# Efficiency of liposomal and micellar emulsifiers during homogenization

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## Abstract

The coalescence of emulsion droplets during the homogenization process is governed by the emulsifier adsorption rate. This is in turn affected by the size and shape of the adsorbing molecule which can be dependant of the aggregation states.

The aim of this thesis was to develop a model system suitable for measuring the efficiency of emulsifier during homogenization and to evaluate the hypothesis that the efficiency is dependant of the emulsifier aggregation state. The idea was to compare the degree of coalescence during emulsification when the emulsifier is adsorbing from a micellar respectively liposomal dispersion. The intension was to do this by using the method of fluorescence. The liposomes used were soybean phosphatidylcholine (PC) and mixed micelles of sodium cholate (NaC) and PC.

The efficiency of liposomal and micellar emulsifiers during homogenization could not be evaluated. The liposomes had no emulsifying properties possibly due to insufficient mixing and in the current case poor solubility of lecithin in the aqueous phase. The mixed micelles could not be formed. The methods used in this work are not to be recommended since the emulsifier must be soluble in the aqueous phase and the desired aggregation state easily prepared.

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# 1. Introduction

An emulsion is a dispersion of two immiscible liquids such as oil in an aqueous phase. The behavior and stability of emulsions are important for both biological and technological processes. Foods such as mayonnaise and milk are examples of emulsions. Therefore the study of emulsions is an important field of food technology.

Emulsions are formed through the process of homogenization as oil droplets are dispersed in the continuous phase by shearing. Emulsion droplets are formed by shear induced disruption of droplets and they are lost by shear induced collisions. Emulsifiers are surface active species that can cover emulsion droplets to prevent coalescence of merging droplets. Thus the thermodynamically unstable emulsions can remain stable for a long period of time.

The emulsifier adsorption rate needs to be fast to prevent coalescence of the newly formed drops. The efficiency of the emulsifier is dependent of the rate of which it reaches the droplet surface and spreads over it. The transports of emulsifiers to the droplet surfaces occur via diffusion or collisions between emulsifier aggregates and surface. Diffusion is fast for small and soluable particles while the collisions are faster for large, unsoluable and unstabile aggregates. The collision of large and stable emulsifier aggregates with the droplet surface is created by currents and eddies originating from the turbulent fluctuations created in the homogenization process[1].

The aim of this thesis is to develop a method to measure the efficiency of emulsifier during homogenization and to evaluate the hypothesis that the efficiency is dependant of the aggregation state. This is done by comparing the degree of coalescence from liposomal and micellar emulsifiers. Water, phosphatidylcholine (PC) and sodium cholate (NaC) were chosen to make up the emulsifier systems. Thus the emulsifiers could have a gradual transition of properties from the PC liposomal- to the NaC/PC mixed micellar state.

# 2. Theory

## 2.1 Emulsions and homogenization

Emulsion droplets are dispersed in the continuous phase by the energy supplied by shearing with a homogenizer. Emulsifiers protect the droplet surface and stabilize the dispersion. Figure 1 illustrates the separated oil and water phase before homogenization and the water-in-oil (W/O) emulsion with droplets covered by emulsifiers after shearing.

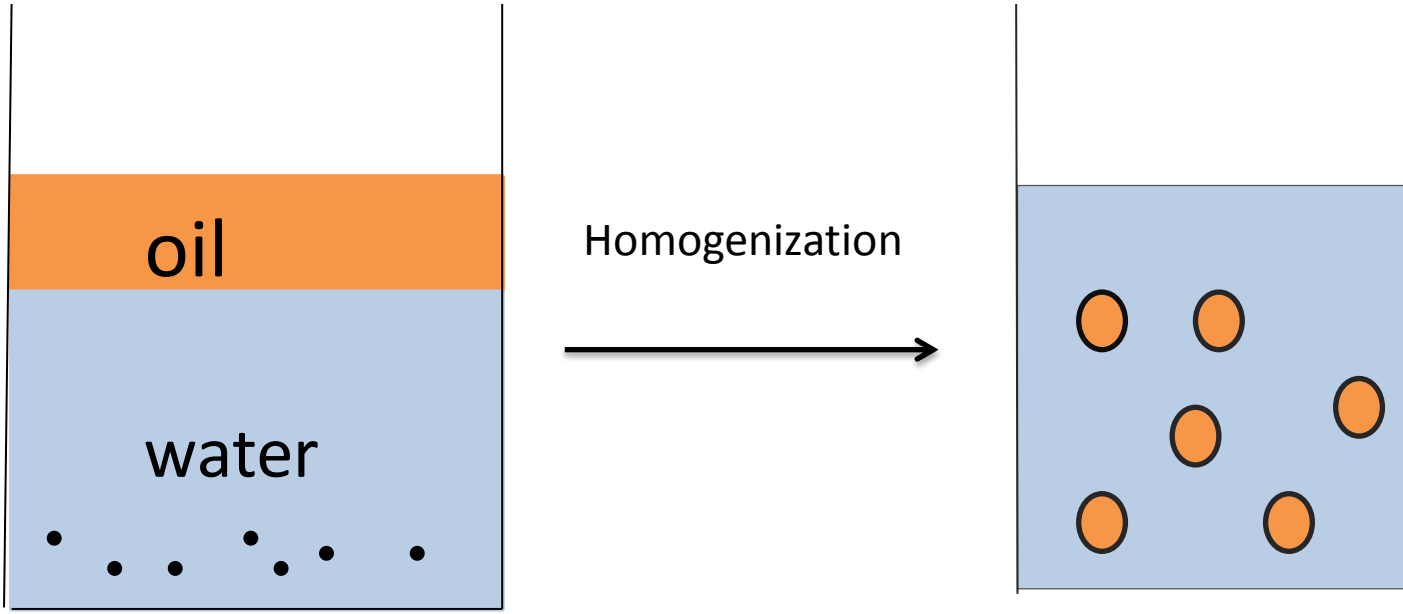


**Figure 1. The left vial represents the separate oil and water phase and emulsifiers before homogenization. The right vial illustrates oil emulsion droplets covered by emulsifiers dispersed in water after homogenization.**

Through the turbulent process of homogenization eddies are formed that both tear droplets apart (droplet formation) and merge unprotected droplets (coalescence). Figure 2 illustrates the events during homogenization. Flocculation and coalescence are two competing processes and the latter is governed by the rate of which emulsifier adsorbs onto and spreads over the surface.

The transports of emulsifiers to the droplet surfaces occur via diffusion or collisions between emulsifier aggregates and droplet. This emulsifier transport is influenced by the emulsifier shape and size. Particles with a hydrodynamic radius smaller than a few nanometers are dominated by diffusion, while collisions of bigger particles with a larger surface area are

controlled by convective forces originating from the turbulent fluctuations in the homogenization[1].

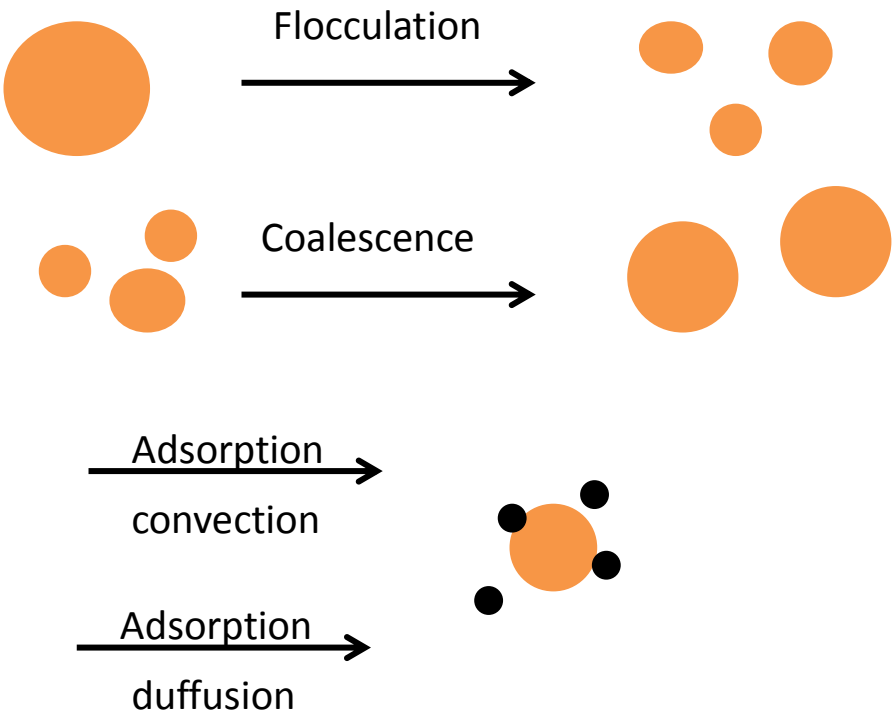


**Figure 2. Events during homogenization. The two upper processes illustrate the competing events, flocculation and coalescence. The coalescence is governed by adsorption of emulsifiers, the lower process, originating from convection or diffusion.**

