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Efecto de emulsionantes en microcápsulas de grasa dura

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TÍTULO:	Effect of emulsifiers on hard fat microcapsules
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RESUMEN

Este trabajo trata sobre la producción de microcápsulas mediante la técnica de emulsificación. Para ello, emulsiones agua-aceite-agua se llevaron a cabo. El proceso de microencapsulación tuvo lugar en platos de agitación y en un sistema rotor-estator (Ultraturrax). Los parámetros estudiados fueron la distribución de tamaño de partícula, morfología, eficiencia de encapsulación y estabilidad. La eficiencia de encapsulación se calculó mediante mediciones de conductividad utilizando una solución de NaCl 10%. Como un primer estudio, diferentes ratios agua interna-aceite fueron probados, resultando un ratio de 1:6 como el más viable en todos los casos. Posteriormente, doce emulsionantes diferentes, tanto lipófilos como hidrófilos, a diferentes concentraciones fueron estudiados, siendo los que proporcionaban una eficiencia de encapsulamiento mayor SPAN 65 y TWEEN 80 (efectividades del 55% y 65% respectivamente). Además se realizó un estudio de estabilidad de las cápsulas en agua en intervalos de tiempo de hasta 4 horas, siendo las cápsulas estabilizadas por SPAN 80 las más estables.

Palabras clave: Microcápsulas, emulsión agua-aceite-agua, emulsionantes, eficiencia de encapsulación, NaCl.

ABSTRACT

In this work they were produced microcapsules by using emulsification process technique. To this aim, water-oil-water emulsions were carried out. Microencapsulation process was made with stirring plates and with a rotor-stator system (Ultraturrax). The studied parameters were particle size distribution, morphology of the capsules, encapsulation efficiency and stability. Encapsulation efficiency was calculated by conductivity measurements using a NaCl 10% solution as internal water phase. As a previous experiment, different ratios internal water-oil were tested, being a 1:6 ratio the most workable. After that, twelve different emulsifiers were studied at different concentrations and as a result, SPAN 65 and TWEEN 80 showed the best values of encapsulation efficiency (55% and 65% respectively). Furthermore, it was made a stability study of capsules in water at time intervals till 4 hours, where capsules stabilized by SPAN 80 showed the most stability.

Keywords: Microcapsules, water-oil-water emulsion, emulsifiers, encapsulation efficiency, NaCl.

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LIST OF SYMBOLS

Latin symbol	Units	Meaning
A	µm^2	Total interfacial area
c_s	kg·m^{-3}	Concentration of the emulsifier in the emulsion
C_t	g·cm^{-3}	Concentration of the tracer in the external water phase
C_w	g·cm^{-3}	Known concentration of added tracer
d	µm	Diameter
$\bar{d}$	µm	Mean diameter
d_i	µm	Diameter in region i
$d_{0.5}$	µm	Median
d_{32}	µm	Sauter mean diameter
d_{43}	µm	Broukere mean diameter
E_{mix}	kg·mol^{-1}	Difference between molecular interaction energy of the mixed and unmixed states
E_v	J·m^3	Energy density
f_i	-	Number frequency
G_{mix}	kg·mol^{-1}	Free energy change
M_H	g·mol^{-1}	Molecular weight of the hydrophilic part
M_L	g·mol^{-1}	Molecular weight of the lipophilic part
N	-	Total number of droplets
n_i	-	Number of droplets in I region
$NaCl_{TOT}$	g	Total weight of NaCl added to the inner water phase
$NaCl_{ext.phase}$	g	Weight of NaCl in the external water phase
$NaCl_{encapsulated}$	g	Weight of NaCl in the internal water phase
P	bar	Laplace pressure
P_H	bar	Pressure difference operating at the nozzle
r	µm	Radius
r_{min}	µm	Minimum droplets radio
R_1, R_2	µm	Two principal radios of curvature in Young-Laplace equation
S_{mix}	Kg·mol^{-1}·K^{-1}	Difference between molecular interaction entropy of the mixed and unmixed states
T	°C, K	Temperature
t_1	min	Desired time in the first W/O emulsion
t_2	min	Desired time in the second W/OW emulsion
V_d	mL	Volume of emulsion droplets
V_ε	mL	Volume of the emulsion
W_1	g	Weight of the inner water phase
W_2	g	Weight of the second water phase
W_t	g	Amount of the total water added to the process
W_w	g	Weight of the external aqueous phase
x_1	wt.-%	Weight fraction of surfactant 1
x_2	wt.-%	Weight fraction of surfactant 2

Greek symbol	Units	Meaning
φ	vol.-%	Dispersed-phase volume fraction
φ_m	wt.-%	Dispersed-phase mass fraction
ρ_c	$g\cdot cm^{-3}$	Density of the continuous phase
ρ_d	$g\cdot cm^{-3}$	Density of the dispersed phase
σ	-	Standard deviation
η_d	$kg\cdot m^{-1}\cdot s^{-1}$	Viscosity of the dispersed phase
η_c	$kg\cdot m^{-1}\cdot s^{-1}$	Viscosity of the continuous phase
Γ_{sat}	$kg\cdot m^{-2}$	Excess surface concentration of the emulsifier at saturation
γ	$N\cdot m^{-1}$	Interfacial tension

ABBREVIATIONS

W/O	Water in oil emulsion
O/W	Oil in water emulsion
W/O/W	Water in oil in water emulsion
EE	Encapsulation Efficiency
HLB	Hydrophilic-Lipophilic Balance
DSC	Differential Scanning Calorimeter
H2O	Water
NaCl	Sodium Chloride
LD_{50}	Amount that causes the death of 50% of a group of test animals

1. INTRODUCTION

Microencapsulation is a process of enclosing a core material as small droplets in another substance called shell. The products obtained are called microcapsules and they have a size range between 3 - 800 µm. This process has application mainly in food, pharmaceutical and cosmetic industries, because microcapsules protect sensitive substances from the external enviroment, screen organoleptic properties as colour, taste and odour of the substance, obtain controlled release of the drug substance, safe handling of the toxic materials, get targeted release of the drug and avoid adverse effects like gastric irritation of the drug [1]. This work is focused on research for food applications.

One of techniques to produce microcapsules is called emulsification process. It utilizes the principles and techniques of emulsion science to enhance the quality of the food supply and the efficiency of food production [2]. It is the unique technique used in this work to make microcapsules. Other microencapsulation techniques are spray-drying, fluid bed coating or coacervation [1].

An emulsion is the dispersion of a liquid as small spherical droplets into another liquid, where both liquids are immiscible between themselves. Usually, the two liquids are oil and water. In food emulsions, droplets generally have a range size between 0.1 and 100 µm and are stabilized by a third component, an emulsifer. If oil is dispersed into water, the system is called oil-in-water emulsion (O/W), as mayonnaise, milk, cream soups and sauces; whereas if water is dispersed into oil, the system is called water-in-oil emulsion (W/O), like margarine, butter and spreads. Parameters such as thermodynamic and kinetic properties, dispersed phase volume fraction, particle size distribution, viscosity of continuous and discontinuous phase, temperature and type and concentration of emulsifier have an influence on the properties of the emulsion [2].

This work is focused on W/O/W emulsions, which consist of water droplets dispersed into larger oil droplets, which are at the same time dispersed in an aqueous continuous phase [2]. Concentration and type of emulsifier, dispersed phase volume fraction and the stability are the studied parameters.

The aim of emulsification is making small and kinetical stable emulsions, but their stability can be affected by physic-chemical mechanisms, which may alter their properties and break the emulsion. Most important mechanisms are creaming and sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion [2]. Also, W/O/W emulsions are affected by interfacial and pressure properties [3].

Emulsifiers are surface-active molecules which absorb to the surface of formed droplets during homogenization[2]. They decrease the interfacial tension, reduce the Laplace pressure and facilitate the droplet disruption [4]. Most of them are amphiphilic molecules, with both lipophilic and hydrophilic regions. They are classified depending on their HLB value in hydrophilic emulsifiers (more water affinity, forms O/W emulsion) or lipophilic emulsifiers (more oil affinity, forms W/O emulsions) [3]. The efectiveness of the emulsifier is related to its chemical structure and its “orientation at the interface” [4]. The most common emulsifiers used in the food industry are amphiphilic proteins, small-molecule surfactants and phospholipids [2].

The method used to produce emulsions with small and uniform droplet size distributions is called homogenization [4]. In food industry, this process is carrying out in devices known as homogenizers, in which mechanical agitation is used to disrupt the dispersed phase into droplets [2]. Most important homogenization devices are stirrer, rotor-stator systems, high pressure homogenizers, membranes and ultrasonic systems [5]. Each method provides different droplet sizes and phisicochemical properties to the final product, is able to work with different volume of production, and disrupts the droplets in a different way [2]. In this work, microcapsules are made with IKA® C-MAG HS 7 stirring plates (small-scale experiments) and with IKA® T 50 ULTRA-TURRAX® system (large-scale experiments).

Characterization of an emulsion according to their particle size distribution, particle morphology, their encapsulated efficiency and their stability is made in Mastersizer 2000, BX-60 Motorized Fluorescence Microscope, conductivity meter 3310 WTW and Digital Orbital Shaker Sea Star devices, respectively.

The main principle of this work is encapsulate a solution of 10% NaCl as inner water phase in oil phase of Witepsol W 31 in order to check the encapsulation efficiency (EE) of the microcapsules and the release of the inner water phase by conductivity measurements. In such a way, it was possible to compare the efectivity of different types and concentration of emulsifiers as well as how the dispersed phase volume fraction affect the encapsulation. The most efficient emulsifiers are SPAN 65 (lipophilic) with an EE of 55%, and TWEEN 80 (hydrophilic), with an EE of 65%. The most stable studied emulsifier in the time is SPAN 80, with a 23% of NaCl released after 4 hours.

1.1 LAYOUT

This work consists of 7 chapters. This one, Chapter 1, gives a brief summary about microcapsules, emulsification process and introduces the research topic. Chapter 2 deals with literature review of the research topic. Chaper 3 is about materials used in the thesis. In Chapter 4 is described the methods used in emulsification process and techniques used to analyse the obtained microcapsules. In Chapters 5 and 6 small and large-scale experiments are shown and explained, respectively. Chapter 7 summarize the obtained results and suggests an outlook for any future work.

2. LITERATURE REVIEW

2.1 MICROENCAPSULATION

Microencapsulation is the process in which a substance, called core material or internal phase, is enclosed by another substance, called shell material or external phase, producing particles with different diameters and structures [1;6].

If the formed particles have a diameter below 1 µm, they are called nanoparticles. Particles with a diameter between 3-800µm are known as microcapsules, whereas diameter particles larger than 1000µm are called macroparticles [1]. In respect of their structure, two main types of encapsulates might be differentiate, the reservoir type and the matrix type (Figure 2.1).

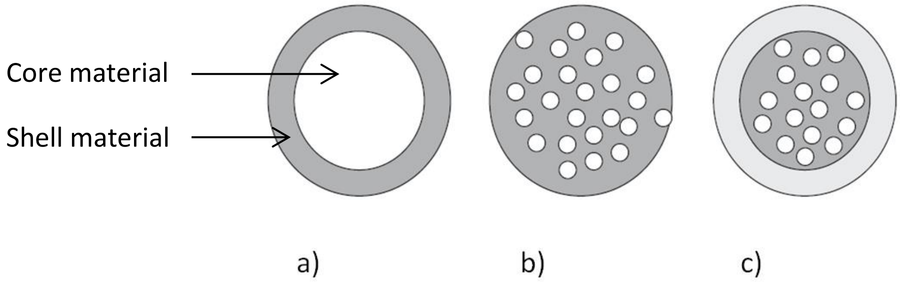


Figure 2. 1 a) Reservoir type, b) Matrix type and c) Coated matrix type [6]

In the reservoir type, the core material is covered by a shell material while in the matrix type, the core material is dispersed into the shell material. Coated matrix type is a blend between the first two. To keep that structure in the matrix type, active agents are usual present at the interphase [6].

2.1.1 Techniques of microencapsulation

Various techniques are used to encapsulate food active agents. They can be divided in three types: chemical methods, physico-chemical methods and physico-mechanical process. The used method depends on the characteristics of the core material and the desired properties of the final product. A brief summary of these techniques is shown below.

Table 2. 1 Common microencapsulation processes [1][6]

Technique	Type of Process	Particle size (μm)	Morphology
Spray drying	Physico-mechanical	10-400	Matrix
Fluid bed coating	Physico-mechanical	5-5000	Reservoir
Encapsulation by using supercritical Fluid	Physico-chemical	10-400	Matrix
Coacervation	Physico-chemical	10-800	Reservoir
Emulsification	Physico-chemical	0.2-5000	Matrix
Interfacial polymerization	Chemical	0.2-5000	Reservoir

In this work, the used technique is emulsification and it will be the one explained in the following sections.

2.1.2 Uses of microencapsulation

Microencapsulation is widely used in the industry because it provides some advantages such as superior handling of the active agent (e.g. conversion of liquid active agent into a powder, which might be dust free, free flowing, and might have a more neutral smell); immobility of active agent in food processing systems; improved stability in final product and during processing (i.e. less evaporation of volatile active agent and/or no degradation or reaction with other components in the food product such as oxygen or water); improved safety (e.g. reduced flammability of volatiles like aroma, no concentrated volatile oil handling); creation of visible and textural effects (visual cues); adjustable properties of active components (particle size, structure, oil- or water-soluble, color); off-taste masking; controlled release (differentiation, release by the right stimulus).

On the other hand, some disadvantages of this technique are the additional costs, increased complexity of production process and/or supply chain, undesirable consumer notice (visual or touch) of encapsulates in food products, stability challenges of encapsulates during processing and storage of the food product.

In any case, encapsulates facilitate the production of food products that are healthier, tastier and more convenient, and the demand for encapsulation has been growing since the last few decades [6].

2.2 EMULSION PROCESSING

2.2.1 Characteristics of an emulsion

An emulsion is a thermodynamically unstable system consisting of at least two immiscible liquid phases, generally water and oil, one of which is dispersed in the other liquid phase, stabilized by the presence of an emulsifying agent. Emulsions can be employed as foods, detergents and cleaning agents, lubricants or crop protection agents. Milk, mayonnaise, water-based paints, creams and ointments are typical examples of emulsions [7].

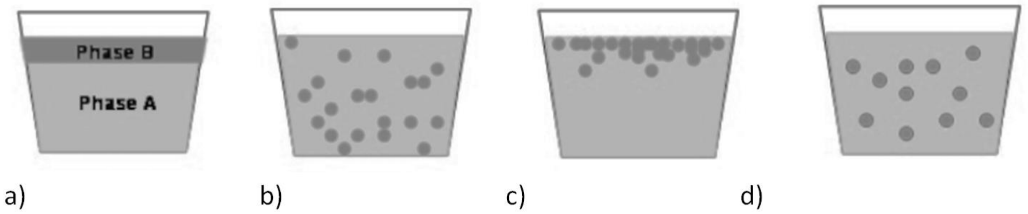


Figure 2.2 a) Two immiscible liquids not emulsified, b) An emulsion of Phase B dispersed in Phase A, c) The unstableemulsion progressively separates, d) The surfactant positions itself on the interfaces between Phase A and Phase B, stabilizing the emulsion [7].

The process by which an emulsion is achieved is called homogenization. For food emulsions, it is usual to carry out this process in homogenizers, devices where the mix of liquids is subjected to mechanical agitation [7]. In such a way, the formation of the emulsion is the net result of two opposite forces: the effect of agitation in the disintegration of the liquid and the tendency of the dispersed phase to re-coalesce. Although both liquids are broken, only one of them tends to coalesce. The main function of an emulsifier is avoiding this coalescence during and after the process [5].

The aim of producing emulsions is to distribute finely the dispersed phase through the continuous phase. For instance, if the dispersed phase is a lipophilic liquid and the continuous phase is a hydrophilic liquid, this is an oil-in-water (O/W) emulsion. If the hydrophilic liquid is dispersed in a lipophilic liquid, it will be a water-in-oil (W/O) emulsion [2;3].

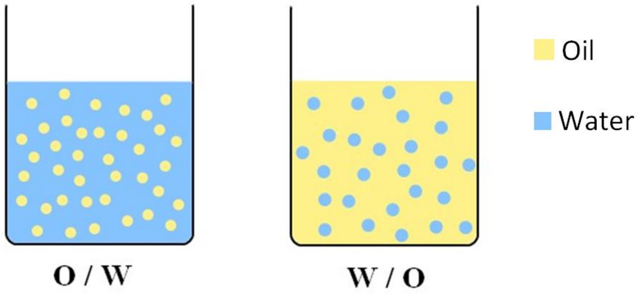


Figure 2.3 Oil-in-water and Water-in-oil emulsion [7]

Usually, in food emulsions, the inner phase contains water-soluble substances as sugars, salts, acids, bases, surfactants, proteins and carbohydrates. The oil phase also include lipid-soluble components as triacylglycerols, monoacylglycerols, free fatty acids, sterols and vitamins. All of these components can form different structures as fat crystals, protein aggregates, air bubbles, liquid crystals or surfactant micelles. Furthermore, changes in temperature, pressure and mechanical agitation during their production, storage and handling affect emulsion properties and also affect the properties of the final product. Because of this, the knowledge of emulsion processing is very complex and there is an important interest in knowing with more detail how these parameters affect the emulsions.

2.2.1.1 Classification of an emulsion

Emulsion type is affected by parameters as the ratio of the oil-water phase, the temperature, the presence of additives, the methodology of preparing emulsions, but mainly it depends on the nature and the concentration of the emulsifying agent. Individual molecules in an emulsion can interact with each other to form a variety of different structural systems [2].

The most important structures in emulsions are described below.

