Universidade de Lisboa

Faculdade de Farmácia

Oil Incorporation in Foamable Mixtures of Phospholipids and Surfactants

Laura Sofia de Amaral Fortunato

Trabalho de Campo orientado pelo Professor Doutor Rolf Daniels, e coorientado pela Professora Doutora Maria Helena Cabral Marques, Professora Associada com Agregação

Mestrado Integrado em Ciências Farmacêuticas

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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Resumo

O presente trabalho tem por base o estudo de espumas potencialmente medicamentosas, obtidas a partir de emulsões que incorporem na sua constituição óleos, fosfolípidos e os tensioativos adequados para obter uma espuma estável e com uma estrutura satisfatória do ponto de vista técnico.

As formulações estudadas neste trabalho, não contêm substância ativa. O objetivo é desenvolver uma espuma formada a partir de uma emulsão contendo um óleo com polaridade variável que seja adequada para veicular uma substância ativa com ação a nível cutâneo. Por exemplo, corticosteroides para o tratamento da psoríase.

As espumas têm interesse a nível farmacêutico devido à comodidade da sua utilização. Permitem uma administração eficaz em zonas de difícil acesso, como a raiz capilar, e, portanto, ajudam à promoção da adesão à terapêutica.

Neste estudo foram desenvolvidas 72 formulações. Todas elas têm por base emulsões em que a fase oleosa inclui um dos seguintes óleos: triglicéridos de cadeia média, parafina líquida ou óleo de rícino, em concentrações de 10 ou 20%. O óleo é disperso em misturas aquosas de fosfolípidos e tensioativos que possuem capacidade de formar espuma. Capacidade previamente comprovada através de um método utilizado, também neste trabalho, para avaliar a formação e a estabilidade das espumas denominado “screening method”, em que três colunas graduadas são dispostas lado a lado. A emulsão que dá origem à espuma é introduzida dentro das colunas e o ar é impulsionado a partir da base das colunas através de um filtro em forma de disco, dispersando-se assim na emulsão.

As espumas foram avaliadas e classificadas segundo: a sua capacidade de serem ou não produzidas; a sua estrutura inicial e ao longo de todo o tempo da experiência; e segundo a sua altura em relação ao líquido. As emulsões que dão origem às espumas foram também avaliadas em relação à sua estabilidade. As que mesmo sendo capazes de produzir uma espuma com uma boa estrutura, se não apresentassem estabilidade, não eram consideradas formulações adequadas.

Com os resultados foi possível concluir que o óleo de rícino não é adequado nestas formulações e que os demais componentes são determinantes no seu comportamento.

Palavras-chave: Espuma, emulsão, óleo, fosfolípido, tensioativo

Abstract

The present work is based on the study of potentially medicated foams, obtained from emulsions that are constituted of oils, phospholipids and the appropriate surfactants in order to produce stable foams, with a technically satisfactory structure.

The studied formulations do not contain an active substance. The objective of the investigation is to develop a foam obtained from an emulsion in which the oil phase can have variable polarity depending on the oil used. This emulsion must be adequate to vehiculate an active substance that acts on the skin. For example, corticosteroids for the treatment of psoriasis.

Foams are of pharmaceutical interest because of the commodity of their utilization. They allow the efficient administration in areas of difficult access, such as the scalp, and therefore they promote patient adhesion to therapy.

In this study, 72 formulations were developed. Each one of them is based on an emulsion in which the oil phase includes one of the following oils: medium chain triglycerides, liquid paraffin or castor oil, in concentrations of either 10 or 20%. The oil is dispersed in aqueous mixtures of phospholipid and surfactants which are known to be capable of producing a foam, this was previously demonstrated using a method also applied in this work to evaluate the foamability and the foam structure called "screening method" in which three graduated columns are arranged side by side. The emulsion that gives rise to the foam is introduced into the columns and the air is driven from the base of the columns through a disc-shaped filter, thus dispersing into the emulsion.

The foams were evaluated and classified according to: their ability to be produced; their initial structure and over the time of the experiment; and their height in relation to the liquid. The emulsions that give rise to the foams were also evaluated according to their stability. Those that, even though they were able to produce a foam with a good structure, if they were not stable, they were not considered adequate formulations.

With the results it was possible to conclude that castor oil is not suitable in these formulations and that the other components are determinant in their behavior.

Keywords: Foam; emulsion; oil; phospholipid; surfactant

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Abbreviatures

a/w – air/water

CO – Castor oil

DSA – Droplet size analysis

H - High

L – Low

M – Medium

MCT – Medium chain triglycerides

O/O – oil-in-oil

O/W – oil-in-water

PC - phosphatidylcholine

PE - phosphatidylethanolamine

PG - phosphatidylglycerol

PI - phosphatidylinositol

PL – Phospholipid

PTT – Phase transition temperature

rpm – rotations per minute

SCG – Sodium coconut glutamate

SCS – Sodium coconut sulfate

SLES – Sodium laureth sulfate

W/O – water-in-oil

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1 Introduction

1.1 Disperse systems

A disperse system is defined as an heterogenous system, constituted by two phases. The internal (dispersed, discontinuous) phase is distributed or dispersed within the continuous (external) phase. They can be classified in various ways, based on the physical state of the two constituent phases or on the size of the dispersed particles within the dispersion medium (1).

The state of the dispersed phase (gas, solid, or liquid) in the dispersion medium defines the system as a foam, suspension, or emulsion (Table 1). Likewise, the particle size of the dispersed phase provides further classification (colloidal dispersion vs. suspension and microemulsion vs. macroemulsion). These definitions are somewhat arbitrary since there isn't a specific particle size at which one type of system ends and the other begins. Furthermore, almost without exception, disperse systems are heterogeneous in particle size (2). A suspension is a solid/liquid dispersion. An emulsion is a liquid/liquid dispersion in which the two phases can be completely immiscible or saturated with each other. A foam is a gas/liquid dispersion and, in the case of aerosols, either a liquid or a solid is dispersed within a gaseous phase. There isn't a disperse system in which both phases are gases (1)

The classification based on the size of the dispersed particles is presented in Table 2 where three classes of dispersions can be distinguished: molecular, colloidal and coarse dispersions.

Table 1 Classification of disperse systems based on the physical state of the dispersed phase and the dispersion medium (1)

Dispersed phase	**Dispersion medium**		
	Solid	Liquid	Gas
Solid	Solid suspension	Suspension	Solid aerosol
Liquid	Solid emulsion	Emulsion	Liquid aerosol
Gas	Solid foam	Foam	Nonexistent

Table 2 Classification of disperse systems based on the particle size of the dispersed phase (1)

Category	Particle dimensions	Properties of the system
Molecular dispersion	<10 nm	Particles invisible by electron microscopy; pass through semipermeable membranes; diffuse rapidly
Colloidal dispersion	10 nm – 1.0 μm	Particles visible by electron microscopy but not by ordinary microscope; pass through paper filter but not by semipermeable membranes; generally slow diffusion
Coarse dispersion	>1.0 μm	Particles visible by ordinary microscopy; can't pass through paper filter or semipermeable membranes

1.1.1 Foams

Foam is a disperse system where a large proportion of gas is dispersed, in the form of bubbles, in a solid, liquid, or semisolid continuous phase. The volume fraction of gas in the foam is mostly between 0.5 and 0.9. The bubble size is usually between 0.1 and 3 mm (3). Foams can be classified into 2 types: liquid and solid foams. Although this study is focused on liquid foams, solid foams can be generated when the liquid phase is changed into gel or solid phase after foam formation (4).

Liquid foams' rheological properties, offer them the unique feature that the same foam may behave like an elastic/plastic solid or like a viscous liquid depending on how it is manipulated (5).

Foams have been widely used in several applications like cosmetic, pharmaceutical, laundry, firefighting, oil recovery, soil remidation or foam fractionation (5).

Emollient foams are emulsion-based so they have a soothing, moisturizing effect. Oil-in-water (O/W) or water-in-oil (W/O) emulsions can be used for the formulation, where the oil phase consists of mineral oil, triglyceride, fatty acid esters, such as isopropyl myristate, or isopropyl palmitate, or essential oil (6).

Foam emulsions are complicated systems which do not form under every circumstances. Slight shifts in the composition, may destabilize the foam. Furthermore, many emulsions do not provide the high foam capacity, foam stability and/or fast-breaking action under stress or temperatures that are desired in a topical foam composition (7).

Foams are characterized by a highly developed and vast interface which tends to reduce itself, making foams thermodynamically and mechanically unstable systems (8). In contrast to other disperse systems, the individual bubbles in the foam contact immediately after its generation, resulting in the formation of foam films that are an essential structural element of the foam, determining its stability (9).

1.1.1.1 Foam structure

Structurally, the bubbles of the foam can vary in their size and shape, ranging from spherical to irregular polyhedral, depending on how they were generated and on the excipients used. Properties like the origin and concentration of the excipients, and environmental factors such as temperature and humidity, influence the viscosity of the liquid phase as well as its pH, and therefore determine the foam structure (8).

When the volume of the gas phase is moderate, the bubbles dispersed in liquid phase are uniform and packed as spheres. At higher phase volumes (>70%), the air bubbles deposited near each other start to deform themselves resulting in polyhedral shapes with partly plane faces (10). The film that separates the faces of two adjacent polyhedral bubbles is called lamellae, and the thicker channels where three lamellae meet are known as plateau borders. The thickness of lamellae can vary between 10 nm and 1µm (8).

When the bubbles have the same size, their boundaries or lamellae meet at an angle of 120◦ (3). The liquid in lamellae is fixed by the molecules of a surfactant that acts as a foaming agent which are positioned at both surfaces of lamellae. This fixation is crucial, otherwise the liquid in vertical lamellae would drain immediately. In spite of the firm fixation, liquid tends to drain into the plateau border region from lamellae as the pressure within this region is lower than in air bubbles and in lamellae. This process causes the lamellae to become thinner. Thin lamellae are unstable and rupture easily because their surface area is too large for their volume (11).

All the essential foam processes, including those determining gas bubble expansion and their lifetime, bear on the thickness, structure and physicochemical properties of foam films (9).

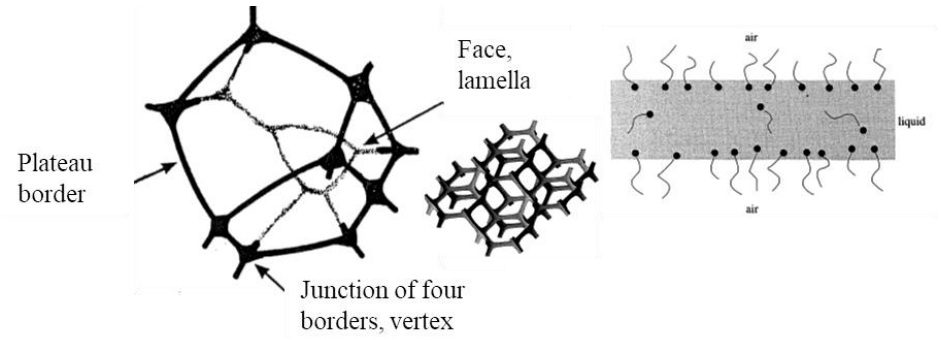


Figure 1 Three-dimensional scheme of foam structure. Adapted from (12).

1.1.1.2 Foam production

Foams can be produced by two basic methods. The most common one, is mechanical and works by dispersing the gas phase in the liquid with beating or shaking. The other way is by the gas-supersaturation of the liquid. The gas can be dissolved under pressure, which is later released, or can be formed in situ (6).

Both process create gas/liquid interfaces of interfacial tension (γ) which, require an energy input of at least

$$U=4\gamma \mathbf{r_B^2} \quad (1)$$

per bubble, where U is the flow velocity. For typical interfacial tensions and bubble sizes, this is many orders of magnitude larger than thermal energies (kT), which means that bubble formation is not a spontaneous process and that it requires a lot of energy into a liquid (5).

1.1.1.3 Foam stability

The presence of surface-active agents is essential to achieving stable foam. These amphipathic molecules position themselves onto the interface of the bubbles that constitute the foam. The polar (hydrophilic) group contacts with the water and the hydrophobic part is oriented towards the gas, creating a monomolecular layer that decrease the surface tension (γ) and therefore reduces the amount of energy required to produce the foam (6).

The lowering of γ per se is, however, not the cause of enhanced foam stability. It can vary with time and location of the surfactant and this variation only occurs if the surfactant is adsorbed.

The relation between surface tension, surface excess Γ and the activity a of the surfactant in the bulk (liquid) phase is given by the Gibbs equation:

$$\mathrm{d}\gamma = -RT\,\Gamma \mathrm{d}\ln a \quad (2)$$

Macromolecules tend to be much more surface active than small-molecule surfactants. This does not imply that macromolecules give a lower surface tension: the opposite is often true. However, far fewer molecules are needed to obtain a certain Γ or a certain lowering of γ (3).

1.1.1.4 Foam instability

The stability of foams has been related to the stability of the thin films. The relation of the Van der Waals' attraction and the electric repulsion potential of the double layer are dominant factors for this stability (8).

Foams can break down through different processes: Ostwald ripening (disproportionation), drainage and film rupture. These processes are not independent actions and often happen concurrently. Ostwald ripening involves the transport of gas between foam bubbles of different sizes, which causes the growth of bubbles and can be explained by the Laplace equation (13).

$$P = Pa + 2\gamma/R \quad (3)$$

where P is the pressure in a gas bubble, Pa is the atmospheric pressure, γ is the surface tension and R is the bubble radius. From equation (3) it can be concluded that the pressure in the foam bubbles is greater than atmospheric pressure. It is also clear that the smaller the radius of the foam bubbles, the greater the pressure in the bubbles, i.e. the smaller bubbles have a higher internal pressure compared to larger bubbles. This is therefore the driving force of Ostwald ripening, i.e. the air diffuses from small bubbles through the liquid film into larger ones.

