 200; A200) was obtained from Evonik
21 Industries (Germany). Sodium stearyl lactylate (SSL) was kindly donated by Danisco
22 (Denmark). Sunflower oil (SFO) was obtained from the local supermarket (Tesco, UK).
23 Materials were used directly from the manufacturer without any further purification. Water was
24 passed through a double-distillation column equipped with a de-ionisation unit. Concentrations
25 (%) are reported as wt% (g/g) unless stated otherwise.

27 2.2. Methods

28 2.2.1. Preparation of emulsifier-containing aqueous phases

29 The precise proportions and preparations of the emulsifier-containing aqueous phases are as
30 follows:

- 31 *i. Sodium stearyl lactylate (SSL):* A 1% SSL aqueous phase was prepared by dispersing
32 bulk SSL powder into water at 50°C under magnetic stirring; this resulted in an opaque
33 solution/suspension containing lamellar SSL aggregates [21]. The suspension was left to
34 stand at ambient temperature until it reached 20°C.
- 35 *ii. Sodium Caseinate (NaCAS):* A 1% NaCAS aqueous phase was prepared in 30 mM sodium
36 acetate buffer adjusted to pH 5 under magnetic stirring. At this pH, NaCAS has been found
37 to exist as partly dewatered particles [22] which formed the desired insoluble complexes
38 upon the addition of chitosan (see section v).
- 39 *iii. Bovine serum albumin (BSA):* A 1% aqueous solution of BSA was prepared by addition
40 of BSA to a pH 5 sodium acetate buffer solution; the pH environment was selected to
41 facilitate complexation with chitosan (see section vi). The solution was left to stir for
42 approximately 2 hours prior to use.
- 43 *iv. Chitosan (Ch):* A stock solution of 1% chitosan (MCh or LCh) was prepared with water
44 and acetic acid to pH 3, with required volumes taken and adjusted under vigorous stirring
45 to pH 5 with 10% sodium hydroxide solution. This was to enable the formation of
46 insoluble complexes when added to NaCAS or BSA aqueous phases at the same pH (see
47 sections v and vi, respectively) [22].

- 1 v. *Sodium caseinate/chitosan complexes* (NaCAS/Ch): Equal quantities of aqueous phases
2 of 1% NaCAS and 1% Ch (MCh or LCh) at pH 5 (see above preparations) were combined
3 using magnetic stirring, spontaneously producing insoluble complexes. Suspensions were
4 subjected to sonication (Viber Cell 750, Sonics, USA) using a 12 mm diameter probe for
5 2 minutes (20kHz, 95% amplitude). This method is described in detail elsewhere [22]. The
6 Ch-to-protein fraction of the resulting NaCAS/Ch complexes was 1/1 and their (z-average)
7 diameter was ~564 nm.
- 8 vi. *Bovine serum albumin/chitosan complexes* (BSA/Ch): BSA/Ch complexes were formed
9 via two different processing methods, following the procedure reported elsewhere [22].
10 Regardless of the processing method employed, the Ch-to-protein fraction in the BSA/Ch
11 complexes was 1/3. Briefly:
- 12 • METHOD 1 (M1). Under M1, 37.5 g of 1% Ch solution was added dropwise to 12.5 g
13 of 1% BSA solution (both at pH 5) while stirring at 20°C. Irrespective of Ch molecular
14 weight (MCh or LCh), M1 yielded transparent dispersions/solutions with no discernible
15 interface present between the biopolymer solutions, suggesting co-solubility. This is in
16 agreement with [22] which concluded that BSA/Ch complexation following the M1
17 protocol yields soluble complexes.
 - 18 • METHOD 2 (M2). For M2, Ch and BSA aqueous solutions (pH 5) of identical
19 compositions as in M1 were combined as above, but the mixture was heated to 90°C
20 (above the denaturation temperature of BSA) [23] then cooled to ambient temperature
21 (20°C). The suspension was then sonicated using a 12 mm diameter probe for 2 min
22 (20 kHz, 95% amplitude). Irrespective of Ch molecular weight (MCh or LCh),
23 sonicating these aggregates yielded sub-micron sized complexes (~667 nm in
24 diameter), as verified previously by dynamic light scattering [22]. This aligns with the
25 findings of [22] which reported that BSA/Ch complexation following the M2 protocol
26 yields insoluble BSA/Ch complexes.
- 27 vii. *Aerosil® 200 Silica* (A200): A200 was dispersed in 30 mM sodium acetate buffer at pH 2
28 (using 10% NaOH for the pH adjustment) to make a 1% suspension. 50 g of A200 (pH 2)
29 dispersion were sonicated for 2 min at 95 % amplitude. This yielded a z-average particle
30 diameter of ~150 nm, as measured by dynamic light scattering (DLS); this is in-line with
31 previous work [24].

32 33 2.2.2. *Preparation of o/w emulsions*

34 The emulsions comprised 20% oil phase and 80% aqueous phase. The oil phase was a
35 solution of sunflower oil (SFO) and dimethyl phthalate (DMP) prepared in a weight ratio of
36 56:6 (SFO:DMP). The emulsifier composition was prepared in the emulsion aqueous
37 (continuous) phase. All emulsions were produced by adding 40 g of the aqueous (emulsifier-
38 containing) phase to 10 g of the oil phase and emulsifying using a Silverson rotor-stator mixer
39 (Silverson Machines, UK) for 5 min at 6000 rpm and at room temperature.

40 41 2.2.3. *Preparation of o/w LbL emulsion*

42 The layer-by-layer (LbL) approach involves the preparation of a primary protein-stabilised
43 emulsion which is then diluted into an aqueous phase of an oppositely charged biopolymer; the
44 pH of both is the same or is adjusted after mixing to promote electrostatic deposition at the
45 interface. The LbL route was investigated only for the NaCAS/Ch system and compared
46 against emulsions stabilised by preformed NaCAS/Ch complexes. To achieve this, a primary
47 emulsion stabilised by 1% NaCAS was first prepared, before the addition of 1% Ch solution at
48 pH 5, under either low shear (LS) magnetic stirring or high shear (HS) Silverson rotor-stator
49 mixing (6000 rpm, 5 min).

1 2.2.4. Emulsion Characterisation

2 *Droplet size analysis.* Average droplet sizes were measured by laser diffraction using a
3 Malvern Mastersizer 2000S (Malvern Instruments, UK) device equipped with a Hydro S
4 dispersion cell. Because of the specific importance of emulsion droplet surface area to
5 interfacial transport, average droplet diameters are reported as the volume-surface weighted
6 mean diameters ($D_{3,2}$) rather than volume mean diameters ($D_{4,3}$).

7 *Interfacial tension (IFT).* Interfacial tension measurements were carried out following a slight
8 variation to the protocol employed by Pichot *et al.* (2009) [25]. Briefly, IFTs were measured
9 via the pendant drop method (Easydrop goniometer Krüss, Germany). Drops were aged to
10 3000 s, whereupon equilibrium IFT was reached; the only exception being A200. In this case,
11 IFT continued to decrease (at a much significantly slower rate) after this time point, an effect
12 that has been previously reported to be due to surface active impurities present in commercial
13 sunflower oil [25]. As such, equilibrium IFT values for all systems reported here were obtained
14 at a droplet age of 3000 s. The interfacial tension between distilled water and the used
15 commercial sunflower oil was monitored throughout this work (at least on a weekly basis); the
16 average equilibrium interfacial tension for this system was 24.61 ± 0.89 mN/m.

17 2.2.5. Release measurements

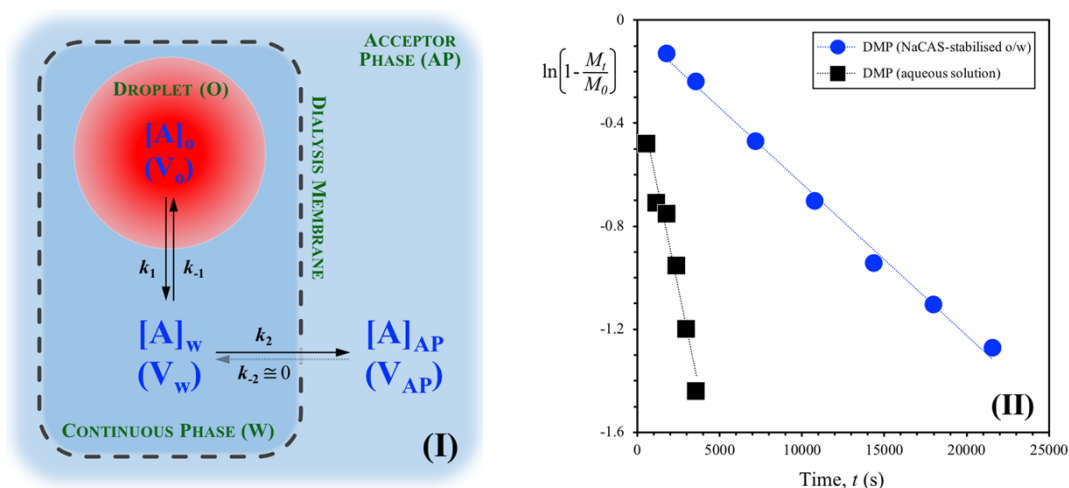
18 1 g of each DMP-containing o/w emulsion was transferred into cellulose dialysis tubing
19 (10 mm \times 150 mm, 14 kDa molecular cut-off), which was hydrated for 48 h prior to use. The
20 emulsion-containing tubing was placed in 200 g of sodium acetate buffer (30 mM, pH 5) in a
21 conical flask (the acceptor phase). Prior to the addition of the dialysis tubing, the acceptor phase
22 was equilibrated to 20°C using a controlled water bath and was then on mildly mixed using a
23 magnetic stirrer. The release was monitored by collecting 3 mL aliquots of the acceptor phase
24 at regular time intervals over a total period of 6 h. The ultraviolet-visible (UV-VIS) absorbance
25 (Libra S12, Biochrom, UK) of these samples was measured at a wavelength of 280 nm in a
26 quartz cuvette. Using predetermined linear DMP calibration curves, the mass of active in the
27 acceptor phase could then be determined. All release experiments were carried out within 48 h
28 following emulsion preparation in order to ensure that destabilisation phenomena do not impact
29 the measurements.
30