- **O/W and W/O macroemulsions:** These usually have a size range of 0.1-5 µm with an average of 1-2 µm. They are kinetically stable in presence of emulsifiers.
- **Nanoemulsions:** These actually have a size range of 20-100 nm. Similar to macroemulsions, there are only kinetically stable [9].
- **Micellar emulsions or microemulsions:** These have micelles with the hydrocarbon tails in contact with oil forming the core and the polar groups in contact with the

aqueous phase or vice versa [10]. These usually have a size range of 5-50 nm. They are thermodynamically stable.

- **Double and multiple emulsions:** These are emulsions of emulsions, W/O/W and O/W/O systems.
- **Mixed emulsions:** These are systems consisting of two different disperse droplets that do not mix in a continuous medium [9].

2.2.1.2 Emulsion properties

A stable emulsion is a system in which the droplets keep their initial character and remain uniformly distributed throughout the continuous phase. Nevertheless, food emulsions are not usually in their most thermodynamically stable state and form structural organizations with the aim of achieve the lowest free energy level.

The stability of the emulsion as well as the emulsification process is influenced by parameters as thermodynamic and kinetic properties, the volume phase ratio, the particle size, the properties of both phases, the type and concentration of emulsifiers, the diminution energy and the power input, the type of homogenizer, time of the emulsion in the homogenizer, thermodynamic changes during the emulsification process (reactions, temperature), order in which constituents are added. Some of them will be explained in the next sections [2].

2.2.1.2.1 Thermodynamic and kinetic properties

When homogenization between pure oil and pure water is produced, the two liquids are separated in two differentiated phases. The system is thermodynamically unstable. It is possible to achieve a kinetically stable emulsion (metastable) for a defined period of time (days, weeks, months or years) by adding substances known as emulsifiers. Main action and nature of emulsifiers will be explained in Chapter 2.2.2.

The interactions of molecules and its behaviour at equilibrium can be described by thermodynamics. Droplets tend to aggregate between themselves to minimize the free energy of the system, that is linked to enthalpy and entropy of it. The enthalpy contribution is related with the molecular interaction energies, while entropy contribution depends on the tendency of the system to be disordered.

$$\Delta G_{mix} = \Delta E_{mix} - T\Delta S_{mix} \tag{2. 1}$$

where ΔG_{mix} is the free energy change, ΔE_{mix} is the difference between molecular interaction energy of the mixed and unmixed states and ΔS_{mix} is the difference between molecular interaction entropy of the mixed and unmixed states [2].

2.2.1.2.2 Dispersed phase volume fraction

Droplet concentration is an important parameter because it affects the appearance, texture, flavour, stability and cost of the emulsion. It is usually used the term of dispersed-phase volume fraction, φ, to refer to the droplet concentration and it is defined as:

$$\varphi = \frac{V_d}{V_\varepsilon} \qquad (2.\ 2)$$

where V_d is the volume of emulsion droplets and V_ε is the volume of the emulsion. Often it is more useful to express the concentration as a mass fraction. Dispersed-phase mass fraction, φ_m, is related with the volume fraction through the equation:

$$\varphi_m = \frac{\varphi\rho_d}{\varphi\rho_d + (1-\varphi)\rho_c} \qquad (2.\ 3)$$

where ρ_c is the density of the continuous phase and ρ_d is the density of the dispersed phase.

As the volume of dispersed phase decrease, the stability of emulsion increase. Another parameter to measure the relation between the amount of oil and water in the emulsion is the ratio of dispersed phase and continuous phase (DP/CP). Encapsulation efficiency and particle size increase as the ratio DP/CP decrease [1; 2].

2.2.1.2.3 Particle size distribution

Droplet size of the dispersed phase in a emulsion is a main parameter in emulsions. Because of this, it is important to reach a homogeneous droplet size and measure the dispersion. If the emulsion is monodisperse (all the droplets have the same size), only one parameter characterize them (diameter, d, or radius, r) whereas if it is a polydispersed emulsion, (droplets are different regarding to the size), other parameters are necessary to specify the emulsion size but in most cases, it is enough to know the average size of the droplets and the distribution. In the representation, it is used to divide the size range into regions and stipulate the frequency of droplets that fall into each one. The number frequency, f_i, is the number of droplets that are in a region related to the total of droplets:

$$f_i = \frac{n_i}{N} \tag{2. 4}$$

Where n_i is the number of droplets are in the i region and N is the total number of droplets. The mean diameter, $\bar{d}$, is a measure of the central tendency of the distribution:

$$\bar{d} = \sum \frac{n_i d_i}{N} \tag{2. 5}$$

where d_i is the region diameter. The standard deviation, σ, represent the amplitude of the distribution and is defined as:

$$\sigma = \sqrt{\sum \frac{n_i(d_i - \bar{d})}{N}} \tag{2. 6}$$

It is also posible to express the mean droplet in other ways as the median ($d_{0.5}$ or d_{50}), the de Broukere mean diameter (d_{43}) or the Sauter mean diameter (d_{32}). The d_{50} is the diameter in that the distribution is divided in such a way that half of the distribution is above and the other half below this diameter.The same meaning is possible to apply to d_{90}or $d_{0.9}$and d_{10}or $d_{0.1}$ [2].

Another value useful the SPAN, and is defined as [12]:

$$SPAN = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \tag{2. 7}$$

Droplet size is a critical factor in emulsion process because it determines, among others, shelf life stability, consistency, rheological properties, colour and possibly the taste of the product. Because of that, droplets as small as possible is usually the aim of emulsification [11]. More information about size distribution is described in the literature.

2.2.1.2.4 Properties of continuous and discontinuous phase

Physicochemical properties of oil and water phase are important in the homogenization process. Viscosity ratio between the dispersed and continuous phase (η_d/η_c) determines the characteristics of the emulsion.

Some oils have surface-active impurities that tend to accumulate in the oil-water interphase decreasing the interfacial tension, helping in the disruption of droplets. The water phase usually contains substances as minerals or sugars that affect rheology, interfacial tension, coalescence stability or adsorption kinetics [2].

2.2.1.2.5 Temperature

Changes in temperature affect the viscosity of both liquid phases. Thus, the viscosity ratio (η_d/ η_c)is a temperature dependent. It could be expected that increases in temperature cause better emulsions because the interfacial tension between the oil and water phases is reduced, generating smaller droplets, but some emulsifiers lose their ability above a certain temperature, or form aggregates. Alterations in temperature also affect their adsorption, origing modifications in the interfacial composition.

The physical state of the oil phase is determined by the temperature. It is necessary an oil liquid phase to carry out the emulsion, because energy input required to disrupt solid oil phase is too high and the homogenization process is more complicated.

Operating above a certain temperature can produce the phase inversion of the emulsion if this temperature is above the phase inversion temperature [2].

2.2.1.2.6 Energy input

The more energy input is introduced in the homogenization, the smaller droplet size of the dispersed phase is obtained, provided that the amount of emulsifier is enough and its properties do not change because of high pressure or excessive heating. A smaller droplet size usually means better stability of the emulsion. Depending on the kind of homogenizer, the energy input can be increased in a different ways. It is important to make sure in W/O/W emulsions that the first emulsion will not be affected by the energy input necessary to form the second emulsion [2].

2.2.1.2.7 Emulsifier type and concentration

In an emulsification process, it can happen that the droplet size of dispersed phase is so small that the amount of emulsifier available is not enough to cover all the interfacial areas. When this happens, the droplets tend to aggregate between them (coalescence). The minimum and stable size of droplets that can be formed in the process depends on the type and concentration of the emulsifier, and the relation assuming monodisperse droplets is given by:

$$r_{min} = \frac{3\Gamma_{sat}\varphi}{c_s} \tag{2. 8}$$

where r_{min} is the minimum droplets radio, Γ_{sat} is the excess surface concentration of the emulsifier at saturation (in $kg \cdot m^{-2}$), ϕ is the dispersed-phase volume ratio and c_s is the concentration of the emulsifier in the emulsion ($kg \cdot m^{-3}$). This equation show that it is possible decrease the droplet size increasing the concentration of emulsifier, decreasing the droplet concentration or using an emulsifer with a smaller Γ_{sat}. A homogenizer has to introduce the input energy and generate high pressure gradients necessary to disrupt the droplets till r_{min} and emulsifiers have to be adsobed quickly in the interface [2].

2.2.1.3 Breakdown processes of simple emulsion

An emulsion is stable when it is able to resist changes in its properties over time. This changes can be produced by physico-chemical mechanism related with the different density of the phases or van der Waals interactions, among others. Most important physical mechanisms are described in the next section [2].

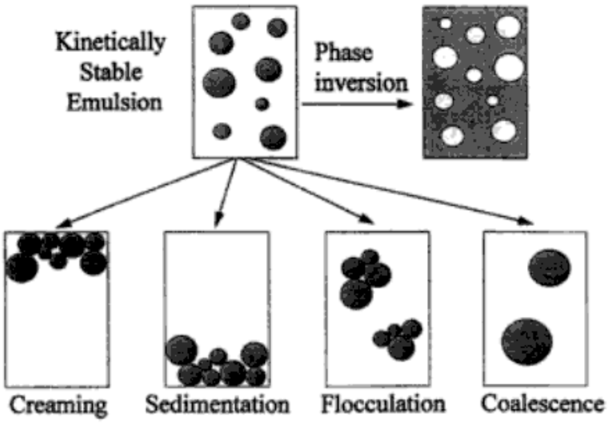


Figure 2.4 Physical mechanisms that affect stability of emulsions [2]

2.2.1.2.1 Creaming and sedimentation

It is produced because of external forces as gravitational or centrifugal and they take place when the densities of dispersed and continuous phase are different. If these forces are high enough to exceed the thermal motion of the droplets (Brownian movement), they cause a large concentration gradient with the larger droplets moving faster to the top of the container if their density is lower than that of the medium (Creaming) or to the bottom if their density is higher than that of the medium (Sedimentation). Small droplets are more resistant to creaming [3; 9].

2.2.1.2.2 Flocculation

This process results when the van der Waals attraction is stronger than the repulsion to keep the droplets apart and it consist in the aggregation of the droplets as a result of collisions between themselves. The outcome is droplet combination in which each one retains their individual integrity [2; 9].

2.2.1.2.3 Coalescence

It is the process in which the liquid film that keeps two droplets apart is broken with the result of join two droplets in one larger. It reduces the surface tension and the surface area of droplets.

2.2.1.2.4 Ostwald Ripening (Disproportionation)

When a collision takes place between two droplets, it might happen to occur between one bigger and one smaller. After a certain time and due to curvature effects, the small droplets are solubilised in the continuous phase. This effect is called Ostwald Ripening.

2.2.1.2.5 Phase Inversion

Phase inversion is the exchange of phases between the dispersion and the continuous medium and is produced by time or change of conditions. In this way, a W/O emulsion becomes in O/W or vice versa [9].

2.2.2 Emulsifiers

Emulsions are stabilized by a third component, an emulsifier, which is strongly adsorbed at the liquid-liquid interface and reduces the surface tension between the two liquids among other things. They can be surfactants (surface active agent) or other substances. Most of them are amphiphiles which means that they present lipophilic (fat affinity, no polar) and hydrophilic (water affinity, polar) properties. It means, they have a structure with both regions, hydrophilic and lipophilic part [13]. For an O/W emulsion droplet, the hydrophobic chain resides in the oil phase, whereas the hydrophilic head group resides in the aqueous phase. For a W/O emulsion droplet, the hydrophilic group(s) resides in the water droplet, whereas the lipophilic groups reside in the hydrocarbon phase [9].

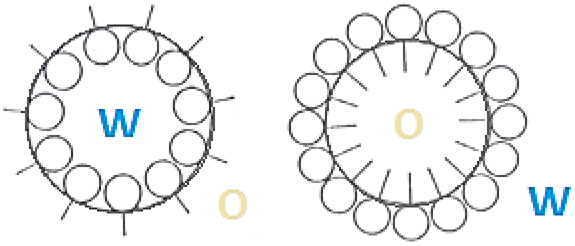


Figure 2.5 Water in oil and oil in water emulsion with the corresponding emulsifier [7]

Amphiphilic molecules are present in everydays life and they are essential in many industrial processes. Examples of applications are their use in cleaning, detergency, as emulsifiers for creams in the cosmetic, food and pharmaceutical industry, and as flotation agents in the mining industry [13].

2.2.2.1 Nature of the emulsifier

The most effective emulsifiers are non-ionic surfactants that can be used to emulsify O/W or W/O emulsions. Also can be applied as emulsifiers simple molecules and ions as OH--, ionic surfactants as sodium dodecyl sulfate or SDS, surfactant mixtures, non-ionic polymers (Pluronics), polyelectrolytes (polymetacrylicacid), mixtures of polymers and surfactants, lamellar liquid crystalline phases or finely divided solid particles with amphiphilic properties as silica (Pickering emulsions) [9].

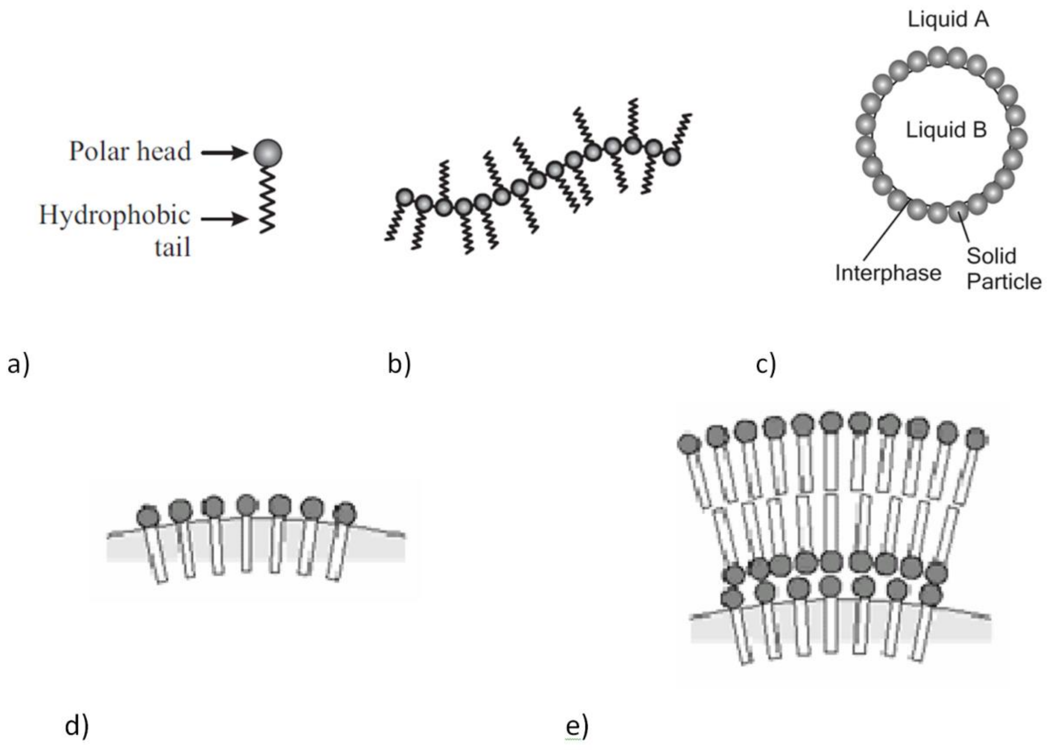


Figure 2. 6 a) Conventional emulsifier, b) Polymeric emulsifier, c) Pickering emulsions, d) monomolecular surfactant film, and e) liquid crystalline surfactant film [9].

The nature of the emulsifying agent controls to a great extent the type of the emulsion that it will be formed (O/W or W/O). In such a way, the Bancroft's rule stated that the phase in which an emulsifier is more soluble constitutes the continuous phase. Thus, for forming O/W emulsions, emulsifying agents have to be more soluble in water than in oil (high HLB value), whereas in O/W emulsions, they should be more soluble in oil than in water (low HLB value). The HLB value will be explained in the next section (Chapter 2.2.2.2 "HLB value").

In addition to reduce the interfacial tension and control the type of the emulsion, more actions that emulsifiers carry out are, for instance, forming a barrier between the phases, which aids the stability of the dispersed phase, facilitate the formation of the emulsion or producing finer droplet size [14].

Emulsifiers have also the property to form aggregation in presence of water, resulting in substances as spherical micelles, cylinders, bilayers, etc. The simplest one is the micelle. It is a dynamic structure because surfactants leave it and go into the solution while other surfactants from the solution enter in the micelle.It is formed because of, at low concentration of emulsifier in water, the polar part of the emulsifier is dissolved as individual ions while its hydrocarbon chains tend to adsorb at the air-water interphase, with its hydrocarbon chains oriented in the direction of the vapour phase. The surface tension decreases strongly with increasing concentration till a certain point, the critical micelle concentration (CMC), above it the surface tension remains almost constant. For the osmotic pressure or the electrical conductance of the solution it is observed comparable dependencies [13].

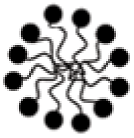


Figure 2. 7 Micelle

2.2.2.2 HLB value

The nature of an emulsifier, water or lipid affinity, is represented by the HLB value (Hydrophilic-Lipophilic-Balance). A high HLB value (10 to 18) means more hydrophilic character, appropriate for O/W emulsions, whereas a low HLB value (3 to 8) means more lipid affinity, appropriate for W/O emulsions [8]. Applications of emulsifiers for intermediate values of HLB are shown in the next table:

Table 2.2 Summary of HLB ranges and their application [9]

HLB	Applications
1-3	Antifoaming agents; inverse micelles
3-8	W/O emulsifiers
7-9	Wetting agents
10-16	O/W emulsifiers
13-16	Detergents
15-18	Solubilizers

The hydrophilic character of a surfactant is determined by the polarity of the head group as amine, quaternary ammonium, ethoxylate, sulfate, phosphate and carboxylate. For non-ionic products, HLB calculation is obtained with the Griffin formula:

$$HLB = 20\left(\frac{M_H}{M_H + M_L}\right) = \frac{wt\%\ hydrophile}{5} \tag{2. 9}$$

where M_H is the molecular weight of the hydrophilic part, M_L is the molecular weight of the lipophilic part. For ionic and non-ionic surfactants, the HLB value of individual surfactant molecules can be also calculated applying the Davies formula [15]:

$$HLB = \sum(Hydrophilic\ group\ contributions) - \sum(Hydrophobic\ group\ contributions) + 7 \tag{2. 10}$$

Group contributions are listed in Apendix I “Group contributions in HLB value”.