Foam drainage is the flow of liquid through channels between the bubbles, which is usually driven by capillary (surface tension) forces and is resisted by viscous forces (14). The thickness of the channels that separate the foam bubbles can be reduced by foam drainage, a phenomenon that can expedite Ostwald ripening and film rupture (15). Rupture of the liquid films separating the bubbles leads to the coalescence of the bubbles and complete collapse of the foam structure. Nevertheless, the presence of surfactants at the interfaces can form a strong interfacial film around the foam bubbles and thus retard coalescence when the bubbles do come into contact. Such interfacial surfactant films may form a diffusion barrier, leading to a low permeability to gas molecules, and thus can decrease the effect of Ostwald ripening on foam stability (16).

1.1.2 Emulsions

Emulsions are a class of disperse systems consisting of two immiscible liquids. The liquid droplets (the disperse phase) are dispersed in a liquid medium (the continuous phase). Several classes may be distinguished: O/W, W/O, and oil-in-oil (O/O) (17). Emulsions are thermodynamically unstable systems. To disperse two immiscible liquids, a third component is required: the emulsifier. Due to the huge energy at the interface of the emulsions, these components are added to decrease this surface energy, as well as to reduce the oil droplet size and modify the flow and electrical properties of the interfacial layer, keeping the oil droplets dispersed in the hydrophilic phase (18).

1.1.2.1 Emulsion production

To prepare an emulsion, oil, water, surfactant and energy input are needed (19). The introduction of energy into the system can be achieved by trituration, homogenization, agitation or heat (1). The procedures that can be applied range from simple pipe flow (low agitation energy L); static mixers and general stirrers (low to medium energy, L–M); high-speed mixers such as the Ultraturrax (M); colloid mills and high-pressure homogenizers (high energy, H); and ultrasounds (M–H) (20).

When using the classical method, the emulsifying agent is dissolved into the phase where it is most soluble, after which the second phase is added, and shear is applied to the mixture using either high speed mixing or vigorous agitation. For oil-in-water (O/W) emulsions, the agitation must be turbulent which is crucial to producing sufficiently small droplets (21). Frequently, after an initial mixing, that originates the "pre-emulsion", a second mixing with very high applied mechanical shear forces is necessary. This latter mixing can be provided by a paddle, propeller or turbine mixer. Frequently a colloid mill or ultrasound generator is employed (20).

1.1.2.2 Emulsion stability

Emulsions can break over time due to the following mechanisms: Gravitational separation, droplet aggregation (or flocculation), Ostwald ripening, and droplet coalescence (22). Gravitational separation occurs because of the density difference between phases. It is referred to as "creaming" when droplets float up to the top, or "sedimentation" when they sink down. Droplet aggregation (or flocculation) occurs

when droplets attract each other, forming a loosely clumped mass of droplets ("flocs")(23). Flocculation is usually referred to as the precursor of the irreversible Ostwald ripening, which is caused by the difference in pressure inside large and small droplets, leading to a mass diffusion from the smaller to the larger droplets (1, 23). This phenomenon proceeds slower when the size distribution of the drops becomes narrower or when the dispersed phase is very insoluble in the continuous phase (24). All these instability mechanisms can then lead to droplet coalescence, which is the irreversible process of two droplets merging by the disruption of the stabilizing layer forming a larger one, eventually leading to the formation of separate oil and water phases (23).

1.2 Components

1.2.1 Oils

Natural oils are liquid products obtained by different techniques from vegetable or animal sources. They are mainly constituted by triglycerides; they also contain other lipophilic substances in low proportions, such as fatty alcohols, hydrocarbons, fatty acids, vitamins, phytosterols, etc. These last components determine in many cases their cosmetical and pharmaceutical activity. The main constituents of vegetable oils are esters of glycerol and fatty acids along with partially glyceridic material such as lecithin and substances such as tocopherol. Their composition will vary according to the species and the use will depend especially upon the variety, type and proportion of fatty acids (25).

Since antiquity, oils have played an important role in the composition of cosmetics, providing emollience, moisture, grooming, and acting as solvents and carriers to other agents.

A prime factor in selecting an oil, is the feel on the skin. This is a subjective matter, but the study of rheology provides a great deal of information. In addition, other factors may play a substantial role. Relative occlusivity on the skin is important to achieve moisturizing. Compatibility, and solubilization of other materials can broaden applicability.

Ability to form stable emulsions can be very significant. Odor and stability to light and oxygen are critical to modern cosmetic formulation. (26)

Lipids perform different functions in cosmetic formulations. They are moisturizing agents that limit water loss through different mechanisms. The first way is occlusion, obtained by placing a waterproof film on the skin to delay water evaporation from the surface. Substances typically used for these aims are hydrocarbons, fatty acids, fatty alcohols, vegetable waxes, phospholipids, and sterols.

Mineral oils and waxes are synthetic chemicals used in cosmetic and pharmaceutical industry. They are stable and dermatologically well-tolerated compounds, used to regulate the viscosity of formulations and for their protective and lubricating properties. They are prepared from natural crude petroleum oil through various refining steps. Moreover, mineral oils are non-allergenic, highly stable and not susceptible to oxidation or rancidity.

Lipids are used as surfactants and emulsifiers, to reduce the surface tension between the skin's surface and product and to keep water and oil blended in a product (27).

In the past, lipids have been used as penetration enhancers in cosmetics and more recently they have been used as nanoparticles such as solid lipid nanospheres, liposomes, nanosomes, and nanostructured lipid carriers for bioactive molecule delivery (28).

Oils affect foam stability. They can be solubilized in the micelles or remain as emulsions. The dependence on surfactant concentration, brine composition, temperature and pressure are also important for the foam stability in presence of an oil. There are four main theories for explaining foam stability in presence of oil:

1. Spreading and entering coefficients

2. Lamella number

3. Bridging coefficient

4. Pseudo-emulsion film theory (29).

By definition, the oil will spread at the surface and break the foam if the spreading coefficient is positive. If the spreading coefficient is negative, the oil will remain as a droplet at the surface. (29) The spreading can be calculated by using interfacial and surface tensions:

$$S = \gamma_{aw} - \gamma_{ow} - \gamma_{ao} \quad (4)$$

The oil droplets dispersed within an aqueous surfactant solution will "enter" the air-water surface. The entry of the droplet can be indicated by the coefficient E:

$$E = \gamma_{aw} + \gamma_{ow} - \gamma_{ao} \quad (5)$$

Where γ_{aw}, γ_{ow}, γ_{ao} are the air-water, oil-water, and air-oil tensions respectively. The entering coefficient E is a thermodynamic property, which determines whether the particular configuration of the oil droplet is energetically favorable or not, but cannot predict the behavior of oil droplets under dynamic conditions (30).

Lamella number represents the tendency of an oil phase to become emulsified and absorbed into a foam lamella (31).

The bridging coefficient is positive if the oil drop is able to enter both the liquid films surfaces, spanning the film and breaking the foam.

A pseudo-emulsion film is the thin liquid film between the oil droplet and the gas phase. If the pseudo-emulsion film is stable, the oil will stay in the lamella. If the pseudo-emulsion film is ruptured, the oil may form a lens at the gas-water interface, and this can break the foam down (29).

The oils used in this experiment vary in their properties, the ones that are the most relevant for the study are summarized in Table 3.

Table 3 Properties of the oil phases (32, 33)

	MCT	**PARAFFIN**	**CASTOR OIL**
Composition	Caproic acid ≤ 2.0% Caprylic acid 50-80% Capric acid 20-50% Lauric acid ≤ 3.0% Myristic acid ≤ 1.0%	Mixture of refined liquid saturated hydrocarbons obtained from petroleum	Ricinoleic acid 87% Oleic acid 7.0% Linoleic acid 3.0% Palmitic acid 2.0% Stearic acid 1.0% Dihidroxystearic acid neglectable
Density	0.93 – 0.96 g/ml	0.825 – 0.850 g/ml	0.955 – 0.968 g/ml
Viscosity	25-33 mPa.s	25-80 mPa.s	1000 mPa.s
Solubility	Soluble in organic solvents, miscible with long chain hydrocarbons and triglycerides. Practically insoluble in water	Soluble in chloroform, ether and hydrocarbons, sparingly soluble in ethanol, practically insoluble in water	Miscible with several organic solvents, soluble in ethanol and ether petroleum, practically insoluble in water and paraffin
Dielectric constant	3.93	1.6 - 2.5	4.4 - 4.7
Surface tension	31.0 – 32.3 mN/m at ~25°C	35 mN/m at 25°C	39.0 mN/m at 20°C;

1.2.1.1 MCT oil

MCT (medium chain triglycerides) oil is a mixture of triglycerides of saturated fatty acids (≥95%). It is a transparent, colorless liquid at room temperature. It is a versatile solubilizer for lipophilic drugs and a skin protectant through moisture retention.

The spreading value of an emollient has an important impact on the skin-feel of the resulting emulsion. The use of fast spreading emollients will result in a smooth feeling on the skin that disappears quickly. MCT oil has a spreading value of 550 mm2, giving it medium emollience properties (34). It does not impede skin respiration, has good penetration properties, doesn't leave a visible film on the skin surface, has good compatibility, good solvent properties and good oxidative stability. Because of that, MCT oil is largely used in ointments, creams and liquid emulsions. It is nontoxic and nonirritant and can be used in many pharmaceutical preparations (oral, parenteral and topical) (32).

MCT oil has medium water permeability, meaning that it retains moisture by creating a barrier that prevents water from evaporating off the skin and also increases the flexibility of the stratum corneum, leading to hydration of the skin (34).

1.2.1.2 Paraffin

Also known as mineral oil, paraffin is a mixture of refined liquid saturated paraffinic and naphthenic hydrocarbons, obtained from petroleum. It is functionally used as an emollient; oleaginous vehicle; solvent; tablet and capsule lubricant and also therapeutic agent. Its emollient properties are exploited in ointment bases for topical pharmaceutical formulations; in transdermal preparations, it functions as a solvent and penetration enhancer. Paraffin is also used in cosmetics and certain food products (35) (32).

1.2.1.3 Castor Oil

Castor oil, produced from castor beans, is used in the manufacturing of soaps, lubricants, and coatings, among others (36, 37). In pharmaceutical industry, it is used to form stable emulsions of nonpolar materials in various aqueous systems in the form of Cremophor EL (38, 39).

1.2.2 Phospholipids

Phospholipids (PLs) are amphiphilic lipids found in all plant and animal cell membranes, arranged as lipid bilayers that function as semipermeable barriers (40). Besides glycolipids and cholesterol, PLs are the main component of eukaryotic membranes (41). They can also take part in crucial processes, which give structural integrity to membranes and assist in the functions of the cell to carry out metabolism-related processes (42). In recent years, PLs have been recognized as intracellular messengers, which proves that they play other roles other besides being structural components (43).

1.2.2.1 Structure

The phospholipid molecule contains a hydrophilic part and a lipophilic part. In the hydrophilic part, the glycerol backbone is esterified in position 3 with phosphate and positions 1 and 2 with fatty acids. The two fatty acids form the lipophilic part, one of them is saturated and the other is unsaturated (42) (Figure 2). The distribution of the substituents in positions 1, 2 and 3 of the glycerol introduce chirality. In typical membrane phospholipids, the phosphate group is further esterified with the alcohols; choline in phosphatidylcholine (PC), ethanolamine in phosphatidylethanolamine (PE), glycerol in phosphatidylglycerol (PG) and inositol in phosphatidylinositol (PI). The phospholipid without esterified alcohol is phosphatidic acid (44).

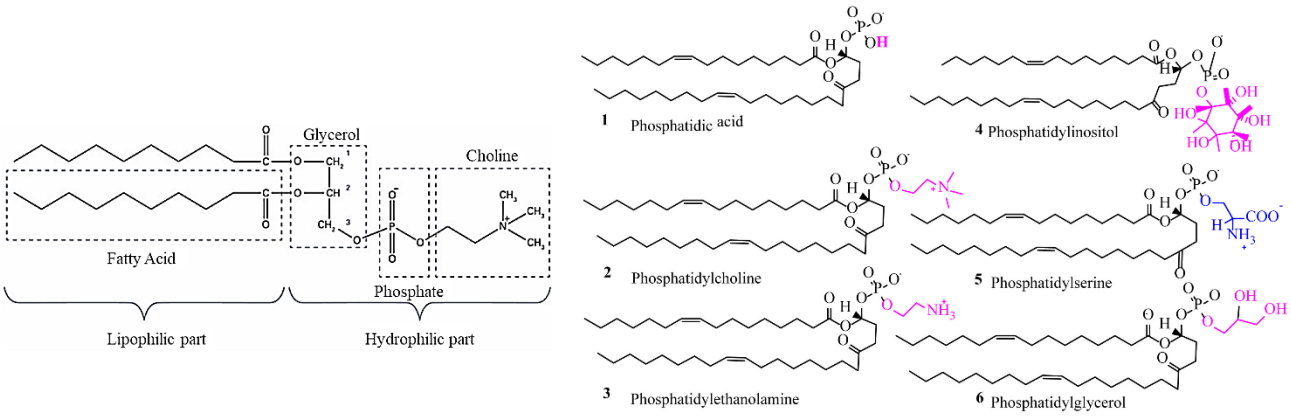


Figure 2 Molecular structures of phospholipids. Adapted from (42) and (44).