## 2.2 Emulsifiers

### 2.2.1 Formation and size

The emulsifier used in this experiment is liposomal soy phosphatidylcholine (PC) and mixed micelles of sodium cholate (NaC) and PC. PC is a phospholipid and one of the main constituents of the food emulsifier lecithin extracted from egg yolk, sunflower oil or soybean oil[2]. The aggregation state of the pure PC is the bicontinuous phase that after addition of water adopts the planar bilayer conformation in the lamellar D – phase. Further dispersion in water makes the bilayer sheets incurve into themselves to form multilamellar vesicles[3]. Through the process of for example ultra sonication the vesicles becomes unilamellar liposomes, the step from bilayer to unilamellar liposome are illustrated in figure 3.

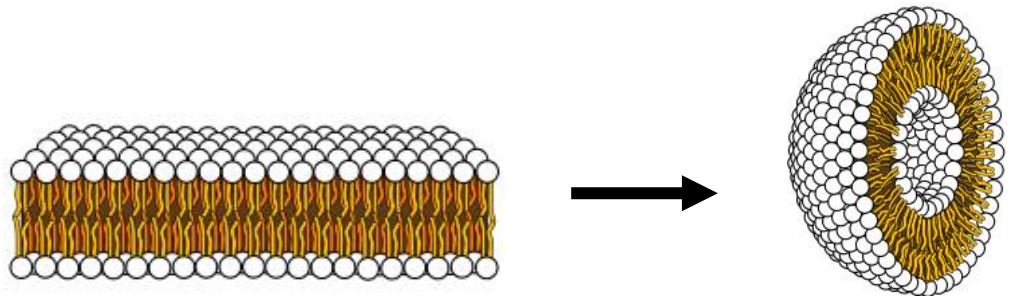

**Figure 3. Transition from bilayer conforamtion to unilamellar liposome. Modified from [4].**

The mixed NaC-PC-micelles are found in the $L_1$- isotropic aqueous liquid phase of the phase diagram in figure 4 (egg yolk PC- sodium cholate- water). As sodium cholate enters the vesicles dispersion the vesicle shape is energetically unfavored. It goes back to the bilayer formation and is further transformed to cylindrical mixed micelles as the bile salt stabilizes the bilayer edges[5] as illustrated in figure 5. Additional NaC converts the cylindrical micelles with a length of 100-300 nm and a diameter of about 3-5 nm in diameter to spheres with diameters of around 6 nm[5].

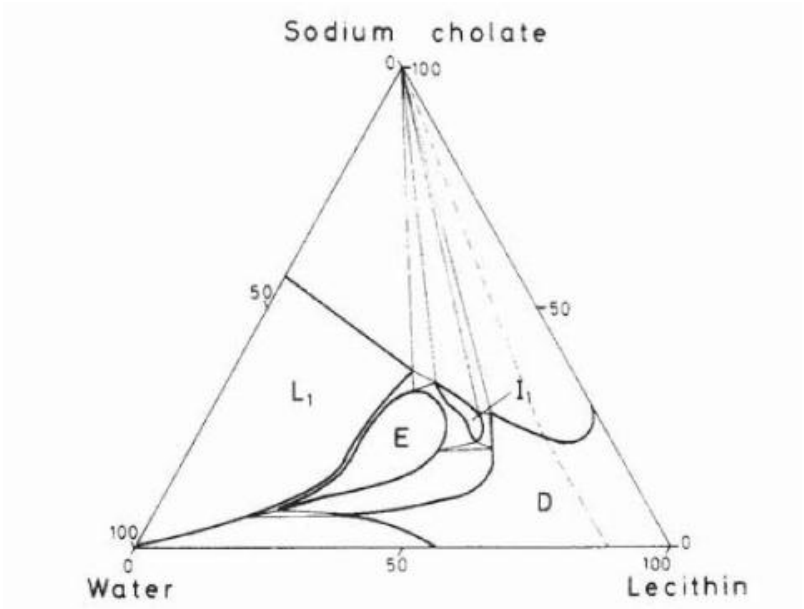


**Figure 4. Phase diagram for egg yolk lecithin- sodium cholate- water at 22°C [redrawn from Small et al. (1966)] . $L_1$- isotropic aqueous liquid, E- the hexagonal, D- the lamellar and I –the cubic liquid- crystalline phases. [6]**

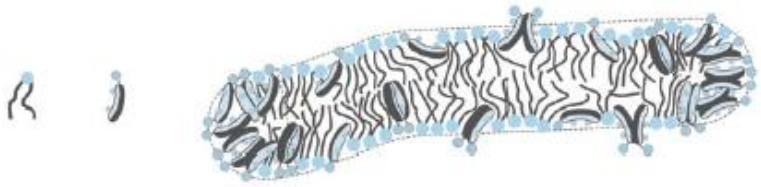

**Figure 5. Schematic structure of possible organization in a lecithin -bile salt mixed micelle (egg yolk lecithin and sodium taurochenodeoxycholate) Lecithin molecules with double chain and bile salt molecule with bulky tail group (modified from 7).**

### 2.2.2 Emulsifier concentration

The concentration of emulsifier needed to cover the oil droplets is seen as the volume of emulsifier surrounding the oil droplet. This is illustrated in figure 6.

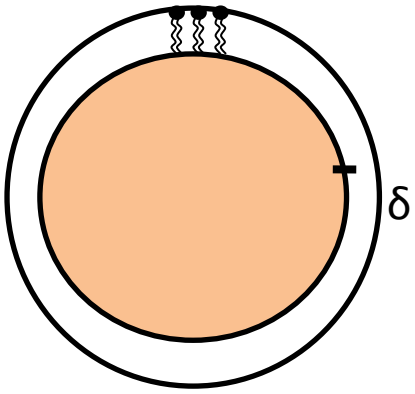


**Figure 6. Oil droplet covered with phospholipid emulsion layer of thickness δ .**

Since the thickness of the emulsion layer is much smaller than the droplet diameter the layer volume is expressed as $V_E = A \cdot \delta$. Equation 1 describes how much the emulsion layer of makes up of the whole emulsion droplet volume.

$$\frac{Volume_{Emulsifier\ droplet}}{Volume_{Oil\ droplet}} = \frac{V_E}{V_{Oil}} = \frac{A \cdot \delta}{\frac{\pi d^3}{6}} = \frac{\pi d^2 \cdot \delta}{\frac{\pi d^3}{6}} = \frac{\delta \cdot 6}{d} \qquad (1)$$

The emulsifier volume needed in the water phase to cover the oil droplets is dependant of the volume fraction of oil, $\varphi_{oil}$ . To investigate the efficiency of micellar emulsifiers the emulsifier concentration must be above the critical micelle concentration (CMC) in the aqueous phase. Adding these terms to equation one gives the minimum volume fraction of emulsifier according to equation 2, where $V_{Em}$ is the emulsion volume.

$$\frac{Volume_{\ emulsifier}}{Volume_{\ H_2O-phase}} = \frac{\delta \cdot 6}{d}\varphi_{oil} + \frac{V_E}{V_{Em}}(1-\varphi_{oil}) = \Phi_{E,H_2O} \qquad (2)$$

Since the density of the emulsifiers NaC and PC are about the same as the density of water, the volume fraction is equal to the mass of emulsifier as explained in equation 3.

$$V_E = |\rho_E = 1\ g/ml| = m_E \qquad (3)$$

The thickness of the emulsifier layer, δ, is set to 2nm for PC and to 1nm for NaC. An oil droplet diameter of five µm is assumed. The volume fraction of emulsifier, $\Phi_{E,H_2O}$, required to cover the oil droplets with PC is 0.0072, 0.036 and 0.072 % for $\Phi_{oil}$ = 1,5 and 10%. In the case of NaC the volume fraction of emulsifier needed to cover the oil droplets, $\Phi_{E,H_2O}$ , is 0.00036 and 0.018% for $\varphi_{oil}$ =1 and 5%. The $\Phi_{E,H_2O}$ needed to cover the oil droplets and still have bile salt at CMC in the bulk is 0.67, 0.68 and 0.70 % for $\Phi_{oil}$ =1, 5 and 10 %.

