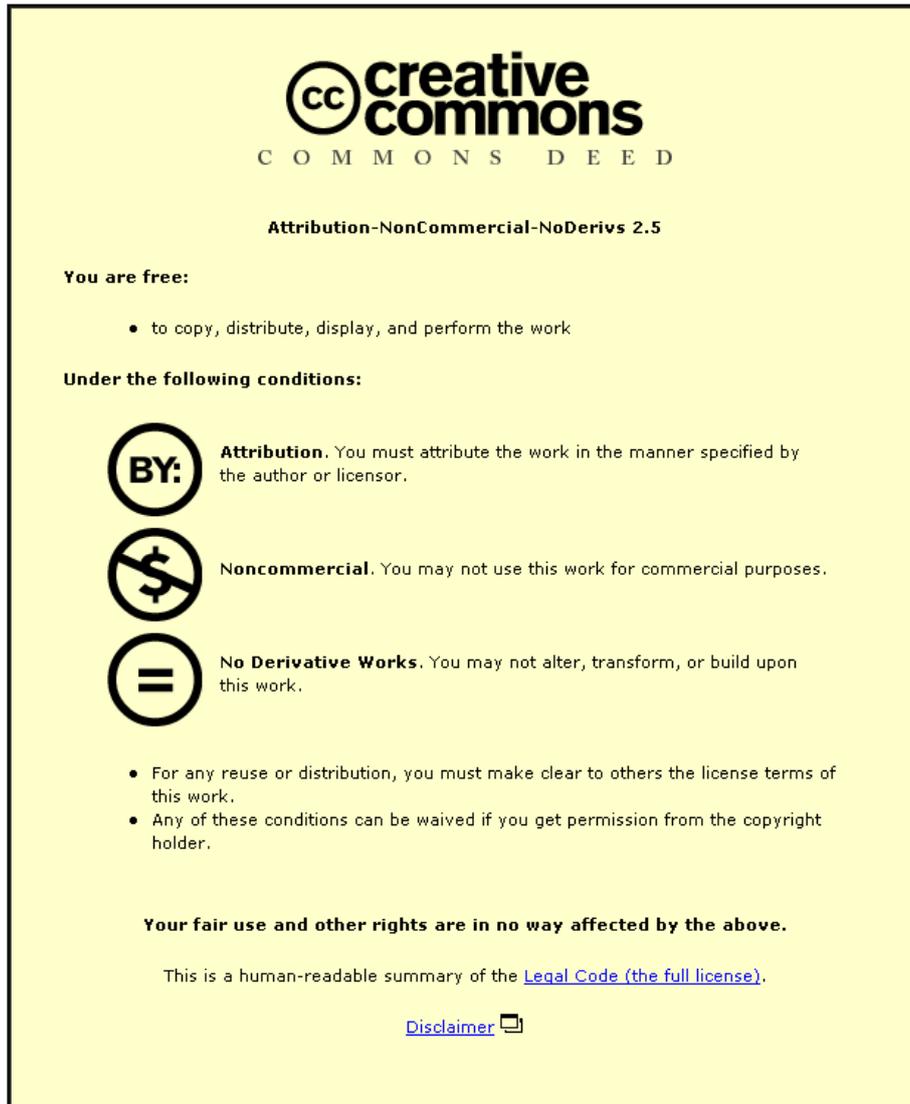


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Modification of interfacial characteristics of monodisperse droplets produced using membrane emulsification by surfactant displacement and/or polyelectrolyte electrostatic deposition

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Abstract

This study examined the possibility of modifying the interfacial characteristics of uniformly sized droplets produced by premix membrane emulsification using surfactant-displacement and/or electrostatic deposition methods. The non-ionic surfactant Tween 20 is particularly effective at producing uniformly sized lipid droplets by premix membrane emulsification, but the droplets produced have a low net charge. The interfacial characteristics of emulsion droplets initially coated by Tween 20 could be made either negative or positive (without altering droplet size) by adding controlled amounts of either anionic emulsifiers (SDS) or cationic emulsifiers (DTAB or β -lactoglobulin) to the continuous phase after homogenisation. In addition, cationic emulsion droplets could be prepared by depositing a cationic biopolymer (chitosan) onto the surfaces of anionic droplets (SDS/Tween coated). These results have important consequences for the design, fabrication and utilisation of uniformly sized lipid droplets with controlled interfacial characteristics.

Keywords: Shirasu porous glass membrane; Membrane emulsification; Layer-by-layer electrostatic deposition; Chitosan; β -lactoglobulin; Multilayered emulsion.

Introduction

Membrane emulsification is a relatively new technique for producing uniform micron-sized drops based on: (i) *in situ* drop generation by permeation of pure dispersed phase through a porous membrane into the continuous phase ('direct membrane emulsification') [1, 2], or (ii) passing a pre-emulsified mixture through a porous membrane ('premix membrane emulsification') [3-6]. The resultant drop size is primarily dictated by the membrane pore size, but many other factors play an important role, such as pore shape (cylindrical, elongated, irregular, etc.), pore spacing, dispersed phase flux, shear stress at the membrane/continuous phase interface, viscosity of the continuous and dispersed phases, surfactant type and concentration, and wetting properties of the membrane used [7]. Hydrophilic membranes are needed for preparation of oil-in-water emulsions to restrict contact between the oil phase and the membrane wall to the region inside the pores and to prevent spreading of the dispersed phase over the membrane surface [1]. In addition, surfactants used to stabilise oil-in-water emulsions must not adsorb to the membrane surface, *e.g.* through electrostatic interactions, since otherwise the membrane would lose its hydrophilic nature and become hydrophobic [8].

Shirasu Porous Glass (SPG) membranes are one of the most widely used microporous media for membrane emulsification [1-6, 8-10] due to their uniformly sized pores [11], uniform internal microstructure with no microscopic voids or cracks [12], and a wide range of commercially available mean pore diameters (0.05-50 μm) [13]. An untreated SPG membrane has a negative zeta-potential of -15 to -35 mV within a pH range of 2–8, due to dissociation of residual silanol groups on the surface and formation of SiO^- sites. Therefore, to prevent deposition of emulsifier onto the surface of a clean untreated SPG membrane, the use of cationic emulsifiers must be

avoided [1]. Zwitterionic surfactants are also unsuitable, even when they carry a net negative charge. For example, lecithin at pH 3 hinders SPG membrane emulsification due to electrostatic interactions between positive groups (*e.g.* $-\text{N}(\text{CH}_3)_3^+$ or $-\text{NH}_3^+$) on the phospholipid molecules and anionic silanol groups on the SPG membrane [14]. Similar fouling problems have also been reported during the production of oil-in-water emulsions by hydrophilic silicon microchannel plates using cationic or zwitterionic surfactants [15, 16]. Consequently, there is a limit to the type of emulsifiers that can currently be used to form stable emulsions with uniformly sized droplets using the SPG membrane method. This means that there is limited scope to design emulsion systems with different interfacial characteristics. One solution to this problem is to prepare emulsions using an emulsifier that is effective at forming uniform droplets using the membrane homogenisation method, and then to use post-homogenisation interfacial engineering methods to subsequently alter the droplet interfacial characteristics.

In this study, we examined the possibility of controlling the interfacial characteristics of uniformly-sized droplets using surfactant-displacement and/or electrostatic deposition methods. The surfactant displacement method simply involves mixing a pre-formed emulsion with an emulsifier solution so that the original emulsifier is partially or fully displaced from the droplet surfaces by the new emulsifier. The electrostatic deposition technique involves depositing polyelectrolytes onto oppositely charged droplet surfaces [17]. This technique offers a promising way to improve the stability of emulsions against environmental stresses such as pH, ionic strength, freezing, and heating [17]. First, a “primary” oil-in-water emulsion is prepared by homogenising an oil and aqueous phase with a charged surfactant. An oppositely charged polyelectrolyte is then added to the system so that it adsorbs to the droplet surfaces and produces a “secondary” emulsion consisting of oil droplets coated by a 2-layer emulsifier-polyelectrolyte

interface. This procedure can be repeated to form oil droplets coated by 3 or more interfacial layers. The charge, thickness, responsiveness to external triggers, and permeability of interfacial layers can be controlled to create delivery systems suitable for protection, encapsulation, and controlled release of different types of active components [18]. Tables 1 and 2 summarise preparation of two-layer and three-layer emulsions by the LbL deposition technique, the ξ -potential of primary, secondary, and tertiary emulsion being denoted by ξ_1 , ξ_2 , and ξ_3 , respectively. Lipophilic active components can be encapsulated within the oil droplets, whereas charged hydrophilic components can be incorporated within the interfacial layers. Potentially, active ingredients can be released at the site of action in response to a specific environmental stimulus. For example, interfacial layers can be detached from the droplet surfaces when the pH is altered or the permeability of the interfacial membrane can be increased as a result of change in pH or ionic strength [34]. Starting with uniformly sized droplets as a template for these multilayer emulsions has the advantage that their functional performance can be more carefully controlled.