31 A schematic of the experimental dialysis membrane set-up is shown in Figure 1(I), where
32 the transfer of active A from a single oil droplet, firstly to the continuous phase of the emulsion
33 and then (after transferal across the dialysis membrane) to the acceptor phase, is depicted. The
34 active is at a concentration of $[A]_o$ within the oil droplet (of volume V_o), $[A]_w$ in the continuous
35 phase (of volume V_w) enclosed in the dialysis tube, and $[A]_{AP}$ in the acceptor phase (of volume
36 V_{AP}); all quantities vary based on the rate of each transfer. As two interfaces are present (the
37 droplet interface and the semi-permeable dialysis tubing) two rate constants must be
38 considered. The rate constant k_1 , describing release across the emulsion interface (the
39 interfacial rate constant) into the continuous phase (with rate constant k_{-1} being relevant to
40 transferal in the opposite direction), and rate constant k_2 , relating to molecular release from
41 continuous phase of the emulsion to the acceptor phase (due to the imposed sink conditions,
42 the reverse transfer rate constant k_{-2} becomes practically zero). Preliminary testing
43 demonstrated DMP adsorption onto the dialysis tubing to be insignificant, which contrasts
44 previous work using dibutyl phthalate [10].

45 One potential issue with the release measurement set-up used here, as cited by
46 Washington [26], is that the carrier phase (the emulsion droplet) does not experience a ‘true’
47 sink environment; i.e. the active does not experience its full driving force to release. This could
48 lead to a situation where the molecular release kinetics are masked by partitioning phenomena;

1 i.e. measurement of an equilibrium feature, rather than a mass transfer coefficient [26]. Such a
 2 masking effect on release kinetics is in-line with a previous study which showed an inverse
 3 relationship between drug release rate and drug partition coefficient using the dialysis set-up
 4 [27]; in this study the release rate from the emulsion was governed by the amount of drug that
 5 was initially partitioned into the aqueous continuous phase, as dictated by the active's oil-water
 6 partition coefficient K_p (k_{-1}/k_1). However, more recent work [27],[28] using model actives
 7 defined by reduced partition coefficients did not show an inverse relationship. This means that
 8 kinetic information can be obtained when using actives or model compounds of appreciable
 9 water solubility. In the present study different release rates were observed despite the use of
 10 the same active and oil phase, suggesting that the partition coefficient was not the only (or even
 11 a dominant) factor for delivery to the acceptor phase. What is more, DMP has an octanol-water
 12 partition coefficient ($K_{p,oct}$) of 33, which means that for o/w emulsions with a 20% oil phase,
 13 the percentage of total DMP partitioned into the continuous aqueous phase is predicted to be
 14 $\sim 12\%$. This concentration may vary somewhat since the partition coefficient could have a slight
 15 interfacial area dependence [29], in addition to the difference in hydrophobicity between
 16 octanol and sunflower oil.

17



18
 19 **Figure 1. (I)** Kinetic scheme for the dialysis membrane set-up used to measure the release rate of active A from
 20 o/w emulsions. $[A]_o$ and V_o , $[A]_w$ and V_w , and $[A]_{AP}$ and V_{AP} , are the concentration of the active in (and volume
 21 of) an oil droplet, the continuous phase of the emulsion, and the acceptor phase (sink). At equilibrium, the
 22 partitioning (K_p) of the active between the oil droplet ($[A]_o$) and the continuous phase ($[A]_w$) is governed by the
 23 ratio of the reverse and forward release rate constants (k_{-1}/k_1); k_1 is the interfacial rate constant. As the (emulsion-
 24 containing) dialysis membrane is placed within the acceptor phase, this equilibrium can be perturbed and the
 25 release of the active can also be affected by k_2 . Concentration $[A]_{AP}$ is then assayed over time to generate the
 26 release profile. **(II)** First order fits to experimental data for DMP release from a NaCAS-stabilised o/w emulsion
 27 (blue circles) and an aqueous DMP solution (no emulsion; black squares). DMP concentration in the latter was
 28 half of the aqueous partitioning concentration; assumed to be that of octanol/water ($K_{p,oct} = 33$). For the emulsion,
 29 M_t is equal to $[A]_{AP}$ at different times t , and M_0 is equal to $[A]_o$ at $t = 0$. For the DMP aqueous solution, M_t is also
 30 equal to $[A]_{AP}$ at different times t , but in this case M_0 is equal to $[A]_w$ at $t = 0$.

31

32 In order to determine the extent to which the dialysis membrane influenced DMP release
 33 kinetics, the fastest DMP-releasing system in this study (a DMP-containing NaCAS-stabilised
 34 o/w emulsion) was compared against an aqueous solution of the active (no emulsion). In the
 35 latter case, DMP was dissolved in water at half its aqueous partitioning concentration (assumed
 36 against $K_{p,oct}$), and then placed in a dialysis bag and released into the acceptor phase under the
 37 exact same conditions used in the release experiments from emulsions. DMP release from these
 38 two systems is shown in Figure 1(II) as first order plots. The slope of the line in each case gives

1 a first order rate constant; for the no emulsion situation this would be the k_2 rate constant. The
 2 data presented in Figure 1(II) demonstrate that the slope for no emulsion is five-times greater
 3 than that for the release of DMP from the emulsion. This clearly suggests that the overall rate
 4 constant for DMP release from the emulsion droplets into the acceptor phase is primarily
 5 governed by the interfacial rate constant (k_1) and not by the rate of mass transfer across the
 6 dialysis membrane (k_2).

7 8 2.2.6. Fractional Release profiles

9 Cumulative fractional release (CFR) profiles for DMP are presented as a function of time t .
 10 CFR data are calculated as the fraction of the cumulative amount of DMP released at time t
 11 (M_t) over that released at infinite time (M_0):

$$\text{CFR} = \frac{M_t}{M_0} \quad \text{Eq. 1}$$

12 M_t is essentially the total mass of active measured in the acceptor phase at time t , while M_0 is
 13 equal to the amount of active entrapped within the emulsion droplets at time $t = 0$.

14 15 2.2.7. Release modelling

16 Release of an active enclosed within emulsion droplets can be considered using two limiting
 17 models [20],[30]. The first relates to release phenomena that are predominantly driven by the
 18 diffusion of the active through the oil core of an emulsion droplet and towards the interface;
 19 active concentration along the droplet radius is in this case complex and time-dependent. It has
 20 been proposed [20] that when no interfacial hindrance is present, active release at long times
 21 is well approximated by:

$$\frac{M_t}{M_0} = 1 - \frac{6}{\pi^2} \exp\left(-\frac{\pi^2 D}{r^2} t\right) \quad \text{Eq. 2}$$

22 or its linear form:

$$\ln\left(1 - \frac{M_t}{M_0}\right) = \ln\left(\frac{6}{\pi^2}\right) - \frac{\pi^2 D}{r^2} t \quad \text{Eq. 3}$$

23 where M_t and M_0 retain their previous meanings as defined for Eq. 1, D is the diffusion
 24 coefficient of the active in the emulsion droplet, and r is the globule radius. Plotting the natural
 25 logarithm term on the left-hand side of Eq. 3 against time should give a straight line, the slope
 26 of which can be used to calculate the diffusion coefficient D . The second model however is
 27 relevant to release behaviour that is mainly dictated by the transfer of the active across the
 28 interfacial barrier around the emulsion droplet; under these circumstances the concentration of
 29 the active within the droplet is now independent of radial distance and uniform at any given
 30 time. A mathematical model describing the release of an active when transport across the
 31 droplet interface is the rate-limiting step has also been proposed [20] and its long-time
 32 approximation is given below:

$$\frac{M_t}{M_0} = 1 - \exp\left(\frac{-3k_1}{r^2} t\right) \quad \text{Eq. 4}$$

33 where k_1 is the interfacial rate constant; all other symbols retain their previous meanings. Eq. 4
 34 can be rearranged to give:

$$\frac{r^2}{3} \ln\left(1 - \frac{M_t}{M_0}\right) = -k_1 t \quad \text{Eq. 5}$$

35 and plotting the term on the left-hand side against time t should yield a straight line, the slope
 36 of which can be used to directly calculate k_1 .