It is important to note that to actually measure the efficiency of liposomal and micellar emulsifiers the mixed micelles must be over the CMC. The mixed micelle will have a lower CMC compared to the NaC-micelle; This is observed for mixed micelles of PC and the bile salt sodium taurochenodeoxycholate (NaTCDC). The CMC of NaTCDC is 2.5-3mM[8] and about 0.7mM in combination with egg yolk phosphatidylcholine [9,10]. Thus the CMC for NaC in the presence of PC are probably around 2.3mM which is equivalent to a $\Phi_{NaC}$ of 0.09wt%.

The cylindrical micelles are known to be formed at the concentration of 9 mM of NaC and PC respectively[6]. This is equivalent to a $\Phi_{E,H_2O}$ of 1.1 wt%. The corresponding volume fraction of NaC, $\Phi_{NaC}$, relative to the total mass of emulsifiers is 35 wt%.

## 2.3 Particle size measurement

Emulsion droplets and liposome diameters are in the order of microns and therefore not visible to the naked eye. The particle size and size distribution is measured from the light scattering evaluated using the Mie theory that describes the light scattering behavior of the particle assuming a small spherical shape. These measurements are made by a laser

diffraction instrument named Mastersizer. By the use of particle and dispersant medium refractive indices it gives statistics of the particle size distribution using the derived diameters method D[m,n]. This method described in equation 4 gives the *volume weighted mean* diameter D[4,3] and *surface weighted mean* diameter D[3,2].

$$D[m,n] = \left[\frac{\sum v_i \, d_i^{m-3}}{\sum v_i d_i^{n-3}}\right]^{\frac{1}{m-n}} \qquad (4)$$

# 3. Materials and methods

## 3.1 Materials

The emulsion oil phase consisted of hexadecane (acros organics) with M= 226 g/mol. The continuous phase was a 10mM imidazole buffer with HCl of pH=7.5. The emulsifier used to prepare liposomes in the continuous phase was soya bean lecithin (Epikuron 200, Cargill inc, Minneapolis, MN ) of 94.8 % phosphatidylcholine with a fatty acid chain length of 18 carbons, M=812 g/mole. PC and sodium cholate of CMC between 9-14mM [11] (AppliChem, GmbH) M=431g/mole were used to produce mixed micelles. The solvents used to create mixed micelles through molecular mixing of NaC and PC were absolute ethanol (99%, VWR), chloroform (Labscan) and diethylether (Merck).

## 3.2 Methods

### 3.2.1 Preparation of vesicles by ultra sonication

The intension was to add the emulsifiers in vesicular form. Thus 9 mM soyabean PC dispersed with a magnetic stirrer in the 10mM imidazole buffer of pH 7.5. The water content was 99 wt% corresponding to the vesicular phase [12]. The solution was then transferred to a v-formed sample tube of 10 ml. The tip of a cup horn ultra sonicator (Branson sonifier B-12 ultrasonic tip) was immersed into the tube and the sample was sonicated at 20 kHz for around ten minutes until the dispersion got an opaque blue toned color. Hearing protection was used to avoid hearing impairments. The surface- and volume mean drop diameter (D[3,2], D[4,3]) of the vesicles was measured with a Mastersizer (Malvern, England) at an obscuration of 10-

15% and at a pump speed of 2030 rpm. The refractive indices (RI) were 1.48[13] for PC, 1.43 for Hexadecane and 1.33 for the continuous water phase.

### 3.2.2 Preparation of emulsions using the emulsifier dispersed in vesicular form

Two sets of test emulsions were made, the first one with an oil fraction of 1% and PC concentrations of 0.3, 3 and 9 mM. In the second test emulsion the oil fraction was increased to 5% and the emulsifier concentration was 0.9, 9 and 30 mM. Oil as added to the vesicle dispersion and the ten milliliter samples were homogenized at 24,000 rpm in homogenizer (Ystral D-79282, Germany) for ten minutes.

### 3.2.3 Preparation of emulsions with sodium cholate

Emulsions were made with an oil fraction of 1 and 5%. The sodium cholate concentrations were 6.4, 13, 30and 31mM. The samples were homogenized for 5, 6 or 8 minutes (Ystral D-79282, Germany). The droplet emulsion size was investigated with an optical microscope (Olympus BX50) with magnification 20x or with the Mastersizer. A set of measurements with 30mM bile salt and $\varphi_{Oil} = 1\%$ was homogenized for 320, 160, 120, 80, 40, 20, 10 and 5 seconds, the droplet sizes was investigated with the microscope.

### 3.2.4 Preparations of emulsions with mixed micelles

Two methods were used to create mixed micelles. In the first one bile salt concentrations of 0.93, 6.3, 9.5 and10 mM was added to PC vesicles dispersion of 9mM. Hexadecane was added to the surfactant mix and the samples were homogenized three minutes at 20,000 rpm.

In the second method PC and NaC were solubilized separately in ethanol and dispersed with a magnet stirrer. The surfactant dispersions were then mixed during continuous dispersion and additional ethanol was added until the mixture was transparent. The solvent was then evaporated under vacuum using a rotor evaporator (BÜCHI R-114, Switzerland). Other solvents evaluated were diethylether and chloroform.

# 4. Results and discussion

## 4.1 Vesicle preparation

The plan was to create an emulsion with the emulsifiers dispersed in liposomal form in the aqueous phase. The assumption was that homogenization could lead to coalescence due to kinetic restrictions of the adsorption rate. Thus the experimental work started with preparing vesicles from PC-water dispersions by varying the sonication time.

The particle size distribution of vesicles prepared by ultra sonication is illustrated in figure 7. Table 1 shows D[3,2] and D[4,3] for different sonication times of 9mM PC vesicles. The crude PC-buffer dispersion is made up by large almost visible multilamellar vesicles that are transformed to more narrowly distributed unilamellar vesicles with increased sonication time. When the time of ultra sonication exceeded more than about ten minutes the dispersion became almost transparent and the sample was almost undetectable in the Mastersizer. Through this process D[3,2] is ranging between 125-0.127 µm and D[4,3] between 48.1-0.382 µm.

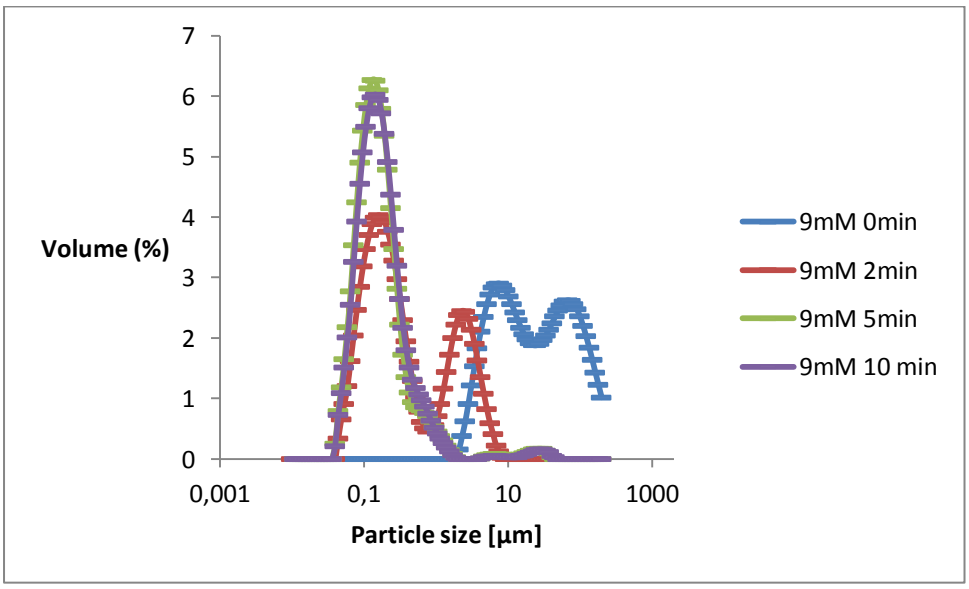


**Figure 7. Particle size of PC vesicles prepared during different sonication times.**

**Table 1. D[3,2] and D[4,3] for different ultra sonication times of 9mM PC vesicles.**

| Conc [mM] | Time [min] | D[3,2] [µm] | D[4,3] [µm] |
|---|---|---|---|
| 9 | 0 | 11.6 | 48 |
| 9 | 10 | 0.13 | 0.38 |
| 40 | 13 | 0.11 | 0.13 |