Nonionic surfactants (Tweens) have been found to be particularly effective at preparing uniform oil-in-water and oil-in-water-in-oil multiple emulsions by premix SPG membrane emulsification [3, 5]. However, oil drops stabilised by nonionic surfactants are not suitable for some applications due to their relatively low net charge. For example, it is difficult to form multilayer emulsions by depositing polyelectrolyte layers onto droplets that initially have a low net charge. Therefore, there is a strong interest to investigate the modification of the charge on droplets initially stabilised by nonionic surfactants using interfacial engineering methods. The main objective of this study was therefore to show that surface charge could be modified using either

surfactant displacement and/or electrostatic deposition methods. The development of these interfacial engineering techniques means that it may be possible to rationally design emulsions with different physicochemical and functional properties.

Experimental

Materials. Polyoxyethylene sorbitan monolaureate (Tween 20), analytical grade sodium dodecyl sulfate (SDS), dodecyltrimethyl ammonium bromide (DTAB), chitosan (medium molecular weight, 75-85% deacetylation, viscosity of 1 wt% solution in 1 wt% acetic acid, 200-800 cps), hydrochloric acid (HCl), sodium azide (NaN₃), sodium chloride (NaCl) and acetic acid were purchased from the Sigma Chemical Company (St Louis, MO). Powdered β -lactoglobulin (β -Lg) ($pK_a \approx 5.2$) was donated from Davisco Foods International (LOT # JE 001-1-922, Le Sueur, MN). As stated by the manufacturer, the β -Lg content in the powder determined by electrophoresis was 98% (the remainder being mostly globulins). Corn oil was purchased from a local supermarket and used without further purification. Distilled, deionised water was used for the preparation of all solutions.

Solutions Preparation. Various surfactant solutions containing 0.5 wt% surfactant (Tween 20, SDS, DTAB and β -Lg) were prepared by dispersing the surfactant in acetic acid solution (100 mM acetic acid and 10 mM sodium chloride, adjusted to pH 3.0 with 1M HCl) containing 0.02 wt% NaN₃ as an antibacterial agent. 2 wt% solution of low molecular weight chitosan (50,000-190,000 Da, acetylation degree = 75-85 %) was prepared by dispersing chitosan in acetic acid

solution, stirring overnight to ensure complete dispersion and filtrating using a vacuum pump to remove any insoluble material from the solution.

Emulsion Preparation. An oil-in-water emulsion was prepared by homogenising 20 wt% corn oil and 80 wt% aqueous surfactant solution. The surfactant solution and oil was mixed together by magnetic stirrer agitation for 2 min and the pre-emulsion so formed was then passed through a batch membrane homogeniser five times at 100 kPa (External pressure type micro kit, MG-20-5, Kiyomoto Iron Works Ltd., Japan). The pressure vessel was filled with 100 g of the premix and the driving pressure of 100 kPa was built-up with compressed nitrogen, as shown in Fig. 1. The emulsion which had been passed through the membrane was collected in a beaker and used as a feed emulsion in the subsequent passes. The beaker was placed on a Fisher electronic balance and the balance was interfaced to a PC computer to collect time and mass data every 2 s using a data acquisition software. The experiments have been carried out at a constant temperature of 297 K. The membrane used was SPG membrane with 8.5 mm inner diameter and 0.8 mm wall thickness supplied from SPG Technology Co., Ltd (Sadowara, Japan). The internal membrane structure determined by x-ray microtomography is shown in Fig. 1(c). The mean pore size was 8.0 μm , the effective length of the membrane tube inside the module was 12 mm, and the effective cross-sectional membrane area was 3.75 cm^2 . The membrane was cleaned by placing in ethanol for 2 days and then in toluene for 2 days, which was followed by heating at 500 $^{\circ}\text{C}$ for 30 min in an electric muffle furnace.

Displacement of surfactants. Oil-in-water emulsions prepared by membrane homogenisation were mixed with an acetic acid buffer solution (100 mM acetic acid, 10 mM sodium chloride, pH

3) at a 1:100 ratio. The diluted emulsion consisted of 0.2 wt% oil and 0.005% Tween 20 was then mixed with 2 wt% solution of displacing surfactant (SDS, DTAB or β -Lg in acetic acid buffer) in different ratios to obtain emulsions with a corn oil content of 0.012 wt%. The concentration of Tween 20 in the continuous phase of these emulsions was 0.0003 wt%, the concentration of displacing surfactant in the continuous phase ranged from 0-0.2 wt% (100 mM acetic acid, 10 mM sodium chloride, pH 3). The ζ -potential in the secondary emulsions was measured without further dilution.

Production of multilayer emulsions. O/W emulsions prepared by membrane homogenisation were mixed with 2 wt% SDS solution (100 mM acetic acid, 10 mM NaCl, and 0.4 g/l NaN_3 at pH 3) to prepare diluted emulsions with 0.2 wt% oil phase and 0.02 wt% SDS in the continuous phase. The diluted emulsion initially stabilised by Tween 20 containing SDS adsorbed onto the droplet surfaces was mixed with 0.2-0.002 wt% chitosan solution to prepare secondary emulsions stabilised by Tween 20–SDS–chitosan interfacial membranes (Figure 2). The corn oil content in the secondary emulsions was kept constant at 0.012 wt%, as well as the ionic strength and pH of the continuous phase. The ζ -potential in the secondary emulsions was measured without any further dilution.

Particle Size Measurements. The particle size distribution of the emulsions was measured by laser light scattering (Mastersizer X, Malvern Instruments Ltd., Malvern, U.K.). The computer software used to analyze the angular dependence of the scattered light intensity identified the particle size distribution that gave the best fit between the experimental measurements and predictions made using (Mie) light scattering theory. A refractive index ratio (refractive index of

particles divided by refractive index of surrounding liquid) of 1.08 was adopted in the calculation of the particle size distributions. The particle size measurements are reported as the average and standard deviation of measurements made on at least two freshly prepared samples, with three readings made per sample.

ξ -Potential Measurements. Emulsions with a droplet concentration of 0.012 wt% were injected into the measurement chamber of a particle electrophoresis instrument (ZEM 5003, Zetamaster, Malvern Instruments, Worcester, U.K.), and the ξ -potential was determined by measuring the direction and velocity of the droplets in the applied electric field. The ξ -potential measurements are reported as the average and standard deviation of measurements made on two freshly prepared samples, with five measurements made per reading and three readings per sample.

Optical Microscopy. Emulsions were gently agitated in a glass test tube before analysis to ensure that they were homogeneous. A drop of emulsion was then placed on a microscope slide and covered by a cover slip. The microstructure of selected emulsions was determined using a conventional light microscopy (Nikon microscope Eclipse E400, Nikon Corporation, Japan). The images were acquired using a CCD camera (CCD-300-RC, DAGE-MTI, Michigan City, IN) connected to Digital Image Processing Software (Micro Video Instruments Inc., Avon, MA) installed on a PC computer.

Results and Discussion

Premix membrane emulsification

The median drop diameter in the premix was in the range between 110 and 170 μm (Fig. 3(a)). After the first pass through the membrane, the drops were still highly polydisperse with a median diameter of 35 μm , as shown in Fig. 3(b), which can be explained by a low transmembrane flux in the first pass due to droplet creaming in the premix before homogenisation. Creaming is more rapid for larger drops and therefore, due to very large drops the premix quickly separates into two layers, the lower one containing the continuous phase almost free of droplets and the upper layer containing closely packed drops. The initial drop concentration was 20 wt% and on the assumption that the drop concentration in the creamed layer was 50 wt% (the volume fraction at which the spherical particles become closely packed is 0.585 [35]), the amount of the clear liquid and creamed layer was 60 and 40 g per 100 g of premix, respectively. Therefore, it is reasonable to expect a relatively high flux until about 60 g of the permeate is collected, followed by a much lower flux for the remaining 40 g. As can be seen in Fig. 4, the flux during first pass was indeed very high until about 60 g of the permeate had been passed and after that the flux rapidly declined due to sudden rise in the drop concentration. In premix membrane emulsification, transmembrane pressure, Δp_{tm} provides energy for flow through the membrane and for droplet breakup [5]:

$$\Delta p_{tm} = \underbrace{\eta(R_m + R_{f,i})J_i}_{\Delta p_{flow}} + \underbrace{C\phi(1/d_i - 1/d_{i-1})\gamma}_{\Delta p_{breakup}} \quad (1)$$

where C is a constant, ϕ is the volume fraction of the dispersed phase, γ is the interfacial tension, η is the emulsion viscosity within the pores, J_i and d_i are the transmembrane flux and final particle size in the i th pass, R_m is the membrane resistance, and $R_{f,i}$ is the fouling resistance in the i th pass. The second term in Eq. (1) is based on the assumption that the energy needed to reduce

the size of a droplet is proportional to the resultant increase in surface area. The fouling resistance is a consequence of the accumulation of drops on the membrane surface (external fouling) and inside the pores (internal fouling). It is reasonable to expect that the drops do not coalesce on the membrane surface because the SPG membrane is highly hydrophilic and the lipid drops do not have a tendency to spread over a hydrophilic surface. In addition, the applied pressure difference of 1 bar is probably too low to induce drop coalescence on the membrane surface. However, it is very likely that the drops in the fouling layer are deformed into non-spherical shapes that offer greater resistance to transmembrane flow than spherical drops.