1 DMP release from the emulsions studied in this work was expected to be dominated by
2 transferal of the active across the emulsion interface and as such it should fall under the
3 considerations within the second of these two models. Fitting the DMP data from the slowest
4 and fastest active-releasing emulsion systems (SSL- and NaCAS-stabilised o/w emulsions,
5 respectively) resulted in diffusion coefficients of 5.14×10^{-18} and $5.64 \times 10^{-16} \text{ m}^2 \text{ s}^{-1}$,
6 respectively. However, at least in theory, these values should be equivalent as they both
7 describe the same phenomenon, i.e. the diffusion of DMP within the same material (sunflower
8 oil), and are normalised to exclude effects arising from differences in the size of the domains
9 (droplets) within which diffusion takes place. Moreover, both these values are significantly
10 lower to the diffusion coefficient for DMP within sunflower oil (a relatively small molecule
11 diffusing through a liquid phase of moderate viscosity) as calculated using the Stoke-Einstein
12 equation ($8.5 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$). As such, the diffusion-limited model is not applicable to describe
13 the release behaviour observed in this study and instead DMP data (at longer times; $t \geq 2 \text{ h}$)
14 were fitted to the model assuming that overall transport of the active is controlled by the nature
15 of the interfacial layer (Eq. 5); best-fit parameters were used to calculate the interfacial rate
16 constants k_I for the discharge of DMP from all o/w emulsion studied in the present work.

17 18 2.2.8. *Statistical analysis*

19 All data are presented as mean values \pm one standard deviation (SD). Statistical significance
20 was determined by performing Student's t-test. Results were considered statistically significant
21 at p -values ≤ 0.05 .

22 23 **3. Results and Discussion**

24 **3.1. Emulsion interface, droplet size and stability**

25 The first part of this work considers the effects of emulsifier adsorption on the interface,
26 droplet size and stability of a range of formed o/w emulsions. A range of emulsifiers were
27 studied here; although these differ in terms of their fundamental structures, they have also been
28 shown to form (under the conditions used in this work) colloidal species that are expected to
29 produce emulsion interfaces with properties akin to those stabilised by hard/solid particles. For
30 all these systems, the relationship between interfacial tension and droplet size as well as two-
31 month stability against droplet-droplet coalescence are presented. The emulsions were also
32 observed for flocculation and creaming, phenomena that are both discussed where relevant; no
33 specific attempts were made to control either of these.

34 35 3.1.1. *The effect on interfacial tension and emulsion droplet size*

36 Figure 2 presents the $D_{3,2}$ and equilibrium IFT data for each emulsifier composition. SSL
37 facilitated the smallest average droplet size despite having a comparatively high IFT
38 ($\sim 14 \text{ mN m}^{-1}$) relative to the other emulsifiers. This was likely due to the higher adsorption rate
39 of SSL at the droplet interface, which during the high shear rate process of emulsification could
40 be expected to provide a faster rate of IFT reduction and thus smaller droplets. Low molecular
41 weight surfactants like SSL are well known to exhibit fast adsorption relative to larger
42 polymers, biopolymer complexes and other colloidal species [31]. SSL has also been postulated
43 to be present in the form of bi- or multilayer aggregates such as lamellas, where the
44 comparatively high equilibrium interfacial tension has been previously explained with
45 reference to this self-assembly [21]. The high IFT is likely to be due to a reduction in cohesive
46 packing energy relative to other anionic and non-ionic surfactants. Sodium dodecylsulfate or
47 Tween 20, for instance, lower the oil-water IFT to 1-5 mN m^{-1} [32],[33].

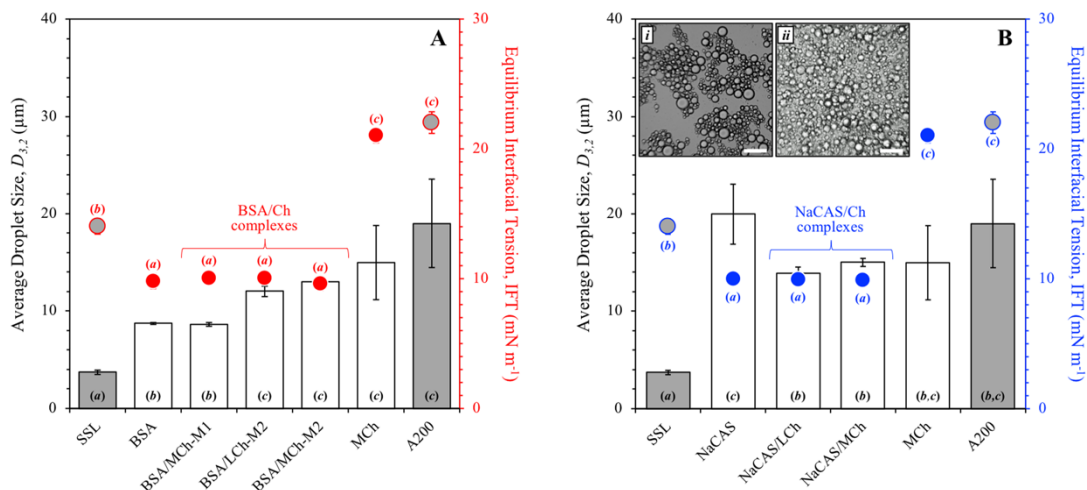


Figure 2. Average droplet sizes ($D_{3,2}$) of o/w emulsions (bars) stabilised by different emulsifiers and corresponding equilibrium interfacial tension (IFT) data (circles). **A.** o/w emulsions stabilised by BSA, MCh and BSA/Ch complexes. **B.** o/w emulsions stabilised by NaCAS, MCh and NaCAS/Ch complexes. SSL- and A200-stabilised o/w emulsions are also presented for comparison. Differences between data marked with the same letter are not statistically significant ($p > 0.05$). Inset micrographs showing o/w emulsions stabilised by NaCAS (i) and NaCAS/MCh complexes (ii) immediately after formation; scale bar is 60 μm .

Emulsifier compositions containing BSA or NaCAS give $\sim 4 \text{ mN m}^{-1}$ lower IFT than SSL with both reaching similar values ($\sim 10 \text{ mN m}^{-1}$). BSA is a globular protein with an isoelectric point at $\sim \text{pH } 4.9$ [34]. BSA self-association taking place near its isoelectric point (pH 5) has been shown to yield colloidal protein aggregates with dimensions of $\sim 650 \text{ nm}$ in diameter [34]. However the impact of the protein on the oil/water interfacial tension as a function of pH is minor. Ghosh and Bull [35] reported that a 0.06% BSA concentration at the water/*n*-octadecane give interfacial tensions of 19.4 and 18.2 mN m^{-1} at pH 6.3 and pH 4.95, respectively. Although at higher protein concentrations (such as those studied here) BSA interfacial tension is further reduced, the low pH sensitivity remains. NaCAS, possesses a disordered structure and although typically associates into spherical micelles of 20–40 nm in diameter under neutral and weakly basic conditions, it can form colloidal particles of dimensions in the order of 200–300 nm at more acidic conditions (pH 5.1) [36] closer to its isoelectric point ($\sim \text{pH } 4.2$) [37]. Sodium caseinate partial aggregation could explain the higher equilibrium IFT values measured here (at pH 5) compared to those reported for the protein ($\sim 3 \text{ mN m}^{-1}$) at its native pH (pH ≈ 6.8) [38]. BSA/Ch and NaCAS/Ch complexes appear to closely follow the IFT reduction caused by their protein components [22]; statistical analysis confirmed that no significant difference ($p > 0.05$) in terms of equilibrium IFT exists between proteins and their equivalent complexes with Ch (Figure 2). Nonetheless, the droplet sizes for emulsions stabilised by proteins or their complexes with Ch do exhibit some variation. BSA- and BSA/MCh-M1-stabilised emulsions possessed practically indistinguishable droplet sizes, while systems formed in the presence of the BSA/LCh-M2 or BSA/MCh-M2 complexes had larger dimensions. It is suggested that the change in droplet sizes primarily relates to the soluble nature of BSA/Ch complexes produced via the M1 processing protocol, as oppose to the insoluble characteristics of those delivered in M2 [22]. On the other hand, droplet sizes stabilised by either of the two NaCAS/Ch complexes were not statistically different. Similarly to the BSA/LCh-M2- and BSA/MCh-M2- stabilised systems, Ch molecular weight in the NaCAS/Ch complexes does not appear to affect emulsion droplet size, providing complexation has been carried out according to the same procedure. NaCAS/Ch-stabilised emulsions though are smaller in comparison to systems formed in the presence of NaCAS alone. The latter systems however did exhibit evidence of flocculation soon after formation; see inset micrograph (i) in Figure 2B. Flocculation in o/w emulsions

1 stabilised by NaCAS/Ch complexes has also been reported to take place, but such events are
2 significantly reduced for biopolymer assemblies with Ch-to-protein fractions equal or greater
3 to 1/1 [22]. This was confirmed for the emulsions stabilised by NaCAS/Ch complexes in the
4 present study (Ch-to-protein fraction of 1/1); see inset micrograph (*ii*) in Figure 2B.

5 In agreement to the data presented here, both MCh (pH 5) [39] and A200 (pH 2) [25] have
6 been shown previously to give relatively high IFT values at the oil/water interface. As a result,
7 both MCh- and A200-stabilised emulsions exhibited the largest final droplet sizes. Despite
8 their deficiency to lower IFT, both species were able to produce o/w emulsions with no free oil
9 phase observed (at 20% oil incorporation). In most cases polysaccharides are not efficient
10 emulsifiers, with notable exceptions including gum-arabic (beverages) and sugar-beet pectin
11 [40]. Chitosan is largely hydrophilic and has been shown to form a network of polyelectrolytic
12 brushes on the water (continuous phase) side of o/w emulsions, thus providing significant steric
13 stabilisation [39]. The emulsifying ability of chitosan depends on pH, where emulsification is
14 facilitated at a pH closer to the amine pKa of chitosan (~6.5-7.0) but low or high enough such
15 that phase separation does not ensue [41]. This was also confirmed in the present study, where
16 the emulsifying capacity of chitosan at pH 5 was all but absent at pH 3.