It is found that all oils, waxes and other materials likely to be incorporated into emulsions have an individual “Required HLB”, but some of them are yet to be studied [16]. Stable emulsions are achieved in a best way with emulsifiers or combination of emulsifiers having HLB values close to that required of the oil phase [16; 17]. For a required HLB value, blend of two surfactants (one with high HLB value and the other with low HLB value) is found the most effective way, where the average HLB number may be calculated from additivity:

$$HLB = x_1 HLB_1 + x_2 HLB_2 \tag{2. 11}$$

where x_1 and x_2 are the weight fractions of the two surfactants with HLB_1 and HLB_2 [9].

HLB can provide a good starting point and generally produces a good emulsion, but does not take into account parameters as temperature, concentration of emulsifier, phase volume ratios, component interactions or fatty alcohols [14].

Table 2.3 HLB values for some food emulsifiers [18]

Emulsifier	**HLB**
Sorbitan tristearate (SPAN 65)	2.1
Glycerol monostearate	3.8
Sorbitan monooleate (SPAN 80)	4.3
Propylene glycol monolaureate	4.5
Sorbitan monopalmitate (SPAN 40)	6.7
Sorbitan monolaureate (SPAN 20)	8.6
Polyoxyethylene sorbitan monostearate (TWEEN 60)	14.9
Polyoxyethylene sorbitan monopalmitate (TWEEN 40)	15.6
Polyoxyethylene sorbitan monolaureate (TWEEN 20)	16.7
Sodium oleate	18.0
Sodium steroyl-2-lacylate	21.0

2.2.3 Multiple emulsions, W/O/W

Multiple emulsions are complex multiphase system in which the dispersed phase of an emulsion is at the same time dispersed into another phase [18]. One of the most common types of multiple emulsions is water-in-oil-in-water (W/O/W) double emulsion. It consists of an oil-in-water emulsion (O/W) in which the dispersed oil phase is a water-in-oil emulsion (W/O), called inner emulsion [3]. It combines properties of both W/O and O/W emulsions and has many different applications in pharmaceutical, cosmetic and food industry for releasing active agents or replacing some of the oil phase by water to reduce fat content in the final product [18; 19].

In W/O/W emulsion, the two aqueous phases are separated by the oil film that acts as a membrane. Nevertheless, it can produce a release from the inner water phase into the external water phase because of diffusion processes or rupture of the oil membrane, which means coalescence between both aqueous phases [18; 19].

It can be getting three types of droplets, as it is shown in Figure 2.8.The same definition is applied to form O/W/O emulsions, but in this work they are not evaluated.

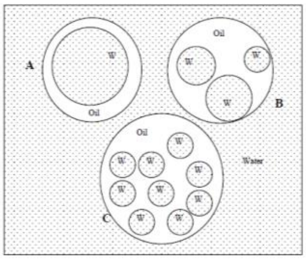


Figure 2.8 Types of droplets in a W/O/W emulsion [19]

2.2.3.1 Formation of W/O/W emulsion

For the production of multiple emulsions, different methods are used. All of them start from the inner emulsion formation commonly by means of applying mechanical energy. If the mechanical energy is kept on, large droplets are formed which consequently are disrupted into smaller droplets while surfactants molecules are transported to the interphase and stabilise the droplets [3]. Getting stable and small W/O droplets (around 1 µm) is critical to achieve W/O/W stable emulsion [20]. The most often used methods are one-step emulsification and two-step emulsification.

In one-step emulsification, part of the initial W/O emulsion is inverted and forms a W/O/W emulsion as an intermediate step before it achieves an O/W emulsion. A gradual addition of an aqueous solution of a hydrophilic emulsifier is needed [3].

In two-step emulsification, the inner W/O emulsion is dispersed in another solution and forms droplets in the continuous phase, as is showed in Figure 2.9.

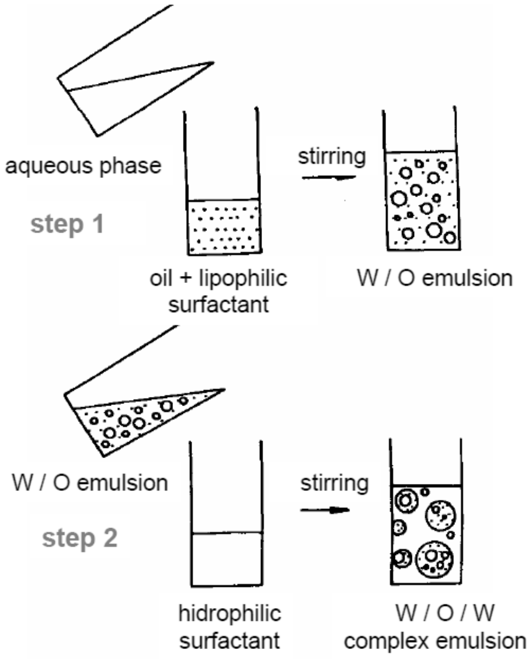


Figure 2. 9 Two-step emulsification method [21]

It has to take into account the first emulsification must not be affect by hydrodynamic forces during the second step, producing the breakdown as it is explained at the beginning of this chapter [3; 18]. Mechanical factors such as the agitation mode, density and viscosity of liquids, interfacial tension and the mass ratio affects the dispersion degree [5]. The mechanical energy can be provided by different types of emulsifying equipment. The most important are stirrer, rotor-stator systems, high pressure homogenizers and membranes [11]. How these equipments operate will be explain in the next Chapter 2.2.4 “Emulsifying equipment in W/O/W emulsion process”. In this kind of technique, generally it is used two both hydrophilic and lipophilic emulsifiers. The lipophilic emulsifier stabilizes the inner W/O emulsion while the hydrophilic emulsifier, the secondary O/W emulsion (Figure 2.10) [19].

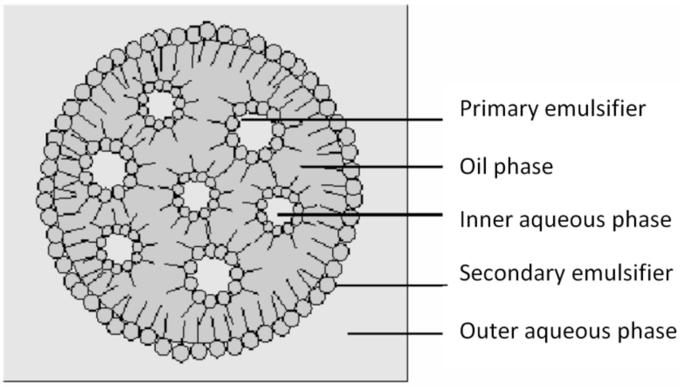


Figure 2.10 Water-in-oil-in-water emulsion [7]

The two-step emulsification method is more used than the one-step method because in the second one, only a percentage of the first emulsion is formed into a W/O/W emulsion. Furthermore, stable multiple emulsions of well-defined composition and reproducible droplet size distribution can be achieved using the two-step emulsification [3].

2.2.3.2 Multiple emulsion Interfacial properties

Multiple emulsions are thermodynamically unstable and kinetically stable. The thermodynamic instability results from the fact that the cohesive forces between the molecules of a single liquid are greater than the adhesive forces between the liquids, which raise an excess free energy in the surface of the emulsion droplets. The interfacial area of the dispersion phase grows spontaneously because of the tendency to come back to the original system (two separated phases), causing the breaking down of the emulsion as well as the reduction of the interfacial tension. In such a way, the interfacial free energy associated is defined by the Gibbs equation as:

$$\Delta G = \gamma \Delta A \tag{2. 12}$$

where G is the interfacial free energy, γ is the interfacial tension, and A is the total interfacial area of the dispersed phase.

As it is said in the previous chapter, an emulsifier is needed to preserve the surface area and prevent the separation, since the interfacial tension is decreased and it allows the formation of smaller droplets with closer size distributions and greater kinetic stability. Techniques of measuring the interfacial tension as a function of time can contribute to categorize the dynamic process of adsorption of emulsifiers and the equilibrium state of the interfacial film. Some of these techniques are the Wilhelmy plate, capillary rise or du Nouy ring methods.

On the other hand, interfacial rheology is the study of the flow properties of liquid interfaces. It is useful to quantify interfacial film strength characteristics as viscous or elastic properties. It has been reported that for long-term emulsion stability the strength of the interfacial film is more important than reduction in interfacial tension [22].

2.2.3.3 Multiple Emulsion Pressure Properties

2.2.3.3.1 Osmotic Pressure

The osmotic pressure is defined as the minimum pressure that is needed apply to a solution to avoid the flow from the inner water through a semipermeable membrane [23]. In W/O/W emulsions, the oil film acts as a membrane and the inner water phase can pass through it depending on the osmotic pressure difference between the internal and external aqueous phases. If the osmotic pressure in the internal water phase is much higher than in the external water phase, the oil membrane will be busted and the content of the inner water phase will be released and mixed with the external aqueous phase, turn into simple emulsions [22].

2.2.3.3.2 Laplace Pressure

The Laplace pressure is the pressure difference between the inside and the outside of a curved surface caused by the surface tension of the interface between two fluids [13]. The Young-Laplace equation relates the pressure difference between the two phases, also called Laplace pressure, ΔP, and the curvature of the surface:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{2. 13}$$

where R_1 and R_2 are the two principal radios of curvature and γ is the interfacial tension. For a spherical particle ($R_1 = R_2 = R$), the Laplace equation is reduced to [13; 22]

$$\Delta P = \frac{2\gamma}{R} \tag{2. 14}$$

It was demonstrated that Laplace pressure acts against the stability of simple emulsions and an addition of electrolyte to the dispersed phase can stabilize this effect [22].

2.2.3.3.3 Balance between Laplace Pressure and Osmotic Pressure

It was demonstrate that the addiction of small amounts of sodium chloride, sodium salicylate or materials other than electrolytes as proteins or sugars in the inner aqueous phase could even out the osmotic pressure in both aqueous phases. Furthermore, an addition of electrolyte to the dispersed phase can stabilize the effect of Laplace pressure, getting a control of multiple emulsion stability. Nevertheless, electrolytes can pass across the oil layer through molecular migration, so it is still not understood at all how and what are the parameters that affect to the kinetics and migration of electrolytes.

In the literature has been reported the demonstration of Walstra's equation that suggest Laplace and osmotic pressures can be counteracted by the addition of a determinate amount of salt concentration. It means that the concentration of electrolytes has to be high enough to counteract the Laplace pressure but sufficiently low to avoid osmotic effects.

In any case, that conclusion is useful if osmotic and Laplace pressures mainly control the droplet stability, but this stability can be affected by other factors such as viscosity [22].

2.2.4 Emulsifying equipment in W/O/W emulsions process

As it is said in Chapter 2.2.3.1 "Formation of W/O/W emulsion", for getting the dispersed phase in an emulsion, applying mechanical energy is the most common way even though the huge energy consumption. Different emulsifying devices to get this mechanical energy are used, as stirrer, rotor-stator systems, high pressure homogenizers, membranes and ultrasonic devices [5]. Each homogenizer has its own advantages and disadvantages. The election of the homogenizer for a particular use depends on the volume of production, the nature of materials, the throughput, the required droplet size and phisicochemical properties of the final product, and cost of purchasing and running the equipment [2].

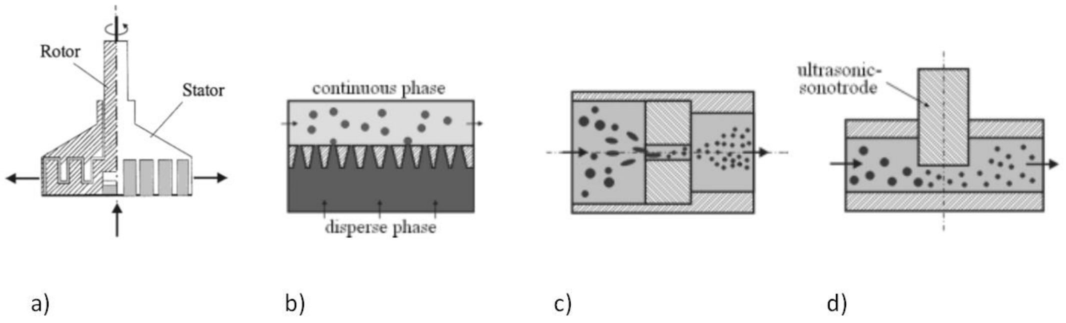


Figure 2.11 a) rotor-stator system b) membrane system c) high-pressure homogenizer d) ultrasonic system [24]

2.2.4.1 Rotor-stator systems

This homogenization system is formed generally by a rotor and an axially fixed stator around it. The breakup is produced by forces of inertia and shearing in turbulent flow. Two different rotor-stator devices can be differentiated according to the operation method, discontinuous or continuous.

For discontinuous process, agitators or gear-rim dispersion are used. In this case, the rotor has at least one axial slotted rim and a concentric fixed stator. The premix emulsion is introduced axially in the middle of the rotor and flows in a radial direction through the rims of the rotor and stator. It is possible to control the strength of the droplet disruption through the number of revolutions, the time of emulsifying and the geometry of the rotor-stator system. Rotation speed can achieve a value of 5000 rpm in big production machines or even above 25000 rpm in laboratory use machines. The minimum droplet size possible to get is approximately 2 µm [24; 25].

Rotor-stator system operating in continuous mode are colloid-mills, gear-rim dispersion machines and intensive mixers. In colloid mills, the disruption of the dispersed phase occurs in a conic annular slit between the rotor and stator. They work as a centrifugal pump which it means that the flow of the emulsion is inbuilt. The rotor and stator can be smooth or toothed. In colloid mills with toothed rotor and stator, laminar flow can also affect the breaking. Typical rotation speed is in a range of 2000-3000 rpm. The achieved droplet size can be a bit smaller that with discontinuous rotor-stator systems (1 µm) [24; 25].

2.2.4.2 High pressure homogenizer

In High pressure homogenizers, dispersed and continuous phases are submitted to different forces as turbulence, shear flows or cavitation. The main mechanism affecting the mixture is turbulence and it is the responsible of disrupting of the dispersed phase into small droplets

[26]. The homogenizing pressure is typically between 50 and 500 bar. High pressures allow decreasing the droplet size of the emulsion and improving the surface activity of the emulsifiers, getting better stability of the emulsion. This is because of the more pressure gradients and flow rates, the more stress is applied on the product. Also another parameter as temperature has to be taken into account [11][26].

These devices mainly are constituted by a high pressure pump and a homogenizing nozzle. The pump is useful to achieve the operating pressure (Homogenizing pressure) whereas in the nozzle it is produced the depressurization and, consequently, the droplet disruption [11]. The flow guidance depends on the kind of homogenization nozzles (standard nozzle, microfluidizer, jet dispersed or orifice valve). They always operate in continuous mode and can be possible get a droplet size lower than 0.1 µm. The difference on droplet size is directly related with the energy input density [24].

The volume specific energy input or the energy density, E_v, is the mechanical energy that is introduced in the system per unit volume of the area where the disruption of the droplets is produced. In High Pressure Homogenizers, the energy density is the pressure difference operating at the nozzle, ΔP_H [11].

$$E_v = \frac{Energy\ input}{Homogenized\ Volume} = \frac{Power\ input}{Volume\ flow\ rate\ of\ emulsion} = \bar{P}_v \cdot \bar{t}_v = \Delta \mathrm{P}_H \qquad (2.\ 15)$$

where $\bar{P}_v$ is the power density input and $\bar{t}_v$ mean the residence time [11]. The energy input in a rotor-stator system is between 104 and 106 Wm^{-3} while in a high pressure homogenizer is about 1012 Wm^{-3} [3]. In such a way, it is possible to relate the mean droplet size with the Energy density while other parameters are kept constant through the next equation:

$$x_{3,2} = \mathrm{C} \cdot \mathrm{E}_v^{-b} = \Delta \mathrm{P}_H \qquad (2.\ 16)$$

Where $x_{3,2}$ is the Sauter mean diameter (average of particle size), C is a constant that depends on the efficiency of droplet disruption and b is another constant that depends on the flow conditions in the homogenized volume. It means, the higher the energy input, the smaller the droplet size. This equation is valid for other emulsifying equipment as rotor-stator systems [11].

For preparing W/O/W emulsions, the two-step method is normally used. The first W/O emulsion is usually prepared using a high speed blender or rotor-stator system. Then, a fraction of this emulsion is mixed with an aqueous surfactant solution and then the blend is introduced in the High pressure homogenizer [27].

2.2.4.3 Microporous membranes

Microporous membranes of glass or ceramic are new continuous emulsification processes, which stand out because of the low energy consumption, a better droplet size distribution and a delicate disruption. The dispersed phase is forced to flow under high pressure through a membrane, which cause its disruption in small droplets. Once it passes through, it is mixed with the continuous phase that is along the membrane. The forces produce the rupture of dispersed phase with both turbulence and laminar shear flow. Droplet size depends on the porous size and the dispersion phase flow. It is possible to achieve homogeneous droplets with a size lower than 0.2 µm. The disadvantage of this method is the low productivity and time consuming [3; 27].

2.2.4.4 Ultrasonic system

With this system, the energy input necessary to break the dispersed phase is introduced through ultrasounds, which produce cavitation and microturbulences. These disruption forces are very strong. It is possible to reach a droplet size of 0.4 µm [27].