Initially, $\varphi \rightarrow 0$ due to droplet creaming and $\Delta p_{flow} \rightarrow \Delta p_{tm}$, i.e. the pressure energy is almost completely used for providing flow through the membrane. A rapid decline in flux during the first pass after about 8 s is due to a sudden increase in φ and R_{fi} , so that according to Eq. (1): $\Delta p_{breakup} \rightarrow \Delta p_{tm}$ and $\Delta p_{flow} \rightarrow 0$, i.e. the pressure energy is almost completely used for droplet breakup. As can be seen in Fig. 4, the flux in the second pass was lower than the flux at the beginning of the first pass because the drop size in the second pass diminished from 35 to 8.6 μm (Fig. 5) and thus, a substantial portion of the total pressure drop was used for droplet breakup. For example, in the second pass about 27 % of the total pressure drop is used for droplet breakup and 73 % for providing flow through the membrane. Another reason for the greater initial flux is that the viscosity of the clear liquid formed by creaming of the premix is lower than the viscosity of the feed emulsion in the second pass. In the subsequent passes the median drop size was progressively less reduced and therefore, a greater and greater fraction of the total pressure drop was used for providing flow through the membrane. For example, in the third pass about 92 % of the total pressure drop was used for providing flow through the membrane. In the fourth and fifth

pass, the median drop size remained virtually constant at about 7 μm with the flux value approaching $79 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$ (Fig. 6), which is equivalent to the production rate of 50 g of emulsion in less than 6 s. The final median drop size was smaller than the membrane pore size, which was the behavior observed earlier using SPG membranes with a mean pore size of 8 and 10.7 μm [3-5]. In Figure 6 the mean transmembrane fluxes are presented as a function of number of passes. The mean transmembrane flux during the first pass was calculated as $V/(tA_m)$, where V is the total permeate volume collected during the first pass, t is the time required for permeation and A_m is the effective membrane area. In the subsequent passes, the transmembrane flux was constant and calculated by dividing the slope of the corresponding V vs. t line of best fit by A_m . The two of these best-fit straight lines for 2nd and 5th pass are shown in Figure 4.

The particle size distribution (PSD) of the emulsion formed after the first pass is presented in Fig. 7(a). This emulsion is composed of a mixture of a relatively monodisperse population of small drops with a diameter in the range of 4-20 μm (peak A) and a polydisperse population of large drops with a diameter ranging from 20 to 200 μm (peak B). The large drops were probably formed at low flux values, i.e. during permeation of the concentrated creamed layer through the membrane. The small uniform drops present in peak A can be separated from the large drops to form surprisingly regular hexagonal arrays on the microscope slide, as shown in Figure 7(b). The drop separation can be achieved by squeezing large drops between a microscope slide and cover slip and inclining the microscope slide to filter small droplets through the bed of squeezed large drops. The average diameter of the drops in Fig. 7(b) is 11 μm , which agrees very well with the maximum of peak A in Fig. 7(a). It can be concluded that highly monodisperse drops with a size

approximately equal to the mean pore size can be obtained by filtering polydisperse emulsions produced by a single pass homogenisation through a SPG membrane.

Modification of interfacial properties by surfactant displacement

The purpose of these experiments was to show that the interfacial properties of uniform droplets could be modified after homogenisation using the surfactant displacement method. Oil-in-water emulsions containing uniform droplets stabilized by emulsifier were prepared using SPG membrane homogenisation, and then these emulsions were mixed with different amounts of emulsifier solutions (SDS, DTAB or β -lactoglobulin) and their electrical characteristics and particle size were measured. For each emulsifier, two types of experiments were carried out: (i) the emulsifier was added to an emulsion initially prepared using Tween 20 as the emulsifier; (ii) the emulsifier was added to an emulsion initially prepared using the same emulsifier.

In the absence of additional emulsifier, the electrical charge on the emulsion droplets coated by Tween 20 was around -12 mV, which can be attributed to the presence of free fatty acids in the corn oil and/or surfactant. As mentioned earlier, a low electrical charge on the droplets poses a problem for electrostatic deposition of oppositely charged polyelectrolytes onto their surfaces. Therefore, it is necessary to increase the charge on the droplets by addition of more highly charged emulsifiers. For emulsions initially coated with Tween 20, the negative charge on the droplets increased from -12 to -105 mV when the SDS concentration in the continuous phase was increased from 0 to 0.2 wt% (Fig. 8), which suggested that SDS molecules adsorbed to the droplet surfaces. Interestingly, there was also an increase in the negative charge on the droplets

initially coated with SDS, when the concentration of additional SDS in the continuous phase was increased from 0 to 0.2 wt% (Fig. 8). This effect can be attributed to a change in the partitioning of SDS molecules between the droplet surfaces and the continuous phase, which alters the surfactant load at the oil-water interface. At low SDS concentrations, some of the SDS moves into the continuous phase, thereby reducing the interfacial surfactant load and droplet charge. At similar higher SDS levels (0.05 – 0.2%), the charge on droplets initially prepared using SDS was similar to that on those initially prepared using Tween 20 (Fig. 8), which suggests that the Tween 20 molecules were completely displaced from the droplet surfaces by SDS.

Similar trends were obtained for the emulsions to which the cationic surfactant DTAB was added. For emulsions initially coated with Tween 20, the charge on the droplets went from –12 to +48 mV when the DTAB concentration in the continuous phase was increased from 0 to 0.2 wt% (Fig. 8), which suggested that DTAB molecules adsorbed to the droplet surfaces. There was also an increase in the positive charge on the droplets initially coated with DTAB, when the additional DTAB concentration in the continuous phase was increased from 0 to 0.2 wt% (Fig. 8). Again, this effect can be attributed to a change in the partitioning of DTAB molecules between the droplet surfaces and continuous phase. At low surfactant concentrations, some of the DTAB moves into the continuous phase, thereby reducing the net droplet charge. At similar higher DTAB levels (0.05 – 0.2%), the charge on droplets initially prepared using DTAB was appreciably more positive than those initially prepared using Tween 20 (Fig. 8), which suggests that the Tween 20 molecules were not completely displaced from the droplet surfaces by DTAB at these levels. As mentioned earlier, emulsions containing cationic droplets cannot usually be produced using SPG membrane emulsification because cationic surfactants foul the membranes.

Nevertheless, these results show that cationic droplets can be produced by forming emulsions with a non-ionic surfactant first, and then adding a cationic surfactant after homogenisation.

Finally, we examined the impact of adding additional β -lactoglobulin to emulsions initially stabilized by either Tween 20 or β -lactoglobulin. β -lactoglobulin (β -Lg) is often used as an emulsifier to stabilize O/W emulsions because it is a natural polymer with good biocompatibility [19-23]. For emulsions initially coated with Tween 20, the charge on the droplets went from -12 to $+20$ mV when the β -lactoglobulin concentration in the continuous phase was increased from 0 to 0.2 wt% (Fig. 8), which suggested that β -lactoglobulin molecules adsorbed to the droplet surfaces. On the other hand, there was no change in the charge on the droplets initially coated by β -lactoglobulin when additional β -lactoglobulin was added to the continuous phase, which can be attributed to the fact that this protein has an extremely high affinity for the droplet surfaces. At similar high β -lactoglobulin levels (0.05 – 0.2%), the charge on droplets initially prepared using β -lactoglobulin was similar to that on those initially prepared using Tween 20 (Fig. 8), which suggests that the Tween 20 molecules were completely displaced from the droplet surfaces by β -lactoglobulin. Thus, even though β -lactoglobulin is not particularly effective at forming uniform-sized droplets using the SPG membrane method itself since it tends foul the membranes [4], it is still possible to form uniform droplets coated by β -lactoglobulin using this surfactant-displacement method.

It is important to note that in all experiments discussed in this section, the initial narrow particle size distribution was not disturbed by the addition of surfactants (data not shown).

Modification of interfacial properties by electrostatic deposition

In these experiments, we examined the possibility of altering the interfacial characteristics after homogenisation using the electrostatic deposition method. Initially, an emulsion containing uniform-sized oil droplets was prepared using Tween 20, and then SDS was added to give the droplets an appreciable negative charge. This emulsion was then diluted with chitosan solutions (pH 3) to promote adsorption of the cationic chitosan molecules to the anionic droplet surfaces.

In the absence of chitosan, the net charge on the emulsion droplets was -53 mV due to the presence of anionic SDS on the droplet surfaces. The electrical charge on the droplets became less negative and eventually changed from negative to positive, as the chitosan concentration in the emulsions was increased (Figure 9). There was no net charge on the droplets when the chitosan concentration was somewhere between 4×10^{-6} and 1×10^{-5} wt%, indicating that droplet charge neutralisation occurred at a mass ratio (R) between 0.003 and 0.008 g of chitosan per gram of SDS. When the chitosan concentration was increased further, the positive charge on the droplets continued to increase, until it reached a constant value (between $+43$ and $+44$ mV) when the chitosan concentration exceeded about 0.005 wt% ($R = 4$ g/g) (Figure 9). The ability of charged polyelectrolytes to adsorb to the surface of oppositely charged colloidal particles and cause charge reversal (“overcharging”) is well-established in the literature [36]. Overcharging occurs because only a fraction of the charged groups on a polymer are required to neutralise the oppositely charged groups on the surface of a colloidal particle. The remainder of the charged polymer groups may protrude into the aqueous solution or may be in contact with uncharged regions on the particle surface [36].