Depending upon the structure of the polar region and pH of the medium, PE and PC are zwitterionic and have a neutral charge at neutral pH values, whereas PG, for example, is negatively charged.

After being mixed with an aqueous phase, PLs can form various structures depending on the number and type of fatty acids esterified to the glycerol backbone and the ratio of the surface areas occupied by the hydrophilic and lipophilic part of the phospholipid molecule. Diacylphospholipids having a cylindrical shape are organized as lipid bilayers (lamellar phase) with the hydrophobic tails lined up against one another and the hydrophilic head group facing the water on both sides (liposomes) (

Figure **3**).

When only one fatty acid is esterified to the glycerol backbone of the phospholipid molecule (monoacylphospholipids, also called lyso-phospholipids), and the polar head group are relatively large, the molecules are cone-shaped, and they can form micelles (also called hexagonal HI phase). When the surface area of the polar head group is small, inverted cones are formed which are upon hydration arranged in the HII phase (Figure 4).

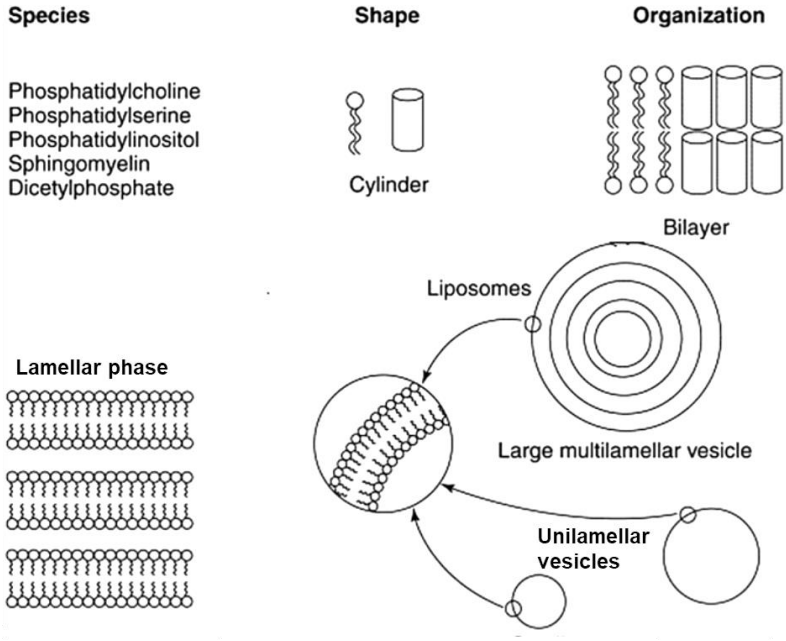


Figure 3 Formation of phospholipid bilayers and liposomes after contact with an aqueous phase (44)

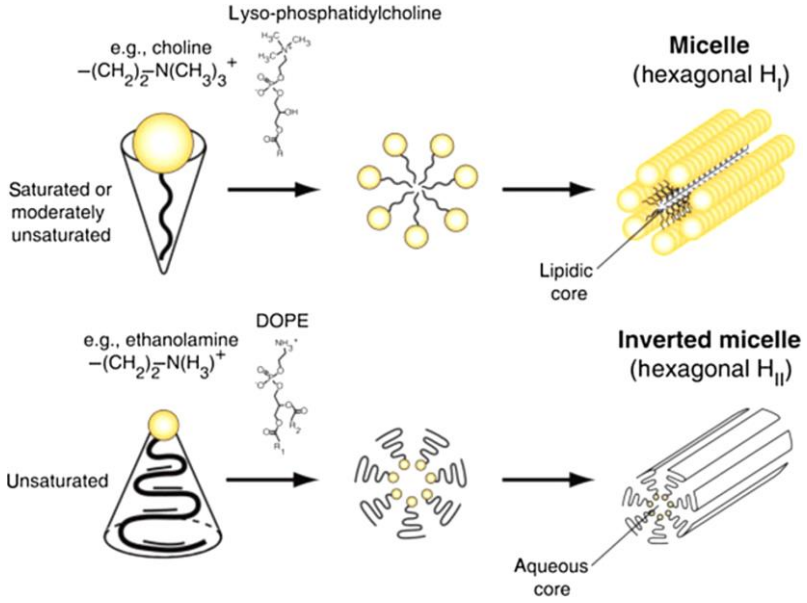


Figure 4 Phospholipid molecules with either cone shape (above) or inverted cone (below) shape, forming upon hydration micelles or inverted micelles, respectively.

Phospholipids can change their mobility, the temperature at which that occurs is determined by the fatty acids that constitute the molecule. Below the phase transition temperature (PTT) (specific for each phospholipid molecule) the fatty acids and the phospholipid molecule are rigid (gel state), whereas above this phase transition temperature they are mobile. PLs with polyunsaturated fatty acids have a very low (below 0 °C) phase transition temperature. When applied on the skin, these lipids are in the liquid state and, upon hydration, structures/liposomes with a flexible membrane. Phospholipids with saturated fatty acids have a higher PTT. At skin temperatures, liposomal dispersions with hydrogenated lipids are in the gel state and are rigid (44).

1.2.2.2 Applications

Due to their amphiphilic character, PLs can adopt various molecular assemblies when dispersed in water, such as bilayer vesicles or micelles, which give them unique interfacial properties and render them very attractive in terms of foam or emulsion stabilization (45). They have attracted much attention in drug delivery development, polymer science, food and cosmetics formulations, and biomedical engineering, etc (43). In cosmetic formulations, they can function as an emulsifier, liposome/lamellar phase former, solubilizer or wetting agent (44).

PLs are also largely used in the food industry, as inhibitors of lipid oxidation or additives (45).

Lecithin is likely the most common form of phospholipids (45). Typically, for pharmaceutical use, lecithins are derived from egg yolk or soybean. Although possessing a polar zwitterionic head group, the hydrocarbon tails result in a surfactant with very low water solubility in the monomer state. The ability of lecithin to form a tough but flexible film between the oil and water phases is responsible for the excellent physical stability. In aqueous media, phospholipids can assemble into concentric bilayer structures known as liposomes. The therapeutic advantage of such a lipid assembly for drug delivery depends upon the encapsulation of the active ingredient either within the interior aqueous environment or within the hydrophobic region of the bilayer (46).

In foams, PLs are able to spread along the air/water interface when it is stretched, due to their unique visco-elastic properties. PL spreading leads to changes in the state of the phospholipids at the interface from solid to gaseous, with various phases co-existing such as liquid expanded and liquid condensed (45).

Mixtures of PLs and co-surfactant or proteins to stabilize emulsions have received a lot of interest. Phospholipids act as an emulsifying agent while the surfactant provides additional strength to stabilize the interface to resist instability phenomena (47).

1.2.3 Surfactants

Surface active agents (usually referred to as surfactants) are amphipathic molecules that consist of a non-polar hydrophobic portion, usually a straight or branched hydrocarbon or fluorocarbon chain containing 8–18 carbon atoms, which is attached to a polar or ionic portion (hydrophilic). The hydrophilic portion can, therefore, be nonionic, ionic or zwitterionic (17), classifying the surfactant. In addition, polymeric surfactants belong to a different class and they have been widely used to stabilize emulsions and suspensions.

Surfactants are important as formulation aids for the delivery of active ingredients in the many pharmaceutical forms, where foams are included. They facilitate the passage of active ingredients across the various membranes (48).

Surfactants are necessary in making foams because the air/water/air interface is intrinsically unstable. They provide elasticity to the surface, making it more resistant to external forces. The elasticity of a material is the force required to change the dimensions by a certain amount. For foams, the elasticity depends on how the surface tension changes with film surface area.

They perform a counter pressure ("disjoining pressure") against the capillary pressure that drives liquid out of the walls of the bubbles into the edges. This can be produced by charges on the surfactant either side of the wall, and/or by steric interactions between surfactant chains.

Other reason that makes surfactants essential, is that they give the foam resistance against Ostwald ripening, drainage, and defects. It is the surfactant that provides the Entry Barrier (49). This barrier impedes the oil droplets from entering the foam bubbles, they stay at the film walls instead.

In this experiment, the anionic surfactants used are sulphated fatty alcohols, which are esters of sulphuric acid. The main component is sodium dodecyl sulphate, $C_{12}H_{25}$-O-SO_3^- Na^+. It is used pharmaceutically as a preoperative skin cleanser having bacteriostatic action against Gram positive bacteria. It is also used in medicated shampoos and toothpaste (as foam producer).

Ether sulphates (sulphated polyoxyethylated alcohols) R-(OCH_2-CH_2)n-O-$SO3^-$ M^+ (n< 6) are other class of anionic surfactants, they have better water solubility than the alkyl sulphates, better resistance to electrolyte and less irritation to the eye and the skin (17).

As anionic surfactants have strong lipid solubilizing abilities and protein denaturing action, they can potentially reduce the barrier function of the skin, resulting in skin irritation (50).

2 Objectives

The aim of this project is the incorporation of different oils, in different concentrations, into previously developed foamable mixtures of phospholipids and surfactants. This incorporation results in oil in water emulsions, which are then evaluated by their stability, foamability and foam structure in order to determine the formulations that work best when it comes to producing high quality foam formulations, that are suitable for application on the skin. Such formulations can be used for the treatment of chronic skin conditions such as seborrheic dermatitis or scalp psoriasis.

Additionally, in this project, different manufacturing approaches were used to evaluate this influence on the emulsions and their foamability as well.

3 Materials and Methods

3.1 Materials

The present work deals with emulsions made of an aqueous phase, phospholipids and anionic surfactants, and an oily phase. Purified water is used as the dispersion medium.

3.1.1 Oils

Three oils with crescent polarities and viscosities were used as the oil phase of the emulsions.

Table 4 List of oils used

Oil	Trade name	Manufacturer
Medium Chain Triglycerides	Kollisolv MCT 70	BASF SE, DE-Ludwigshafen am Rhein
Liquid paraffin, light	Paraffinum perliquidum	Ceasar & Loretz GmbH, DE-Hilden
Castor oil	Oleum Ricini raffinatum	Ceasar & Loretz GmbH, DE-Hilden

3.1.2 Phospholipids

A total of six different phospholipids, that cover a large part of this class, were used. They come from two product lines: LIPOID and PHOSPHOLIPON from the manufacturer Lipoid GmbH, DE - Ludwigshafen am Rhein. They are based on the fatty acids contained in unsaturated, hydrogenated phospholipids and lyso-phospholipids.

Table 5 Composition of the phospholipids with unsaturated fatty acids

	LIPOID S20	LIPOID S75	PHOSPHOLIPON 90G
Phospholipids [%]	≥ 97.0		
Phosphatidylcholine	≥ 20	≥ 70.0	94.0 - 102.0
Phosphatidylethanolamine		7-11	
Phosphatic acid		≤ 3.0	
Phosphatidylinositol		≤ 1.5	
Lysophosphatidylcholine	≤ 3.0	≤ 3.0	≤ 4.0
Non-polar lipids [%]	≤ 3.0		≤ 3.0
Triglycerides		≤ 3.0	
Free fatty acids	≤ 0.5	≤ 0.5	
DL-α-tocopherol		0.1-0.2	≤ 0.3
Phosphorus	3.1 - 3.3	3.4 - 3.7	

Table 6 Composition of the phospholipids with hydrogenated fatty acids

	LIPOID P75-3	PHOSPHOLIPON 80H
Phospholipids [%]		
Phosphatidylcholine	≥ 62.0	≥ 70.0
Lysophosphatidylcholine	≤ 5.0	≤ 6.0
Non-polar lipids [%]		
Triglycerides	≤ 3.0	
Free fatty acids	≤ 0.5	
Phosphorus	3.4-3.7	

Table 7 Composition of the lyso-phospholipids

	LIPOID P LPC 80
Phospholipids [%]	
Phosphatidylcholine	≤ 20.0
Lysophosphatidylcholine	≥ 80.0
Glycerophosphocholine	≤ 5.0
Non-polar lipids [%]	
Natural mixed tocopherols	≤ 0.4
Other substances	≤ 5

3.1.3 Surfactants

Surfactants are amphiphilic molecules made up of a hydrophilic and a lipophilic group. The hydrophilic head can be ionic or non-ionic. The classification of ionic surfactants is based on the structural elements that form the molecule.

The surfactants used in this experiment were all anionic surfactants.

Table 8 List of surfactants used

Surfactant	Trade name	Manufacturer
Sodium coconut sulfate	-	Alexmo cosmetics GmbH, DE - Stuhr
Sodium Cocoglutamate	Plantapon ACG 50	BASF SE, DE-Ludwigshafen am Rhein
Sodium Lauryl Ether Sulfate	Texapon NSO UP	BASF SE, DE-Ludwigshafen am Rhein

3.2 Equipment

All the physical and informatic equipment used in the experiment is listed in the next sections.