2.2.4.5 Summary

Table 2.4Methods of emulsification process [25; 27]

EMULSION PROCESSING						
	Throughput	**Relative Energy Efficiency**	**Minimum Droplet Size**	**Sample Viscosity**	**Product stress**	**Production**
High-Speed Blender	Batch	Low	2 µm	Low to medium	Middle to high	Industrial production
Colloid Mill	Continuous	Intermediate	1 µm	Medium to high	Very high	Industrial prouction
High Pressure System	Continuous	High	0.1 µm	Low to medium	Very high	Industrial production
Membrane Systems	Continuous	High	0.1 µm	Low to medium	Low	Laboratories
Ultrasonic Systems	Batch	Low	0.4 µm	Low to medium	Very high	Laboratories

2.2.5 Encapsulation efficiency in W/O/W emulsions

To check the efficiency of encapsulation in an emulsion it is used a parameter called Encapsulation Efficiency, EE. It is defined as the fraction of the water phase in the first emulsion that it is retained as first water phase in the final emulsion. To that end, it is added a water-soluble trace into the dispersed phase of the primary W/O emulsion. The measure of EE could be calculated measuring the amount of the marker in the second water phase, as:

$$\mathrm{EE}(\%) = 100\left(1 - \frac{C_t W_t}{C_w W_w}\right) \tag{2. 17}$$

where C_t is the concentration of the tracer in the external water phase, W_t is the amount of the total water added to the process, C_w is the known concentration of added tracer and W_w is the weight of the external aqueous phase. 0% of EE means that all amount of tracer is in the external water phase ($C_t \cdot W_t = C_w \cdot W_w$) and therefore there is nothing encapsulated, whereas 100% of EE means all the tracer is in the inner water phase. EE is mostly affected by the second step of the process. Once the emulsion process is completed, the EE depends on its stability [3].

2.2.3.4 Methods to determine encapsulation Efficiency in W/O/W.

Different methods are used to measure the EE in a W/O/W emulsion. They can be divided in indirect and direct measurements techniques. Most usual indirect techniques are electrical conductivity measurements, photometric measurements and rheology test, while Differential Scanning Calorimeter (DSC) is the most important direct technique.

In electrical conductivity measurements, an electrolyte is dissolved in the inner water phase and the conductivity of the external water phase is measured by employing a Conductivity meter. The more conductivity has the external water phase, the less amount of electrolyte has been encapsulated.

In photometric measurements, a dye is used as a tracer in the internal aqueous phase. The colouring substance shows an absorbance at a specific wavelength that can be measured in a UV-Vis spectroscope.

In rheological test, the amount of dispersed phase is estimated by viscosity measurements. For low dispersed phase contents it is possible to relate the viscosity of an emulsion with its dispersed phase content. The less amount of the inner water phase, the more amount of the continuous phase.

For all these indirect measurements, a calibration curve is needed in order to correlate the measure data with the Encapsulation Efficiency.

Differential Scanning Calorimetry (DSC) determines the amount of internal aqueous phase in a direct way. It is based on the heat release during a freezing process at a constant temperature rate. Internal and external aqueous phase freeze at different temperatures so it is possible to differentiate them. The heat release of a substance is directly correlated with its mass, thus the amount of inner water phase can be calculated using the latent heat of freezing.

Nevertheless, all of them have limitations. In tracer measurements, methods assume that the release of water and marker occur at the same time, which is not true. For instance, in electrical conductivity measurements, ions can be transported through an oil membrane independently from water transport. This means that the release of ions and therefore the measured change in electroconductivity is not necessarily directly correlated to the amount of released water. In rheological test, the method is only valid for monodispersed emulsions and it is considerable affected by changes in droplet size. In DSC, changes in droplet size also suppose a difficulty because they affect freezing temperature and the latent heat of freezing. Furthermore, the measurement takes some time (30 minutes) and the sample has to be stable during the analysis [28].

3. MATERIALS

The aim of this work is to produce microcapsules as stable as possible. To this end, three kinds of substances are used, shell material, core material and emulsifiers (See Chapter 2.1, Microencapsulation).

3.1 SHELL MATERIAL

In this work, it is used only one type of shell material, Witepsol W31. Its properties are described below.

3.1.1 Witepsol W 31

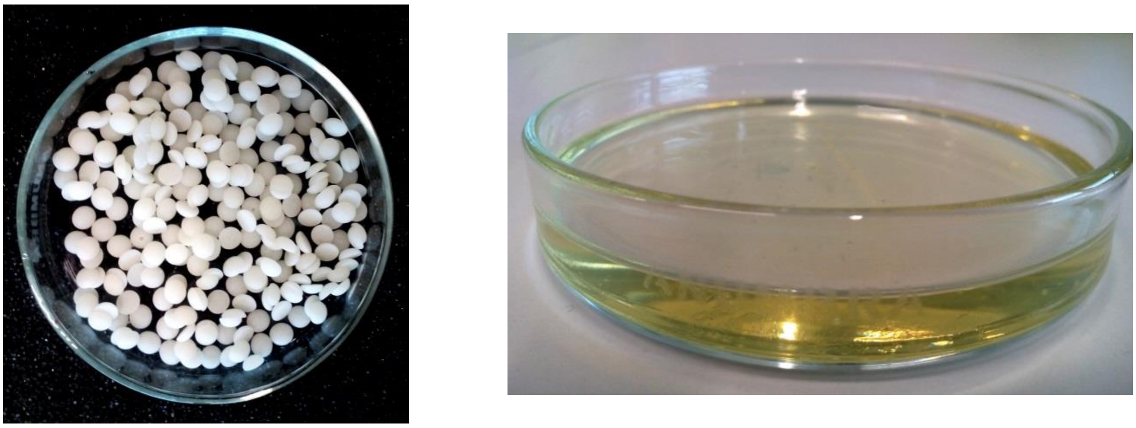


Figure 3. 1 Appearance of Witepsol W31 (At 25°C and at 50°C)

Witepsol W31 is a hard fat, white solid at ambient temperature and yellow when it is melted. It belongs to Witepsol W grades, which are characterized by a high hydroxyl value, between 20 and 50. It is mainly composed of triglycerides (65-80%), although it also contains diglycerides (10-35%) and monoglycerides (1-5%). They are obtained by transesterification of natural vegetable fatty acids with glycerol. Melting and solidification temperatures of Witepsol W 31 are not the same, which allows it to have more resistance against sharp cooling [29]. It is mainly used in the production of suppositories. Some of their properties are shown in Table 3.1.

Table 3. 1Properties of Witepsol W 31 [29]

WITEPSOL W 31	
INCI Name	Hydrogenated Coco-Glycerides
Melting Point	35.5-37 °C
Hydroxyl number	31 mg KOH/g
Acid number	0.2 mg KOH/g
Saponification number	228 mg KOH/g
Density	0.95 g/cm^3
Ash content	< 0.05 %
Unsaponifiables	< 0.5 %

3.2 CORE MATERIAL

Core material is the main substance in microencapsulation technology. In this work, Sodium Chloride solution and Indigo Carmine solution are used to test the quality of encapsulation. Their characteristics are described below.

3.2.1 Water

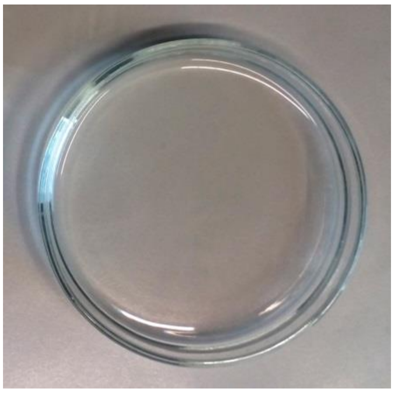


Figure 3. 2 Appearance of Water

Water is a colourless odourless and tasteless chemical substance with H_2O as chemical formula. It is liquid at ambient temperature and pressure and it is the main solvent primarily due to its properties. It is one of indispensable substances to life and it is necessary also to photosynthesis process. Among other properties, it is a polar molecule, water molecules link with each other by hydrogen bonds, it has a high surface tension and adhesion properties, and has less density once it is frozen [30]. Some properties of water are shown below (Table 3.2).

Table 3. 2Properties of Water [31]

WATER	
Melting Point (°C)	0 °C
Chemical formula	H_2O
Molecular Weight	18.02 g/mol
Viscosity (at 25°C)	890 cps
Bulk density (at 25°C)	1 g/cm^3
pH Value at 25 °C	6.0-8.0
Water Solubility	Completely miscible
Toxicological information	Not toxic

3.2.2 Sodium Chloride

It is a colourless white small crystal substance, which is soluble in water but slightly soluble in most other liquids. It is characterized by its specific taste and it is also called salt. It is an ionic compound and it has the same amount of positive charges of sodium and negative charges of chloride ions. Dissolved salt is a conductor of electricity and it can be decomposed in sodium and chlorine by electrolysis [32]. The next table (Table 3.3) summarize some properties of Sodium Chloride.

Figure 3. 3 Appearance of Sodium Chloride

Table 3. 3 Properties of Sodium Chloride [33]

SODIUM CHLORIDE	
Melting Point (°C)	801 °C
Chemical formula	NaCl
Molecular Weight	58.44 g/mol
Viscosity (at 25°C)	No data
Bulk density (at 25°C)	2.165 g/cm^3
pH Value (5%aq. Sol. 20ºC)	5.0-8.0
Water solubility at 20°C	360 g/L Soluble
Toxicological information	LD_{50}rat > 3000 mg/kg, LD_{50}mouse > 4000 mg/kg, LD_{50}rabbit > 10 mg/kg

Sodium Chloride usually is used in W/O/W emulsions for balancing the Laplace pressure by increasing the osmotic pressure in the inner droplets and in this work it will be used as a marker to determine the encapsulation efficiency by electroconductivity measurements [28]. It was supplied by Fisher Chemical.

3.2.3 Indigo Carmine

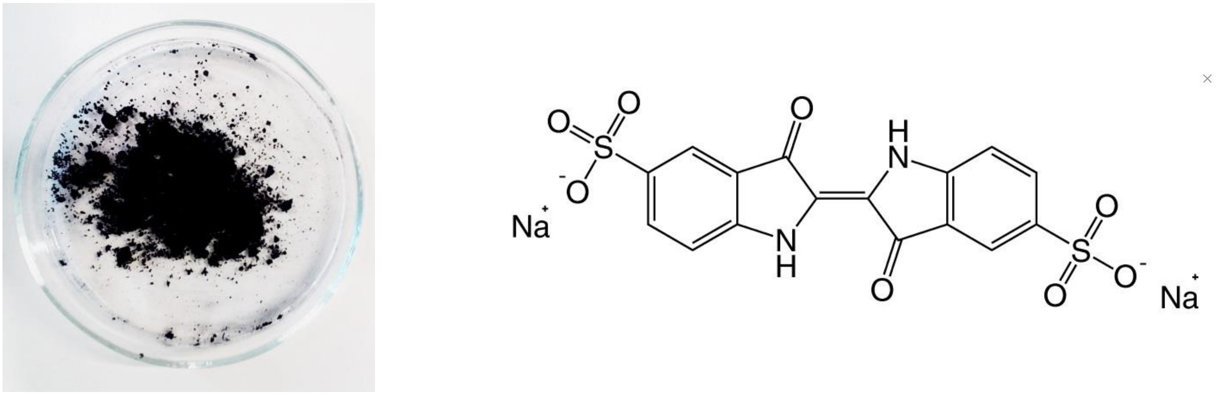


Figure 3. 4 Appearance and Structure of Indigo Carmine [34]

Indigo carmine or Indigotine is a dark blue and odourless powder. It is usually used as a pH indicator (it is blue at a pH value of 11.4 and yellow at 13.0) and it is approved to use as a food colorant with the E number of E132 [9]. Some properties of Indigo carmine are in Table 3.4.

Table 3. 4 Properties of Indigo Carmine [36]

INDIGO CARMINE	
Chemical Description	disodium 5,5'-(2-(1,3-dihydro-3-oxo-2H-indazol-2-ylidene)-1,2-dihydro-3H-indol-3-one)disulphonate
Melting Point (°C)	>300 °C
Chemical formula	$C_{16}H_8N_2Na_2O_8S_2$
Molecular Weight	466.36 g/mol
Viscosity	No data
Bulk density (at 20°C)	700-900 g/cm^3
pH Value (10g/L, 25°C)	5.3
Water solubility at 10% concentration and 25°C	12g/L Soluble
Toxicological information	LD_{50}rat > 2000 mg/kg

It was used as a marker for photometric measurements in order to observe the encapsulation of the double emulsions and it was provided by Carl Roth.

3.3 EMULSIFIERS

As it is said in Chapter 2.2.2, it is necessary another component to stabilize the emulsion, a emulsifier. They usually are classified according to their HLB value in lipophilic emulsifiers (more oil affinity) or hydrophilic emulsifiers (more water affinity). Down below, the emulsifiers used in this work are described. As lipophilic emulsifiers, Span 80, Span 20, Span 65, Sisterna SP10-C, Cutina GMS V and Tego Alkanol S 2 P were tested, whereas hydrophilic emulsifiers, Tween 80, Sisterna SP50-C, Cutina E24, Tego SML 20, Tego SMS 60 and Tego Alkanol S 20 P were chosen.

3.3.1 Lipophilic emulsifiers

3.3.1.1 SPAN 80

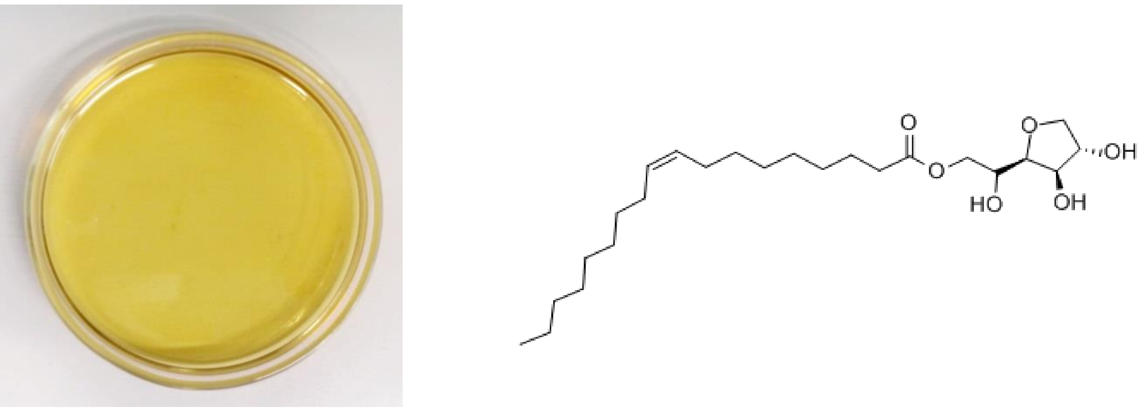


Figure 3. 5 Appearance and Structure of SPAN 80 [37]

Span 80 is an amber yellow and viscose oily liquid. It is insoluble in water and soluble in organic solvents. It is used as nonionic W/O emulsifier, solubilizer, stabilizer, softener, anti-static agent among others and it has application in areas as medicine, cosmetics, textiles, paints and foods [38]. In this work, Span 80 was supplied by Sigma Aldrich and some properties are summarized in Table 3.5.

Table 3. 5 Properties of SPAN 80 [39; 40]

SPAN 80	
INCI Name	Sorbitan monooleate
HLB	4,3
Melting Point	No data
Formula	$C_{24}H_{44}O_6$
Molecular Weight	428.62 g/mol
Viscosity (at 25°C)	1000 cps
Density (at 25°C)	0.994 g/mL
Acid Value	<8 mgKOH/g
Flash point	>113 °C
Water solubility at 10% concentration and 25°C	Insoluble
Toxicological information	LD50 rat > 39.8 g/kg

3.3.1.2 SPAN 20

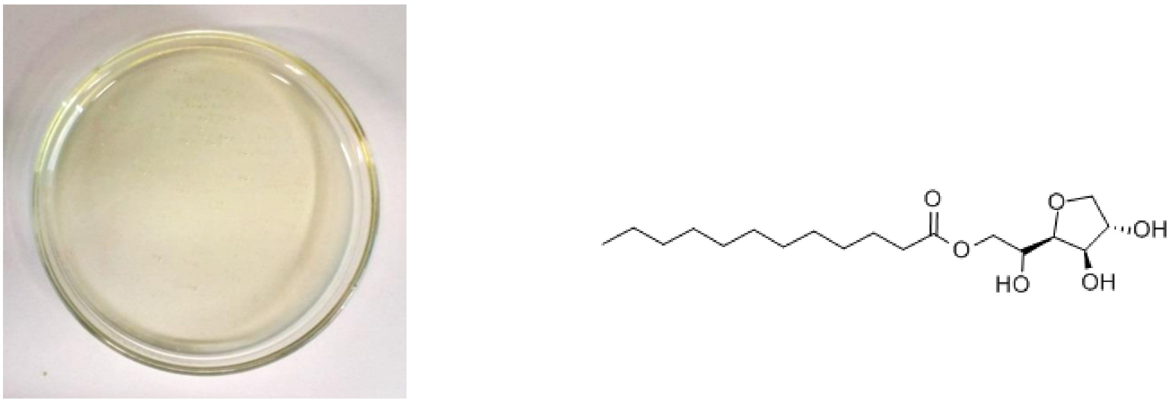


Figure 3. 6 Appearance and Structure of SPAN 20 [41]

Span 20 is a slightly yellow and very viscous liquid that is mainly used as non-ionic W/O emulsifier, wetting agent and lubricant in medicine, cosmetics, textiles, foods among others. For instance, it has been used in a study to improve the solubility and oral bioavailability of itraconazole and to investigate the oxidation of unsaturated lipids in O/W emulsions [42]. It was provided by Sigma Aldrich and some properties are shown below (Table 3.6).