17 18 3.1.2. *The effect on emulsion stability*

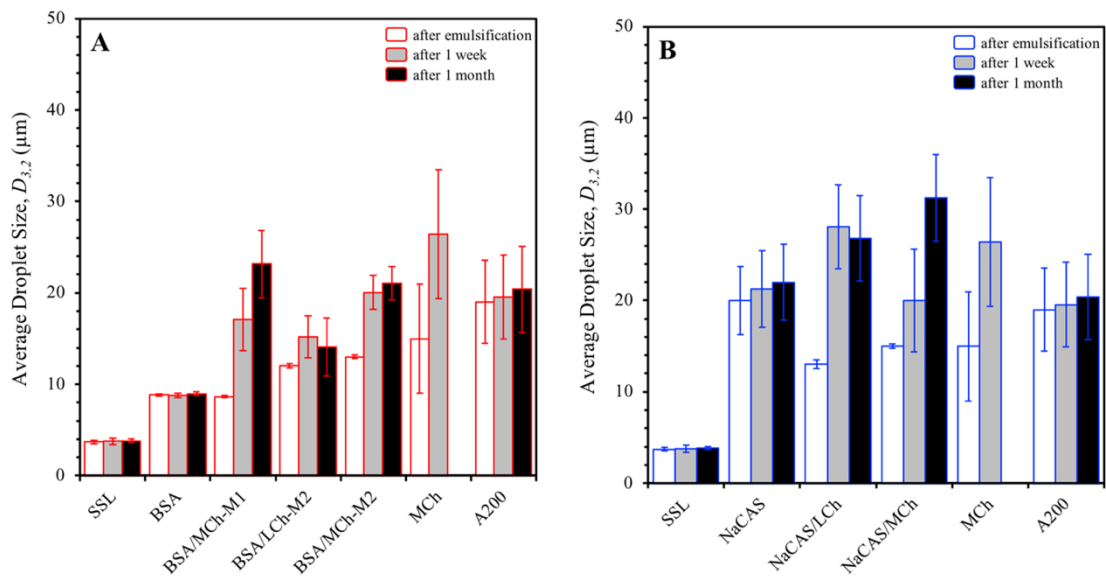
19 Stability is a key performance attribute of any emulsion-based product. All o/w emulsions
20 produced in this study were stored at 20°C and 40°C. Changes to droplet size distribution data
21 were monitored over a month in order to determine variances in coalescence rates between
22 systems stabilised by different emulsifier species.

23 The evolution of the average volume-surface weighted droplet size data ($D_{3,2}$) of o/w
24 emulsions stored over a one month period at 20°C is presented in Figure 3. With the exception
25 of MCh, emulsions stabilised with single emulsifiers (*i.e.* SSL, BSA, NaCAS, or silica)
26 exhibited minimal changes to their original droplet sizes. This shows that these emulsifiers
27 confer stability to coalescence, with little anticipated change in the structure of droplet
28 interfaces over time. Even the NaCAS-stabilised emulsions, which as discussed earlier did
29 initially displayed some level of flocculation, were able to maintain a constant droplet
30 diameter; although it has been reported that the emulsion structure of these systems collapses
31 at ~ pH 4.5 [37]. Ch-stabilised emulsions were the only structures that fully collapsed after one
32 month of storage and were thus unmeasurable. It should be noted that all emulsions prepared
33 in this work exhibited creaming, albeit at different rates. All had appeared to have fully creamed
34 after the one-month storage time; with the exception of SSL, which displayed turbidity in its
35 lower phase suggesting the presence of droplets and/or non-adsorbed emulsifier.

36 Aside from systems formed only in the presence of MCh, emulsions stabilised by
37 protein/chitosan biopolymer complexes exhibited the highest degree of droplet size changes
38 over the quiescent storage period at 20°C (Figure 3). During laser diffraction measurements,
39 the recorded size data could potentially arise from droplet flocs. If the interaction forces
40 stabilising the flocs can withstand the shear forces exerted within the dispersion unit of the
41 measurement device, then the resulting droplet size measurement will be indicative of flocs.
42 This behaviour has been described in previous studies with emulsions, ultimately reporting that
43 measurement errors as a result of the phenomenon are significantly higher than for non-
44 flocculated systems [42]. It has been observed that emulsions stabilised with mixed biopolymer
45 films are prone to flocculation resulting from bridging of the biopolymers across the droplets
46 [6],[42]. Visualisation (using light microscopy) of the emulsions stabilised by protein/Ch
47 complexes in this work after only a few days of storage also revealed floc formation. However,
48 examination of these systems (protein/Ch-stabilised emulsions) directly after emulsification
49 using microscopy and droplet size measurements, did not show signs of flocculation, thus

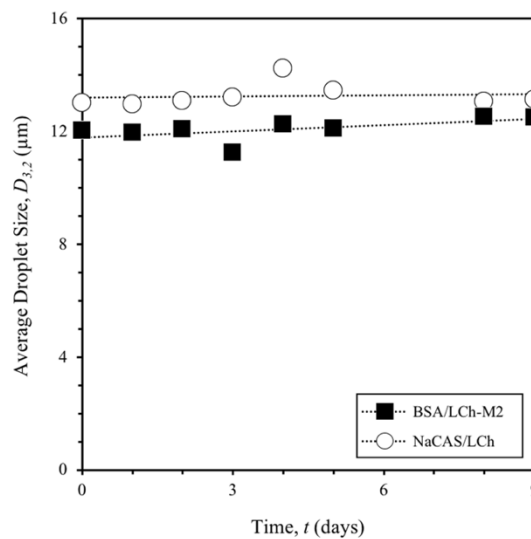
1 suggesting that the phenomenon is exacerbated by creaming (and the close contact of droplets
 2 present within the formed cream layer) and the use of an anti-settling agent could potentially
 3 ameliorate this issue. In order to test this hypothesis, o/w emulsions stabilized by BSA/LCh-
 4 M2 or NaCAS/LCh complexes were stored (immediately after production) under gentle mixing
 5 (inhibiting creaming) at 20°C and their droplet sizes were monitored over a period of nine days;
 6 Figure 4 presents the average droplet sizes measured throughout this time. The data clearly
 7 demonstrates that, in contrast to their quiescently stored counterparts, both these systems
 8 maintain their initial average droplet sizes, thus confirming that restricting droplet-to-droplet
 9 contacts (which are otherwise maximized when the globules are present at a cream layer) by
 10 gentle agitation essentially eliminates flocculation.

11



12 **Figure 3.** Evolution of the average droplet sizes ($D_{3,2}$) of o/w emulsions stabilised by different emulsifiers over a
 13 one month storage period at 20°C. **A.** o/w emulsions stabilised by BSA, MCh and BSA/Ch complexes. **B.** o/w
 14 emulsions stabilised by NaCAS, MCh and NaCAS/Ch complexes. SSL- and A200-stabilised o/w emulsions are
 15 also presented for comparison.
 16

17

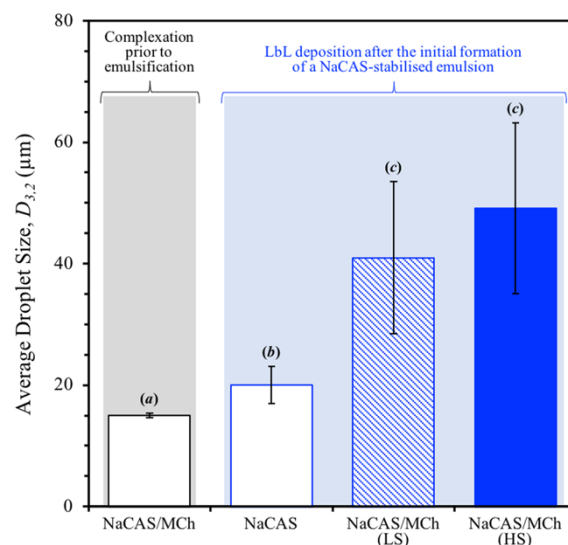


18 **Figure 4.** Evolution of the average droplet sizes ($D_{3,2}$) of o/w emulsions stabilised by either BSA/LCh-M2 or
 19 NaCAS/LCh protein/chitosan complexes and stored under gentle mixing for 9 days. Best-fit lines to the data for
 20 each system are only shown to guide the reader's eye.
 21

1 The o/w emulsions shown in Figure 3 were also stored for one month at 40°C in the presence
 2 of 0.03% sodium azide (NaN₃) to limit microbial activity. The trends in terms of emulsion
 3 stability at 40°C were comparable to those for systems stored at 20°C with one important
 4 difference; the microstructure of the SSL-stabilised o/w emulsion fully collapsed (complete
 5 phase-separation) after one week of storage. It has been previously shown [21] that sunflower
 6 oil-in-water emulsions stabilised by SSL exhibit a melting transition at ~42°C (equivalent to
 7 that of bulk SSL; 44.2°C) but recrystallise at a much lower temperature of ~25°C (significantly
 8 below that of bulk SSL; 43.2°C). As such, it is clear that the crystalline structure of SSL confers
 9 its emulsions a great level of stability which practically disappears at temperatures that promote
 10 the transition of these structures into a liquid-like state.