In figure 8 the PC concentration is increased to 40mM and similar values of D[3,2] and D[4,3], 111 and 0.130µm indicates somewhat monodisperse vesicles. Further increase of concentration was not tested due to the amount of sample needed to produce and detect the vesicles. The vesicle size and distribution was not only dependant of time and concentration but also of the placing and alignment of the sonicator tip in the sample tube. This is clear when comparing the two samples sonicated during five minutes in figure 8.

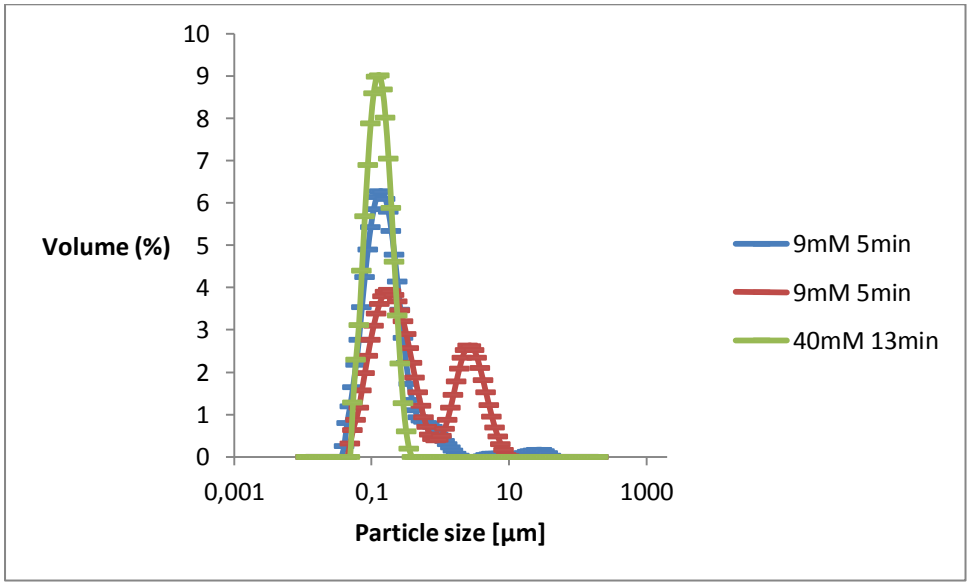


**Figure 8. Particle size of PC vesicles prepared during different sonication times.**

Cryo-TEM detected vesicles prepared by ultra sonication are reported to be about 15-40 and 80-200 nm in diameter [6,14]. Cryo-TEM detected vesicles prepared by extrusion were about 100 nm in diameter and many of them were multilamellar[6]. Other PC vesicles prepared by sonication have a reported diameter of 44.1, 82.8 and 121.8 nm observed by light scattering [15].

Based on the result in figure 7 and 8 we conclude that the sonication of 9mM PC for about ten minutes generates reproducible vesicles. To which extent the vesicles prepared in this

experiment really are multilamellar is hard to say without access to equipment such as Cryo-TEM or dynamic light scattering.

## 4.2 Attempts to make emulsions with the emulsifier dispersed in the aqueous phase in liposomal form

The aim of this experiment was to make emulsions with the obtained PC vesicles as emulsifiers. The volume faction of oil and the amount of emulsifier were varied to see how these parameters affected the size of emulsion droplets. The hypothesis was that the excess quantity of emulsifier relative to the oil would give an emulsion while insufficient emulsifier would not.

All vesicle was prepared from sonication of 9mM PC with a D[3,2] mean diameter of around 130nm . The tested volume fractions of oil were 1, 5 and 10 % respectively as presented in table 2. The micrograph in figure 9 of 9mM vesicles solution with $\Phi_{oil}$=1% shows air bubbles only.

**Table 2. Parameters in attempts to make PC- hexadecane emulsions. Volume fraction of oil, $\varphi_{oil}$, homogenization times, t. Volume fraction of emulsifier in the emulsion water-phase, $\Phi_{E,H_2O}$. Volume of emulsifier relative oil volume, $\frac{V_E}{V_{oil}}$ and droplet diameter.**

| $\varphi_{oil}$ % | t [min] | $\Phi_{E,H_2O}$% | $\frac{V_E}{V_{Oil}}$ % | Droplet diameter [µm] |
|---|---|---|---|---|
| 1 | 10 | 0.025 | 2.5 | - |
| 1 | 10 | 0.25 | 25 | - |
| 1 | 10 | 0.74 | 74 | - |
| 5 | 10 | 0.074 | 0.14 | - |
| 5 | 10 | 0.74 | 1.4 | - |
| 5 | 10 | 2.5 | 4.7 | - |
| 10 | 10 | 0.023 | 0.023 | - |
| 10 | 10 | 0.23 | 2.3 | - |

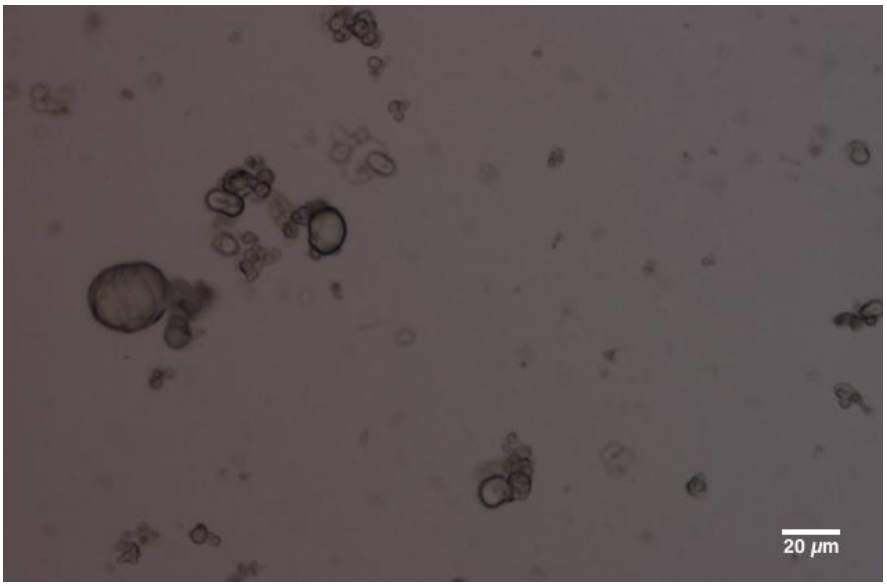


**Figure 9. Microscope image of unsuccessful PC- hexadecane emulsion, $\varphi_{oil}$ =1%. Volume fraction of emulsifier relative to the oil phase, $\frac{V_E}{V_{Oil}}$, is 1.4%. The photo is taken using an objective with 20x magnification.**

A ratio of emulsifier to aqueous phase ranging from 0.023 to 2.5 % did not result in any emulsions even though it was with the exception of 0.023% more than enough to cover the emulsion droplets for droplet sizes of about 1-10µm. The ratio of emulsifier to oil volume is ranging from 0.023-73%, the standard value of $\frac{V_E}{V_{Oil}}$ is 3%. The quantity of emulsifier with respect to oil is sufficient to stabilize the oil droplets which requires 0.0072, 0.036 and 0.072 % for $\varphi_{oil}$ = 1, 5 and 10% .