Table 3. 6 Properties of SPAN 20 [41]

SPAN 20	
INCI Name	Sorbitan mololaureate
HLB	8,6
Melting Point (°C)	-64 to 22 °C
Formula	$C_{18}H_{34}O_6$
Molecular Weight	346.47 g/mol
Viscosity (at 25°C)	No data
Density (at 25°C)	1.032 g/mL
Acid Value	< 8 mgKOH/g
Flash point	>113 °C
Water solubility at 10% concentration and 25°C	Insoluble
Toxicological information	LD_{50}rat > 33600 mg/kg

3.3.1.3 SPAN 65

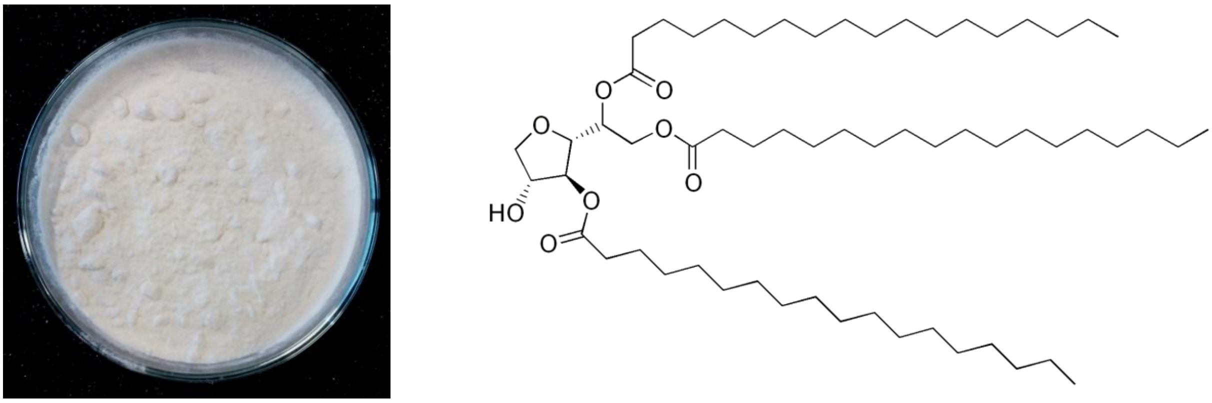


Figure 3. 7 Appearance and Structure of SPAN 65 [43]

Span 65 is a white off solid powder and it is a biodegradable surfactant based on a natural fatty acid (stearic acid) and the sugar alcohol sorbitol [44]. It is usually used as non-ionic emulsifiers in W/O emulsions, thickeners, stabilizers, lubricants, softener, antistatic agents, finishing agents or spinning additives in cosmetics, textiles, pharmaceuticals, oil and other industries [45]. Table 3.7 gives some properties of Span 65. In this work, it was provided by Sigma Aldrich.

Table 3. 7 Properties of SPAN 65 [45; 46]

SPAN 65	
INCI Name	Sorbitan tristearate
HLB	2,1
Melting Point (°C)	50-56 °C
Formula	$C_{60}H_{114}O_8$
Molecular Weight	963.55 g/mol
Viscosity (at 25°C)	No data
Density (at 25°C)	No data
pH value (in 100g/L)	No data
Flash point	>150 °C
Water solubility at 10% concentration	Soluble in hot water
Toxicological information	No data

3.3.1.4 SISTERNA SP10-C

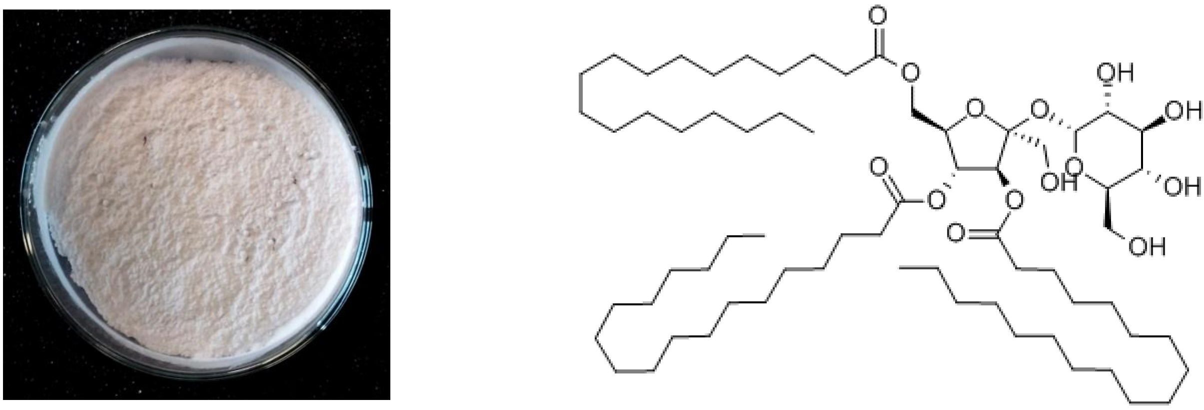


Figure 3. 8 Appearance and Structure of SISTERNA SP10-C [47]

Sisterna SP10-C is a non-ionic surfactant sucrose polystearate. Its appearance is like off-white powder with a characteristic and faint odour. It is based on sucrose and vegetable fatty acids and it is used in the personal care industry, improving smoothness, emolliency and moisture level to the skin, and also is approved and widely used in the food industry [48]. It is supplied by Sisterna B.V. and some properties are summarized in Table 3.8.

Table 3. 8 Properties of SISTERNA SP10-C [49]

SISTERNA SP10-C	
INCI Name	Sucrose polystearate
HLB	2
Melting Point	59-67 °C
Formula	$C_{66}H_{124}O_{14}$
Molecular Weight	1141.68 g/mol
Viscosity (at 25°C)	No data
Bulk density (at 25°C)	300 kg/m^3
pH value (in 100g/L)	7.8
Flash point	230 °C
Water solubility at 10% concentration and 25°C	Insoluble
Toxicological information	LD_{50}rat >2000 mg/kg

3.3.1.5 CUTINA GMS V

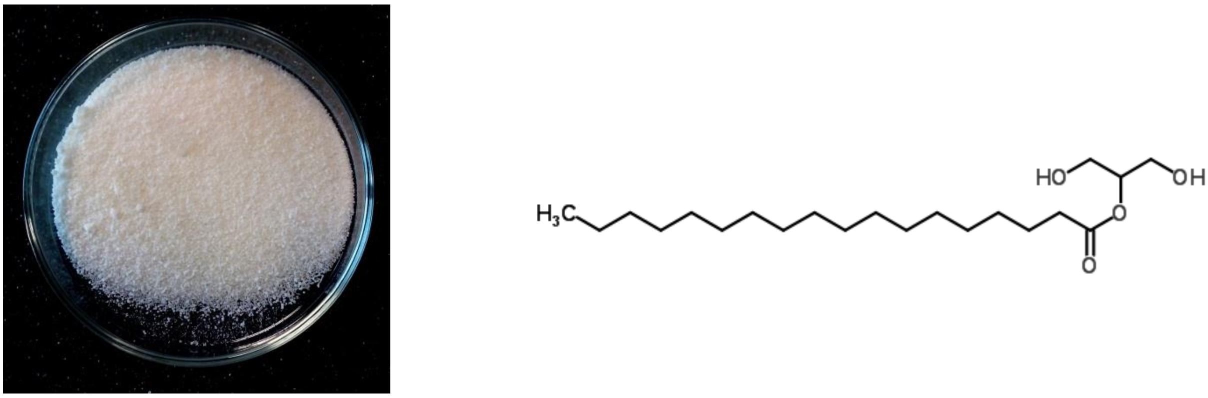


Figure 3. 9 Appearance and Structure of CUTINA GMS V [50]

Glyceryl stearate is the monoester of glycerin and stearic acid and it has a form of off white flakes with faint odour. It is an useful emulsifier for making W/O emulsions [51]. On account of its consistency it is mainly used for the viscosity adjustment in cosmetic O/W emulsions [52]. It was provided by Care Chemicals and some properties are shown in Table 3.9.

Table 3. 9 Properties of CUTINA GMS V [52]

CUTINA GMS V	
INCI Name	Glyceryl Stearate
HLB	3,8
Melting Point	58-59 °C
Formula	$C_{21}H_{42}O_4$
Molecular Weight	358.55 g/mol
Viscosity (at 25°C)	No data
Density (at 25°C)	No data
Acid value	<2 mgKOH/g
Flash point	No data
Water solubility at 10% concentration and 25°C	Insoluble
Toxicological information	No data

3.3.1.6 TEGO® ALKANOL S 2 P

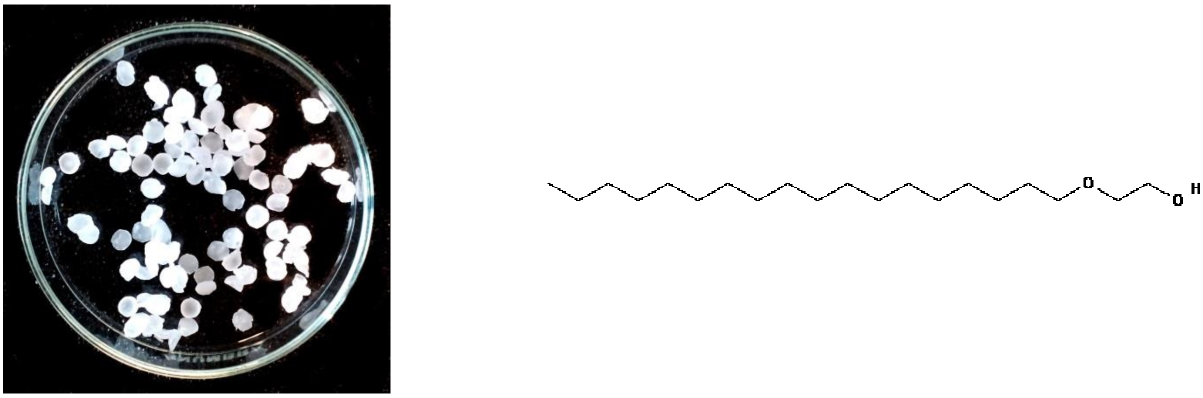


Figure 3. 10 Appearance and Structure of TEGO® ALKANOL S2P [53]

Tego Alkanol S2 Pellets are a white waxy substance with a characteristic odour. It is a non-ionic emulsifier usually used in W/O emulsions and can be used over a wide pH range. In combination with other emulsifiers, creams and lotions can be formulated [54]. They were supplied by Evonik Industries and Table 3.10 shows some of their properties.

Table 3. 10 Properties of TEGO® ALKANOL S2P [55]

TEGO® ALKANOL S2P	
INCI Name	Steareth-2, PEG-2 Stearyl ether
HLB	5
Melting Point	43 °C
Formula	$C_{20}H_{42}O_2$
Molecular Weight	314.55 g/mol
Viscosity (at 25°C)	No data
Density (at 25°C)	0.970 g/cm^3
pH value (in 100g/L)	5-8
Flash point	>149 °C
Water solubility at 10% concentration and 25°C	Insoluble
Toxicological information	No data

3.3.2 Hydrophilic emulsifiers

3.3.2.1 TWEEN 80

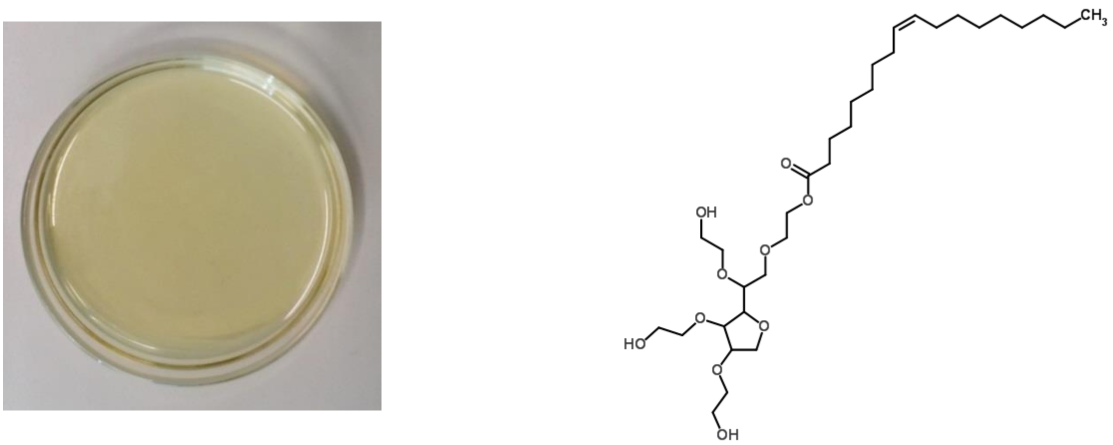


Figure 3. 11 Appearance and Structure of TWEEN 80 [56]

Tween 80 is a yellow viscous non-ionic emulsifier that is widely used as solubilizer of proteins, isolator of nuclei from cells in culture, grower of tubercule bacilli and as emulsifier and disperser of substances in medicinal and food products [57]. In this work, it is provided by Sigma Aldrich. In Table 3.11 some of its properties are summarized.

Table 3. 11 Properties of TWEEN 80 [58]

TWEEN 80	
INCI Name	PEG-20 Sorbitan Monooleate, Polysorbate 80
HLB	15
Melting Point	-20 °C
Formula	$C_{64}H_{124}O_{26}$
Molecular Weight	1310 g/mol
Viscosity (at 25°C)	400-620 cps
Density (at 25°C)	1.07 g/cm^3
pH value (in 100g/L)	5,5-7,2
Flash point	> 113 °C
Water solubility at 10% concentration and 25°C	Soluble
Toxicological information	LD_{50}rat > 25000 mg/kg

3.3.2.2 SISTERNA SP50-C

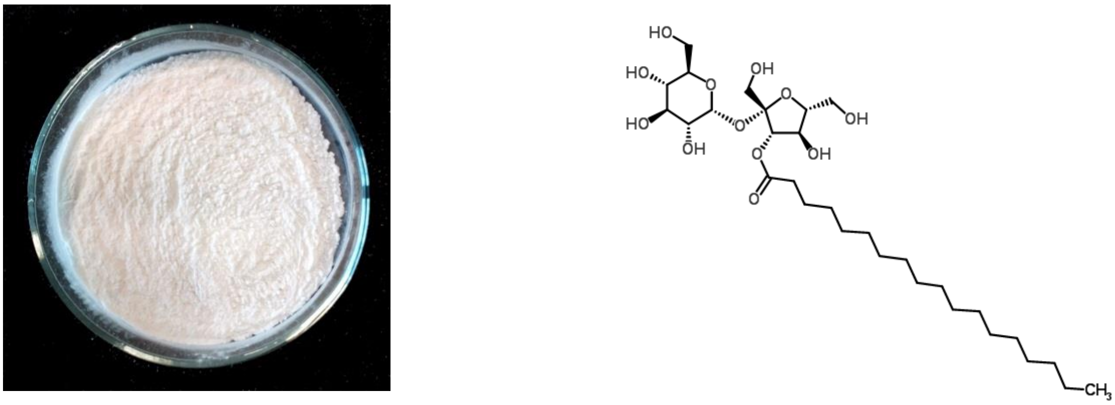


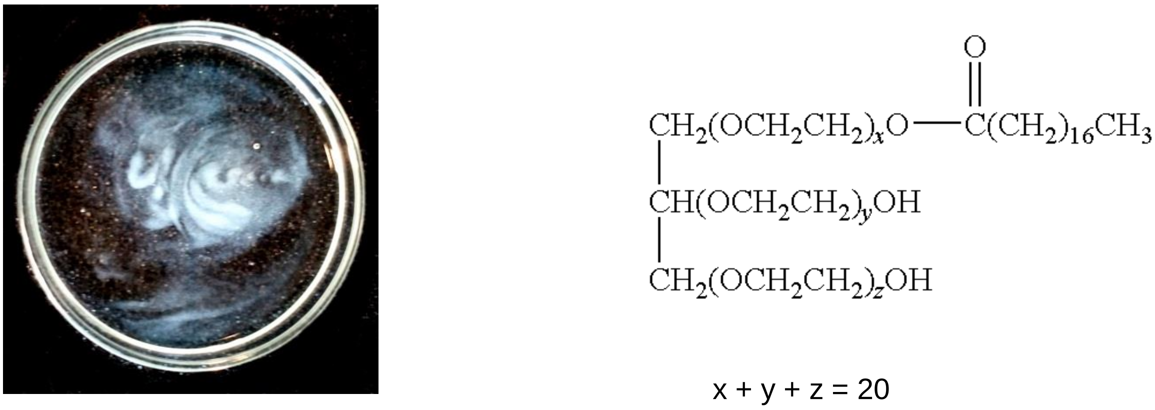
Figure 3. 12 Appearance and structure of Sisterna SP50-C [59]

Sisterna SP50-C is an off-white powder with a very faint and characteristic odour. It is a non-ionic surfactant sucrose stearate, is based on sucrose and vegetable fatty acids and it has the same applications in care industry as Sisterna SP10-C. It also is used as O/W emulsifier [60]. It is supplied by Sisterna B.V. and some properties are shown in Table 3.12.

Table 3. 12 Properties of SISTERNA SP50-C [61]

SISTERNA SP50-C	
INCI Name	Sucrose Stearate
HLB	11
Melting Point	51-59 °C
Formula	$C_{30}H_{56}O_{12}$
Molecular Weight	608.76 g/mol
Viscosity (at 25°C)	No data
Bulk density (at 25°C)	450 kg/cm^3
pH value (in 100g/L)	7.8
Flash point	> 149°C
Water solubility at 10% concentration and 25°C	Partly soluble
Toxicological information	LD_{50}rat > 2000 mg/kg

3.3.2.3 CUTINA E24



$$\begin{array}{l} \overset{\displaystyle O}{\overset{\|}{}} \\ CH_2(OCH_2CH_2)_xO - C(CH_2)_{16}CH_3 \\ | \\ CH(OCH_2CH_2)_yOH \\ | \\ CH_2(OCH_2CH_2)_zOH \end{array}$$

$x + y + z = 20$

Figure 3. 13 Appearance and Structure of CUTINA E24 [62]

Cutina E24 has a liquid-paste consistency with a characteristic odour. It is commonly used as a non-ionic emulsifier for the preparation of cosmetic O/W emulsions. Due to its consistency, it is easy to dose and process [63]. In Table 3.13 some properties about Cutina E24 are summarized and in this work it is supplied by Care Chemicals.