The mean particle diameter of secondary emulsions containing 1 wt% corn oil was measured 24 h after chitosan was mixed with the secondary emulsions (Figure 10). The chitosan concentrations in the continuous phase were higher than those shown in Figure 9 because the emulsions were more concentrated. In the absence of chitosan, the mean particle diameter (d_{50}) was 6.9 μm and a relative span of the particle size distribution was 0.886. At a chitosan concentration from 0.02 to 0.03 wt %, there was a large increase in d_{50} to over 280 μm followed by an increase in span to over 3, which was attributed to extensive droplet aggregation. It might have been expected that these emulsions would have been stable to droplet aggregation, because of relatively strong electrostatic repulsive interactions between the droplets as a result of a high net charge on the droplet surfaces of +43 mV. Observation of the emulsions by optical microscopy indicated that the droplets were highly flocculated (data not shown). It is likely that the droplets were held together by chitosan molecules that were adsorbed to the surface of more than one droplet [37]. This is not surprising, since chitosan was known to induce bridging flocculation of oppositely charged emulsion droplets with mean particle sizes below 1 μm , produced using high pressure valve homogenisers [25]. During the initial stages of mixing of the chitosan solution with the primary emulsion, there would have been many droplets present with completely negative surfaces; hence, it is likely that chitosan molecules could adsorb to the surface of two or more of these droplets simultaneously [34]. At relatively high chitosan concentrations (≥ 0.6 wt%), the measured mean drop diameters (d_{50}) were below 11 μm , which is fairly similar to the initial mean drop diameter, but the span was still around 2.5. The fact that the mean particle diameter was relatively small at higher chitosan concentrations can be attributed to the fact that the emulsion droplets were saturated with chitosan and therefore bridging flocculation was less likely to occur.

The production of cationic droplets has a number of potential advantages for certain applications in the food industry. It has been shown that positively charged droplets are much less susceptible to destabilisation by multivalent cations, such as calcium and iron [38, 39]. In addition, the lipids in positively charged droplets are much less susceptible to iron-catalysed oxidation because of the electrostatic repulsion between the droplet surface and the iron [40, 41].

Conclusions

This study has shown that the surface characteristics of uniformly sized Tween 20-stabilised oil droplets produced by membrane emulsification can be changed by adding various anionic and cationic surfactants to the continuous phase without affecting particle size distribution of the emulsion produced. The surfactants fully or partly displace the original non-ionic surfactant molecules from the droplet surfaces, thereby introducing a higher droplet charge. The interfacial characteristics can also be altered using an electrostatic deposition method, which involves depositing polyelectrolytes onto oppositely charged droplet surfaces. The information obtained in this study is particularly useful for the design and production of oil-in-water emulsions containing relatively large uniform droplets with controlled interfacial properties. Multilayer emulsions with a mean particle size of around 1 μm have previously been produced using high-pressure valve homogenisation [30]. This work is one of the first attempts to produce multilayer emulsions with relatively large uniform droplets of oil using the membrane emulsification technique.

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Table 1. Preparation of bilayer emulsions by LbL deposition. “Primary emulsion” containing electrically charged droplets coated with a surfactant was mixed with an oppositely charged biopolymer to prepare a “secondary” emulsion. In some cases, primary emulsion was prepared at a pH where there was virtually no adsorption of the biopolymer to the droplets. In such cases the pH was varied to change the ξ -potential, the variation being marked in the table with an arrow sign, so that the biopolymer could adsorb to the droplet surfaces through electrostatic attraction.

| Primary emulsion | Secondary (bilayer) emulsion containing droplets coated by two interfacial layers | Source |
|---|--|--------|
| DP: 5% corn oil. CP: 0.25% β -Lg, pH 3.5 ($\xi_1 = +54$ mV) | DP: 5% corn oil. CP: 0.25% β -Lg, >0.1% pectin , pH 3.5 ($\xi_2 = -13$ mV) | [19] |
| DP: 10% corn oil. CP: 1% β -Lg, pH 3 ($\xi_1 = +60$ mV) | DP: 5% corn oil. CP: 0.5% β -Lg, 0.15% ι or λ carrageenan , pH 3 ($\xi_2 = -10$ or -37 mV) | [20] |
| DP: 0.1% corn oil. CP: 0.009% β -Lg, pH 7 ($\xi_1 = -70$ mV) | DP: 0.1% corn oil. CP: 0.009% β -Lg, 0.004% alginate , pH 7 \rightarrow 3 ($\xi_1 \rightarrow +70$ mV, $\xi_2 = -32$ mV) | [21] |
| DP: 0.2% corn oil. CP: 0.018% β -Lg, pH 7 ($\xi_1 < 0$) | DP: 0.1% corn oil. CP: 0.009% β -Lg, 0.03% gum arabic , pH 7 \rightarrow 3 ($\xi_1 \rightarrow +60$ mV, $\xi_2 = -20$ mV) | [22] |
| DP: 1% corn oil. CP: 0.05% β -Lg, pH 3 ($\xi_1 = +60$ mV) | DP: 1% corn oil. CP: 0.05% β -Lg, 0.01% chitosan , pH 3 \rightarrow 6 ($\xi_1 \rightarrow -16$ mV, $\xi_2 \rightarrow +11$ mV) | [23] |
| DP: 15% tuna oil. CP: 3% lecithin , pH 3 ($\xi_1 = -52$ mV) | DP: 5% tuna oil. CP: 1% lecithin , 0.2% chitosan , pH 3 ($\xi_2 = +57$ mV) | [24] |
| DP: 3% corn oil. CP: 3 mM SDS , pH 3 ($\xi_1 = -47$ mV) | DP: 3% corn oil, 3 mM SDS . CP: 0.05% chitosan , pH 3 ($\xi_2 = +50$ mV) | [25] |
| DP: 20% corn oil. CP: 0.46% SDS , pH 3.0 ($\xi_1 = -40$ mV) | DP: 10% corn oil. CP: 0.23% SDS , 2% fish gelatin , pH 3.0 ($\xi_2 = +30$ mV) | [26] |
| DP: 1% corn oil. CP: 0.15% casein , pH 7 ($\xi_1 = -40$ mV) | DP: 1% corn oil. CP: 0.15% caseinate , 0.2% alginate , pH 7 \rightarrow 3.5 ($\xi_1 \rightarrow +35$ mV, $\xi_2 = -37$ mV) | [27] |
| DP: 30 vol% n-tetradecane. CP: 0.75% sodium caseinate , pH 6 ($\xi_1 = -32$ mV) | DP: 20 vol% n-tetradecane. CP: 0.5% sodium caseinate , 1% dextran sulfate , pH 6 ($\xi_2 = -50$ mV) | [28] |

DP – dispersed phase, CP – continuous phase. % denotes wt%.

Table 2. Preparation of three-layer emulsions by LbL deposition. The term primary emulsion refers to oil-in-water emulsion stabilised by a single charged surfactant. The term secondary emulsion refers to emulsion containing droplets coated by two oppositely charged layers formed by mixing primary emulsion with a polyelectrolyte solution. The term tertiary emulsion refers to emulsion containing droplets coated by three oppositely charged interfacial layers formed by mixing secondary emulsion with another polyelectrolyte solution or another emulsion [31].

| Primary emulsion | Secondary (bilayer) emulsion | Tertiary (three-layer) emulsion | Source |
|---|--|---|--------|
| DP: 5% corn oil. CP: 5mM SDS , pH 3 ($\xi_1 = -60$ mV) | DP: 1% corn oil. DP: 1mM SDS , 0.024% chitosan , pH 3 ($\xi_2 = +59$ mV) | DP: 0.2% corn oil. CP: 0.2 mM SDS , 0.0048% chitosan , 0.04% pectin , pH 3 ($\xi_3 = -14$ mV) | [29] |
| DP: 10% corn oil. CP: 0.50% β-Lg , pH 7 ($\xi_1 = -30$ mV) | DP: 5% corn oil. CP: 0.225% β-Lg , 0.2% pectin , pH 7 \rightarrow 4 ($\xi_1 \rightarrow +27$ mV, $\xi_2 = -10$ mV) | DP: 2.5% corn oil. CP: 0.1125% β-Lg , 0.1% pectin , 0.25% chitosan , pH 4 ($\xi_3 = +36$ mV) | [30] |
| DP: 5% corn oil. CP: 0.50% β-Lg , pH 6 ($\xi_1 = -43$ mV) | DP: 5% corn oil. CP: 0.50% β-Lg , 0.1% ι-carrageenan , pH 6 ($\xi_2 = -55$ mV) | DP: 5% corn oil. CP: 0.50% β-Lg , 0.1% ι-carrageenan , 0.2% gelatin , pH 6 ($\xi_3 = -38$ mV) | [31] |
| DP: 3% corn oil. CP: 0.97% lecithin , pH 3 ($\xi_1 = -51$ mV) | DP: 3% corn oil. CP: 0.57 % lecithin , 0.06% chitosan , pH 3.0 ($\xi_2 = +61$ mV) | DP: 3% corn oil. CP: 0.27% lecithin , 0.06 % chitosan , 0.4% pectin , pH 3 ($\xi_3 = -5$ mV) | [32] |
| DP: 4% corn oil. CP: 0.02% β-Lg , pH 4, $d_{32} \sim 0.6$ μm ($\xi_1 = +20$ mV) | DP: 2% corn oil, CP: 0.01% β-Lg , 0.1% pectin , pH 4 ($\xi_2 = -22$ mV) | DP: 1.2% corn oil. CP: 0.8% of small droplets ($d_{32} \sim 0.2$ μm) stabilised by β-Lg , pH 4 ($\xi_3 = +25$ mV) | [33] |