3.2.1 Eletronic equipment

Table 9 List of electronic equipment used

Classification	Designation	Manufacturer
Analytical Scale balance	Mettler AE 200-S	Mettler Toledo GmbH GE - Giessen
Scale balance	Pioneer PX3202	Ohaus Europe GmbH Greifensee, Switzerland
Electronic Stirrer	Heidolph RZR 2102 control	Heidolph Instruments GmbH & Co. KG, GE-Schwabach
Magnetic Stirrer	Heidolph MR HEI-TEC Ø145 (EU)	Heidolph Instruments GmbH & Co. KG, GE-Schwabach
Magnetic Stirrer	Heidolph MR 3001 K	Heidolph Instruments GmbH & Co. KG, GE-Schwabach
Temperature sensor	Heidolph Pt1000 (AISI 316Ti)	Heidolph Instruments GmbH & Co. KG, GE-Schwabach
High-performance dispersing device	T 25 digital ULTRA-TURRAX	IKA-Labortechnik JANKE & DUNKEL GMBH & CO. KG, GE-Staufen
High-performance dispersing device	T 18 digital ULTRA-TURRAX	IKA-Labortechnik JANKE & DUNKEL GMBH & CO. KG, GE-Staufen
Ultrasonic Processor	UP200S	Hielscher Ultrasound Technology GmbH GE - Teltow
Water purifier	PURELAB option Q	ELGA LabWater
Static testing *machine*	Zwick/Roell BDO-FB0.5TS	ZwickRoell GmbH & Co. KG, GE - Ulm
Particle size analyzer	Malvern Mastersizer 2000 with Hydro2000s	Instruments Ltd., UK-Malvern
Digital camera	Honor V10	Huawei
Stopwatch	Big Digit Timer C5079	Carl Roth GmbH+Co.KG, GE - Karlsruhe

3.2.2 Software

Table 10 List of software used

Function	Software	Manufacturer
Material testing	testXpert	ZwickRoell GmbH & Co. KG, GE - Ulm
Particle Size analysis	Malvern Application 5.60	Malvern Instruments Ltd., UK-Malvern
Graph construction	GraphPad PRISM 8.2.1	GraphPad Software Inc., US-San Diego
Text and data processing	Microsoft Office 365	Microsoft Corporation, US-Redmond

3.3 Methods

3.3.1 Preparation of the surfactants solutions

Bulk solutions of the selected surfactants, sodium coconut sulphate (SCS), sodium laureth sulphate (SLES) and sodium coconut glutamate (SCG), were prepared to facilitate the further preparation of the pre-mixes, which also contain the phospholipids.

The pure surfactants were dispersed in purified water, creating bulk-solutions with concentrations of either 5 or 10%. These would be later used to prepare the pre-mixes.

To prepare the SCG solution, the solid form of the surfactant was weighted and dispersed in purified water with the aid of a magnetic stirrer at approximately 300rpm, the temperature was set at 45 °C and the mixture was stirred until complete dissolution (i.e. clear appearance) and cooled down to room temperature.

As SCS and SLES are liquids at room temperature, the final solutions were prepared by dilution to the final concentrations using a magnetic stirrer at approximately 300 rpm.

The SCS/SCG bulk solution was prepared by mixing equal parts of SCS and SCG solutions with equal concentrations of each.

3.3.2 Preparation of the pre-mixes

The pre-mixes are the aqueous continuous phase of the emulsions intended for foaming. Thus, formulations (Table 11) were previously developed and evaluated according to their foaming capacity and quality of the produced foam.

There were 12 pre-mixes formulations, which were considered the best ones. They were produced according to the methods described in the previous investigation's report (51).

The respective phospholipid listed in 3.1.2. was weighted and added to the appropriate quantity of water inside a tall beaker. The mixture was then stirred with a paddle stirrer at 300-600 rpm. The velocity was adjusted to allow an efficient dispersion. The temperature was set at the respective phospholipid phase transition temperature, which was 45 ºC for all used phospholipids but for S20, whose PTT is 25 ºC. The pre-mix containing this phospholipid was prepared at room temperature. When the mixture reached 45 ºC, it was stirred for 15 minutes more.

After this time, letting the mixture cool until room temperature was optional. Then the appropriate amount of surfactant bulk solution was added, and the mixture was stirred with a magnetic stirrer at 300 rpm for 5 minutes. It was then stored in a glass screw top bottle.

Table 11 Formulation of the pre-mixes

	Formula		
Pre-mix	Phospholipid	Surfactant	
	C= 0.5%		%
1	S75	SCS/SCG	1.0
2	S75	SCS	1.0
3	80H	SCS	1.0
4	S20	SCS/SCG	1.0
5	PLPC80	SCS/SCG	0.5
6	S20	SCS	0.5
7	S75	SCS/SCG	0.5
8	S75	SLES	0.5
9	90G	SCS	1.0
10	P75-3	SLES	0.5
11	80H	SLES	0.25
12	PLPC80	SCS	0.5

3.3.3 Preparation of the emulsions

Six emulsions were prepared for each of the twelve pre-mixes listed in Table 11. For each pre-mix, three different oils were incorporated, in concentrations of 10 and 20%. Resulting in a total of 72 emulsions with distinct formulations. The composition of these emulsions as well as the nomenclature used to describe them is shown in Table 12.

The production process was divided in three major steps. Firstly, the phospholipid and surfactant mixture was homogenized with an ULTRATURRAX at 9000 rpm while the oil was slowly added with a disposable syringe, over a period of 4 minutes. Then, the speed of rotation was increased to 13400 rpm and the pre-emulsion was manually moved round and up and down for two more minutes. The first two steps consisted of the assembling of the pre-emulsion. The third and final step involved homogenization by the means of an ultrasonic processor equipped with a sonotrode needle using an amplitude of 50% and 0.5 cycle, for two minutes, moving the emulsion in every direction so the energy input was well distributed in the entire volume of the emulsion.

The emulsions were then transferred to labeled 50 ml plastic tubes and they were stored in a cupboard protected from light.

Table 12 Quantitative formulations of the emulsions

Pre-mix												
Nº	Phospholipid C= 0.5%	Surfactant	%	Oil	%	Emulsion	Oil	%	Emulsion	Oil	%	Emulsion
1	S75	SCS/SCG	1.0	MCT	10	1MCT10	Par	10	1Par10	Castor oil	10	1CO10
					20	1MCT20		20	1Par20		20	1CO20
2	S75	SCS	1.0	MCT	10	2MCT10	Par	10	2Par10	Castor oil	10	2CO10
					20	2MCT20		20	2Par20		20	2CO20
3	80H	SCS	1.0	MCT	10	3MCT10	Par	10	3Par10	Castor oil	10	3CO10
					20	3MCT20		20	3Par20		20	3CO20
4	S20	SCS/SCG	1.0	MCT	10	4MCT10	Par	10	4Par10	Castor oil	10	4CO10
					20	4MCT20		20	4Par20		20	4CO20
5	PLPC80	SCS/SCG	0.5	MCT	10	5MCT10	Par	10	5Par10	Castor oil	10	5CO10
					20	5MCT20		20	5Par20		20	5CO20
6	S20	SCS	0.5	MCT	10	5MCT10	Par	10	5Par10	Castor oil	10	5CO10
					20	6MCT20		20	6Par20		20	6CO20
7	S75	SCS/SCG	0.5	MCT	10	7MCT10	Par	10	7Par10	Castor oil	10	7CO10
					20	7MCT20		20	7Par20		20	7CO20
8	S75	SLES	0.5	MCT	10	8MCT10	Par	10	8Par10	Castor oil	10	8CO10
					20	8MCT20		20	8Par20		20	8CO20
9	90G	SCS	1.0	MCT	10	9MCT10	Par	10	9Par10	Castor oil	10	9CO10
					20	9MCT20		20	9Par20		20	9CO20
10	P75-3	SLES	0.5	MCT	10	10MCT10	Par	10	10Par10	Castor oil	10	10CO10
					20	10MCT20		20	10Par20		20	10CO20
11	80H	SLES	0.25	MCT	10	11MCT10	Par	10	11Par10	Castor oil	10	11CO10
					20	11MCT20		20	11Par20		20	11CO20
12	PLPC80	SCS	0.5	MCT	10	12MCT10	Par	10	12Par10	Castor oil	10	12CO10
					20	12MCT20		20	12Par20		20	12CO20

3.3.4 Evaluation of the emulsions

The emulsions were evaluated by the observation of their aspect through time, as well as by investigation of the droplet size distribution, which determines how they behave and makes it possible to predict whether or not they will maintain their stability.

3.3.4.1 Macroscopic evaluation

To macroscopically evaluate the emulsions stability, pictures were taken at the day they were produced, after 3 days, after one week, after two weeks and finally, after one month of being prepared. In Figure 9 it is possible to observe an example of a formulation of an emulsion that maintained its stability for an entire month. In contrast, the other emulsion revealed instability right after being produced.

3.3.4.2 Droplet size analysis

The method used was the dynamic light scattering technology and the parameters used are listed in Table 13.

Each sample was measured 9 times. Three measuring cycles were conducted for each sample and in each cycle the sample was measured 3 times, in the end of the cycle, the results were automatically averaged.

Table 13 Parameters set for particle size distribution analysis on the mastersizer 2000

Result range	0.020 – 2000 µm
Level sensor threshold	64%
Stirrer	1750 rpm
Sample	Oil
Refractive index	1.449
Dispersant	Water
Result Calculation Calculation sensitivity Particle shape	General purpose Normal Spherical
Measurement times Sample Background	 30 seconds 10 seconds
Number of snaps Sample Background	 30 000 10 000
Laser intensity	75-80%
Obscuration limits	2-6%
Cycles Measurements Delay	3 per sample 3 15 seconds between each measurement

3.3.5 Evaluation of the emulsions foamability

An optimized screening method was used to macroscopically test the foamability of the emulsions.

The apparatus consisted of three glass columns with a glass filter plate inserted at their bottom (Figure 5). These columns were connected, by a rubber pipe, to a pressing machine that pressed the air through the filter plates and into the columns where the sample was placed. The liquid emulsion was therefore transformed into a foam by gas dispersion, where the gas dispersed was simple, natural air.

Each column was filled with 12 ml of the sample, measured with a disposable syringe. The foaming process began by starting the pressing machine on the software. At this point, the pistons on the pressing machine begin to move down, making the air pass through the filter plates, this step takes 10 seconds. After this time, the produced foam stabilizes for 15 seconds, and then the valves that connect the columns to the syringes are closed.

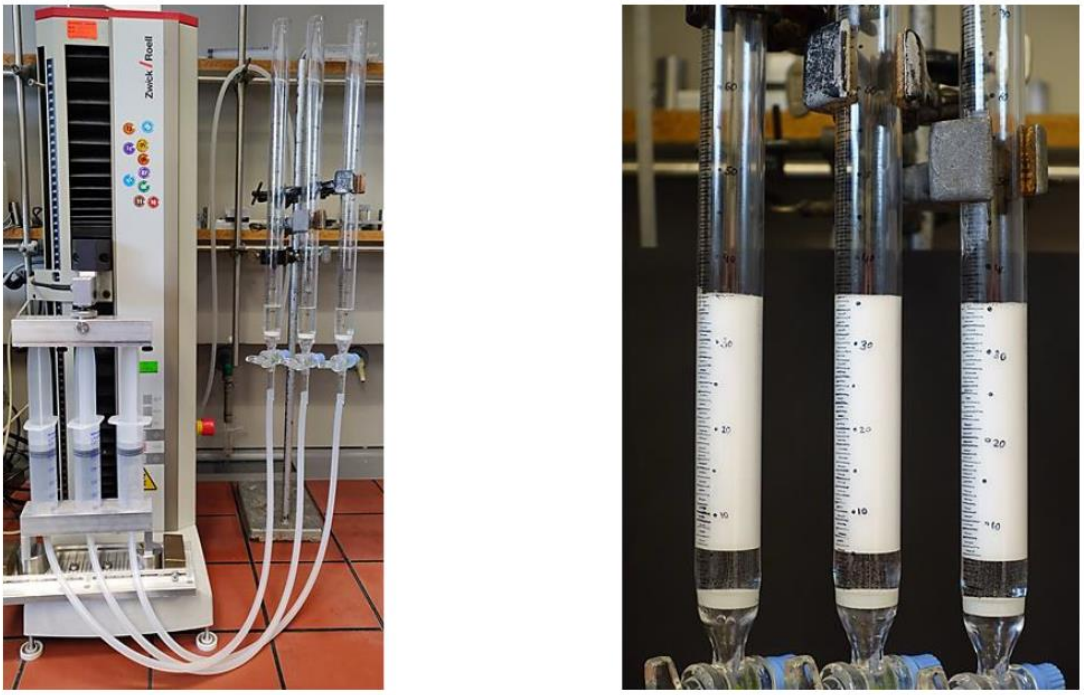


Figure 5 Entire set up of the screening method (left) and columns during a measurement.

The following seconds consist of the time when the foam is evaluated. Consequently, the moment when the valves are closed, counts as t0. Pictures are taken at the defined

time points: t0, t30, t60, t150 and t300 (Figure 6). The pictures taken are stored for later observation and evaluation of the foam.

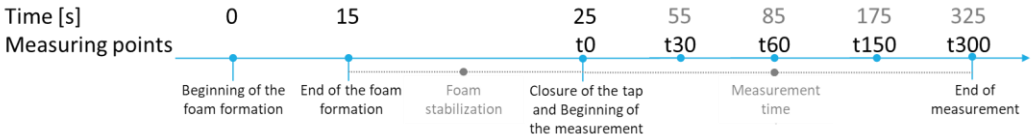


Figure 6 Schematic representation of the foaming process from the beginning to the end of the measurement

3.3.6 Evaluation of the foam stability

To characterize the foamability and foam stability, the foam volume is measured at the beginning and over the course of the experiment. The total volume can be measured directly, using the graduated column. The foam volume is calculated by subtracting the liquid volume from the total volume of the sample.

$$\text{Foam volume [ml]} = \text{total volume [ml]} - \text{liquid volume [ml]} \quad (6)$$

Drainage is a parameter that describes foam stability and it can be assumed as the observed liquid volume.

$$\text{Drainage [ml]} = \text{liquid volume [ml]} \quad (7)$$

The absolute value of the drainage is related to the original volume of the sample and can't assume any higher values than this. Drainage can, therefore, also be described as a relative quantity.