12 3.1.3. Layer by Layer (LbL) Emulsions

13 In the biopolymer-stabilised emulsions discussed so far in this study, protein/polysaccharide
 14 complexation was performed prior to emulsification; i.e. pre-fabricated complexes were used
 15 to stabilise the emulsions. However, another approach often described in literature [17],[43]
 16 involves adding the biopolymers to a pre-formed emulsion in a sequential manner; this is
 17 collectively known as layer-by-layer (LbL) deposition. This methodology has been previously
 18 demonstrated to generate emulsions with modified physicochemical properties, such as
 19 improved stability to heat [17] and freeze-thaw cycles [43]. However, the final volume fraction
 20 of the dispersed phase in LbL emulsions produced in this way is typically low (0.1-1%), as
 21 otherwise widespread flocculation (most commonly bridging flocculation) phenomena can
 22 take place upon addition of the secondary biopolymer to the primary emulsion [17].



23 **Figure 5.** Average droplet sizes ($D_{3,2}$) of o/w emulsions stabilised by NaCAS/MCh complexes (formed prior to
 24 emulsification) and those stabilised by layer-by-layer (LbL) deposition of MCh at low shear (LS) and high shear
 25 (HS) following the initial formation of a NaCAS-stabilised o/w emulsion. Differences between data marked with
 26 the same letter are not statistically significant ($p > 0.05$).
 27

28
 29 Here, the LbL method was only tested for the NaCAS-MCh system and the resulting
 30 emulsions were compared against those stabilised by (preformed) NaCAS/Ch complexes.
 31 Investigation of NaCAS-Chitosan stabilised LbL emulsions indicated a large and statistically
 32 significant ($p > 0.05$) increase in measured droplet sizes (upon formation) as compared to the
 33 dimensions of either their protein-stabilised precursors or those of equivalent systems formed
 34 in the presence of NaCAS/Ch complexes (see Figure 4). As determined by light microscopy,
 35 this large increase in apparent droplet size was indicative of flocculation rather than

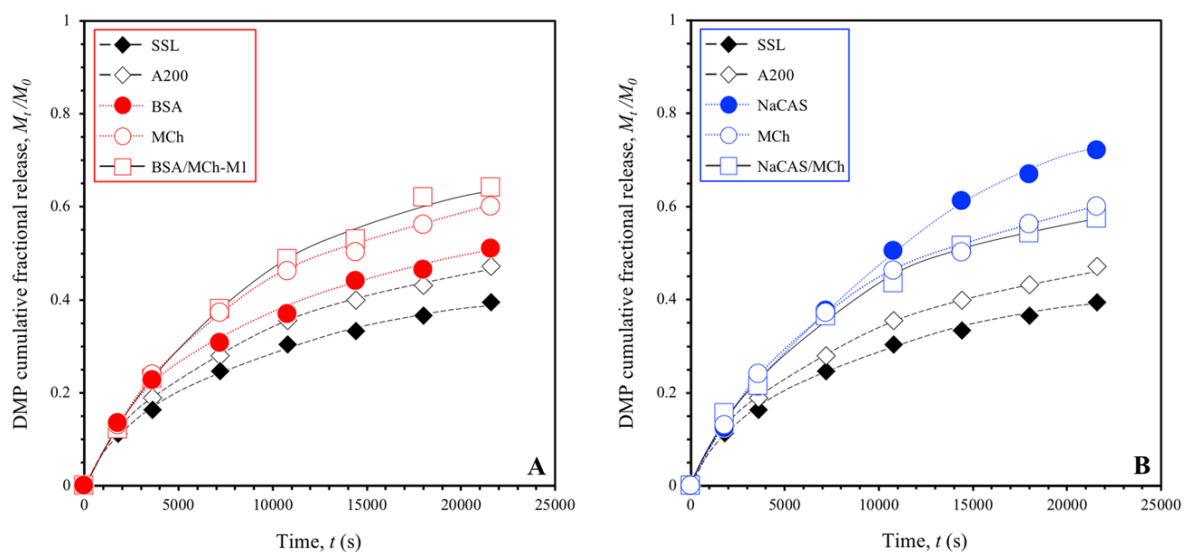
1 coalescence events, specifically occurring following the MCh deposition step. What is more,
 2 the data suggests that there is no statistical difference ($p > 0.05$) in the ‘perceived’ (due to
 3 flocculation) droplet (or floc) dimensions produced by carrying out the deposition of MCh
 4 within a low or high shear environment (Figure 5). This demonstrates that whilst the overall
 5 concentration of each of the biopolymers added to the emulsions can be equivalent for each
 6 processing method, the result in terms of microstructure formation is very different. Preformed
 7 complexes appear to possess the advantage of limiting flocculation phenomena during high
 8 shear mixing (emulsification); an important consideration that is all the more relevant for the
 9 large-scale manufacture of such emulsions.

10

11 3.2. The role of emulsifier on DMP release kinetics

12 The data for the cumulative fractional DMP release from o/w emulsions stabilised by either
 13 BSA, NaCAS, MCh, or selected BSA/Ch and NaCAS/Ch complexes are shown in Figure 6;
 14 DMP release from SSL- or A200-stabilised emulsions are also shown for comparison. Overall,
 15 the release profiles for all systems varied between those for the SSL-stabilised emulsion, which
 16 demonstrates the most sustained DMP release (35% released in 6 hours), and NaCAS, which
 17 released 70% of its payload within 6 hours. DMP cumulative fractional release data (at longer
 18 times; $t \geq 2$ h) for all studied emulsions were fitted to Eq. 5 (Figure 7); droplet radii were taken
 19 as half of the (previously obtained) corresponding $D_{3,2}$ values. The gradient of the produced
 20 linear fits (in all cases with $R^2 \geq 0.97$) is equal to $-k_1$; which allowed the calculation of rate
 21 constants for the transfer of DMP across o/w emulsion interfaces stabilised by a range of
 22 emulsifier species (Table 1).

23



24

25

26

27

28

29

Figure 6. Dimethyl phthalate (DMP) cumulative fractional release profiles from o/w emulsions stabilised by: **A.** BSA, MCh, or their BSA/MCh-M1 complexes, and **B.** NaCAS, MCh, or their NaCAS/LCh complexes. In both cases, DMP cumulative fractional release profiles from o/w emulsions stabilised by SSL or A200 are also shown for comparison. Curves shown to guide the reader's eye.

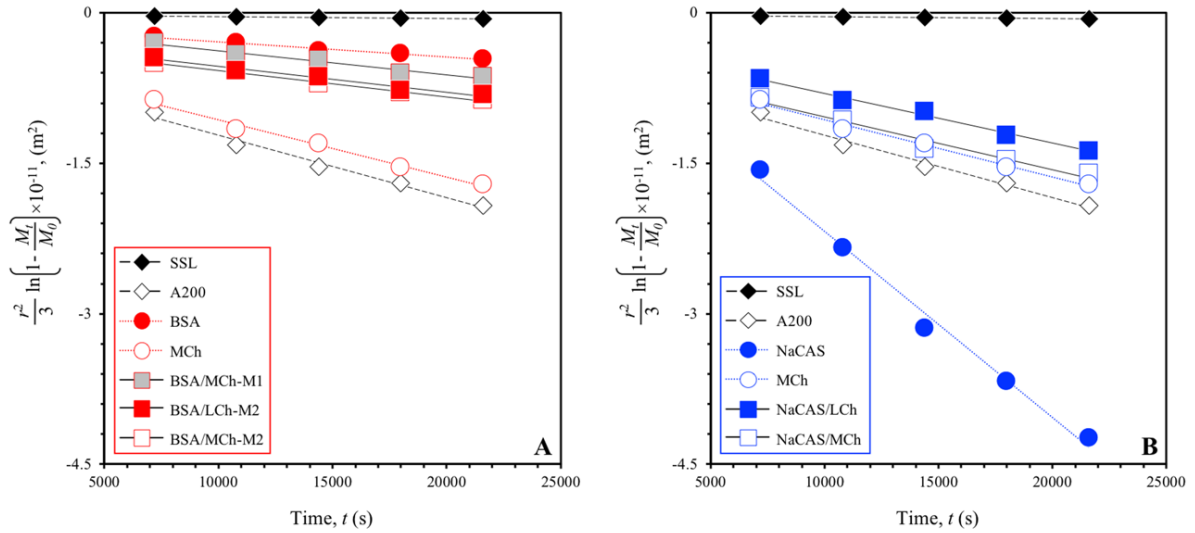


Figure 7. First order fits to data (for times $t \geq 2$ h or 7200 s) for the dimethyl phthalate (DMP) cumulative fractional release from o/w emulsions stabilised by: **A.** BSA, MCh, or their complexes, and **B.** NaCAS, MCh, or their complexes. In both cases, first order fits to data for DMP cumulative fractional release from o/w emulsions stabilised by SSL or A200 are also shown for comparison. Lines shown are best fits to Eq. 5.

	$k_1 \pm \text{SD} \text{ (nm}^2 \text{ s}^{-1}\text{)}$	R^2
SSL	17 ± 2^a	0.986
BSA	153 ± 19^b	0.982
NaCAS	1855 ± 161^f	0.991
MCh	575 ± 53^e	0.990
BSA/MCh-M1	241 ± 37^c	0.974
BSA/LCh-M2	262 ± 21^c	0.992
BSA/MCh-M2	260 ± 37^c	0.977
NaCAS/LCh	497 ± 45^d	0.991
NaCAS/MCh	$528 \pm 89^{d,e}$	0.967
A200	625 ± 72^e	0.985

Table 1. Interfacial rate constants (k_1) calculated by fitting experimental data for the release of dimethyl phthalate (DMP) from o/w emulsions stabilised by different species, to Eq. 5; differences between k_1 values marked with the same superscripted letter are not statistically significant ($p > 0.05$). SD: Standard Deviation.