Table 3. 13 Properties of CUTINA E24 [63]

CUTINA E24	
INCI Name	PPG-5-Laureth-5, PEG-20 Glyceryl Stearate
HLB	15
Melting Point	18-21 °C
Formula	$C_{61}H_{122}O_{24}$
Molecular Weight	1239.61 g/mol
Viscosity (at 25°C)	No data
Density (at 25°C)	1.0170-1.0220 g/cm^3
pH value (in 100g/L)	6-7.5
Flash point	> 149°C
Water solubility at 10% concentration and 25°C	Soluble
Toxicological information	LD_{50}rat > 30000 mg/kg

3.3.2.4 TEGO® SML 20

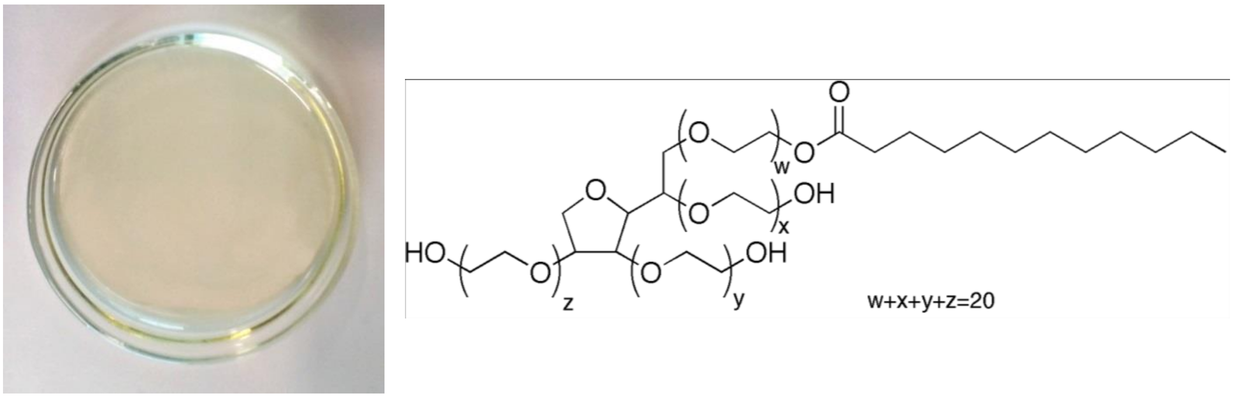


Figure 3. 14 Appearance and Structure of TEGO® SML 20 [64]

Tego SML 20 is a yellow liquid polysorbate surfactant which its odour is very characteristic. Its properties allow it to be used as a surfactant for hair and skin cleansing products, solubilizer for lipophilic substances and as hydrophilic emulsifier for O/W emulsions [65]. It was supplied by Evonik Industries and some of its properties are shown in Table 3.14.

Table 3. 14 Properties of TEGO® SML 20 [66]

TEGO® SML 20	
INCI Name	Polysorbate 20
HLB	17
Melting Point	No data
Formula	$C_{58}H_{114}O_{26}$
Molecular Weight	1227.54 g/mol
Viscosity (at 25°C)	300-500 mPa·s
Density (at 25°C)	1-1.2 g/cm^3
pH value (in 100g/L)	5.5-7.0
Flash point	> 149°C
Water solubility at 10% concentration and 25°C	Soluble
Toxicological information	LD$_{50}$rat > 30000 mg/kg in Rates

3.3.2.5 TEGO® SMS 60

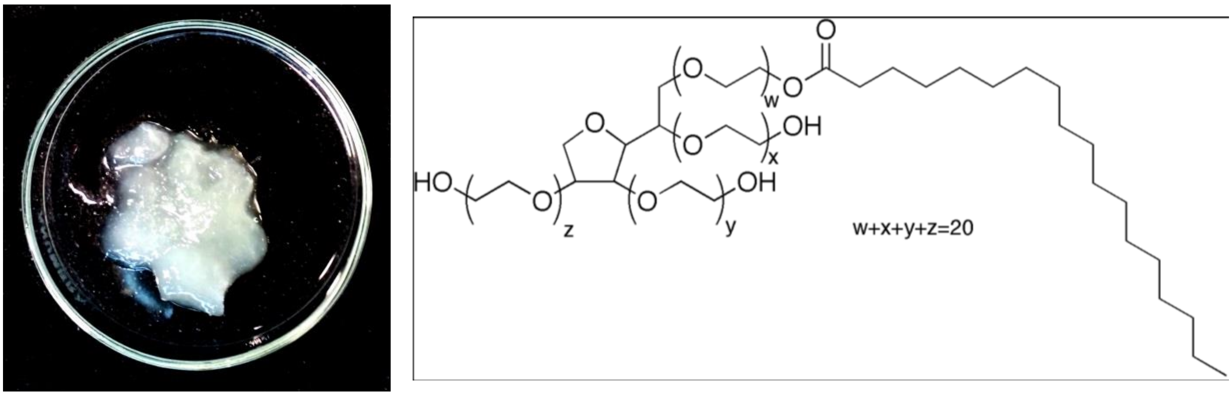


Figure 3. 15 Appearance and Structure of TEGO® SMS 60 [67]

TEGO SMS 60 is a mild nonionic surfactant which has an appearance like slightly yellow gel and its odour is weak. It can be used as a mild non-ionic surfactant in shampoos, shower and foam baths, solubilizer for fatty oils and perfume oil in all aqueous preparations and as hydrophilic emulsifier for O/W emulsions [68]. In this work it was provided by Evonik industries and some properties are summarized in Table 3.15.

Table 3. 15 Properties of TEGO® SMS 60 [69]

TEGO® SMS 60	
Chemical identity	Polysorbate 60
HLB	15
Melting Point	No data
Formula	$C_{64}H_{126}O_{26}$
Molecular Weight	1311.68 g/mol
Viscosity (at 25°C)	400-800 mPa·s
Density (at 25°C)	1.1 g/cm^3
pH value (in 100g/L)	5.5-6.3
Flash point	> 149°C
Water solubility at 10% concentration and 25°C	Soluble
Toxicological information	LD_{50}rat > 38000 mg/kg in Rates

3.3.2.6 TEGO® ALKANOL S20P

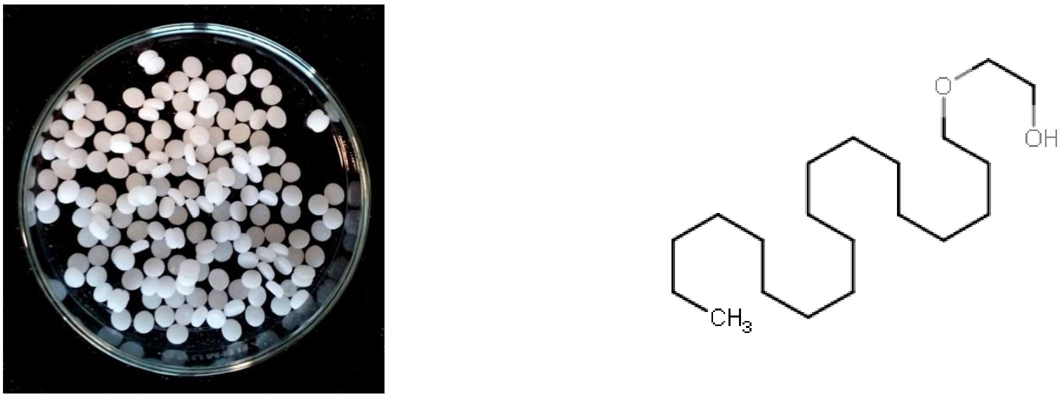


Figure 3. 16 Appearance and Structure of TEGO® ALKANOL S20P [70]

TEGO Alkanol S 20 P is a white and solid emulsifier usually used in the formulation of cosmetic O/W and W/O emulsions. It is also suitable for skin care creams and lotions, deodorant and antiperspirant sprays and roll-ons, hair conditioner and wet wipes [71]. It was supplied by Evonik Industries and in Table 3.16 some of its properties are shown.

Table 3. 16 Properties of TEGO® ALKANOL S20P [72]

TEGO® ALKANOL S20P	
Chemical identity	Staereth-20, Polyoxyl stearyl ether
HLB	15
Melting Point	No data
Formula	$C_{20}H_{42}O_2$
Molecular Weight	313.542 g/mol
Viscosity (at 25°C)	No data
Density (at 25°C)	1.09 g/cm^3
pH value (in 100g/L)	5-8
Flash point	> 149°C
Water solubility at 10% concentration and 25°C	Soluble
Toxicological information	LD_{50}rat > 2070 mg/kg in Rates

4. APPARATUS AND METHODS

In this work, emulsion processing is made in two kinds of devices, one of them for small-scale experiments (stirring plate) and the other one for large-scale experiments (Ultra-turrax). Furthermore, devices used for characterize the droplet size of the capsules, their morphology, their efficiency and their stability will be presented in this chapter.

4.1 COMPOSITE MANUFACTURING

Depending on production and droplet size of capsules, in this work two kinds of manufacturing devices were used, stirring plates (small-scale experiments) and Ultra-turrax (large–scale experiments). Each method will be explained below.

4.1.1 Stirring plates

In small-scale experiments, production of W/O/W emulsions is carried out in IKA® C-MAG HS 7 stirring plates (Figure 4.2, left). They work in a speed range from 100 to 1500 rpm adjustable through speed control from 0 to 6 steps and their heat temperature range is from 50 to 500 °C. For getting a better control of temperature, an external sensor IKA® ETS-D5 is used, which allows control the temperature in a range between -50 and 450 °C (Figure 4.1, right)[73; 74]. Specifications of both devices are given in Table 4.1 and 4.2, respectively.

Figure 4. 1 IKA® C-MAG HS 7 (left) [15] and IKA® ETS-D5 (right) [16]

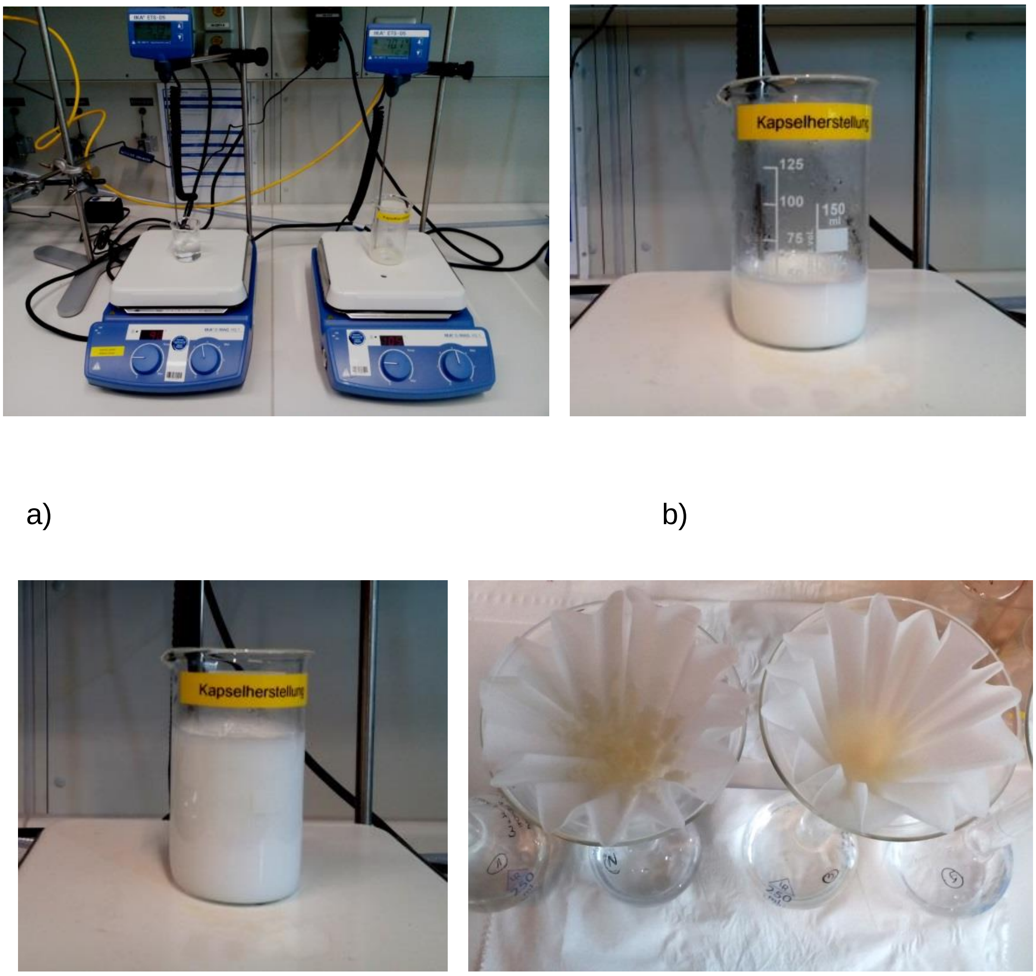
Table 4. 1 Specifications of IKA® C-MAG HS 7 [73]

IKA® C-MAG HS 7	
Speed range	100 – 1500 rpm
Speed control	0 – 6
Heating temperature range	50 – 500 °C
Heat control	LED
Heat control accuracy	10 ± K
Fixed safety circuit	550 °C
Connection for ext. Temperature sensor	ETS-D5
Set-up plate material	ceramic
Set-up plate dimensions	180 x 180 mm
Weight	5 kg

Table 4. 2 Specifications of IKA® ETS-D5 [74]

IKA® ETS-D5	
Temperature measuring range	-50 – 450 °C
Temperature measuring resolution	0.1 K
Accuracy of temperature measurement	± 0.2
Immersion depth max.	200 mm
Dimensions (W x H x D)	82 x 83 x 22 mm
Weight	0.2 kg

To this aim, two-step emulsification method is used. Emulsion takes place in 150 mL beakers and the used magnetic stirrer is 2cm · Ø 5mm size. First of all, oil phase is melted and heated to the operative temperature, 70 °C. The emulsifier type is added and stirred for a time to the oil phase (lipophilic emulsifier) or to the inner water phase (hydrophilic emulsifier) before blending the two phases. Once the oil and the inner water phase are ready, inner water phase is added to the oil phase and the mix continue stirring at chosen speed for the desired time (t_1) (Figure 4.2 a), right). When this time ends, second warm water phase (Figure 4.2 a), left) is added, and the mix is still stirred until the second desired time (t_2) (Figure 4.2 b)). After that, a cold water phase is added to the W/O/W emulsion for cooling down the mixture, solidify the oil phase and get microcapsules (Figure 4.2 c)). Final temperature is measured. Droplets are separated from water by filtering the solution through MN 615 ¼ · Ø 185 mm filters from Macherey-Nagel (Figure 4.2 d)). Capsules were left to dry for one day before collecting them in sample bottles. Each sample bottle is identify by what it contains, who was working with it and a number with the date and the number of sample (for instance, sample nummer 150302_1, where 15 is the year, 03 is the month, 02 is the day and 1 is the sample number that was done in that date).



a) b)

c) d)

Figure 4. 2 Steps during emulsification process in small-scale. a) Warming of second water phase (left) and first W/O emulsion (right) c) W/O/W emulsion d) Cool down of W/O/W emulsion e) Filtering of microcapsules

4.1.2 Ultraturrax

Figure 4. 3 IKA® T 50 ULTRA-TURRAX® (left) [75] and jacketed cooper container (right)

For large-scale experiments, IKA® T 50 ULTRA-TURRAX® system is used (Figure 4.3, left). It works in a speed range from 4,000 to 10,000 rpm adjustable in 6 steps. Specifications of this device are given in Table 4.3. Dispersion S 50 N – G 45 F was used as a rotor-stator geometry, which allows to produce droplets in a size range from 1 to 10 µm [75]. A jacketed and cilindrical cooper container whose working fluid is distilled water is used to retain the required temperature during the emulsification process (Figure 4.2, right)). The container has a total volume of 500 mL. A water thermostatic bath is used to heat the heating water.

Table 4. 3 Characteristics of IKA® T 50 ULTRA-TURRAX® [76]

IKA® T 50 ULTRA-TURRAX®	
Motor Rating Input / Output	1,100 / 700 W
Volume Range (H^2O)	0.25 – 30 L
Speed Adjustment	Stepless
Speed Range	4000 – 10,000 rpm
Noise Motor	72 dB (A)
Dimensions (W x D x W)	125 x 120 x 367 mm
Weight	6 kg

For carrying out large-scale experiments, also two-step emulsification method is used in the same way as in small-scale experiments. Oil phase, inner water and external water phase are preheated in the stirring plates. When emulsifier is added, it is also stirred with its phase corresponding before being added to the container. A mobile plataform is used for elevating the container till a chosen working height, taking into account that the minimum space between the bottom of the container and the rotor-stator has to be 20 mm. The thermostatic bath is switched on some time before for preheating the container. Once the W/O/W emulsion is done, the cool down is made in a 2 L beaker full of cold water and the formed capsules are filtered through MN 615 ¼ · Ø 320 mm filters from Macherey-Nagel. Capsules were left to dry for one day before collecting them in sample bottles. Each sample bottle is identify in the same way as in small-experiments.

4.2 COMPOSITE CHARACTERIZATION

Once capsules are made, it is necessary to characterize them accordingly to their particle size, particle morphology, the encapsulation efficiency (EE) and how stable are. To this aim, devices as Mastersizer 2000, BX-60 Motorized Fluorescence Microscope, Conductivity meter 3310 WTW and Digital Orbital Shaker Sea Star are used.