3.3.7 Evaluation of the foam structure

The pictures taken during the screening method measurement were carefully observed and a score was used to classify the foam in every time point.

The scoring ranges from 1 to 4. In the cases where the result of the experiment cannot be considered as a foam, it is attributed the classification "out of score". An emulsion that resulted in an "out of score" classification was considered not foamable or with very poor foamability.

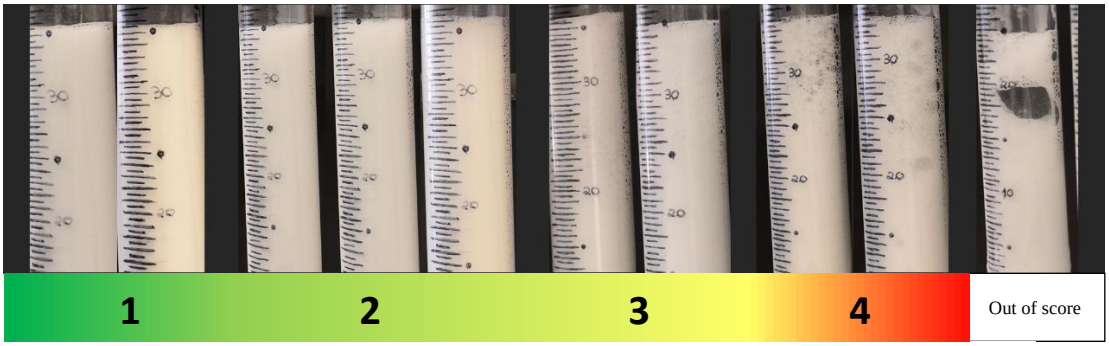


Figure 7 Scoring scale (1 to 4) with illustrative examples of each

4 Results

4.1 Validation of the screening method

4.1.1 Preparation of the validation

A screening method based on the Dynamic Foam Analyzers (DFA 100) from Krüss GmbH is used to access the foamability of the formulations. This method was developed to allow a faster characterization of the foam behavior due to a simultaneous triplicate measurement (n = 3).

Before starting the assessment of individual formulations, the standard deviation of the three columns was determined and compared with each other. For this purpose, a solution with sodium lauryl sulfate (SLS), in a concentration of 0.50%, is used as a reference solution.

Before the measurements, three fitting columns, that were expected to yield similar results were selected and they were tested with the reference solution. They showed slight differences in the foaming behavior. For example, column number 1 was the first to start producing foam, resulting in a higher foam. Column nº3 had a higher drainage, so it was replaced with another column, marked with number 4. But at t150 and t300, it was very clear that there were variations in the foam structure. Column 1 and 2 maintained a really good foam, while column 4 revealed the worst foam structure.

Columns 1, 2 and 3 were the final three selected columns. They were scaled with a graduation of 0.5ml and validated afterwards.

4.1.2 Validation results

The three selected columns were placed next to each other and aligned by the filter plates in the screening method apparatus. The first procedure was testing them with 11mL of water to confirm that the system was working properly and that the plates were clean with no residues of other surfactants.

After being dried, the columns were filled with 12ml of the reference solution and the system was run. Pictures were taken at t0, t150 and t300 to register the volume of the foam and the resulting drainage

The verification was carried out with six measurements per column and the three columns were tested in parallel each time. Thereby, it was possible to estimate both the reproducibility of a single column, as well as the deviation of the columns among each other.

The total volume of the sample at time t0 can be used to assess the foamability of the reference solution. In Figure 8, the average of the total volume (t0) of the three columns in all six measurements is represented. The standard deviation (SD) works as an absolute measure and the coefficient of variation (V (x)) is used as a relative measure of the data (Table 14)

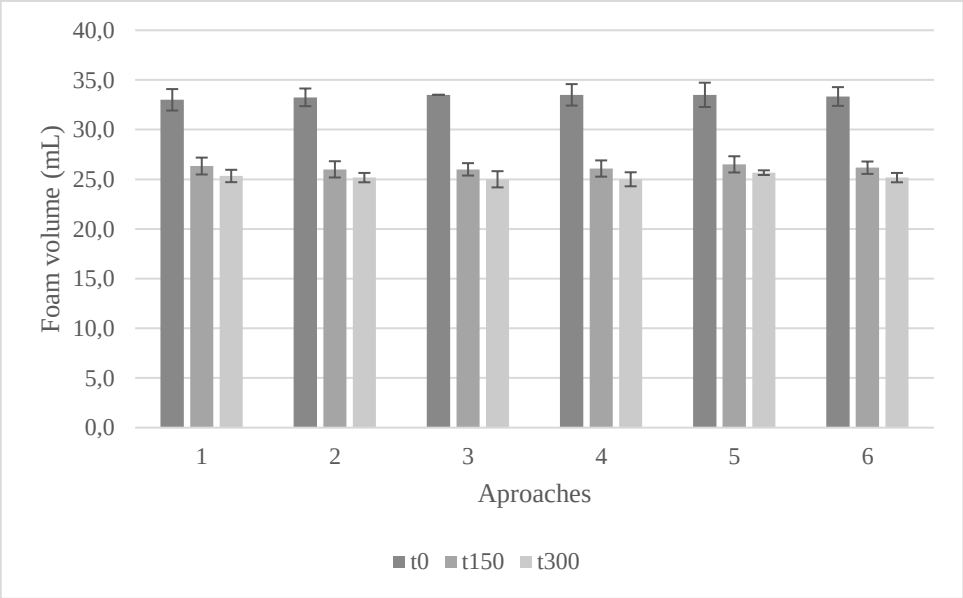


Figure 8 Foam volume for the time points t0, t150 and t300 in the 6 measurements

Table 14 Scatter parameters for investigating the reproducibility of the columns as well as their deviation from each other

		SD	V(x) [%]
Column 1	t0	0.37	1.03%
	t150	0.37	1.03%
	t300	0.29	0.80%
Column 2	t0	0.24	0.65%
	t150	0.00	0.00%
	t300	0.00	0.00%
Column 3	t0	0.37	1.06%
	t150	0.29	0.82%
	t300	0.34	0.98%

Due to the graduation of the columns, the maximum reading accuracy of the volume and thus the accuracy of the screening method is defined as 0.5 ml. Minor differences are, therefore, not detectable with the method and standard measurements (n = 3) may have standard deviations of zero.

The standard deviations were determined to compare the columns. The coefficient of variation is always below 1.5%. which means that the deviations between the pillars are small enough to be considered negligible. They are considered identical, whereby a simultaneous triple measurement (n = 3) is possible.

4.2 Emulsion's stability

The stability of the emulsions was mostly characterized qualitatively, by direct observation. From the macroscopical observation of the produced emulsions in several time points, it was evident that the formulations containing MCT, as an oil phase, were the ones that could maintain their stability better, showing no signs of phase separation. Creaming could be observed in some formulations after a few days, but overall, they could be considered stable emulsions.

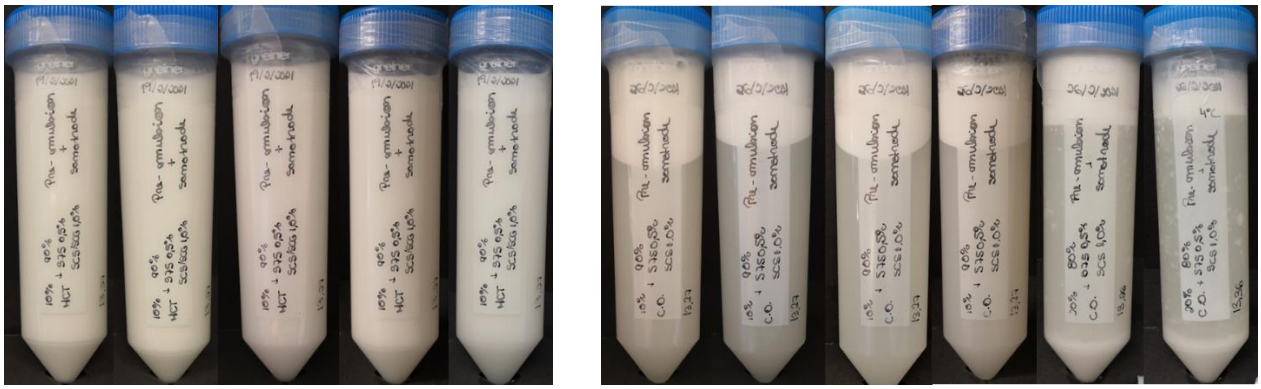
The formulations with paraffin were less stable. In all of them, creaming could be observed after a few hours or on the next day. They could easily be reconstituted.

When castor oil constituted the oil phase, the formulations could not maintain their stability after a few moments of being produced. In some of them, like 8CO20 [0.5% S75; 0.5% SLES; 20% CO] there were visible droplets of oil indicating coalescence and posterior phase separations. These emulsions could not be reconstituted, and it made it difficult to analyze them. From all the CO emulsions, 9CO10 [0.5% 90G; 1.0 %SCS; 10% CO] appeared to be more stable, it could be easily reconstituted by inversion and didn't reveal any noticeable coalescence or oil droplets. 10CO10 [P 75-3; 0.5% SLES; 10% CO] is also an example of a CO emulsion, that despite the evident creaming, could be easily reconstituted by inversion and didn't show visible signs of coalescence.

The percentage of oil in the formulation did not seem to influence the emulsions stability. Formulations with the same pre-mix usually behaved similarly after being produced.

The formulations containing SLES as surfactant, regardless of the phospholipid also incorporated in the pre-mix, and the added oil, generally revealed a deposit at the bottom of the tube where they were stored. They had to be homogenized with more inversions than the other emulsions.

In Figure 9 there is an example of an emulsion that maintained its stability through an entire month, it contains 10% MCT oil and 90% of the premix with 0.5% phospholipid s75 and 1% of the surfactant SCS/SCG. On the contrary, the other emulsion, with CO, was not stable from the beginning, and after a few weeks, besides the creaming, it also showed flocculation and coalescence. A sample was stored in the fridge, at 4 ºC, the conditions where the castor oil is also stored. This approach did not improve the stability. On the contrary, the flocculation in this sample is more evident.



Day 0	Day 1	Week 1	Week 2	Week 4

Day 0	Day 1	Week 1	Week 2	Week 4	Week 4 4ºC

Figure 9 1MCT10 (left) and 2CO10 (right) emulsions through time

4.3 Droplet size distribution

The droplet size analysis was conducted as described in section 3.3.4.2. The data from each sample was extracted from the software and compared with the samples containing the same oil. In Figure 10, the droplet size distribution of the samples containing MCT, paraffin and castor oil can be observed. In the case of MCT, for most formulations, 90% of the droplets had a diameter smaller than 10 micrometers, it was consistent in all formulations and the standard deviations are very low. When observing the graph that represents the samples containing paraffin, it is clear that the droplet size is considerably larger than the formulations with MCT and that, in most formulations, the diameter of the droplets doesn't fall in a size range that indicates that an emulsion has the capacity of holding its stability, leading to instability phenomena like the observed creaming.

As it could be confirmed with visual observation, the droplet size of the emulsions with CO indicates that these emulsions are highly unstable. The droplets' diameter is too large, causing coalescence and later, phase separation. From the graph, it is also possible to observe that the standard deviations have a high value. This is due to the extreme heterogeneity of the samples regarding the size of the oil droplets that could be easily seen with naked eye.

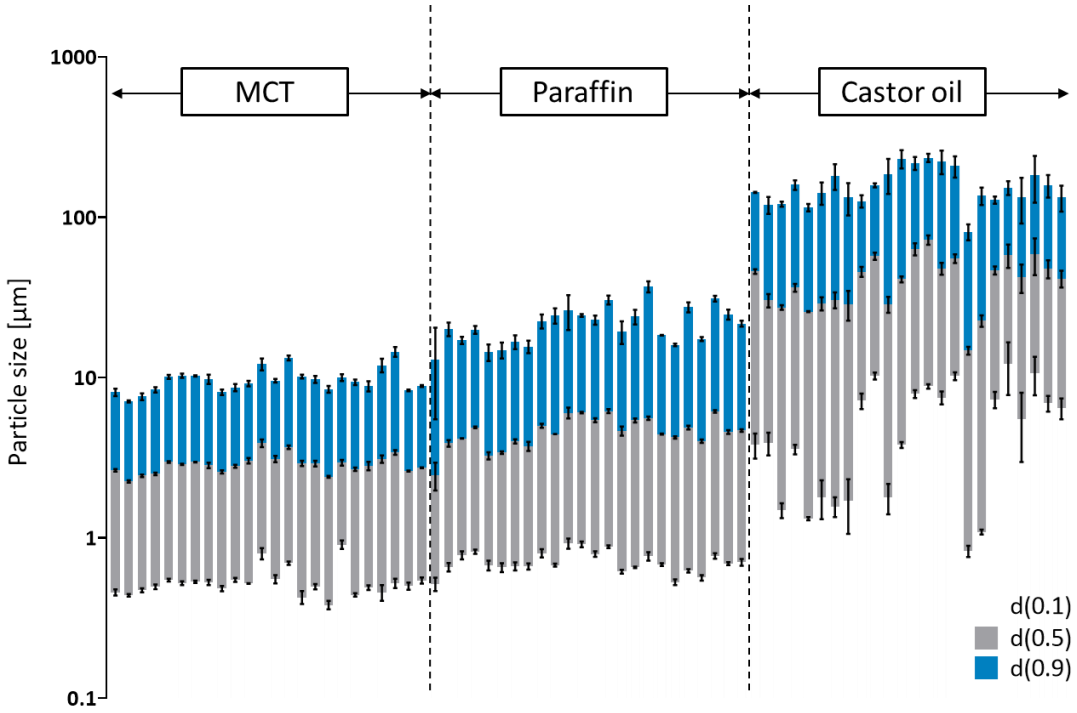


Figure 10 Droplet size diameter of all the emulsions tested on the particle size analyzer

4.4 Foamability

The foamability of the formulations was tested using the screening method. During the time of the assessment, the samples were being observed and pictures were taken at defined time points for further evaluation.