3.2.1. Interfaces stabilised by SSL or proteins

Rate constants k_1 for all systems varied by almost two orders of magnitude from $17 \text{ nm}^2 \text{ s}^{-1}$ for the o/w emulsion exhibiting the slowest DMP release (SSL-stabilised emulsion) to $1857 \text{ nm}^2 \text{ s}^{-1}$ for the system with the fastest release (NaCAS-stabilised emulsion). There was a 10-fold difference in the k_1 values between the slowest two systems (SSL and BSA) and then an equally large variance in the emulsions exhibiting the second slowest (BSA) and highest overall (NaCAS) interfacial rate constants. This suggests that there is an interfacially-relevant structural feature in the SSL-stabilised o/w emulsions that gives rise to a significant retardation of DMP molecular release, relative to all other systems. In previous work [21], SSL was shown to produce a highly structured emulsion interface containing bilayer aggregates at room temperatures (20°C), which are hypothesised control the slow DMP release rates measured here.

1 In terms of the protein-stabilised emulsions, BSA-only systems exhibited slower DMP
2 release kinetics to those formed in the presence of NaCAS. Barbosa *et al.* [44] have shown that
3 BSA retains its globular structure unaltered at pH 4.0 up to 9.0 (and at concentrations relevant
4 to those used here) without any significant conformational change. It has been also previously
5 demonstrated by interfacial rheology experiments that BSA forms more compact elastic surface
6 films originating from its globular structure [45]. This would indicate that the globular structure
7 of BSA, although potentially losing at least part of this higher order structure upon adsorption,
8 creates a denser and higher (DMP) release energy barrier than NaCAS. What is more, if the
9 aggregates that both proteins are expected to form at pH 5 persist during emulsification and
10 take part in the interfaces created, then their dissimilar dimensions could potentially give rise
11 to barriers of different thicknesses, with the BSA larger colloidal structures thus expected to
12 more efficiently retard active migration [34],[36].

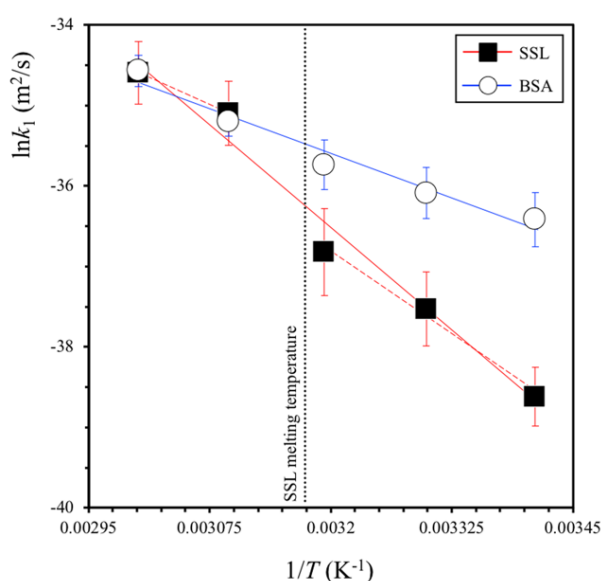
13 The values of interfacial rate constants determined here are largely within the same range
14 of values reported in other (albeit limited) literature. Washington and Evans [20] obtained
15 release rates of model acidic solutes (cupric, caprylic, and arachidic acid) from submicron
16 triglyceride emulsions ($d < 200\text{nm}$) using a range of poloxamer and poloxamine block
17 copolymer emulsifiers and lecithin. Interfacial rate constants determined in this study were
18 within the range of $0.29 - 610 \text{ nm}^2 \text{ s}^{-1}$, with the release of arachidic acid from Pluronic F68-
19 stabilised emulsions and that of chlorpromazine from lecithin-stabilised systems, giving the
20 lowest and highest k_1 values, respectively [20].

21 In an attempt to further explore the large difference in the transport rates of surfactant- and
22 protein-stabilised interfaces, the activation energy (E_A) for DMP release from an SSL emulsion
23 droplet layer was determined following an Arrhenius approach and compared to the equivalent
24 E_A value for systems stabilised by BSA; this approach has been previously used to study the
25 mechanism of molecular release from emulsions [10],[20]. DMP release measurements from
26 emulsions stabilise by either of the two emulsifiers were carried out at different temperatures
27 (20, 30, 40, 50, and 60°C) and in each case the resulting interfacial constants k_1 were calculated.
28 If an Arrhenius relationship exists, plotting the natural logarithm of these k_1 values (at each
29 temperature T) as a function of $1/T$ should produce a straight line of slope equal to $-E_A/R$;
30 where E_A has units of J mol^{-1} and R ($\text{J mol}^{-1} \text{ K}^{-1}$) is the gas constant. Deviation from linearity
31 gives information about the mechanism of release; for example, a change in interfacial
32 composition or structure (*e.g.* hydration or desorption of particles) as a function of temperature
33 [10].

34 The Arrhenius plots for the release of DMP from SSL- and BSA-stabilised o/w emulsions
35 are presented in Figure 8. In the case of BSA, the relationship between $\ln k_1$ and $1/T$ exhibits
36 good linearity with a calculated E_A value of 37 kJ mol^{-1} . Following the same process, the
37 activation energy for DMP transport across the SSL-stabilised interface was calculated at
38 85 kJ mol^{-1} ; clearly suggesting that SSL provides a greater barrier to DMP release than BSA.
39 What is apparent in this case however is that the linear correlation applied across the full
40 temperature range (20-60°C) is somewhat disturbed above 40°C. Moreover, SSL and BSA data
41 $>40^\circ\text{C}$ appear to more or less overlap. If these two linear portions are considered separately
42 then E_A values of 68.8 and 45 kJ mol^{-1} can be determined for the 20-40°C and 50-60°C
43 temperature regions, respectively. This temperature-induced deviation from the initial
44 Arrhenius behaviour and the close alignment of this shift to the melting temperature for SSL
45 (42°C), further reinforce the hypothesis that the unique interfacial structure of this emulsifier
46 controls release.

47 Interfacial activation energies determined here align well with those reported in literature
48 for the release of small hydrophobic actives from emulsions or other relevant colloidal

1 structures. A study of guanosine release from polyethylene glycol-modified liposomes reported
 2 an increase in the activation energy required for the transport of the active across the lipid
 3 bilayer from 14 to 22 kJ mol⁻¹ [46]. In emulsions stabilised by Pluronic F-68, capric acid
 4 release was associated with an E_A value of 52.8 kJ mol⁻¹ [20]. In another study, dexamethasone
 5 release from composites consisting of polylactic-co-glycolic acid microspheres coated with
 6 poly(vinyl alcohol) gave an activation energy of 116 kJ mol⁻¹ [47]. Finally, work [10]
 7 investigating the release of dibutyl-phthalate from polydimethylsiloxane-in-water emulsions
 8 stabilised by hydrophobic nanosilica particles, reported much higher E_A values of 580 and
 9 630 kJ mol⁻¹; at NaCl concentrations of 10⁻³ and 10⁻¹ M, respectively. The authors propose
 10 that under these salt concentrations, a thick multilayer coating of fully aggregated hydrophobic
 11 silica nanoparticles (at 10⁻³ M NaCl) or a thick interfacial wall of hydrophobic nanoparticles
 12 (at 10⁻¹ M NaCl) is expected to be formed, and conclude that such nanoparticle coatings offer
 13 a more significant barrier for molecular transport from emulsion droplets than adsorbed
 14 polymer layers [10].
 15



16
 17 **Figure 8.** Arrhenius plots for the release of DMP from SSL- and BSA-stabilised o/w emulsions; solid lines
 18 represent best-fits to experimental data. In the case of SSL-stabilised o/w emulsions, separate linear fits to data
 19 above and below the melting temperature of the emulsifier (~42°C; dotted line) are also presented as dashed lines
 20 (red).

21
 22 **3.2.2. Interfaces stabilised by protein/chitosan complexes or hydrophilic silica particles**

23 Overall, the interfacial rate constants for DMP release from emulsions stabilised by BSA/Ch
 24 complexes were found not to be statistically dissimilar ($p \leq 0.05$) from one another, with k_1
 25 values in between those determined for either of the corresponding BSA- and Ch-only
 26 stabilised systems (Table 1). As such, no significant effects arisen from changes in the
 27 complexation method (M1 or M2) and/or the molecular weight of the Ch utilised. Despite the
 28 larger proportion of Ch-to-protein (3/1) in the formed complexes, interfacial transport of DMP
 29 appears to be principally controlled by BSA. On the contrary, although the k_1 values for the
 30 release of DMP from emulsions stabilised by NaCAS/Ch complexes were also found to be very
 31 comparable and similarly not sensitive to Ch molecular weight ($p \leq 0.05$), interfacial transport
 32 was shown to be more akin to that of Ch alone rather than the protein-only systems (Table 1).
 33 It might be that the higher fraction of Ch-to-protein in these systems (1/1) amplifies the
 34 polysaccharide contribution much more so than in the case of BSA/Ch complexes (Ch-to-
 35 protein fraction of 3/1). However, a different hypothesis could be put forward suggesting that

1 interfacial rate constants of such complexes are more extensively influenced by their
2 constituent that possess the highest capacity to lower k_1 when used by itself; which is the BSA
3 component for the BSA/Ch complexes and the Ch constituent for the NaCAS/Ch structures.