4.2.1 Particle size measurements

As it is said in Chapter 2.2.1.2 “Emulsion properties”, droplet size is a very important parameter in microencapsulation and it is usual to display it as a distribution function.

In this work, laser diffraction analysis is the used technique to find out the particle size distribution. It is possible to measure droplet size of particles from 0.02 to 2000 µm. Particles are dispersed in water or in air and they are passed through a focused laser beam (See Figure 4.5). The light is scattered by the particles at an angle that is inversely proportional to their size. Larger particles scatter light at smaller angles than small particles. The angular intensity of the scattered light is then measured by a series of photosensitive detectors and the particle size is calculated by using Fraunhofer method and Mie theory of light scattering (unlike Fraunhofer method, Mie theory needs some optical properties of the sample and dispersant to carry out the measurement). The laser beam consists of two light sources (He-Ne) having different wavelength, which provides more sensitivity in a wide range size. A short wavelength blue light source is used in conjunction for enhanced sizing performance [77][78][79].

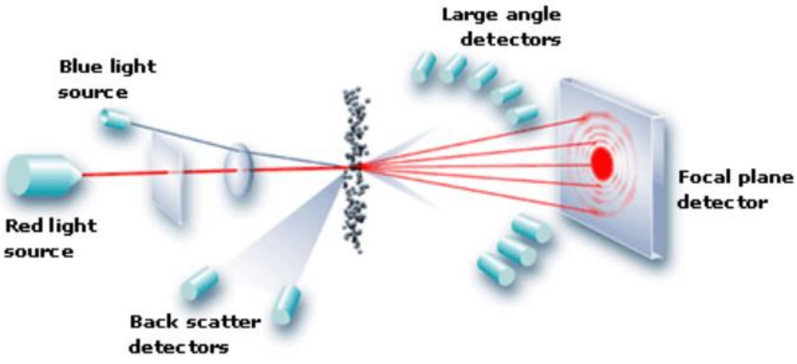


Figure 4. 4 Laser diffraction technique

In this work, Mastersizer 2000 from Malvern is the used device for particle size measurements by laser diffraction (Figure 4.6). It has two operation modules, wet (Hydro 2000MU) and air (Scirocco 2000) dispersion and it is the most widely used particle sizing instrument in the world. Mastersizer software allows to apply Standar Operating Procedures (SOP). The used SOP depends on the dispersion unit type (wet or dry), material to be measured and dispersant (with optical properties) and measurent time, among others [79][80]. Some technical specifications of Mastersizer 2000 are summarized in Table 4.4.

Figure 4. 5 Mastersizer 2000 from Malvern with wet module (left) [78] and a schematic of how laser diffraction technique is placed inside (right) [81]

Table 4. 4 Technical specifications of Mastersizer 2000 [79]

MASTERSIZER 2000	
Size range	0.02 µm to 2000 µm
Measurement principle	Mie scattering
Light sources	Red light: Helium-neon laser Blue light: Solid-state light source
Mastersizer Weight	31.0 kg
Mastersizer Dimensions (W x D x H)	1293 x 255 x 375
Hydro 2000MU Weight	15.4
Hydro 2000MU Dimensions (W x D x H)	320 x 375 x 335/490

To avoid agglomerations, Hydro 2000MU is used (wet dispersion type)(See Figure 4.7). The used SOP is Velten-Feucht-Wachs, based on Velten's work [82]. First of all, 800 mL of water is added in a 1000 mL beaker and it circulates through a cell where laser diffraction takes place. Circulation of water is caused by a turbine, whose speed can be adjusted (in this thesis, 2500 rpm). A measure of the background is done first to avoid that impurities of the dispersant or in the windows and lenses and also electrical noise affect the scattering pattern from the sample. Then, a necessary amount of capsules is added, without stopping the turbine. A 2% Tween solution is also added to avoid agglomerations between capsules. The Mastersizer determines the correct concentration of the sample by measuring the amount of laser light that has been lost by passing it through the sample (obscuration). For a proper measurement, obscuration value has to be between 10 and 20 %. The particle size is reported as a volume equivalent sphere diameter, the measure of each experiment is made three times and the average is provided. Apart from cumulative and distributive graphics, values of span, d_{10}, d_{50} and d_{90} as well as $d_{4,3}$ and $d_{3,2}$ are also given [80].

Figure 4. 6 Hydro 2000MU Module of Mastersizer 2000

4.2.2 Particle morphology observation

Studying the morphology of particles is important to know how homogeneous and spherical they are. If the capsules are large enough, the human eye is useful to test them but in microencapsulation technology, a microscope is necessary.

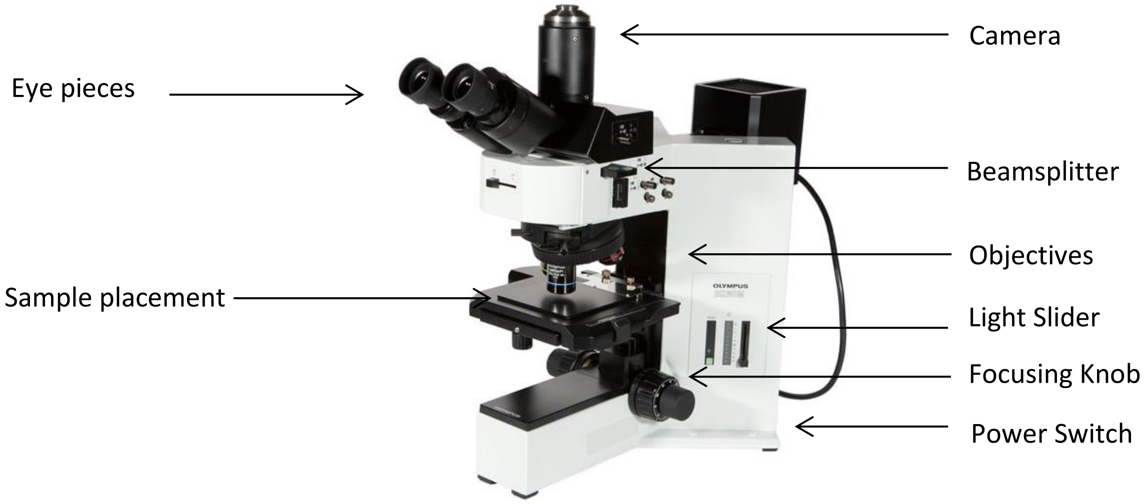


Figure 4. 7 BX-60 Motorized Fluorescence Microscope. Modified from [83]

In this work, Olympus BX60 Microscope in transmitted and reflected light way was used (Figure 4.8). An incorporated camera allows watching samples in the computer. The sample is placed on plates before putting under the microscope. The light intensity and focus can be adjusted to get a better image of the samples. Three different kinds of objectives were used: 5x (red), 20x (green) and 50x (blue). Table 4.5 summarize some characteristics of this microscope but further information can be found in literature [83][84].

Table 4. 5 Characteristics of BX-60 Motorized Fluorescence Microscope [83]

BX-60 MOTORIZED FLUORESCENCE MICROSCOPE	
Height	17mm
Travel Range	102mm x 102 mm
Weight	3.95 kg
Encoder Resolution	0.1 µm
Speed	30 mm/sec
Objectives	UMPlanFl 5X/0.15 BD ∞/0
	UMPlanFl 20X/0.46 BD ∞/0
	UMPlanFl 50X/0.80 BD ∞/0

4.2.3 Encapsulation efficiency testing

The used method for testing the encapsulation efficiency (EE) of microcapsules is conductivity measurements. To this aim, a known concentration solution of Sodium Chloride is encapsulated. The EE value is calculated by two different ways. In the first one, the conductivity of the external water phase is measured. The more salt in the external water phase, the greater its conductivity. It means less salt in the internal water phase and, therefore, less encapsulation efficiency. The formula used to know the value is:

$$EE = \frac{NaCl_{TOT} - NaCl_{ext.phase}}{NaCl_{TOT}} \tag{4. 18}$$

where, NaCl $_{TOT}$ is the total concentration of NaCl added to the inner water phase and NaCl $_{ext.\ phase}$ is the measured conductivity of the external water phase.

The other way is based on the conductivity of an amount of capsules after being melted. To this aim, 1 g of capsules is mixed in 100 g of water and melted at 50 °C for 30 minutes. After that, the solution is left to cool at room temperature. It is used the next formula, taking into account that in each experiment it is obtained approximately 5 g of capsules:

$$EE = \frac{NaCl_{encapsulated} \cdot 5}{NaCl_{TOT}} \tag{4. 19}$$

where NaCl$_{encapsulated}$ is the measured conductivity of salt in water after melting the capsules.

The unit of conductivity is S/cm (µS/cm, mS/cm) and it is measured by using a conductivity cell to make a measurement of the electrical resistance [3]. A calibration curve that relates conductivity of the solution and salt concentration is necessary (Figure 4.9).

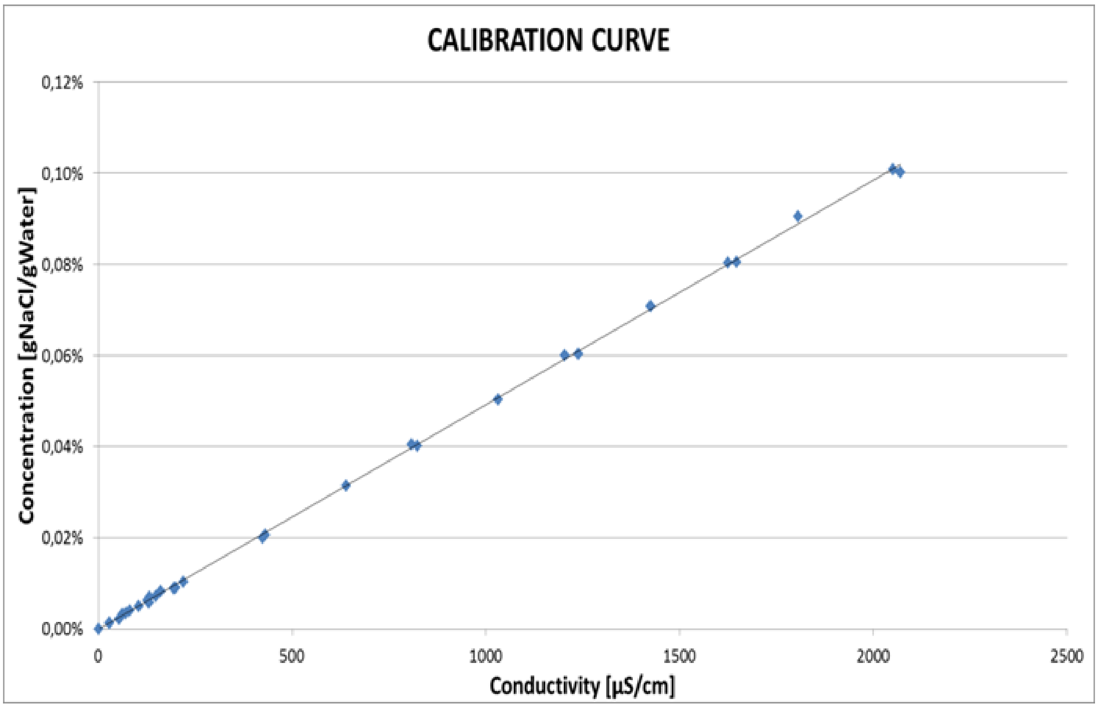


Figure 4. 8 Calibration curve

The relation between the concentration of NaCl (gNaCl/gWater) and their conductivity is given by Equation 4.1.

$$y = 4.929 \cdot 10^{-7}x - 1.401 \cdot 10^{-6} \quad ; \qquad R^2 = 0.9997 \tag{4. 20}$$

Figure 4. 9 Conductivity meter 3310 WTW [85] (left) and TetraCon 325 probe [88] (right)

In this work, the Cond 3310 brand WTW is used as a conductivity meter (Figure 4.9, left). It has a time-controlled data logging function, ideal for long test [85; 86]. The used conductivity probe is a TetraCon 325 conductivity cells (Figure 4.9, right), whose measuring principle is a 4-graphite-electrode conductivity cell measurement. An alternating voltage is applied to one electrode which causes a flow of ions between all the electrodes. The current is measured by the conductivimeter [87]. In Table 4.6 some properties of Cond 3310 are shown.

Table 4. 6 Specifications of Cond 3310 [86]

COND 3310	
Conductivity	0 µS to 1000 mS ± 0.5 % full-scale
Temperature	-5.0 °C to 105.0 °C ± 0.1 °C
Salinity	0 to 70.0 ppt ± 0.2 % full scale
TDS	0 to 200 ppt ± 0.5 % full scale
Display	LCD
Interface	USB
Waterproof	Yes

4.2.4 Particle stability testing

Stability of capsules was made by adding them to a determined amount of water and the conductivity during time intervals was measured. The more conductivity over time, the less stability. In this work, 1g of capsules was added to 100 mL of distilled water in a 150 mL beaker. To get a homogeneous blend, the capsules solution is shaken in a Digital Orbital Shaker Sea Star (Figure 4.10) at 150 rpm. After the desired time, the solution is filtered through MN 615 ¼ · Ø 185 mm filters from Macherey-Nagel and the conductivity is checked.

Figure 4. 10 Digital Orbital Shaker Sea Star [89]

Digital Orbital Shaker Sea Star is moved with circular movements whose speed can be adjusted. Furthermore, beakers, petri dishes or culture bottles can be fixed by using rubber straps. Some of characteristics of this device are given below (Table 4.7).

Table 4. 7 Characteristics of Digital Orbital Shaker Sea Star [89]

DIGITAL ORBITAL SHAKER SEA STAR	
Speed control range	20 to 300 rpm
Shaking amplitude (stroke)	19 mm
Timer	1 min to 49 h or continuous operation
Shaking surface area	279 x 279 mm
Dimensions (W x D x H)	314 x 305 x 183 mm
Loading Capacity	4.5 kg

5. SMALL SCALE EXPERIMENTS

Multiple $W_1/O/W_2$ emulsions were prepared in stirring plates and microcapsules were obtained at a laboratory production. There are several important factors that could affect the conditions of the emulsions as stirring time, stirring speed, temperature, kind of emulsifiers and their concentration, dispersed phase mass ratio and viscosity ratio between the others. Some of these parameters will be studied.

As it is said in Chapter 2.2.2 “Emulsifiers”, the effectiveness of the surfactant is related to its chemical structure and its “orientation at the interphase”. Furthermore, there is a surfactant at which it is achieved the maximum effectiveness of emulsifier at the interphase (surface saturation) [4]. Hence, it could be said that there should be an optimal emulsifier giving high stability and yield of preparation of the multiple emulsions [90].

The purpose of this Chapter is to study the difference between capsules obtained by multiple emulsions stabilized by various emulsifiers and the influence that the dispersed phase volume fraction has on the encapsulation efficiency. Furthermore, the stability of capsules stabilized by SPAN 80, SPAN 65, TWEEN 80 and without emulsifiers will be observed.

5.1 DISPERSED PHASE VOLUME FRACTION

5.1.1 Parameters and experiments done

As a preliminary investigation, the effect of dispersed phase mass fraction (φ) and the concentration of emulsifier on $W_1/O/W_2$ emulsion are examined. Encapsulation efficiency and particle size distribution were calculated. To this aim, SPAN 20 and SPAN 80 were used as emulsifiers, 10% NaCl solution as inner water phase, 5 g of Witepsol W31 as oil phase and 30 g of second water phase (W_2) were chosen. Capsules were prepared following two-step emulsification method explained in Chapter 4.1.1 “Stirring plates”. The operative temperature was selected at 45 °C, enough to keep melted the Witepsol. 80 g of cold water around 4 °C was necessary to decrease the temperature and solidify the hard fat. First and second emulsions were carried out for 5 minutes each and were magnetically stirred at 750 rpm. These parameters are summarized in Table 5.1.

Table 5. 1 Parameters used in influence of dispersed phase volume fraction experiments

PARAMETERS	
Concentration of NaCl	10%
Witepsol [g]	5
W_2 [g]	30
Cold water [g]	80
Temperature [°C]	45
t_1 [min]	5
t_2 [min]	5
Stirring speed [rpm]	750

Dispersed phase mass fraction was changed from 1:6 to 4:6 in four intervals for concentrations of each emulsifier (Concentration of emulsifiers: 5%, 10% and 20%). Encapsulation efficiency was calculated by measuring the conductivity of the external water phase after filtering the capsules (See Chapter 4.2.3, “Encapsulation efficiency testing”, Equation 4.2). Particle size distributions as well as d_{50} value were obtained by laser diffraction technique in Mastersizer 2000 one day after their preparation (See Chapter 4.2.1. “Particle size measurements”).

Each experiment was done at least twice, to give consistency to the results. In Table 5.2 are summarize the experiments done.