The foamability of a formulation can, in this case, be defined as the capacity of a sample to form a foam with a stable structure that can hold its shape for a long time. Some samples like 8MCT20 and 10 Par10 for example, produced a foam that appeared satisfactory in the beginning of the experiment, while showing inferior results at later stages of the measurement when compared to other formulations. These formulations are described as having a low foamability. None of the tested formulations was considered non foamable. Since these emulsions were based on pre-

tested foamable mixtures, all of them produced some foam in the screening method, even if it collapsed after few seconds.

The foaming behavior was similar in most formulations. The average foam volume at t0 of the formulations was 35.69 mm, with a standard deviation of 0.43 mm. The formulation containing the pre-mix composed of 0.5% 80H and 0.25% SLES was excluded from the calculation of this average value, since it is the only one that revealed the least foamability, especially when the oil included in the formulation was MCT. With paraffin and CO, its foamability increased significantly, as it can be easily observed in Figure 11 where all the foam volumes that resulted from the testing on the screening method are represented.

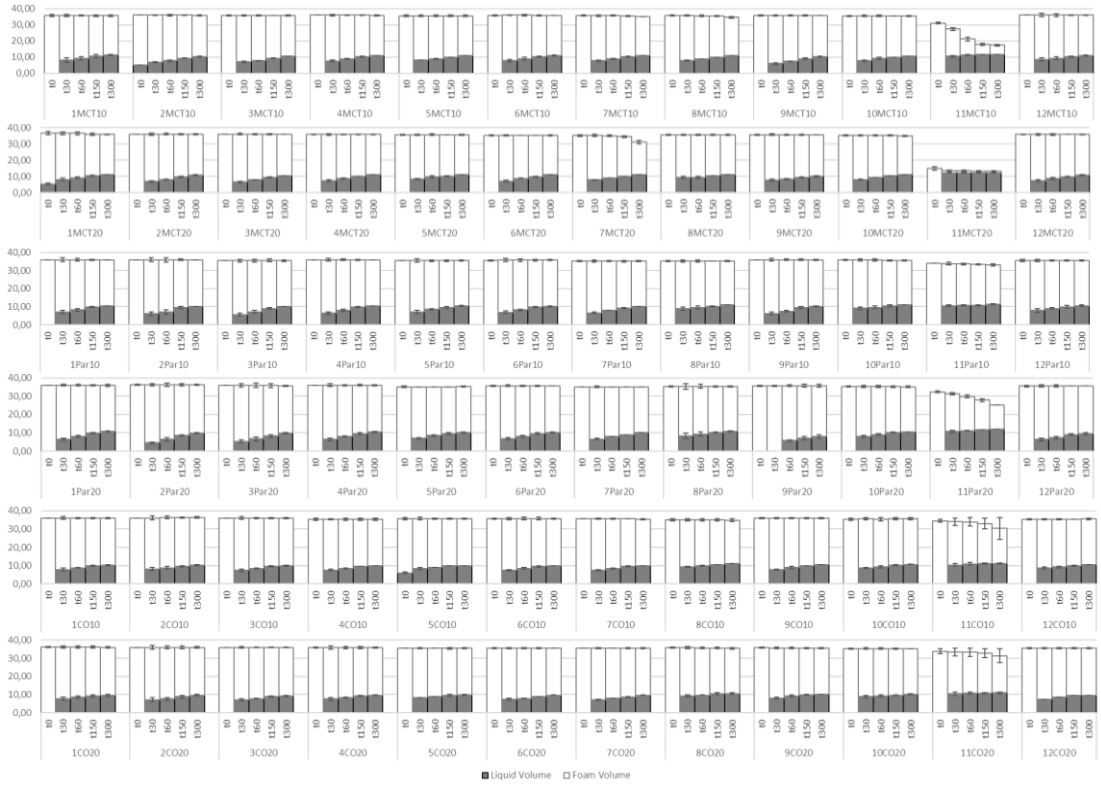


Figure 11 Representation of total volume, drainage volume and foam volume of all the emulsions tested

4.5 Foam structure

The foam structure is the shape and dispersity of the bubble-network over the course of the experiment. It changes and loses its shape over time. The best foam structure for all formulations is right at the beginning of the experiment, after the air stops flowing through the filter plates. In this moment, the air bubbles are smaller and there is little to no drainage.

The foam structure was evaluated using a score ranging from 1(best foam structure) to 4 (worst foam structure). In some cases, the foam produced could not be considered an actual foam, therefore, the classification could not fall in the scoring scale. The classification attributed in these cases was “out of score” (OOS).

In the heat map represented in Figure 12 it is possible to see that the quality of the foam decreases during the time of the measurement with all the formulations. From this representation, it is also possible to pick the ones with the best structured foams.

	t0	t30	t60	t150	t300
1MCT10	2	2	3	3	4
1MCT20	2	2	3	4	4
2MCT10	2	2	3	3	4
2MCT20	2	2	3	3	3
3MCT10	2	2	3	3	4
3MCT20	2	3	3	4	4
4MCT10	2	2	3	4	4
4MCT20	2	3	3	4	4
5MCT10	2	2	3	3	4
5MCT20	2	3	3	4	4
6MCT10	2	3	3	4	4
6MCT20	2	2	3	4	4
7MCT10	2	2	3	4	4
7MCT20	2	3	4	4	4
8MCT10	2	2	2	3	4
8MCT20	2	2	3	3	4
9MCT10	1	1	2	3	4
9MCT20	2	2	2	3	4
10MCT10	1	2	2	2	3
10MCT20	2	2	2	3	3
11MCT10	3	OOS	OOS	OOS	OOS
11MCT20	OOS	OOS	OOS	OOS	OOS
12MCT10	2	2	2	3	3
12MCT20	1	2	2	2	3

	t0	t30	t60	t150	t300
1Par10	2	2	3	4	4
1Par20	2	2	3	3	4
2Par10	1	2	2	3	4
2Par20	2	2	3	3	4
3Par10	1	1	2	3	3
3Par20	1	2	2	3	3
4Par10	1	2	2	3	4
4Par20	2	2	3	3	4
5Par10	3	3	3	4	4
5Par20	3	3	4	4	4
6Par10	1	2	2	3	4
6Par20	2	2	3	3	4
7Par10	2	2	3	3	4
7Par20	2	3	3	4	4
8Par10	2	3	3	4	4
8Par20	2	2	3	3	4
9Par10	2	2	2	3	3
9Par20	2	2	2	2	3
10Par10	2	2	2	3	4
10Par20	2	2	3	4	4
11Par10	3	3	3	3	3
11Par20	2	4	4	4	OOS
12Par10	2	2	2	2	3
12Par20	2	2	2	3	3

	t0	t30	t60	t150	t300
1CO10	2	3	3	4	4
1CO20	2	3	3	3	4
2CO10	2	2	3	4	4
2CO20	2	2	3	3	4
3CO10	2	2	3	3	4
3CO20	2	2	3	3	3
4CO10	2	2	3	3	4
4CO20	2	3	3	3	4
5CO10	2	3	3	4	4
5CO20	2	3	3	4	4
6CO10	2	2	2	3	4
6CO20	2	3	3	4	4
7CO10	2	2	3	4	4
7CO20	2	3	3	4	4
8CO10	2	2	3	3	3
8CO20	2	2	3	3	3
9CO10	2	2	2	3	3
9CO20	2	2	3	3	3
10CO10	2	2	2	3	3
10CO20	2	2	3	3	3
11CO10	3	4	4	OOS	OOS
11CO20	3	4	4	4	OOS
12CO10	2	2	2	3	3
12CO20	2	2	2	3	3

Figure 12 Heat map of the scoring attributed to the foams during the experiments on the screening method

4.6 Droplet size analysis in-between steps of manufacture

Six selected formulations were re-produced and samples were collected after each production step in order to identify the crucial steps to achieve a stable emulsion. The step with the biggest impact on the droplet size was the final homogenization with the ultrasonic processor. In the graph represented in Figure 13 it is evident that after that step, the droplet size decreases considerably. Annex 1 comprises the graphs of the droplet size distribution of these selected emulsions in each step of their production.

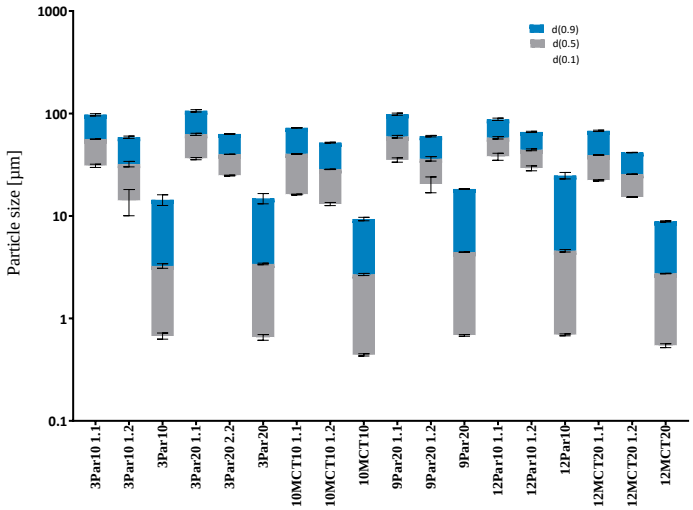


Figure 13 In-between step analysis of droplet size of the six best formulations
1.1 refers to pre-emulsion 1; 1.2 refers to pre-emulsion 2 and the formulation designations with no numeration refer to the final emulsions

4.7 Production Variations

To test the effect of the manufacturing procedure on the foamability and foam structure of the emulsions, different approaches were also tested. The assessment of the resulting products of these experiments was similar to the procedures used with the emulsions produced by the process previously described. They were also photographed, tested on the screening method, and analyzed in the particle size analyzer.

4.7.1 Variation of the order of the addition of surfactant

The first variation tested, was the order in which the surfactant was added to the mixture. In the original process, the surfactant is already incorporated in the aqueous phase, in combination with the respective phospholipid. In this new approach, the aqueous phase only has the phospholipid incorporated, then the oil is added slowly with a disposable syringe while the mixture is homogenized with the ULTRATURRAX. Only after the oil is added entirely, the right amount of surfactant bulk solution is added, and the mixture is stirred with a magnetic stirrer for 5 minutes at room temperature. The final step to complete the assemblance of the emulsion is the homogenization with the ultrasonic sonotrode. Figure 14 illustrates both production schemes.

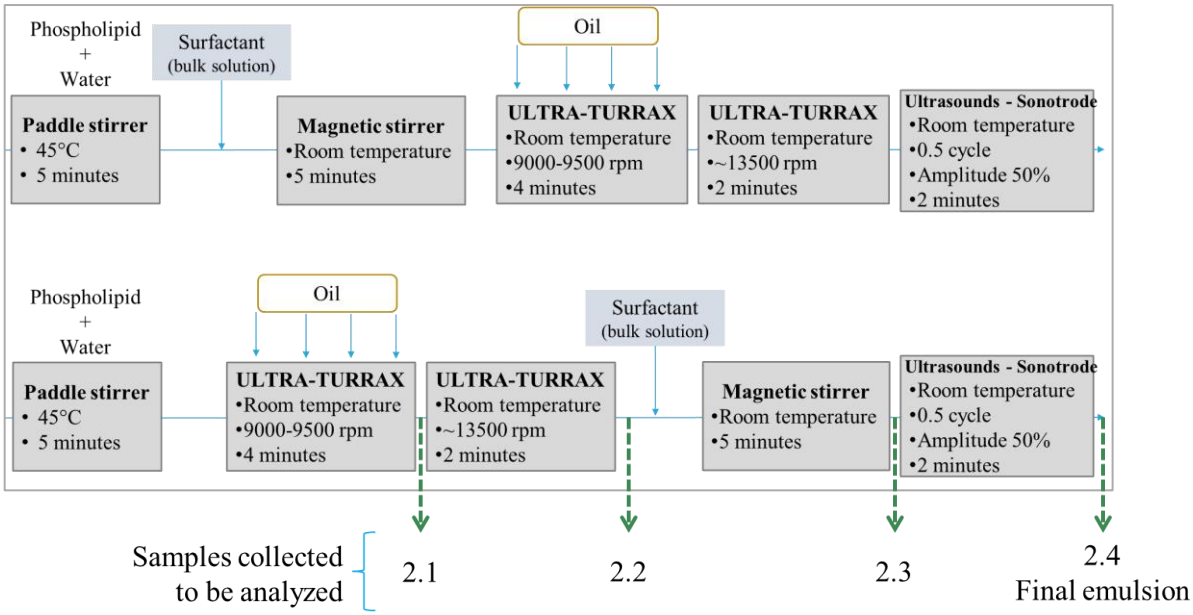


Figure 14 Comparison between the two production methods

For this experiment, only the six best formulations were selected and produced again according to the new manufacturing approach. In each step of the production, samples were collected and their droplet size was evaluated using the Mastersizer 2000, the final emulsions were also foamed and evaluated in the screening method.