4 A study [10] on the release of dibutyl-phthalate (DBP) from o/w emulsions stabilised by
5 either hydrophilic or hydrophobic silica nanoparticles reported (only for systems stabilised by
6 hydrophobic silica; Aerosil[®] R974) very low k_1 values; $0.3 \text{ nm}^2 \text{ s}^{-1}$ and $0.05 \text{ nm}^2 \text{ s}^{-1}$, at NaCl
7 concentrations of 10^{-3} and 10^{-1} M, respectively. These values are significantly lower than the
8 rate constants determined in the current study for hydrophilic silica (Aerosil[®] 200) stabilised
9 interfaces. However, analysis by the present authors of the data presented for emulsions
10 stabilised by hydrophilic particles (Aerosil[®] 380) in the same study [10], reveals an interfacial
11 rate constant of $15.3 \text{ nm}^2 \text{ s}^{-1}$; although still lower, it is much more in line with the findings
12 reported here. The main reasons hypothesised to account for this difference are the smaller
13 emulsion droplet sizes studied in [10] and the higher hydrophobicity of DBP; DBP has an
14 octanol/water partition coefficient of 4.68 and an aqueous solubility of $1.0 \text{ mg}/100 \text{ mL}$ [10],
15 while for DMP these are 33 and $0.43 \text{ g}/100 \text{ mL}$, respectively.

16 It is worth noting that the k_1 values for all BSA/Ch and only the NaCAS/LCh complexes
17 were lower than the interfacial rate constants determined for the silica-stabilised systems; no
18 statistical difference was found in the case of the NaCAS/MCh complexes ($p \cong 0.066$). Besides
19 the obvious differences in the dimensions of the two classes of colloidal systems and the
20 potential impact of these on the thickness of the interfacial barriers that they can create, the soft
21 matter nature of the biopolymer assemblies provides them with a unique interfacial advantage
22 over harder particles. Microgel particles, for example, have been known to, amongst others,
23 exhibit great deformability once positioned at liquid interfaces [48]. This can lead to the
24 formation of highly dense barriers around emulsion droplets [48]. Although this has been
25 principally studied in terms of emulsion stability provision [49], it should also impact on the
26 rate of interfacial transportation and thus on release phenomena. It could be argued that if the
27 biopolymer assemblies studied here possess some level of deformability, this would enable
28 them to form dense interfaces that exhibit lower interfacial transport rates than those populated
29 by inflexible particles such as colloidal silica.

31 **4. Conclusions**

32 The present work explores a range of food-relevant emulsifiers known to form colloidal
33 particulates. The emulsifying performance of these species is firstly discussed, and it is
34 highlighted that equilibrium interfacial tension is not necessarily the most appropriate predictor
35 of final average droplet size obtained via high shear mixing. Instead, a measure of dynamic
36 interfacial tension is recommended, in line with the timescales of droplet formation and
37 coalescence phenomena experienced during processing. Further evidence for the propensity of
38 protein/polysaccharide complexes to provide stable emulsions is also presented and the
39 superiority of these structures over those formed via a layer-by-layer deposition approach is
40 demonstrated. The same series of emulsifier species are also studied in terms of their capacity
41 to modulate the release of a model hydrophobic active (DMP). The rate of DMP molecular
42 release from the internal phase of an emulsion is shown to be controlled, to some extent, by the
43 emulsifier chosen to stabilise the interface of the system. However, emulsions stabilised by
44 hard inorganic particles do not always offer the slowest release. Depending on the protein
45 component utilised, protein/polysaccharide complexes are shown to be more effective than
46 hydrophilic silica nanoparticles. Even then, the lowest rate constant is provided by the SSL-
47 stabilised emulsions; this is hypothesised to be a consequence of bi- or multilayer aggregate
48 formation at the SSL interface.

1 Overall, the present study promotes current understanding onto how emulsion microstructure,
2 and more specifically interfacial architecture, can be controlled to arrive at tailored molecular
3 release capabilities. Although directly relevant to foods, the ability to enable controlled release
4 functionality into emulsions using non-toxic, biodegradable structuring materials, such as those
5 presented in this work, is becoming increasingly important in many other research settings.
6 Previous work [11],[50] has shown that sintering and cross-linking of species at the interface
7 can be employed to control the rate of active release from emulsions. Future research in this
8 area would benefit from further development of quantitative relationships between interfacial
9 material characteristics (e.g. interfacial rheology) and the corresponding release rate
10 performance.

12 Acknowledgements

13 The authors would also like to thank the Engineering and Physical Research Council (EPSRC,
14 UK), Innovate UK (UK) and Syngenta for funding the work presented here. The authors would
15 also like to thank Pat Mulqueen at Syngenta (Jealott's Hill International Research Centre) for
16 useful discussions.

18 References

- 19 [1] Madene A., Jacquot M., Scher J., Desobry S., 2006. Flavour Encapsulation and Controlled Release – A
20 review. *International Journal of Food Science & Technology*, **41**: 1-21.
- 21 [2] Mohan A., Rajendran S.R.C.K., He Q.S., Bazinet L., Udenigwe C.C., 2015. Encapsulation of food protein
22 hydrolysates and peptides: A review. *RSC Advances*, **5**(97): 79270-79278.
- 23 [3] Shchukin D.G., Grigoriev D.O., Möhwald H., 2010. Application of Smart Organic Nanocontainers in
24 Feedback Active Coatings. *Soft Matter*, **6**: 720-725.
- 25 [4] Spyropoulos F., Kurukji D., Taylor P., Norton I.T., 2018. Fabrication and Utilization of Bifunctional
26 Protein/Polysaccharide Coprecipitates for the Independent Codelivery of Two Model Actives from Simple
27 Oil-in-Water Emulsions. *Langmuir*, **34**(13): 3934-3948.
- 28 [5] Dickinson E., 2010. Food Emulsions and Foams: Stabilization by Particles. *Current Opinion in Colloid &
29 Interface Science*, **15**: 40-49.
- 30 [6] Dickinson E., 2009. Hydrocolloids as Emulsifiers and Emulsion Stabilizers. *Food Hydrocolloids*, **23**:
31 1473-1482.
- 32 [7] Quintero Quiroz J., Rojas J., Ciro G., 2018. Vegetable Proteins as Potential Encapsulation Agents: A
33 review. *Food Research*, **2**: 208-220.
- 34 [8] Al-Omran M.F., Al-Suwayeh S.A., El-Helw A.M., Saleh S.I., 2002. Taste Masking of Diclofenac Sodium
35 Using Microencapsulation. *Journal of Microencapsulation*, **19**: 45-52.
- 36 [9] Gibbs B.F., Kermasha S., Alli I., Mulligan C.N., 1999. Encapsulation in the food industry: A
37 review. *International Journal of Food Sciences and Nutrition*, **50**(3): 213-224
- 38 [10] Simovic S., Prestidge C.A., 2007. Nanoparticle Layers Controlling Drug Release from Emulsions.
39 *European Journal of Pharmaceutics and Biopharmaceutics*, **67**: 39-47.
- 40 [11] Frasc-Melnik S., Norton I.T., Spyropoulos F., 2010. Fat-Crystal Stabilised W/O Emulsions for Controlled
41 Salt Release. *Journal of Food Engineering*, **98**: 437-442.
- 42 [12] Garrec D.A., Frasc-Melni, S., Henry J.V.L., Spyropoulos F., Norton I.T., 2012. Designing colloidal
43 structures for micro and macro nutrient content and release in foods. *Faraday Discussions*, **158**: 37-49.
- 44 [13] Pawlik A., Kurukji D., Norton I.T., Spyropoulos F., 2016. Food-Grade Pickering Emulsions Stabilised
45 with Solid Lipid Particles. *Food & Function*, **7**: 2712-2721.
- 46 [14] Ghosh S., Rousseau D., 2011. Fat Crystals and Water-in-Oil Emulsion Stability. *Current Opinion in
47 Colloid & Interface Science*, **16**: 421-431.
- 48 [15] Wang L.-Y., Gu Y.-H., Zhou Q.-Z., Ma G.-H., Wan Y.-H., Su Z.-G., 2006. Preparation and
49 Characterization of Uniform-Sized Chitosan Microspheres Containing Insulin by Membrane
50 Emulsification and a Two-Step Solidification Process. *Colloids and Surfaces B: Biointerfaces*, **50**: 126-
51 135.