The volume of oil in an emulsion volume of 10 ml of $\varphi_{oil}$ =1% might be small enough to get lost in the homogenizer blades. But increasing $\varphi_{oil}$ to 10% still left the oil phase on top of the emulsion solution.

The adsorption of emulsifier onto droplet surfaces was not fast enough to achieve an emulsion. The explanation could be low solubility of lecithin in the aqueous phase, electrostatic repulsion between oil droplet surface or emulsifier aggregates being too stabile to be affected by convective forces. The reason to the formation of large aggregates could be hydrophobic interactions between the PC fatty acid chains due to the hydrophobic effect counteracting an increase in free energy[16]. A method to avoid this would be to disperse PC in the oil phase and then add the water phase gradually during the homogenization [17,18]. With

this method would not be suitable as the emulsifier would reach the droplet surface from within the oil phase instead of a collision driven adsorption in the continuous phase.

The homogenization also has an effect on the formation of emulsions. When considering the homogenization process used in this experiment it is likely to be sufficient. The mixing rate at 25,000 rpm during ten minutes is significantly exceeding the 10,000 rpm one minute homogenization used when mixing the pre dissolved oil and PC phase with the water phase as described above. A way to succeed in mixing PC, water and oil together could be to have an even stronger homogenizer or to try ultra sonication[19]. Worth mentioning is the fact that soya bean PC while egg yolk PC and sunflower seed lecithin were used in the reference articles.

Despite variation of emulsifier and oil content, attempts to make O/W emulsions with PC vesicle emulsifier did not succeed as seen in figure one and table two. From this it is clear that a high ratio of emulsifier with respect to oil is limited by the low solvubility of PC in the aqueous phase and the stability of emulsifier aggregates.

## 4.3 Emulsions with sodium cholate

After the unsuccessful PC emulsions the intension was to evaluate the ability to form emulsions using sodium cholate. This gave an idea of droplet size and the opportunity to use sodium cholate as a starting point to create a system with gradual transition of properties. The emulsifier content, volume fraction of oil and homogenization times was varied to get an overall feeling of how these changes might influence emulsions.

Sodium cholate emulsions are illustrated in figure 10 and 11. Table 3 shows that the diameter of the emulsion droplets is in the magnitude of 5µm independent of $\varphi_{oil}$, concentration and homogenization times. The time independence is exemplified by table 4 with homogenization times ranging from 5-320s for sodium cholate concentrations of 30mM and $\varphi_{oil}$ =1%. According to table 2, 3 and 4 the values of $\frac{V_E}{V_{oil}}$ are much higher in the case of sodium cholate than of phosphatidylcholine. The reason to this is that the sodium cholate concentration needed to be above the CMC.

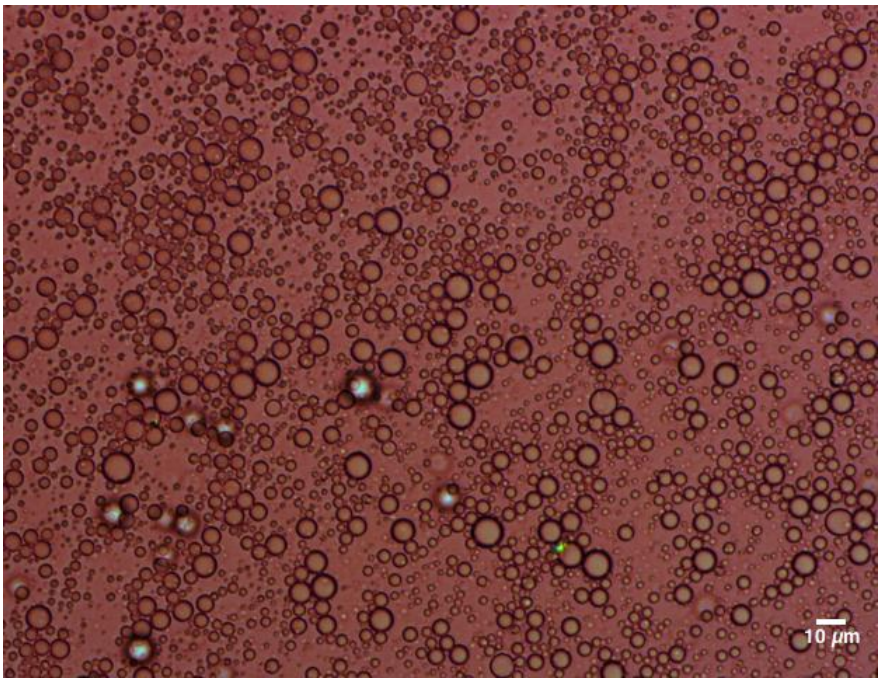


**Figure 10. Sodium Cholate- hexadecane emulsion. [NaC]=13mM $\varphi_{oil}$ =5% homogenization time 5min. The photo is taken using an objective of of 20x magnification.**

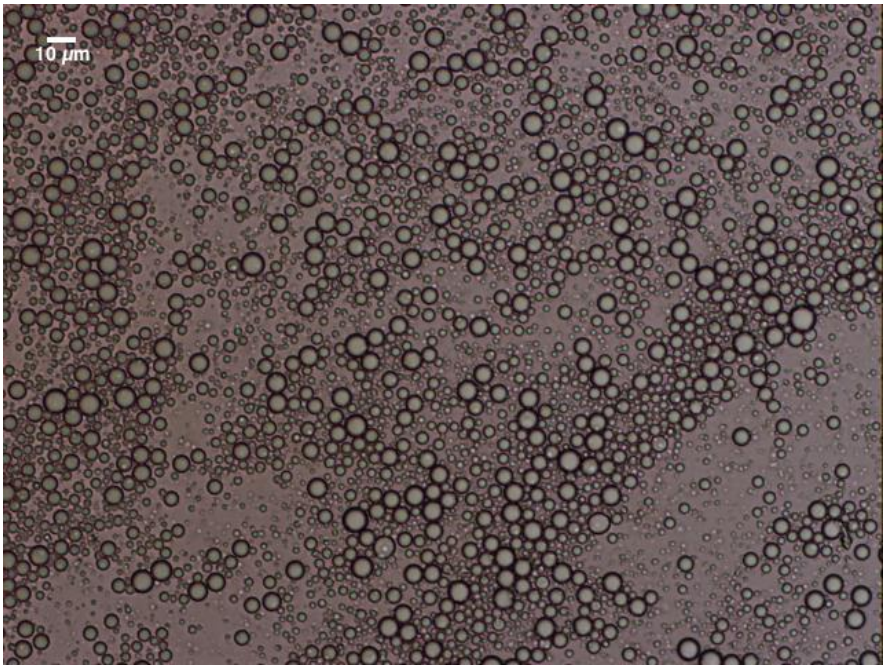


**Figure 11. Sodium Cholate- hexadecane emulsion. [NaC]=31mM $\varphi_{oil}$ =5% homogenization time 5min. The photo is taken using an an objective of of 20x magnification.**

**Table 3. Parameters in different sodium cholate- hexadecane emulsions. Volume fraction of oil , $\varphi_{oil}$ and homogenization times, t. Volume fraction of emulsifier in the emulsion water-phase, $\Phi_{E,H_2O}$. Volume of emulsifier relative oil volume $\frac{V_E}{V_{oil}}$ and droplet diameter.**

| NaC [mM] | $\Phi_{oil}$ % | $\Phi_{E,H_2O}$ % | $\frac{V_E}{V_{oil}}$ % | t [min] | Droplet diameter [µm] | |
|---|---|---|---|---|---|---|
| | | | | | Mastersizer measurement | Microscope measurement |
| 31 | 1 | 1.3 | 130 | 6 | 4.8 | |
| 13 | 1 | 0.55 | 54 | 8 | 4.2 | |
| 12 | 5 | 0.51 | 51 | 5 | 5.1 | 4.2 |
| 31 | 5 | 1.3 | 26 | 5 | 4.8 | 4.2 |
| 6.4 | 10 | 0.48 | 48 | 5 | | 5 |

**Table 4. Parameters in different sodium cholate- hexadecane emulsions. Volume fraction of oil , $\varphi_{oil}$, homogenization times, t. Volume fraction of emulsifier in the emulsion water-phase, $\Phi_{E,H_2O}$. Volume of emulsifier relative oil volume $\frac{V_E}{V_{oil}}$ and droplet diameter.**

| NaC [mM] | $\varphi_{oil}$ % | $\Phi_{E,H_2O}$ % | $\frac{V_E}{V_{oil}}$ % | t [s] | Droplet diameter [µm] Microscope measurement |
|---|---|---|---|---|---|
| 30 | 1 | 1.3 | 128 | 320 | 4.2 |
| 30 | 1 | 1.3 | 128 | 120 | 4.2 |
| 30 | 1 | 1.3 | 128 | 80 | 4.4 |
| 30 | 1 | 1.3 | 128 | 40 | 4.9 |
| 30 | 1 | 1.3 | 128 | 20 | 5.3 |
| 30 | 1 | 1.3 | 128 | 10 | 5.8 |
| 30 | 1 | 1.3 | 128 | 5 | 5 |

Thus the making of NaC emulsions gave a good idea of how emulsions are made but the results also indicate that the different characteristic of NaC and PC makes the systems far from comparable.