Figure 15 represents the evolution of droplet size in every step of the production and in Annex 2 the droplet size distributions for each formulation are represented.

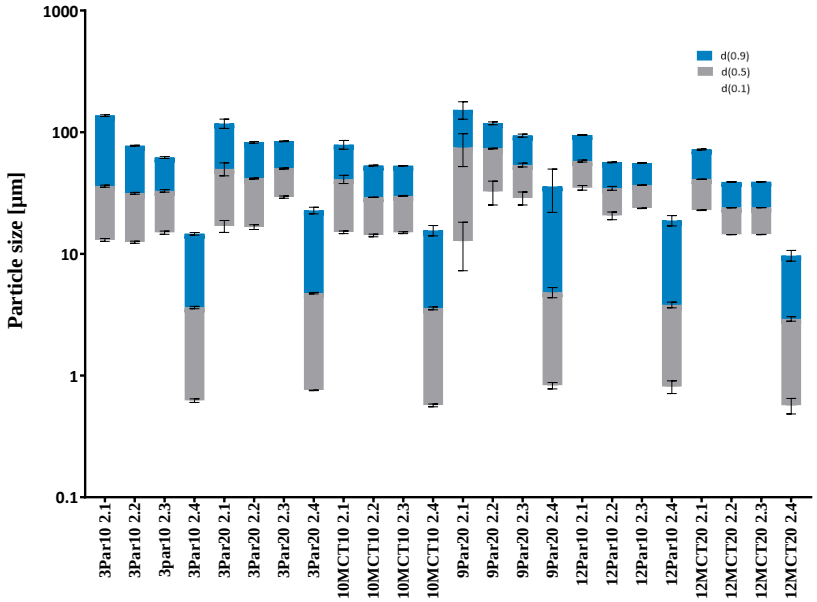


Figure 15 In-between step analysis of droplet size of the six best formulations produced according to the new approach

4.7.2 Increase of the duration of the emulsion stabilization step

The time that the emulsion was subjected to the ultrasonic processor was not optimized for the volume of emulsion produced. In order to evaluate the effect of the increased duration of this step in the manufacturing process, two selected formulations were produced again using 5 minutes instead of the 2 minutes previously defined.

The results are represented in Figure 16 and Figure 17, where the curves shifted to the left correspond to the emulsions treated with ultrasounds for a longer period. Further investigations were not conducted but in theory, the achieved droplet size can be considered too small and lead to coalescence.

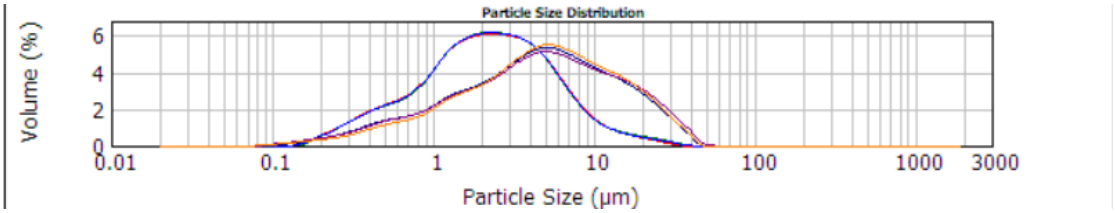


Figure 16 12Par10

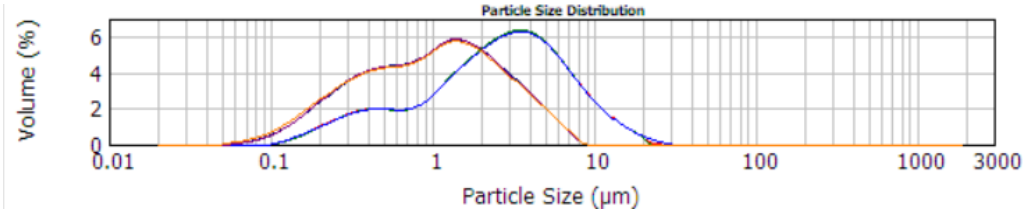


Figure 17 10MCT10

5 Discussion

The primary aim of this project was to evaluate the foamability of emulsions created with a continuous aqueous phase, that contains a phospholipid and a surfactant, as well as a dispersed oil phase. The oil phases used (MCT, Paraffin and Castor Oil) present different polarities and viscosities, parameters that are known to affect the stability of the emulsions. The higher the dielectric requirement for oil solubilization, the higher its apparent polarity is. The polarity of oils has a direct influence on their interfacial tension with water: the lower the oil polarity, the higher the interfacial tension (52). The density of the oil also influences the dependence of the dielectric constant on temperature. A smaller number of molecules per unit volume means that there is less interaction with the electric fields and therefore a decrease in the dielectric constant. As the temperature increases, the density decreases and hence the dielectric constant of the oil also decreases(53).

The oil with the highest polarity and highest viscosity was castor oil. As it was predicted, the formulations containing this oil were not stable, but they were foamable. This could be explained by the phase separation that occurred. When the broken emulsion was foamed in the screening method, the oil rapidly drained to the base of the column, and therefore, only the aqueous phase was foamed.

From the results obtained, the best formulations were selected according to the following criteria, in the respective order:

1. **Foamability**

   All the formulations, excluding the ones containing the pre-mix nº 7 [0.5% S75 + 0.5% SCS/SCG] and nº 11 [80H + 0.25% SLES], were sufficiently foamable

2. **Foam Structure**

   The foams with the best classification in the heat map (Figure 12) were selected. These were: 3Par10, 3Par20, 10MCT10, 12MCT20, 9Par20 and 12Par10.

3. **Droplet size**

   The results are represented in a frequency curve which is calculated by differentiating the result-under/cumulative undersize curve. The frequency curve is particularly useful for displaying the peaks in the graph. The peak of

the frequency curve gives the modal diameter. Several peaks in the graph indicate that there are distinct sizes of droplets within the sample. It is also useful to compare results from different measurements.

The statistics of the distribution are calculated from the results using the derived diameters D[m,n]. D(v, 0.5), D(v, 0.1) and D(v, 0.9) are standard percentile readings from the analysis. D(v, 0.5) is the size in microns at which 50% of the sample is smaller and 50% is larger. Also known as the Mass Median Diameter (MMD) or the median of the volume distribution. D(v, 0.1) is the size of droplet below which 10% of the sample lies and (v, 0.9) is the size of droplet below which 90% of the sample lies (54).

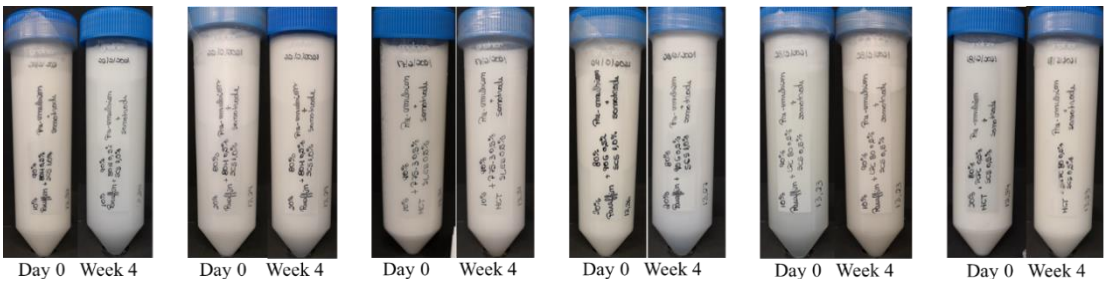
Table 15 Selection of the best formulations, based on the droplet size

Formulation	**Droplet size (μm)**		
	d (0,1)	d (0,5)	d (0,9)
9MCT20	0.909	2.944	10.007
10MCT10	0.441	2.683	9.351
12MCT20	0.543	2.742	8.861
12MCT10	0.501	2.611	8.317
3Par10	0.674	3.255	14.38
3Par20	0.654	3.404	14.844
9Par20	0.683	4.441	18.367
12Par10	0.692	4.574	24.773

4. Emulsion stability

Emulsion stability becomes lastly in the criteria because despite of being crucial to the selection of a suitable formulation, it is not the focal point. Even if a formulation is perfectly stable, if does not foam, it is useless.

The formulations with better stability are 3Par10, 3Par20, 10MCT10, 12MCT20, 9Par20 and 12Par 10.



3Par10 **3Par20** **10MCT10** **9Par20** **12Par10** **12MCT20**

Figure 18 4-week difference of the 6 best formulations

The six best emulsions are, therefore, 3Par10, 3Par20, 10MCT10, 9Par20, 12Par10 and 12MCT20 and their formulations are described in Table 16.

Table 16 6 best formulations

Emulsion	Pre-mix			Oil	%
	Phospholipid (0.5%)	Surfactant	%		
3Par10	80H	SCS	1.00	Paraffin	10
3Par20	80H	SCS	1.00	Paraffin	20
10MCT10	P75-3	SLES	0.50	MCT	10
9Par20	90G	SCS	1.00	Paraffin	20
12Par10	P LPC 80	SCS	0.50	Paraffin	10
12MCT20	P LPC 80	SCS	0.50	MCT	20

5.1 Importance of the production steps

As it can be observed in the graphs that show the droplet size in every step of the production of the emulsions (Figure 13 and Figure 15), the droplet size decreases from step to step. The step where it decreases more significantly is when the preparation is subjected to the sonortrode. Changing the order of when the oil is added, does not seem to have a significant impact on the droplet size, and therefore on the emulsion's stability. As long as the procedure where the emulsion is stabilized is included in the preparation, the stability remains mainly dependent on the formulation. Figure 19 shows the resulting foam, at t300, obtained from the emulsions produced according to the new approach. Only 10MCT10 revealed a decrease in foam height, probably caused by the alteration in the method of production. From the six tested formulations, it is the only one containing the surfactant SLES. One possibility is that, since it was not dissolved in the aqueous phase, it might not have dissolved completely at the time when it was added. From the graph represented in Figure 20, the droplet size increased for this particular formulation.

Oils can work as antifoams, which is not what it is intended when foaming an O/W emulsion. An essential criterion for a substance to be an antifoam, is that it needs to be highly hydrophobic, and needs to be present as small drops/particles, otherwise it would sit as a film along the surface. But as surfactants are used in the formulations, it is possible to form emulsions. If the right surfactant is selected, the oil is dispersed and becomes a hydrophilic drop, thanks to the protective shell of the surfactant (49).

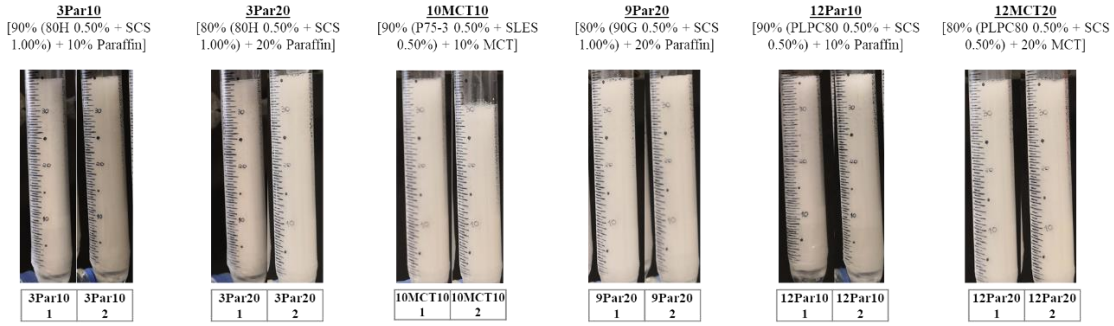


Figure 19 Comparison between the foams obtained from the emulsions produced with the new approach (t300)

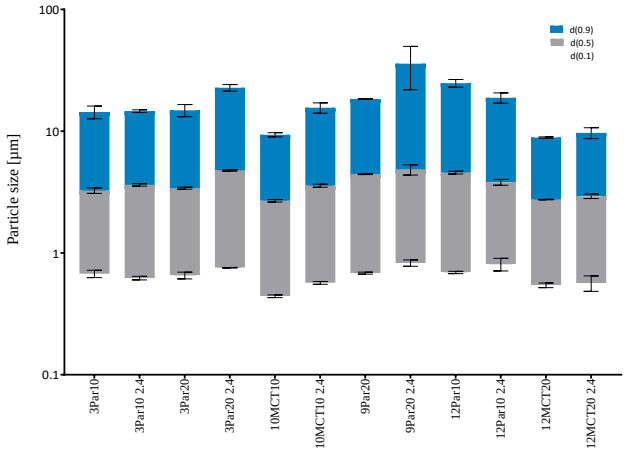


Figure 20 Comparison of the droplet size of emulsions prepared by the two different tested methods

The different method of preparation was only tested on the emulsions that were considered the best ones, the ones that foamed better. They were selected because they also revealed good stability in the form of emulsions. Perhaps, testing the change in the order of when the oil is added in not so stable emulsions (e.g. emulsions with castor oil as oil phase) would result in more diverse results.

6 Conclusions

6.1 Conclusion

Based on the results obtained, it is possible to conclude that, castor oil is not a suitable oil phase for foamable emulsions, due to the observed instability.