- 1 [16] Sato A.C.K., Perrechil F.A., Costa A.A.S., Santana R.C., Cunha R.L., 2015. Cross-Linking Proteins by
2 Laccase: Effects on the Droplet Size and Rheology of Emulsions Stabilized by Sodium Caseinate. *Food*
3 *Research International*, **75**: 244-251.
- 4 [17] Aoki T., Decker E.A., McClements D.J., 2005. Influence of Environmental Stresses on Stability of O/W
5 Emulsions Containing Droplets Stabilized by Multilayered Membranes Produced by a Layer-by-Layer
6 Electrostatic Deposition Technique. *Food Hydrocolloids*, **19**: 209-220.
- 7 [18] Kokelaar J.J., Garritsen J.A., Prins A., 1995. Surface Rheological Properties of Sodium Stearoyl-2-
8 Lactylate (SSL) and Diacetyl Tartaric Esters of Mono (and Di) Glyceride (Datem) Surfactants after a
9 Mechanical Surface Treatment in Relation to Their Bread Improving Abilities. *Colloids and Surfaces A:*
10 *Physicochemical and Engineering Aspects*, **95**: 69-77.
- 11 [19] Husband F.A., Ridout M.J., Clegg P.S., Hermes M., Forth J., Poon W.C.K., Wilde P.J., 2014. The Impact
12 of the Interfacial Behaviour on Emulsion Rheology: A Potential Approach to Reducing Fat Content in
13 Emulsified Foods. In: *Gums and Stabilisers for the Food Industry 17: The Changing Face of Food*
14 *Manufacture: The Role of Hydrocolloids*, P.A. Williams, G.O. Phillips (Eds.), RSC: Cambridge, 230-237.
- 15 [20] Washington C., Evans K., 1995. Release rate measurements of model hydrophobic solutes from submicron
16 triglyceride emulsions. *Journal of Controlled Release*, **33**(3): 383-390.
- 17 [21] Kurukji D., Pichot R., Spyropoulos F., Norton I.T., 2013. Interfacial Behaviour of Sodium
18 Stearoyllactylate (SSL) as an Oil-in-Water Pickering Emulsion Stabiliser. *Journal of Colloid and Interface*
19 *Science*, **409**: 88-97.
- 20 [22] Kurukji D., Norton I.T., Spyropoulos F., 2016. Fabrication of Sub-Micron Protein-Chitosan Electrostatic
21 Complexes for Encapsulation and pH-Modulated Delivery of Model Hydrophilic Active Compounds.
22 *Food Hydrocolloids*, **53**: 249-260.
- 23 [23] Borzova V.A., Markossian K.A., Chebotareva N.A., Kleymenov S.Y., Poliansky N.B., Muranov K.O.,
24 Stein-Margolina V.A., Shubin V.V., Markov D.I., Kurganov B.I., 2016. Kinetics of Thermal Denaturation
25 and Aggregation of Bovine Serum Albumin. *PLoS One*, **11**: e0153495-e0153495.
- 26 [24] Pichot R., Spyropoulos F., Norton I.T., 2009. Mixed-Emulsifier Stabilised Emulsions: Investigation of the
27 Effect of Monoolein and Hydrophilic Silica Particle Mixtures on the Stability against Coalescence. *Journal*
28 *of Colloid and Interface Science*, **329**: 284-91.
- 29 [25] Pichot R., Spyropoulos F., Norton I.T., 2012. Competitive adsorption of surfactants and hydrophilic silica
30 particles at the oil-water interface: Interfacial tension and contact angle studies. *Journal of Colloid and*
31 *Interface Science*, **377**(1): 396-405.
- 32 [26] Washington C., 1989. Evaluation of Non-Sink Dialysis Methods for the Measurement of Drug Release
33 from Colloids - Effects of Drug Partition. *International Journal of Pharmaceutics*, **56**: 71-74.
- 34 [27] Sasaki H., Takakura Y., Hashida M., Kimura T., Sezaki H., 1984. Antitumor Activity of Lipophilic
35 Prodrugs of Mitomycin C Entrapped in Liposome or O/W Emulsion. *Journal of Pharmacobio-Dynamics*,
36 **7**: 120-130.
- 37 [28] Dowding P.J., Atkin R., Vincent B., Bouillot P., 2005. Oil Core/Polymer Shell Microcapsules by Internal
38 Phase Separation from Emulsion Droplets. II: Controlling the Release Profile of Active Molecules.
39 *Langmuir*, **21**: 5278-5284.
- 40 [29] Zoldesi C.I., Imhof A., 2005. Synthesis of Monodisperse Colloidal Spheres, Capsules, and Microballoons
41 by Emulsion Templating. *Advanced Materials*, **17**(7): 924-928.
- 42 [30] Guy R.H., Hadgraft J., Kellaway I.W., Taylor M.J., 1982. Calculations of drug release rates from particles.
43 *International Journal of Pharmaceutics*, **11**(3): 199-207.
- 44 [31] Spyropoulos F., Duffus L.J., Smith P., Norton I.T., 2019. *Langmuir*, **35**(47): 15137-15150.
- 45 [32] Lloyd D., Norton I.T., Spyropoulos F., 2014. Processing Effects During Rotating Membrane
46 Emulsification. *Journal of Membrane Science*, **466**: 8-17.
- 47 [33] Lloyd D., Norton I.T., Spyropoulos F., 2015. Process Optimisation of Rotating Membrane Emulsification
48 through the Study of Surfactant Dispersions, *Journal of Food Engineering*, **166**: 316-324.
- 49 [34] Li G., Huang J., Chen T., Wang X., Zhang H., Chen Q., 2017. Insight into the interaction between chitosan
50 and bovine serum albumin, *Carbohydrate Polymers*, **176**: 75-82.
- 51 [35] Ghosh S., Bull H.B., 1963. Adsorbed films of bovine serum albumin: Tensions at air-water surfaces and
52 paraffin-water interfaces. *BBA - Biochimica et Biophysica Acta*, **66**(C): 150-157.
- 53 [36] Xi Y., Liu B., Jiang H., Yin S., Ngai T., Yang X., 2020. Sodium caseinate as a particulate emulsifier for
54 making indefinitely recycled pH-responsive emulsions. *Chemical Science*, **11**(15): 3797-3803.
- 55 [37] Ma H., Forssell P., Partanen R., Seppänen R., Buchert J., Boer H., 2009. Sodium caseinates with an altered
56 isoelectric point as emulsifiers in oil/water systems. *Journal of Agricultural and Food Chemistry*, **57**(9):
57 3800-3807.

- 1 [38] Zafeiri I., Norton J.E., Smith P., Norton I.T., Spyropoulos F., 2017. The role of surface active species in
2 the fabrication and functionality of edible solid lipid particles. *Journal of Colloid and Interface Science*,
3 **500**: 228-240.
- 4 [39] Payet L., Terentjev E.M., 2008. Emulsification and stabilization mechanisms of O/W emulsions in the
5 presence of chitosan. *Langmuir*, **24**(21): 12247-12252.
- 6 [40] Nakauma M., Funami T., Noda S., Ishihara S., Al-Assaf S., Nishinari K., Phillips G.O., 2008. Comparison
7 of Sugar Beet Pectin, Soybean Soluble Polysaccharide, and Gum Arabic as Food Emulsifiers. 1. Effect of
8 Concentration, pH, and Salts on the Emulsifying Properties. *Food Hydrocolloids*, **22**: 1254-1267.
- 9 [41] Liu H., Wang C., Zou S., Wei Z., Tong Z., 2012. Simple, Reversible Emulsion System Switched by pH on
10 the Basis of Chitosan without any Hydrophobic Modification. *Langmuir*, **28**: 11017-11024.
- 11 [42] Dickinson E., Pawlowsky K., 1997. Effect of ι -Carrageenan on Flocculation, Creaming, and Rheology of
12 a Protein-Stabilized Emulsion. *Journal of Agricultural and Food Chemistry*, **45**: 3799-3806.
- 13 [43] Thanasukarn P., Pongsawatmanit R., McClements D.J., 2006. Utilization of Layer-by-Layer Interfacial
14 Deposition Technique to Improve Freeze–Thaw Stability of Oil-in-Water Emulsions, *Food Research
15 International*, **39**: 721-729.
- 16 [44] Barbosa L.R.S., Grazia Ortore M., Spinozzi F., Mariani P., Bernstorff S., Itri R., 2010. The Importance of
17 Protein-Protein Interactions on the pH-Induced Conformational Changes of Bovine Serum Albumin: A
18 Small-Angle X-Ray Scattering Study. *Biophysical Journal*, **98**: 147-157.
- 19 [45] Murray B.S., Dickinson E., 1996. Interfacial Rheology and the Dynamic Properties of Adsorbed Films of
20 Food Proteins and Surfactants. *Food Science and Technology International, Tokyo*, **2**(3): 131-145.
- 21 [46] Er Y., Barnes T.J., Fornasiero D., Prestidge, C.A., 2009. The encapsulation and release of guanosine from
22 PEGylated liposomes. *Journal of Liposome Research*, **19**(1): 29-36.
- 23 [47] Shen J., Burgess D.J., 2012. Accelerated in Vitro Release Testing of Implantable PLGA Microsphere/PVA
24 Hydrogel Composite Coatings. *International Journal of Pharmaceutics*, **422**: 341-348.
- 25 [48] Deshmukh O.S., Van Den Ende D., Stuart M.C., Mugele F., Duits M.H.G., 2015. Hard and soft colloids at
26 fluid interfaces: Adsorption, interactions, assembly & rheology. *Advances in Colloid and Interface Science*,
27 **222**: 215-227.
- 28 [49] Schmidt S., Liu T., Rütten S., Phan K.-H., Möller M., Richtering W., 2011. Influence of microgel
29 architecture and oil polarity on stabilization of emulsions by stimuli-sensitive core-shell poly(N-
30 isopropylacrylamide-co-methacrylic acid) microgels: Mickering versus Pickering behavior? *Langmuir*,
31 **27**(16): 9801-9806.
- 32 [50] Yow H.N., Routh A.F., 2009. Release Profiles of Encapsulated Actives from Colloidosomes Sintered for
33 Various Durations. *Langmuir*, **25**, 159-166.