Emulsions with NaC and PC as the emulsifiers gave totally opposite emulsifying results. The molecules have a great difference in aqueous solubility. The CMC of NaC is 9-14 mM[11] whereas the solubility of PC is about $10^{-10}$ M[20]. The two molecules look quite different as illustrated in figure 12. The sodium cholate molecule has hydrophilic groups and is smaller than the long phosphatidylcholine molecule with long hydrophobic fatty acid chains [21,22]. The micelle aggregation number is 2-4[23] and hence the micelle diameter is in the order of

around 1.40 nm in contrast to the vesicle diameter of about 100 nm. The small size of the NaC molecule makes it very dynamic and diffusive which facilitates adsorption onto the oil droplet.

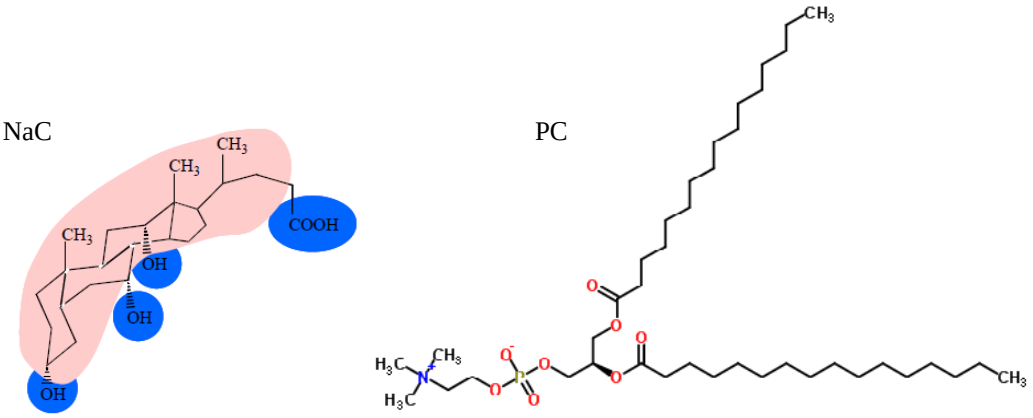


**Figure 12. The sodium cholate- and phosphatidylcholine molecules. The hydrophobic part of the sodium cholate molecule is displayed in pink and the hydrophilic part in blue, the NaC figure is modified from [23]. The PC figure is from [24].**

To sum up, the sodium cholate emulsion system gave a good sense of droplet size range and the effect of homogenization times. The system seemed like a good starting point to create a system with gradual transition of properties

## 4.4 Attempts to make emulsions with mixed micelles

One of the aims of this thesis was to evaluate the hypothesis that the emulsifier efficiency during homogenization is dependant of the aggregation state. The aggregation states in question are the liposomal and the micellar state. This experiment was done to prepare emulsifier in the micellar state, namely mixed PC-NaC- micelles, to see if the micelle shape and size would influence the emulsion droplet size.

### 4.4.1 Simple mixing of NaC and PC

The first method to prepare mixed micelles was to simply add sodium cholate to the vesicle dispersions. The mass fraction of sodium cholate relative to the total emulsifier mass was varied[5] to prepare mixed micelles of different shapes.

Figure 13 presents the relative size distribution for emulsions of mixed PC- NaC micelles and hexadecane oil. The size distribution is similar for all mass fractions of sodium cholate and a

mean droplet diameter of about 6µm is given in table four. According to figure 13 the maximum volume percent around 10µm decreases with $\Phi_{NaC}$ while the volume percent at 1.5 and 0.18µm increases. At $\Phi_{NaC}$ = 5wt% no mixed micelles should be generated and there is simply micelles and vesicles in coexistence[5]. Figure 13 indicate that larger particles as emulsion droplets make up a smaller part of the total particle volume for for $\Phi_{NaC}$= 5 %. As no mixed micelles are formed at this concentration vesicles should be among the detected particles. The observation of the decrease in large particles might be an indication of the smaller vesicles making up a larger part of the sample. Since the vesicles according to table 2 are no good emulsifiers the emulsifying effect is likely due to sodium cholate.

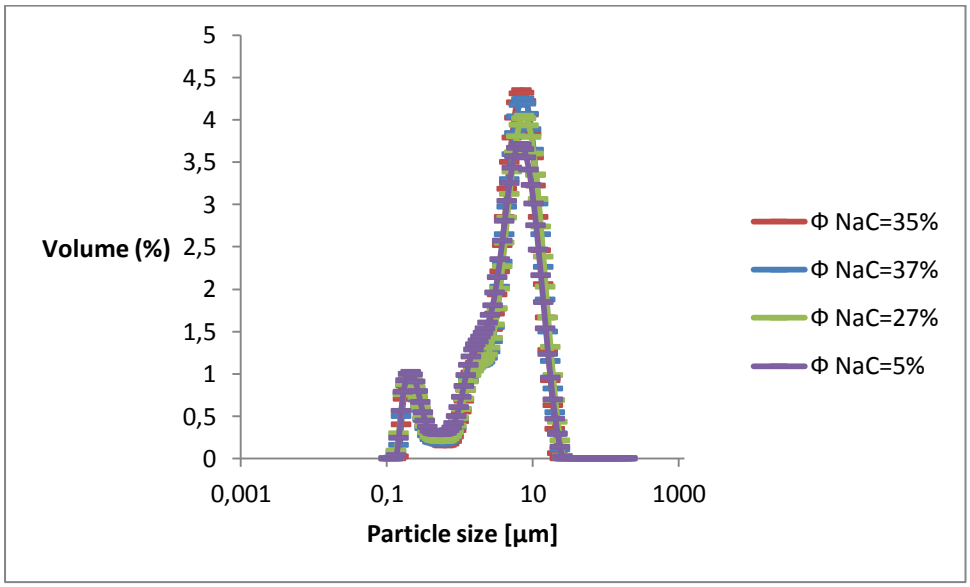


**Figure 13. Emulsions with lecithin and sodium cholate micelles and hexadecane. Volume fraction of oil, $\varphi_{oil}$ =5% and mass fraction of sodium cholate, ΦNaC= 5, 27, 35 and 37%.**

**Table 5. Parameters in mixed micelles- hexadecane emulsions. Mass fraction of NaC relative to the total amount of emulsifier, $\Phi_{NaC}$. Volume fraction of oil, $\varphi_{oil}$, homogenization times, t. Volume fraction of emulsifier in the emulsion water phase ΦE, $H_2O$, volume of emulsifier relative oil volume $\frac{V_E}{V_{oil}}$ and droplet diameter.**

| $\Phi_{NaC}$ % | $\varphi_{oil}$ % | Droplet diameter | t[min] | $\Phi_{E,H_2O}$ % | | | $\frac{V_E}{V_{oil}}$ % |
|---|---|---|---|---|---|---|---|
| | | | | Tot | NaC | PC | |
| 37 | 5 | 6.0 | 3 | 1.1 | 0.43 | 0.75 | 22 |
| 35 | 5 | 6.2 | 3 | 1.1 | 0.41 | 0.75 | 22 |
| 27 | 5 | 6.3 | 3 | 0.97 | 0.28 | 0.75 | 19 |
| 5 | 5 | 5.6 | 3 | 0.75 | 0.04 | 0.75 | 15 |

Figure 14 is a zoom in of the NaC-Lecithin-Water phase diagram from figure 4 with $\Phi_{NaC}$=37, 35, 27 and 5% marked. L1 is the micellar phase and the area below is mostly containing vesicles and coexistence of vesicles and micelles. The composition of NaC and PC at $\Phi_{NaC=}$37and 35% should give mixed micelles[5]. But according to the phase diagram the points for $\Phi_{NaC}$=35 and 27% are very near the area below $L_1$.