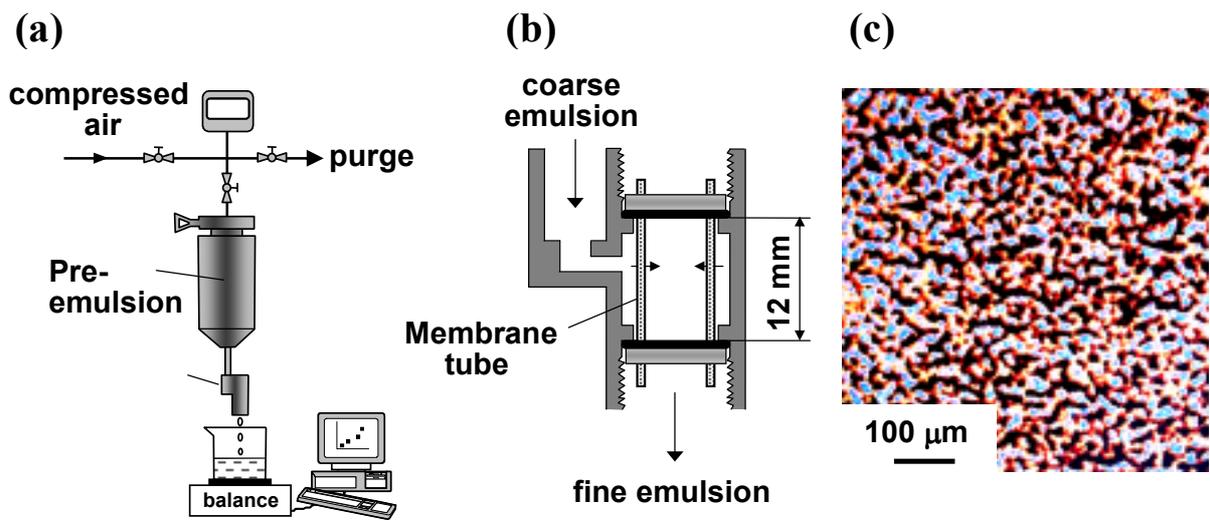


Figure 1. (a) Schematic diagram of the membrane homogeniser used; (b) Flow directions of premix and fine emulsion in the membrane module; (c) X-ray microtomograph of Shirasu porous glass (SPG) membrane showing uniform internal microstructure with interconnected pores.

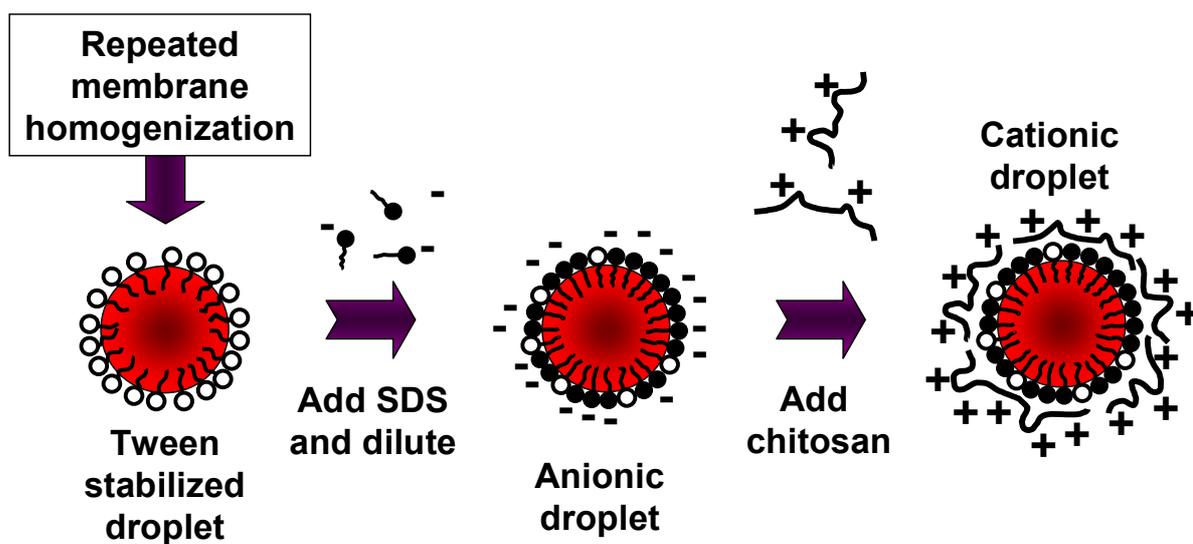


Figure 2. Preparation of secondary emulsions stabilised by Tween 20–SDS–chitosan interfacial layers using repeated membrane homogenisation.

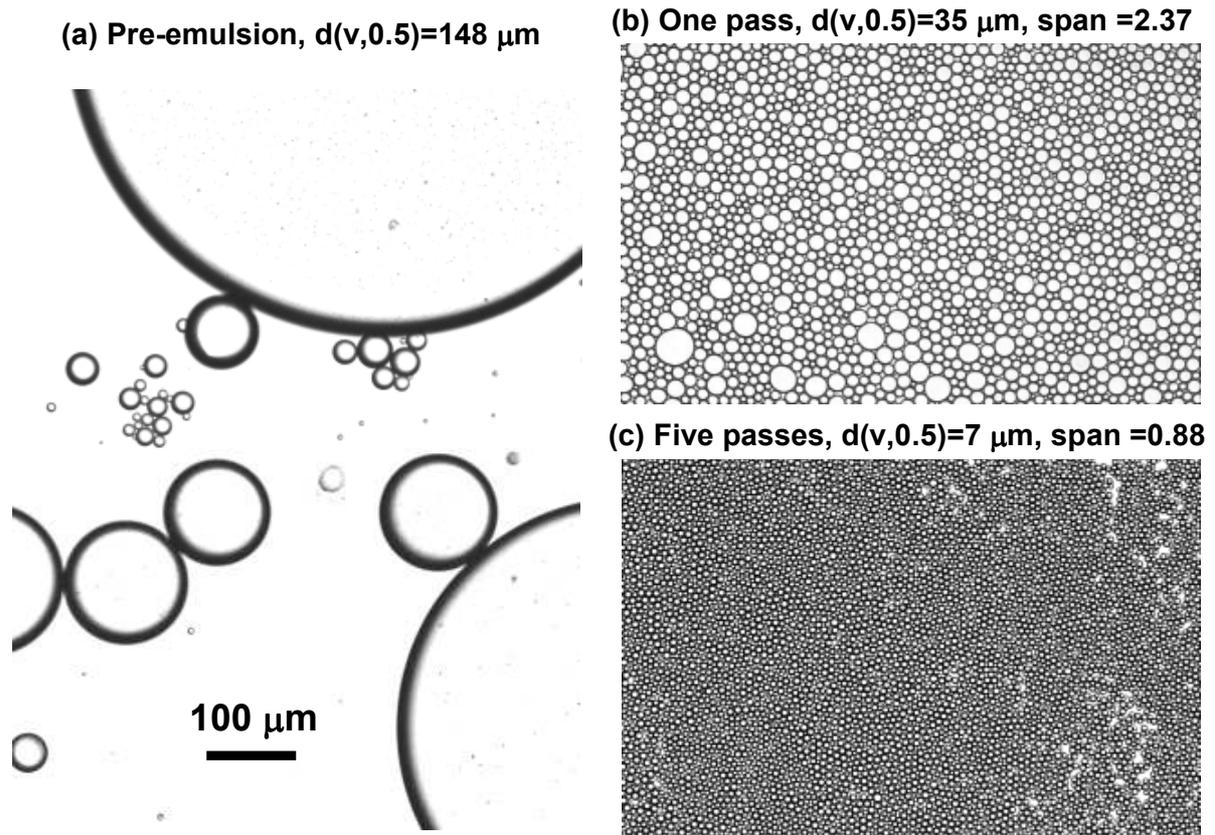


Figure 3. Micrographs of droplets in pre-emulsion and fine emulsions after one and five passes through the membrane. The scale bar shown is applicable to all pictures. Surfactant: 0.5wt% Tween 20, dispersed phase content: $\varphi = 20 \text{ vol}\%$, mean pore size: $d_p = 8.0 \mu\text{m}$, transmembrane pressure: $\Delta p_{tm} = 100 \text{ kPa}$.