The right amount of surfactant is essential for the foamability. Lower concentrations negatively influence the resulting foam.

The stability of disperse systems highly depends on how they were assembled. Every parameter can have an impact in avoiding instability phenomena and/or in delaying it for as long as possible. With the additional investigations on the manufacture procedure, it was evident that every step of the manufacturing is important to reach the desirable droplet size, but the ultrasounds treatment revealed to be especially crucial.

Modifying the point at which the surfactant is added to the formulation, although there were slight changes, it does not appear to change the foaming behavior or emulsion stability.

6.2 Future work

To further characterize the resulting foams of the developed formulations, it would be relevant to evaluate them on the Dynamic Foam Analyzer (DFA) and compare the results with the ones obtained with the screening method. They could also be observed microscopically.

Although the tested formulations behaved as expected; considering the pre-mixes that were already known to develop satisfying foams and the different polarity of the oils used; the procedures selected to produce the emulsions have potential to be further explored and become more efficient. Another approach that could be tested would be adding the surfactant to the oil phase and the phospholipid to the aqueous phase, and then combining the two phases.

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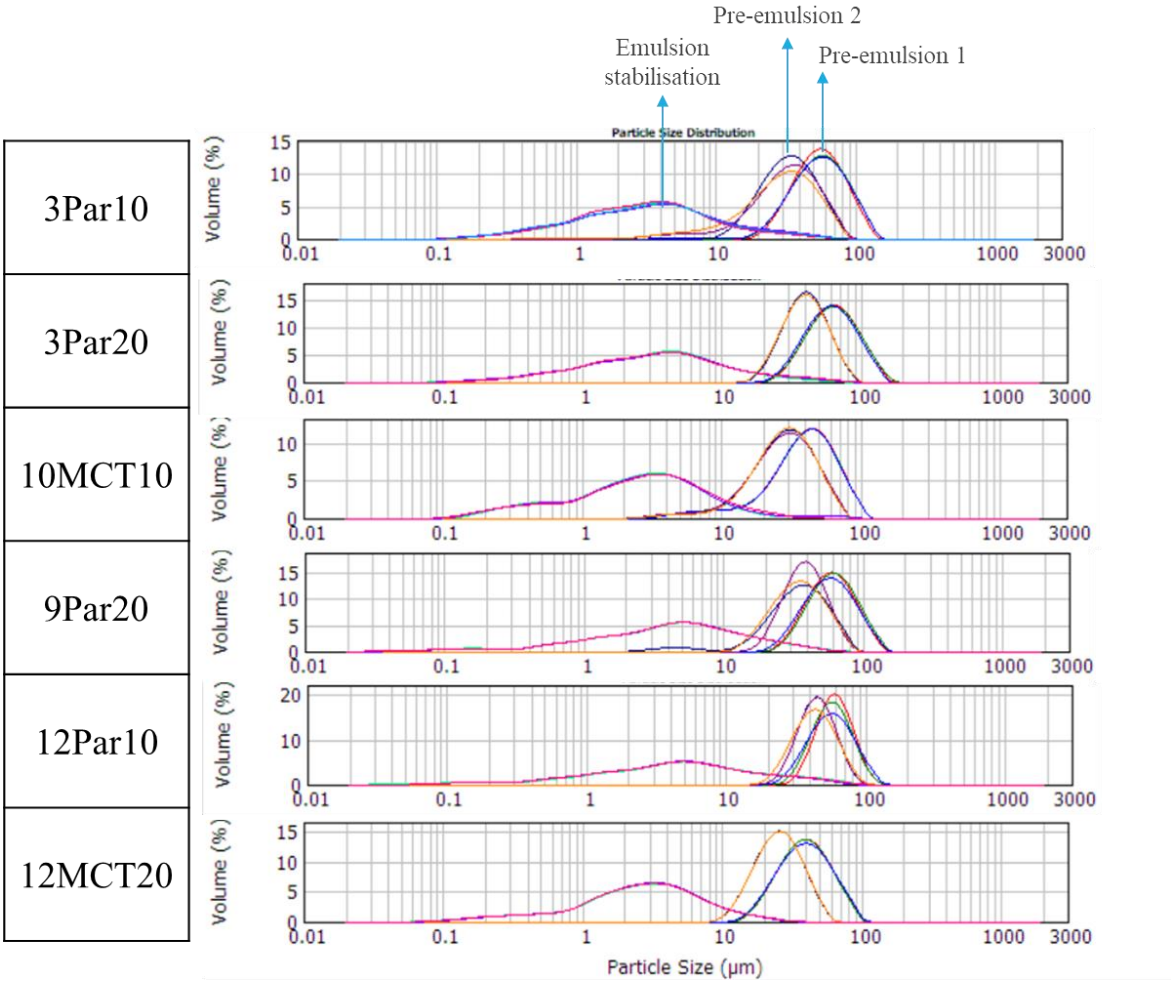
Annexes

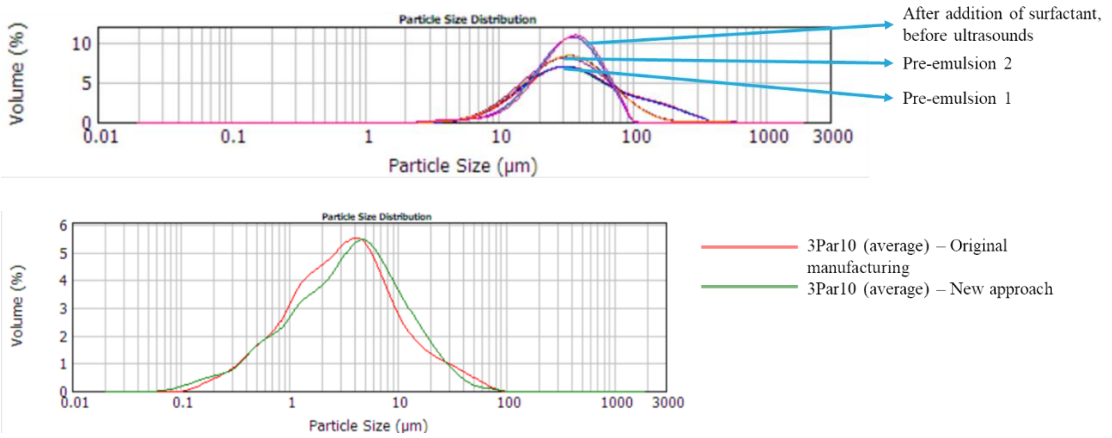
A1. Droplet size distribution of the 6 best emulsions



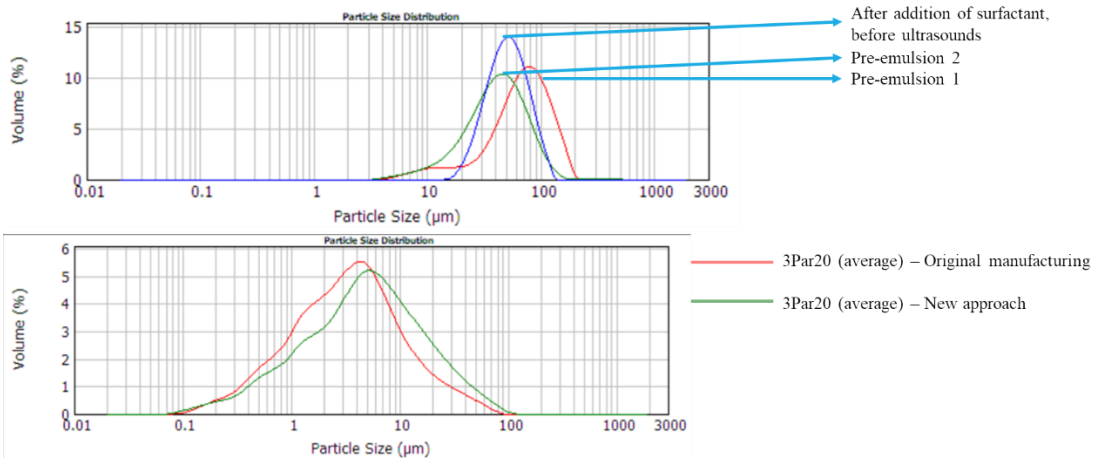
A2. Droplet size distribution of the 6 best emulsions produced with the alternative approach

Each of the six best formulations are represented in the first graph by their droplet size distribution after each step of the alternative production approach. The graphs below compare the final emulsions produced with the two different methods.

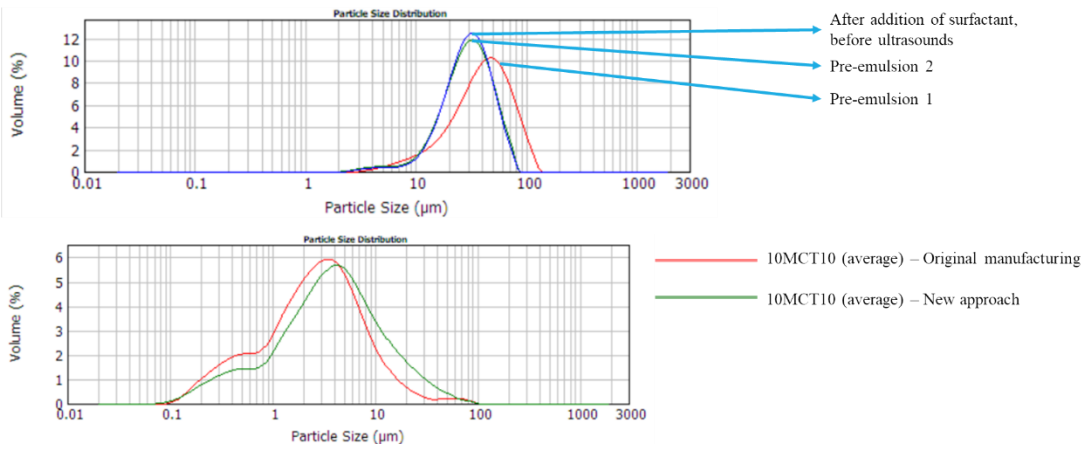
- **3Par10:**



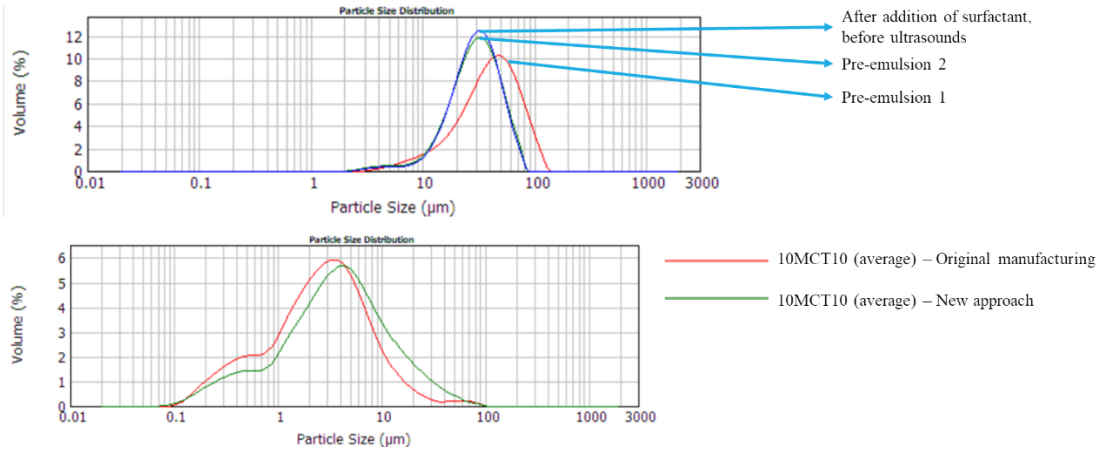
- **3Par20:**



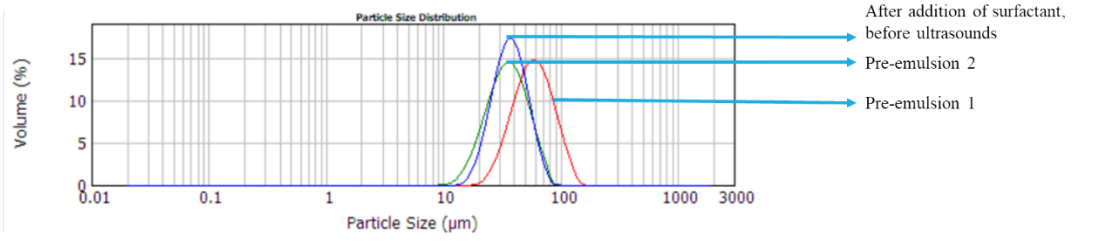
- **10MCT10:**

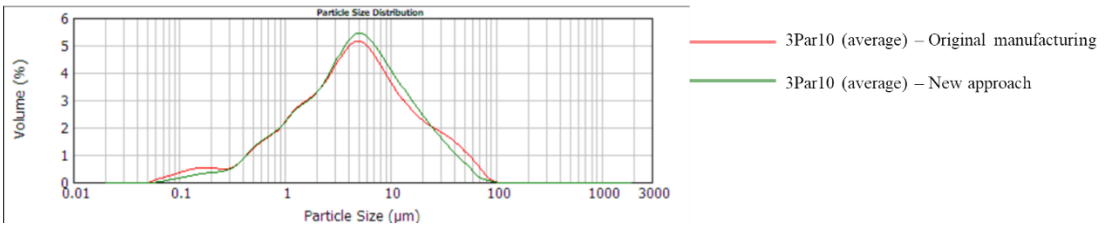


- **9Par20:**



- **12Par10:**





- **12MCT20:**