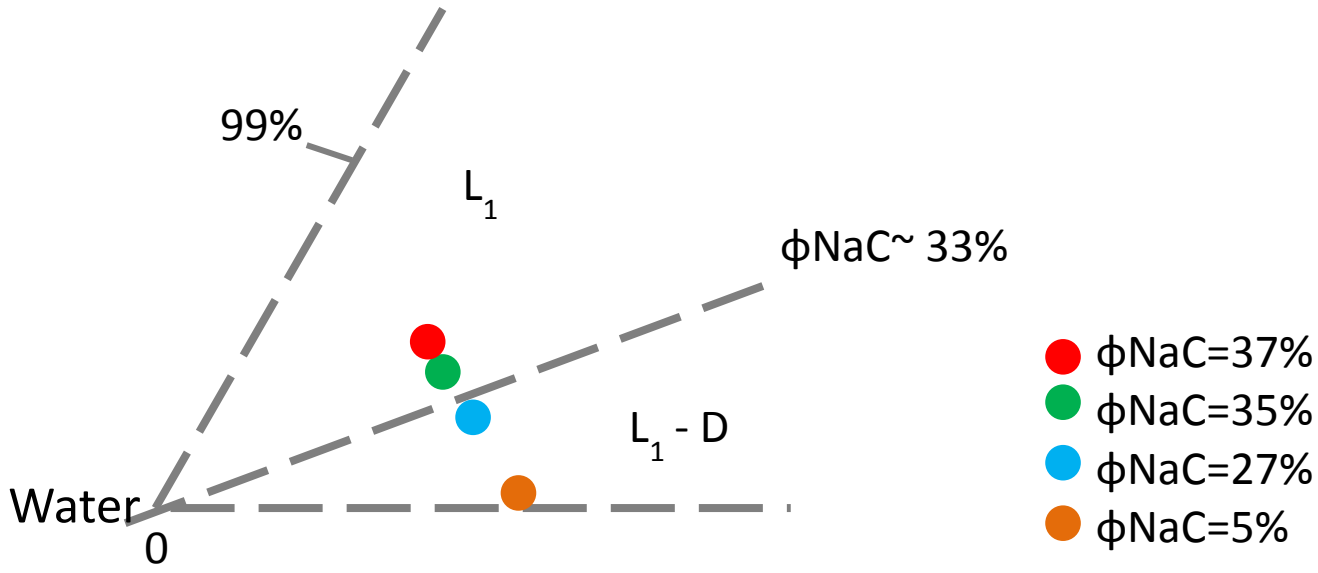


**Figure 14. Zoom in of the NaC-Lecithin-Water phase diagram. ΦNaC, is the massfraction of NaC relative to the total mass of emulsifier. ΦNaC for 37, 35, 27 and 5% is indicated in the figure as explained by the ledend. L1 is the isotropic aqueous phase. The figure is modified from [6].**

To get a further indication of if mixed micelles was formed the above result was compared with the result from the sodium cholate emulsions described in previous section. The idea was that if the particle size distribution would be similar that might indicate the same emulsifier.

Figure 15 is a comparison of the 35wt% sodium cholate mixed micelle from figure 13 and the emulsion with plain sodium cholate micelles described in previous section, all oil volume fractions, $\varphi_{oil}$, are 5%. The particle size distribution looks about the same and that could either be due to both emulsifier reacting similarly to flow from the homogenizer or that the droplets are covered with the same type of emulsifier.

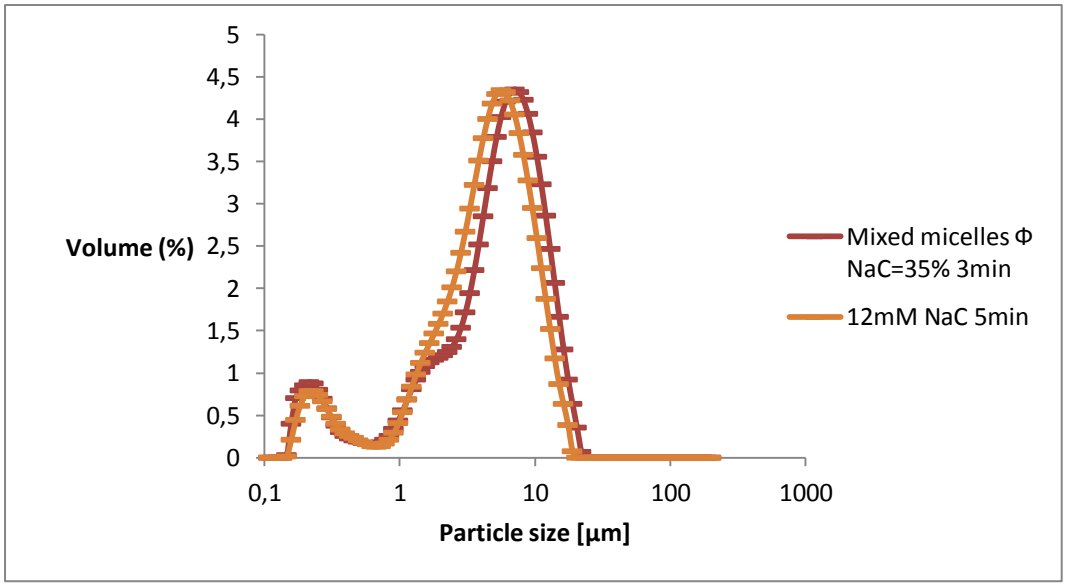


**Figure 15. A comparision of hexadecane emulsions with lecithin-sodium cholate mixed micelles and sodium cholate micelles, $\varphi_{oil}$ =5%. Mixed micelles phi NaC= 35%. Sodium cholate micelles [NaC]= 13mM.**

Since the observations in figure 14 and 15 did not give any crucial information about the existence of mixed micelles the particle size distribution in the continuous phase of the $\Phi_{NaC}$ = 35% mixed micelle emulsion was measured.

Figure 16 shows the size distributions for mixed lecithin- sodium cholate micelles in aqueous solution with $\Phi_{NaC}$ = 35%. The figure shows that a large part of the total particle volume is made up by particles with a diameter of 100-300 nm. There are also a segment of particles with a diameter of 80-150 µm. There is on the contrary almost no particles with a diameter of around 6-10 µm.

In comparison to vesicle size distribution in figure 7 the size distribution clearly indicates PC vesicles rather than mixed micelles and excludes the presence of mixed micelle emulsions. The explanation could be that as the sodium ion of the head groups of NaC are solubilized in the emulsion aqueous phase the molecules becomes negatively charged. If a bile salt molecule reaches a vesicle the vesicle will also be negatively charged and repulsion between vesicles and sodium cholate molecules or micelles suppresses formation of mixed micelles.