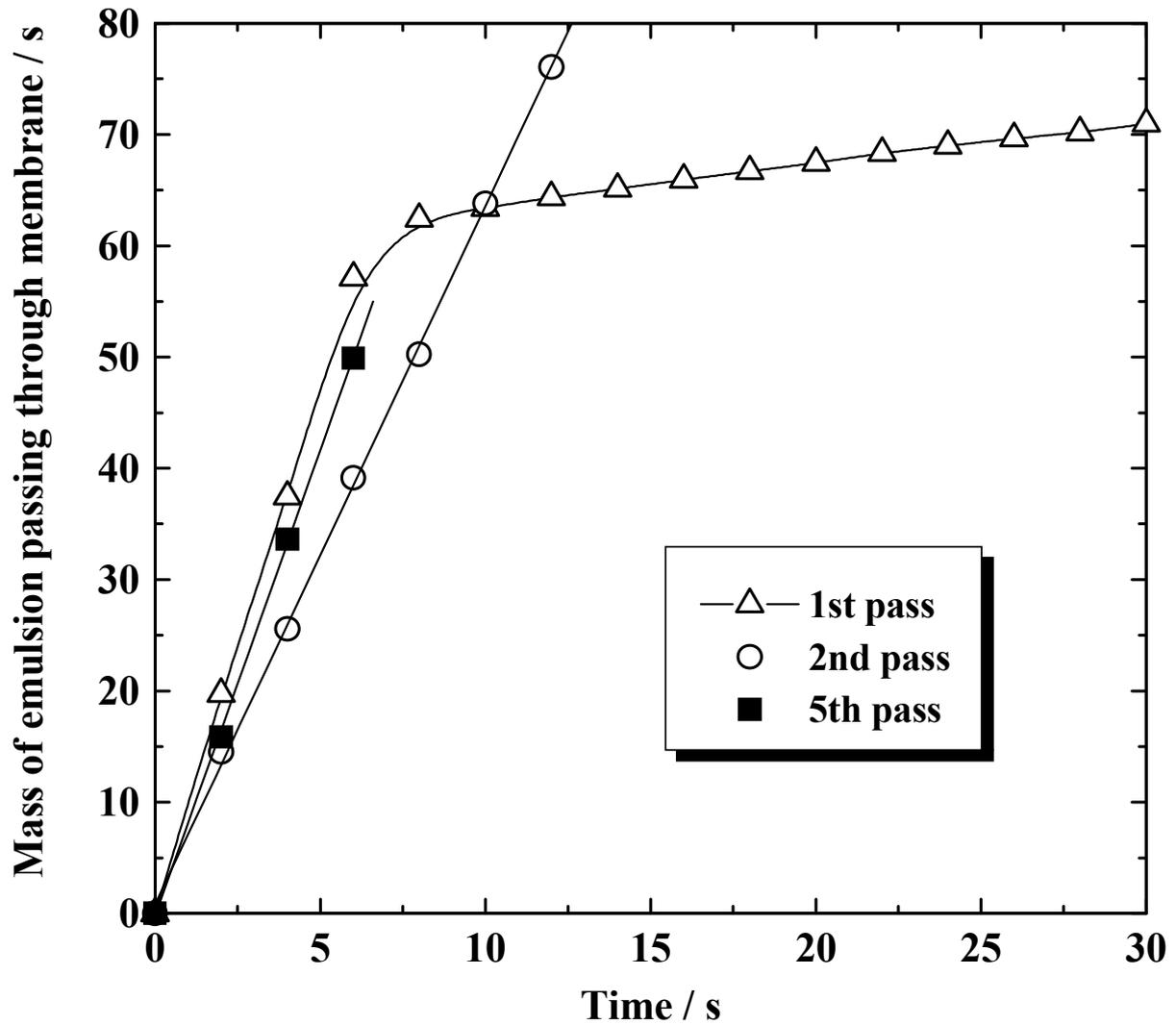


Figure 4. Dependence of the mass of feed emulsion passing through the membrane on time for different passes. Surfactant: 0.5wt% Tween 20, dispersed phase content: $\varphi = 20$ vol%, mean pore size: $d_p = 8.0 \mu\text{m}$, transmembrane pressure: $\Delta p_{tm} = 100$ kPa.

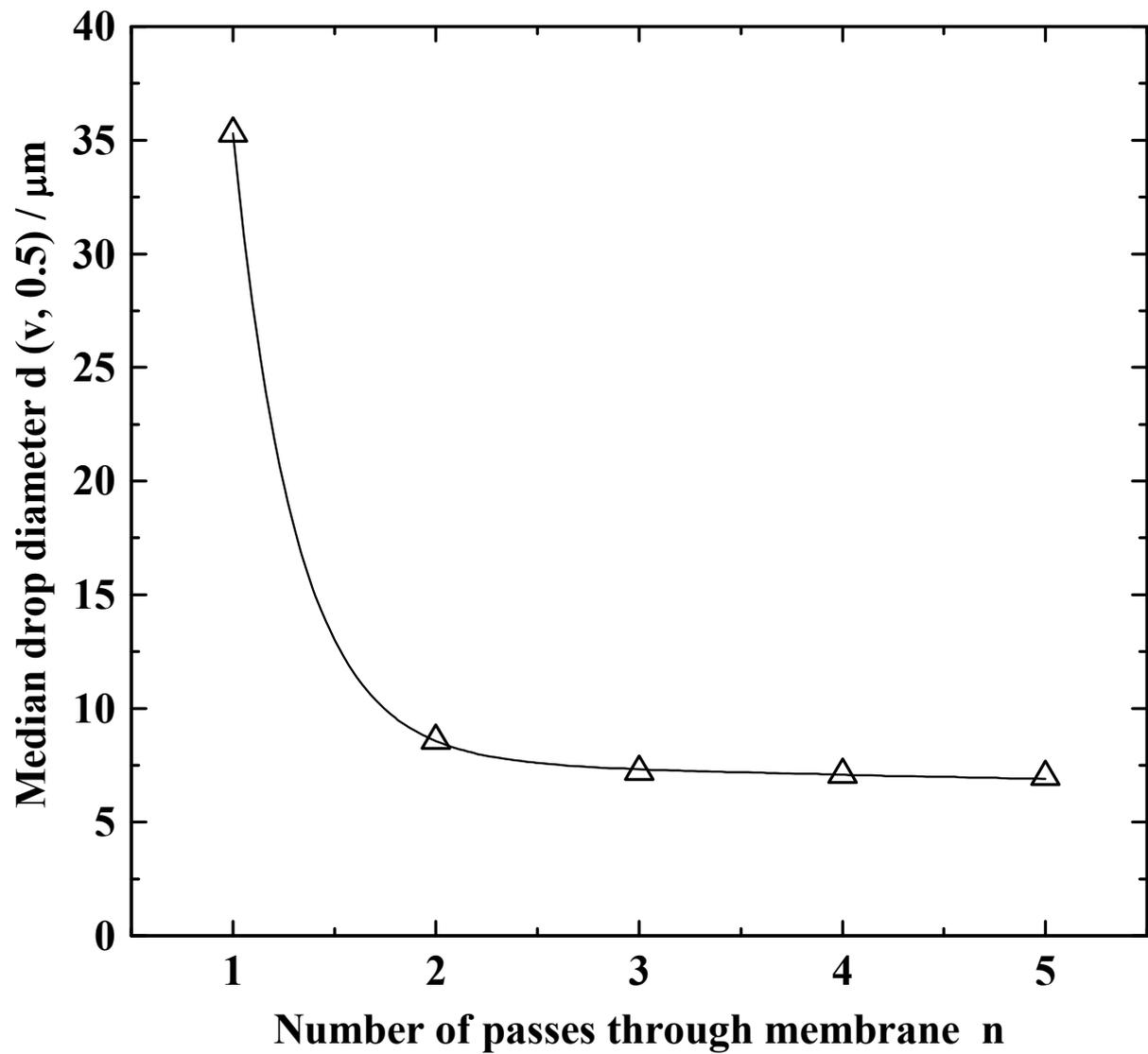


Figure 5. The median diameter of homogenised drops vs. number of passes through the membrane. Surfactant: 0.5wt% Tween 20, dispersed phase content: $\varphi = 20$ vol%, mean pore size: $d_p = 8.0 \mu\text{m}$, transmembrane pressure: $\Delta p_{tm} = 100$ kPa.

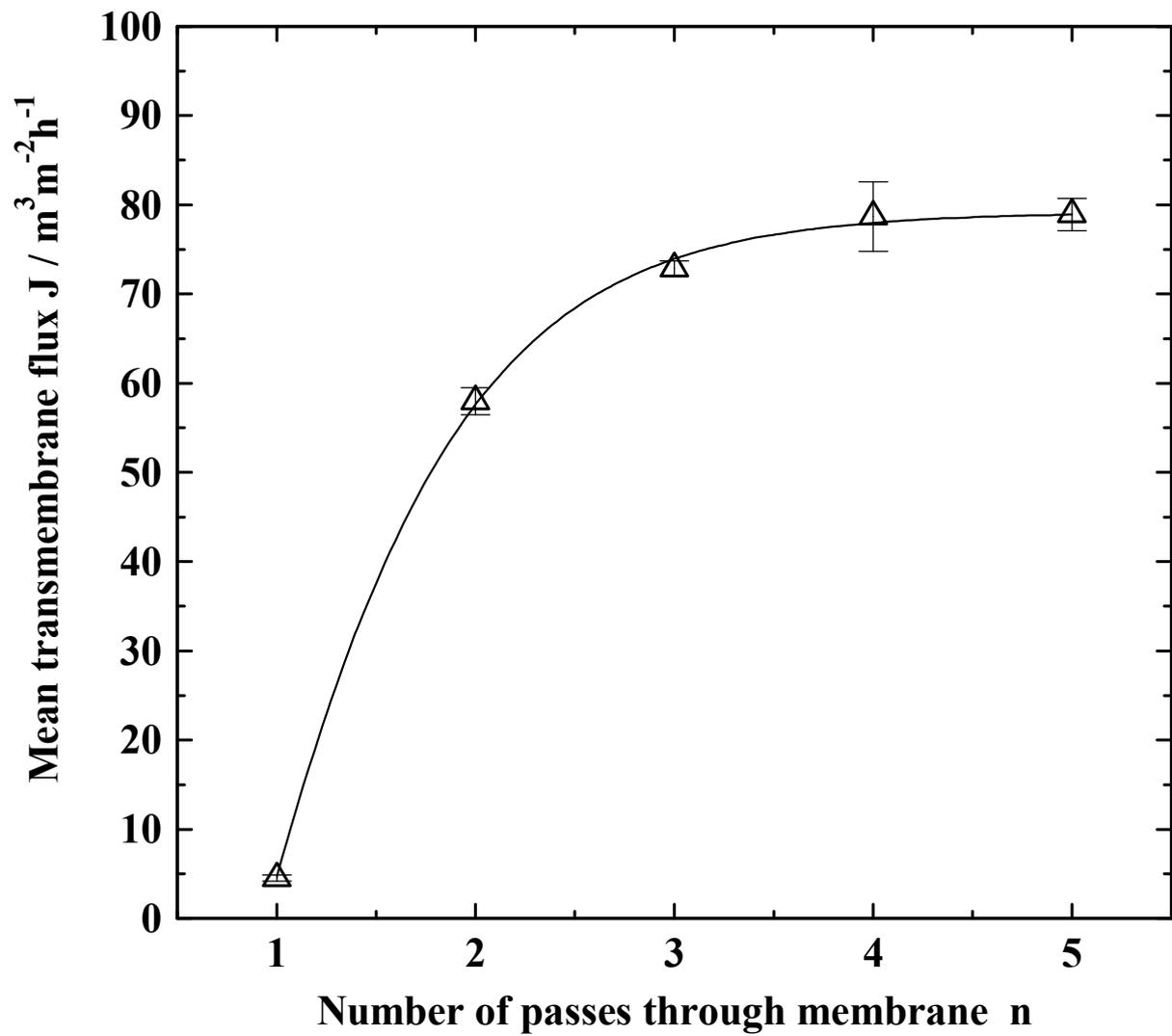


Figure 6. The mean transmembrane flux vs. number of passes through the membrane. Surfactant: 0.5wt% Tween 20, dispersed phase content: $\varphi = 20$ vol%, mean pore size: $d_p = 8.0 \mu\text{m}$, transmembrane pressure: $\Delta p_m = 100$ kPa.

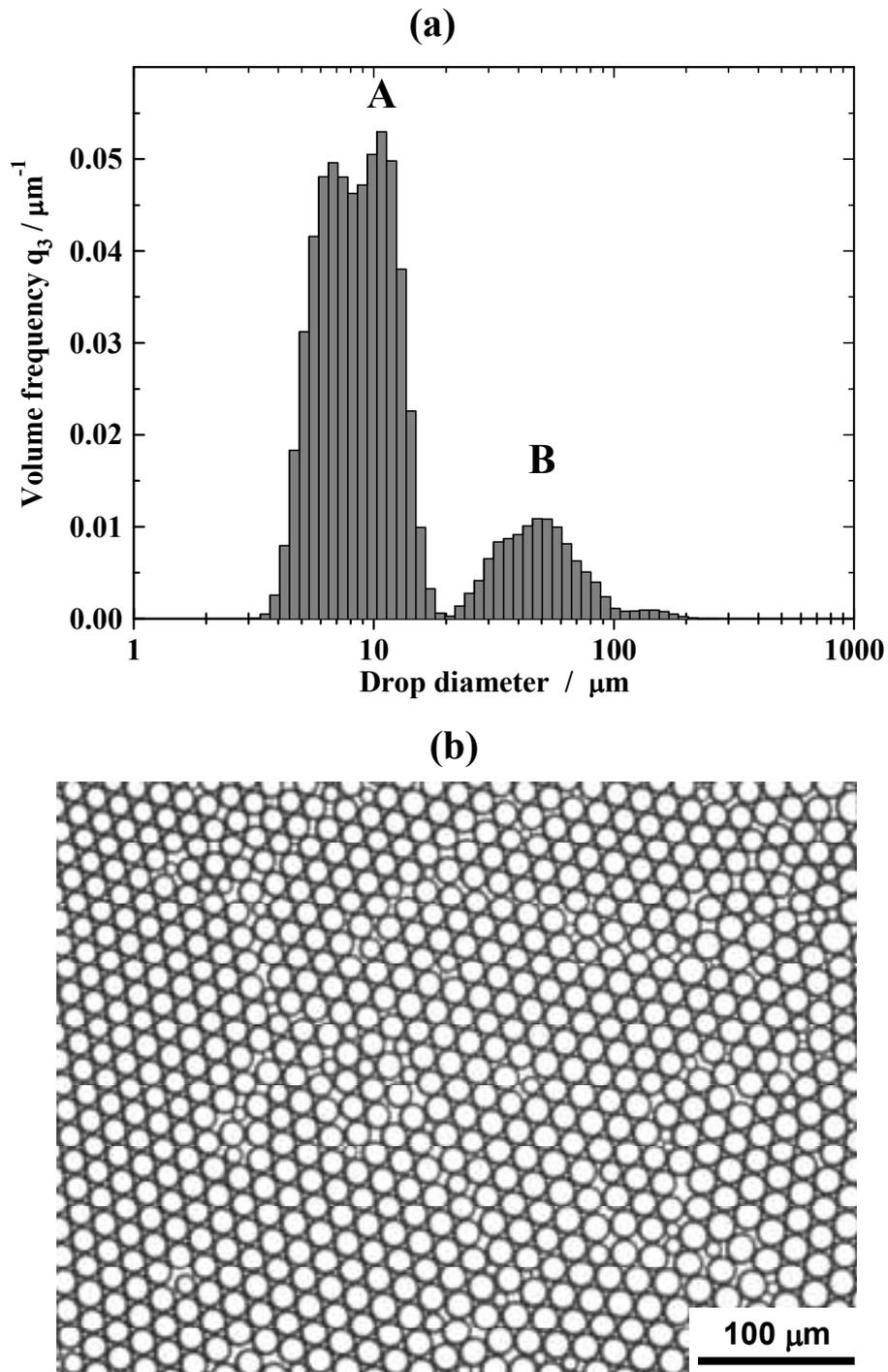


Figure 7. (a) Particle size distribution of the emulsion shown in Figure 3(b); (b) Hexagonally ordered uniform drops with a diameter of 11 μm formed by separation of the emulsion drops on the surface of slightly inclined microscope slide. The drops in peak B are retained between the microscope slide and cover slip due to their large size. The picture indicates that the drops in peak A are very uniform in size, although the emulsion as a whole is highly polydisperse.