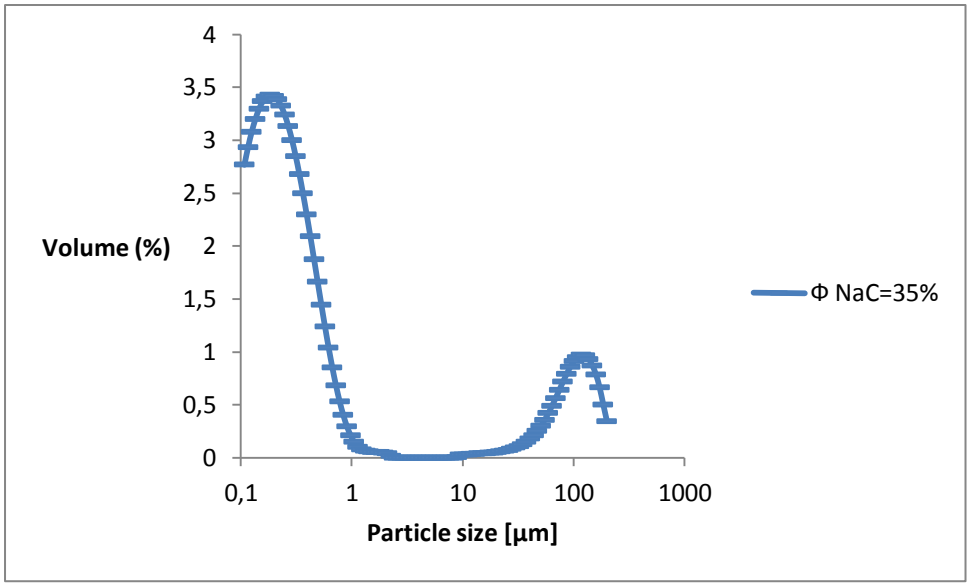


**Figure 16. Particle size measurement of mixed micelles, mass fraction of sodium cholate, ΦNaC, is 35%, $\varphi_{oil}$= 5%.**

**Table 6. Surface- , D[3,2], and volume weighted mean particle sizes ,D[4.3], from size distribution measurements of lecithin-Sodium cholate mixed micelles.**

| $\Phi_{NaC}$ | t [min] | D[3,2] [µm] | D[4,3] [µm] |
|---|---|---|---|
| 35% | 3 | 0.220 | 15.751 |

Another way to create mixed micelles would have been to add small concentrations of the bile salt at a slower rate as in reference 5 instead of just adding the powder directly. In this way the NaC concentration would be low and so would the risk of repulsion between vesicles and sodium cholate micelles.

Perhaps the addition of oil and the homogenization started too shortly after NaC was added to the vesicle solution. This might have led to competition between the opening of PC bilayer and covering of oil droplets, with the latter process being faster. The process of opening up bilayer might have been slowed down further by multilamellar vesicles. A simple but unfortunate explanation could be that the PC-NaC-Water composition is too close to the region below $L_1$. Despite that this composition is proven to produce mixed micelles.

In summary the simple mixing of NaC and PC was not a good method to create mixed micelles.

### 4.4.2 Evaporation of dissolved NaC and PC

The next method evaluated the creation of mixed micelles by dissolving the surfactants in a solvent to achieve mixing on a molecular level. The idea was that after evaporation of the solvent the molecules would organize spontaneously in mixed micelles at the addition of water. The tested solvents were ethanol, chloroform and diethylether

The bile salt was insoluble in diethylether and chloroform despite extensive mixing. NaC was most soluble in ethanol, probably due to hydrogen bonding between OH-groups in both molecules. The PC had a better solubility in the solvents. A mixture of 35 wt% NaC and 65 wt% PC was dissolved in ethanol to an almost clear solution. Thus we assume that a molecularly mixed system was formed. The emulsifier mixture was dispersed in water and used in an emulsification experiment.

Figure 17 illustrates this emulsion together with the 35 wt% NaC mixed emulsion dissolved in water from figure 15. Table 8 explains the emulsion parameters. The figure shows two very similar particle size distributions. A small part of the total particle volumes are 0.15-0.25 nm in diameter and a large part of the particles have diameter of 5-10 µm. The mean droplet diameter of particles prepared with the molecular mixing method and simple mixing method is 5.9 and 6.2 respectively. The results suggests the molecularly mixed NaC and PC after evaporation is not likely to have formed mixed micelles as emulsification of the reference system is similar to the concentration of pure NaC.

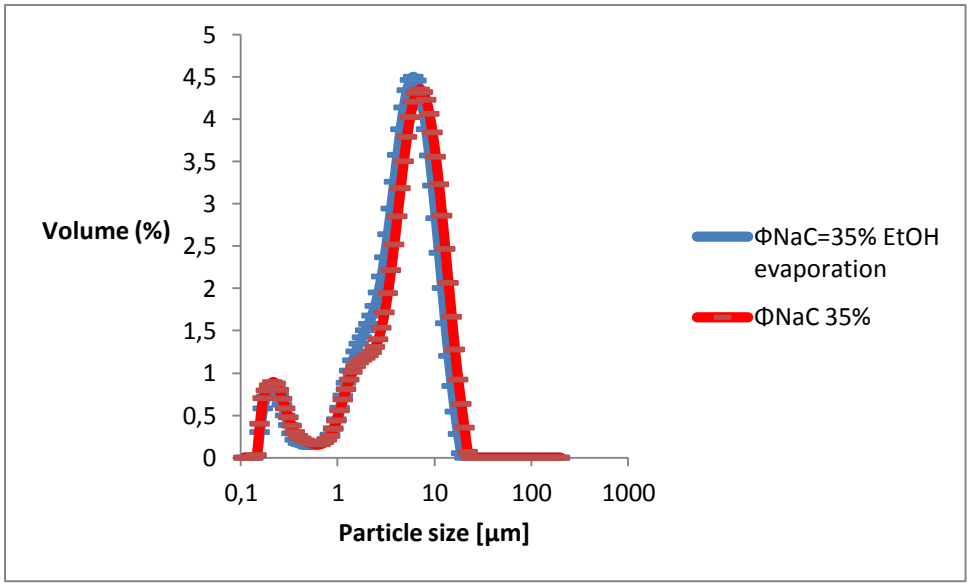


**Figure 17. Emulsions with phosphatidylcholine (PC), sodium cholate (NaC) and hexadecane obtained from evaporation or plain mixing of PC and NaC. $\Phi_{NaC}$ is the massfraction of sodium cholate, $\varphi_{oil}$= 5%.**

**Table 7. Parameters in emulsion of phosphatidylcholine. Sodium cholate and hexadecane. $\varphi_{oil}$-volume fraction of oil, t-homogenization times. $\Phi_{E,H_2O}$ -volume fraction of emulsifier in the emulsion water phase. $\frac{V_E}{V_{oil}}$ - volume of emulsifier relative oil volume and droplet diameter.**

| $\Phi_{NaC}$ % | $\varphi_{oil}$ % | t[min] | Droplet diameter [µm] | $\Phi_{E,H_2O}$ % | $\frac{V_E}{V_{oil}}$ % |
|---|---|---|---|---|---|
| 35% | 5 | 3 | 5.9 | 4.8 | 97% |

To sum up evaporation of ethanol, diethylether or chloroform dissolved NaC and PC was not a satisfying method to prepare mixed micelles. Since both methods failed the desired mixed micellar aggregation state could not be reached and its efficiency during homogenization could not be investigated.

# 5. Conclusion

The intension of this thesis was to develop a method to measure the efficiency of emulsifier during homogenization and to achieve results indicating that the efficiency is dependant of the aggregation state. The plan was to prepare liposomal and mixed micellar emulsifiers of phosphatidylcholine (PC) and sodium cholate (NaC) to evaluate the efficiency during homogenization by fluorescence measurements.

PC liposomes were prepared by ultra sonication and were used in attempts to make O/W emulsions. The liposomes failed as emulsifiers due to aggregates being too stable to be affected by convective forces formed during homogenization. A possible explanation is electrostatic repulsion between oil droplet surface and emulsifier. The low solubility of PC in the aqueous phase can also explain the lack of emulsion. Efforts were made to produce NaC and PC mixed micelles through the method of simple mixing of surfactants in the continuous phase. The other method was dissolution in ethanol, diethylether and chloroform to achieve molecular mixing between PC and NaC. None of the methods produced mixed micelles as the emulsifying properties in both systems were due to NaC only.

The inability to create mixed micelles was perhaps due to repulsion between NaC molecules attached on vesicles and in solution. The opening of vesicles to create mixed micelles is maybe slower than the process of spreading over droplets surfaces. The shortcomings of the molecular mixing method are probably due to the wrong type of solvent. The difficulties of producing mixed micelles could also be explained by the composition of NaC and PC being too close to the coexistence region of micelles and vesicles.

In summary, if a method of investigating the efficiency of liposomal and micellar emulsifiers is to be developed, the emulsifier must be soluble enough in the aqueous phase and the desired aggregation state must be easily prepared. The methods used in this work are therefore not to be recommended.

# 6. Aknowledgements

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