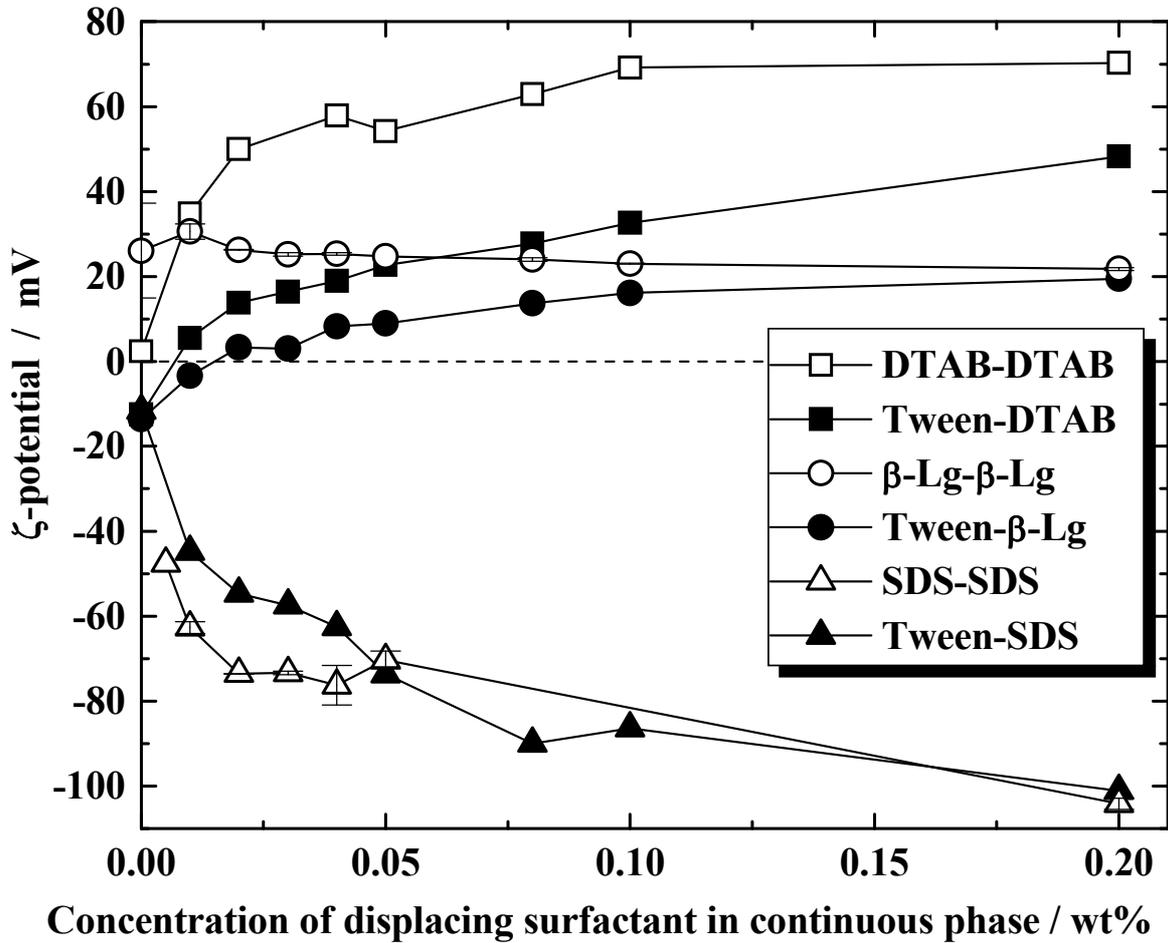


Figure 8. Particle electrical charge (ζ -potential) of the emulsions as a function of concentration of displacing surfactant in the continuous phase. Starting emulsion: 20 wt% corn oil, 0.5 wt% surfactant, 100 mM acetic acid, 10 mM NaCl (pH 3.0). Mixed emulsion: 0.012 wt% corn oil, 0.0003 wt% original surfactant, 100 mM acetic acid, 10 mM NaCl, and 0-0.20 wt% displacing surfactant (pH 3.0).

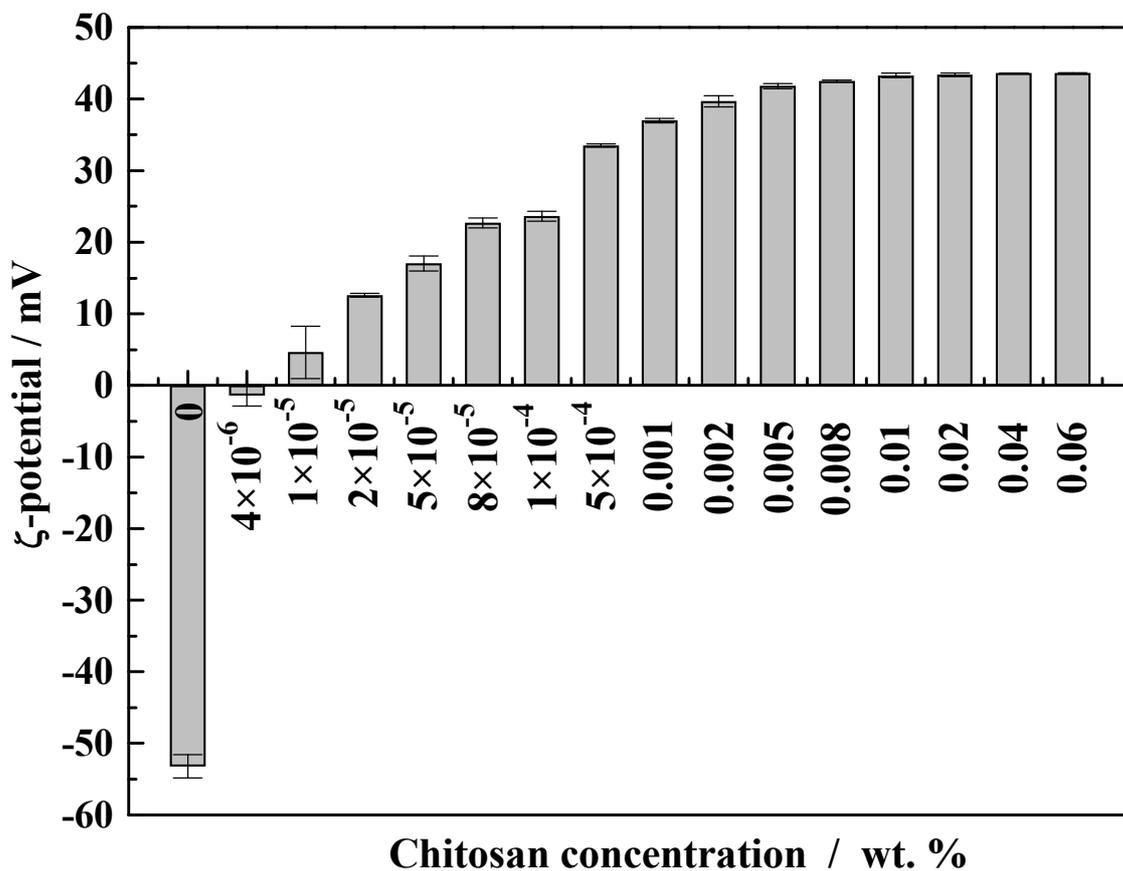


Figure 9. Dependence of droplet ζ -potential on chitosan concentration for secondary emulsions stabilised by Tween 20-SDS-chitosan multilayered interfacial membrane. Starting emulsion: 20 wt% corn oil, 0.5 wt% Tween 20. Mixed emulsion: 0.2 wt% corn oil, 0.005 wt% Tween 20 and 0.02 wt% SDS. Secondary emulsion: 0.012 wt% corn oil, 0.0003 wt% Tween 20, 0.0012 wt% SDS and 0-0.06 wt% chitosan (100 mM acetic acid, 10 mM NaCl, pH 3).

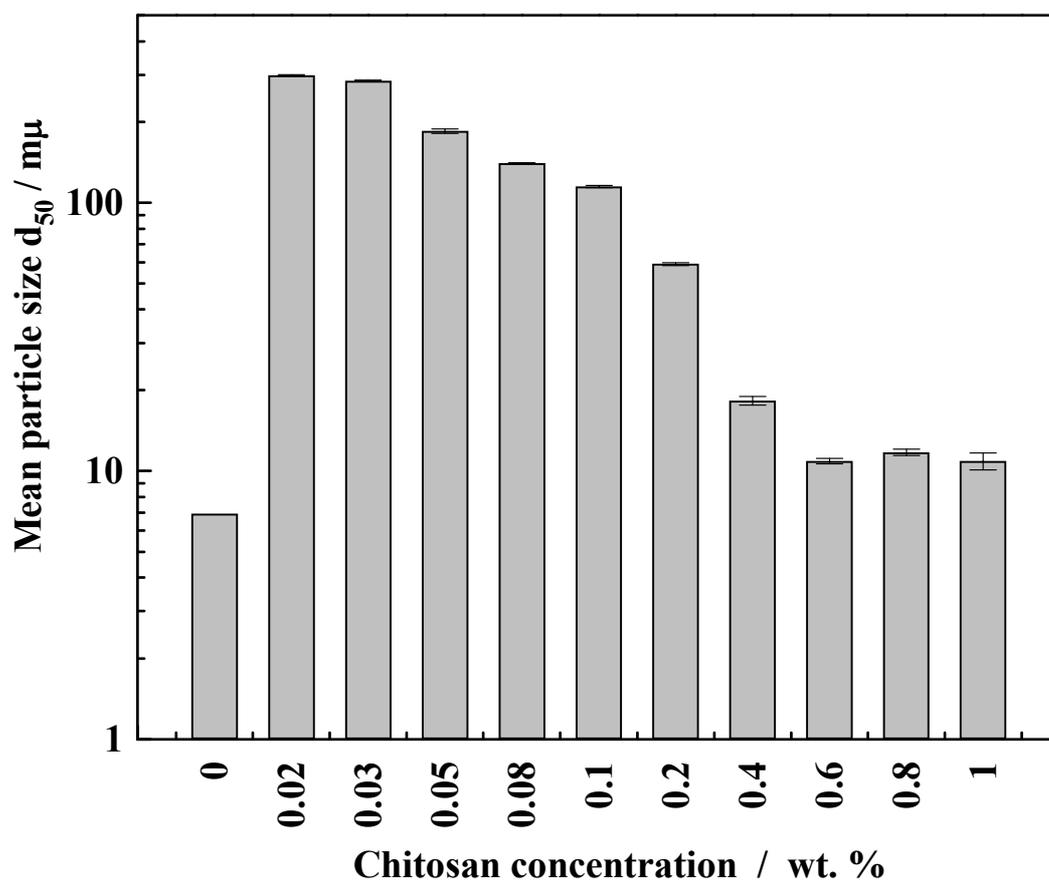


Figure 10. Dependence of mean particle diameter on chitosan concentration for secondary emulsions stabilised by Tween 20-SDS-chitosan multilayered interfacial membrane. Starting emulsion: 20 wt% corn oil, 0.5 wt% Tween 20. Mixed emulsion: 2 wt% corn oil, 0.05 wt% Tween 20 and 0.04 wt% SDS. Secondary emulsion: 1 wt% corn oil, 0.025 wt% Tween 20, 0.02 wt% SDS and 0-1 wt% chitosan (100 mM acetic acid, 10 mM