Table 5. 2 SPAN 80 experiments which have been done in influence of dispersed phase mass fraction

SAMPLE	EMULSIFIER		Φ
150203_1; 150203_2; 150203_3	SPAN 80	5%	1:6
150309_5; 150309_6	SPAN 80	5%	2:6
150310_1; 150310_2	SPAN 80	5%	3:6
150313_1; 150313_2	SPAN 80	5%	4:6
150122_1; 150320_1	SPAN 80	10%	1:6
150122_2; 150320_2	SPAN 80	10%	2:6
150122_3; 150320_3	SPAN 80	10%	3:6
150122_4; 150320_4	SPAN 80	10%	4:6
141218_2; 150108_8; 150116_1	SPAN 80	20%	1:6
141218_6; 150108_3; 150116_2	SPAN 80	20%	2:6
150108_4; 150113_1; 150116_3	SPAN 80	20%	3:6
150108_5; 150113_4; 150116_4	SPAN 80	20%	4:6

Table 5. 3 SPAN 20 experiments which have been done in influence of dispersed phase mass fraction

SAMPLE	EMULSIFIER		Φ
150205_1; 150205_2; 150205_3	SPAN 20	5%	1:6
150313_3; 150313_4	SPAN 20	5%	2:6
150318_1; 150318_2	SPAN 20	5%	3:6
150318_3; 150318_4	SPAN 20	5%	4:6
150127_1; 150320_5	SPAN 20	10%	1:6
150127_2; 150320_6	SPAN 20	10%	2:6
150127_3; 150320_7	SPAN 20	10%	3:6
150127_4; 150120_8	SPAN 20	10%	4:6
141218_4; 150108_7; 150120_1; 150127_5	SPAN 20	20%	1:6
141218_5; 150108_8; 150120_2; 150127_6	SPAN 20	20%	2:6
150108_9; 150113_2, 150120_3; 150127_7	SPAN 20	20%	3:6
150108_10; 150113_3; 150120_4; 150127_8	SPAN 20	20%	4:6

5.1.2 Results and comments

In Tables 5.4 and 5.5 the results for the previous mentioned experiments are shown, where ϕ is the parameter used to refer the dispersed phase volume fraction, encapsulation efficiency (EE) is given in % and d_{50} in μm. As it was said in Chapter 2.2.1.2.3 “Particle size distribution”, d_{50} diameter indicates that the 50% of the total volume of the microcapsules is obtained by particles with a diameter equal or less than d_{50}. The reproducibility of EE and d_{50} is also given.

Table 5. 4 Results of SPAN 80 experiments in influence of dispersed phase mass fraction

SAMPLE	EMULSIFIER		Φ	EE [%]	d_{50} [μm]
150203_1; 150203_2; 150203_3	SPAN 80	5%	1:6	36 ± 8	171.9 ± 13.9
150309_5; 150309_6	SPAN 80	5%	2:6	27 ± 19	141.1 ± 32.9
150310_1; 150310_2	SPAN 80	5%	3:6	14 ± 7	156.4 ± 7.6
150313_1; 150313_2	SPAN 80	5%	4:6	13 ± 5	138.5 ± 15.5
150122_1; 150320_1	SPAN 80	10%	1:6	38 ± 20	122.5 ± 32.8
150122_2; 150320_2	SPAN 80	10%	2:6	33 ± 15	139.8 ± 12.9
150122_3; 150320_3	SPAN 80	10%	3:6	28 ± 18	155.9 ± 7.1
150122_4; 150320_4	SPAN 80	10%	4:6	15 ± 5	155.4 ± 28.6
141218_2; 150108_8; 150116_1	SPAN 80	20%	1:6	38 ± 11	250.3
141218_6; 150108_3; 150116_2	SPAN 80	20%	2:6	27 ± 21	312.2
150108_4; 150113_1; 150116_3	SPAN 80	20%	3:6	17 ± 15	358.2
150108_5; 150113_4; 150116_4	SPAN 80	20%	4:6	13 ± 11	756.5

Table 5. 5 Results of SPAN 20 experiments in influence of dispersed phase mass fraction

SAMPLE	EMULSIFIER		Φ	EE [%]	d50 [μm]
150205_1; 150205_2; 150205_3	SPAN 20	5%	1:6	37 ± 16	145.2 ± 23.4
150313_3; 150313_4	SPAN 20	5%	2:6	19 ± 5	165.3 ± 4
150318_1; 150318_2	SPAN 20	5%	3:6	13 ± 0	147.9 ± 3.3
150318_3; 150318_4	SPAN 20	5%	4:6	6 ± 1	157.3 ± 4.8
150127_1; 150320_5	SPAN 20	10%	1:6	37 ± 18	156.9 ± 18.5
150127_2; 150320_6	SPAN 20	10%	2:6	39 ± 12	143.5 ± 1.8
150127_3; 150320_7	SPAN 20	10%	3:6	27 ± 11	153.2 ± 19.7
150127_4; 150120_8	SPAN 20	10%	4:6	16 ± 2	185.9 ± 75.4
141218_4; 150108_7; 150120_1; 150127_5	SPAN 20	20%	1:6	42 ± 12	173.1
141218_5; 150108_8; 150120_2; 150127_6	SPAN 20	20%	2:6	38 ± 14	193.2
150108_9; 150113_2, 150120_3; 150127_7	SPAN 20	20%	3:6	21 ± 15	193.2
150108_10; 150113_3; 150120_4; 150127_8	SPAN 20	20%	4:6	2 ± 12	177.8

Average of EE and their corresponding error bars according to the type of emulsifier, dispersed phase volume fraction and concentration of emulsifiers are representing in Figures 5.1 and 5.2.

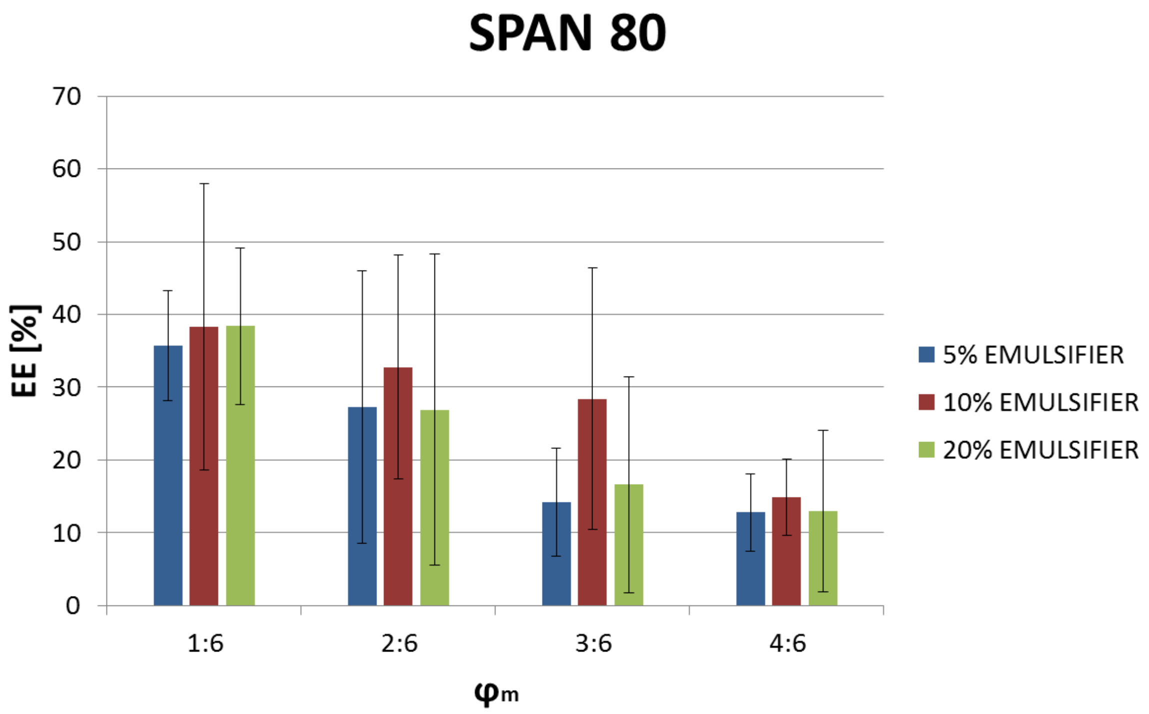


Figure 5. 1 Influence of dispersed phase volume fraction (ϕ) and concentration of SPAN 80 in Encapsulation Efficiency

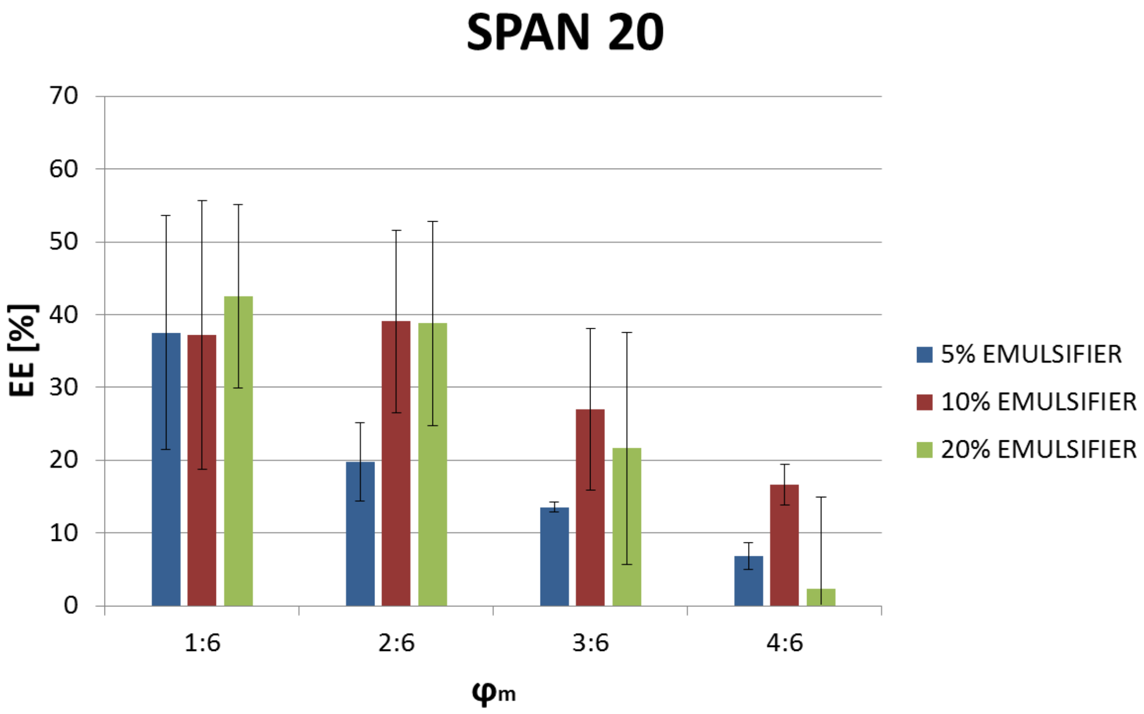


Figure 5. 2 Influence of dispersed phase volume fraction (ϕ) and concentration of SPAN 20 in Encapsulation Efficiency

It is observed that EE decreases as dispersed phase volume fraction increase, which has concordance with the literature review [91] (EE values less than 20% in all cases for ϕ=4:6). It could be expected that by increasing the amount of emulsifier, the emulsion quality improves without introducing additional mechanical energy. Nevertheless, capsules stabilized by 10% of emulsifiers show the best EE in almost all the experiments except on when ϕ=1:6. It may be because at a 20% of emulsifier the value is higher than the concentration that gives the maximum effectiveness, and micelles of emulsifiers can be formed [4].

Looking at the particle size, it appears to be no relation between particle size, concentration of emulsifier and dispersed phase volume fraction.

Regarding to the kind of emulsifier, there is not a significant difference between SPAN 80 and SPAN 20. With both emulsifiers, EE values are similar in most of the cases. It could be said that the mean particle size of capsules stabilized by SPAN 80 is bigger and more heterogeneous than those who were stabilized by SPAN 20. Anyway, the particles obtained in both cases are so big to be considered microcapsules.

Despite the 10% concentration of emulsifiers give the best EE values, it is a too high concentration to be used in food industry. Because of this, all the experiments from now will be done at a concentration of emulsifier of 5% or less than it.

5.2 HYDROPHILIC AND LIPOPHILIC EMULSIFIERS

5.1.1 Parameters and experiments done

For evaluating how emulsifiers affect the encapsulation efficiency, capsules made with different emulsifiers are evaluated according to their encapsulation efficiency, particle size distribution and appearance. Capsules were made in the same way as the previous experiments. In this case, 5% of emulsifier and 1:6 of dispersed phase volume fraction (ϕ) was selected. Experiments were carried out at 70 °C due to the melting point of emulsifiers (the highest melting point is 59-67 °C, SISTERNA SP 10C). These parameters are presented in Table 5.6.

Table 5. 6 Parameters used in lipophilic and hydrophilic experiments

PARAMETERS	
Mass fraction of NaCl	10%
Witepsol [g]	5
W_1 [g]	1
W_2 [g]	30
ϕ	1:6
Cold water [g]	80
Temperature [°C]	70
Emulsifier [%]	5
t_1 [min]	5
t_2 [min]	5
Stirring speed [rpm]	750

As lipophilic emulsifiers, SPAN 80, SPAN 65, SPAN 20, TEGO ALKANOL S2P, CUTINA GMS V and SISTERNA SP10-C were used, while as hydrophilic TWEEN 80, TEGO ALKANOL S20P, CUTINA E24, TEGO SML 20, TEGO SMS 60 and SISTERNA SP50 were employed. Their characteristics were described in Chapter 3.3 “Properties of emulsifiers”, and in Table 5.7 in given each corresponding HLB value.

Table 5. 7 Used lipophilic and hydrophilic emulsifiers with their corresponding HLB value.

LIPOPHILIC EMULSIFIERS	
PRODUCT NAME	**HLB**
SPAN 80	4.3
SPAN 65	2.1
SPAN 20	8.6
TEGO ALKANOL S2P	5
CUTINA GMS V	3,8
SISTERNA SP 10	2

HYDROPHILIC EMULSIFIERS	
PRODUCT NAME	**HLB**
TWEEN 80	15
TEGO ALKANOL S20P	15
CUTINA E 24	15
TEGO SML 20	17
TEGO SMS 60	15
SISTERNA SP 50	11

5.2.2 Conductivity of emulsifiers

As a previous experiment, conductivity of lipophilic and hydrophilic emulsifiers was measured to support that they do not influence the conductivity of the external water phase. In such a way, 0.25 g of lipophilic emulsifier was magnetically stirred and melted (when necessary) in 5 g of Witepsol W31 and 30 g of distilled water (which means 5% of emulsifier according to the oil phase) for 30 minutes. After that, the conductivity was measured. For hydrophilic emulsifier, 1.5 g was stirred and melted in 30 g of distilled water, which means 5% concentration of emulsifier in water, and the same procedure was applied. Results of conductivity of lipophilic and hydrophilic emulsifiers are shown in Tables 5.8 and 5.9, respectively.

Table 5. 8 Conductivity of lipophilic emulsifiers

LIPOPHILIC EMULSIFIERS			
EMULSIFIER	**EMULSIFIER/OIL PHASE RATIO**	**CONDUCTIVITY [µs/cm]**	**TEMPERATURE [°C]**
SPAN 80	5%	26.9	26
SPAN 20	5%	30.8	25.1
SPAN 65	5%	19.3	23.3
SISTERNA SP10	5%	16.3	26.3
CUTINA GMS V	5%	11.9	25.4
TEGO ALKANOL S2P	5%	16.0	25.8

Table 5. 9 Conductivity of hydrophilic emulsifiers

HYDROPHILIC EMULSIFIERS			
EMULSIFIER	**EMULSIFIER/WATER PHASE RATIO**	**CONDUCTIVITY [µs/cm]**	**TEMPERATURE [°C]**
TWEEN 80	5%	60.8	27.8
CUTINA E24	5%	60.6	33.1
SISTERNA SP50*	5%	NO DATA	NO DATA
TEGO ALKANOL S20P	5%	26.9	42.8
TEGO SML 20	5%	66.4	28.4
TEGO SMS 60	5%	225	39.9

*Sisterna SP50 showed a sticky aspect, it wasn't possible to measure the conductivity with the conductivity meter.

Hydrophilic emulsifiers exhibit more conductivity than lipophilic emulsifiers, because it was added more amount of emulsifier to carry out the test. It was checked that values are not high enough to reject the experiments, except for TEGO SMS 60, which 225 µS/cm can influence the results.

5.2.3 Results and comments

In Tables 5.10 and 5.11 are represented the encapsulation efficiency (EE) and the d_{50} of capsules made by different kind of emulsifiers, as well as their reproducibility. Also their morphology was observed using BX60 Microscope as it was explained in Chapter 4.2.2 "Particle morphology observation" after one day of their preparation. Each experiment was done three times. EE value was calculated through the first method presented in Chapter 4.2.3 "Encapsulation Efficiency testing", Equation 4.1.

Table 5. 10 EE and d50 of capsules stabilized by lipophilic emulsifiers at a concentration of 5%

LIPOPHILIC EMULSIFIERS					
SAMPLE	**EMULSIFIER**		**Φ**	**EE [%]**	**d_{50} [µm]**
150209_1; 150209_2; 150209_3	NO EMULSIFIER	-	1:6	40 ± 16	242.6 ± 54.9
150203_1; 150203_2; 150203_3	SPAN 80	5%	1:6	45 ± 7	206.2 ± 60.2
150203_7; 150203_8; 150203_9	SPAN 65	5%	1:6	55 ± 4	118.9 ± 18.8
150205_1; 150205_2; 150205_3	SPAN 20	5%	1:6	38 ± 16	145.2 ± 23.4
150130_4; 150130_5; 150130_6	TEGO ALKANOL S2P	5%	1:6	33 ± 18	188.1 ± 29.9
150129_1; 150129_2; 150129_3	CUTINA GMS V	5%	1:6	7 ± 9	108.3 ± 2.4
150128_1; 150128_2; 150128_3	SISTERNA SP 10	5%	1:6	53 ± 1	98.2 ± 12.4

Table 5. 11 EE and d50 of capsules stabilized by hydrophilic emulsifiers at a concentration of 5%

HYDROPHILIC EMULSIFIERS					
SAMPLE	**EMULSIFIER**		**Φ**	**EE [%]**	**d_{50} [µm]**
150216_1; 150216_2; 150216_3	TWEEN 80	5%	1:6	63 ± 3	136.7 ± 9.4
150302_3; 150302_4; 150302_5	TEGO ALKANOL S20P	5%	1:6	0 + 6	191.2 ± 25.6
150216_7; 150216_8; 150216_9	CUTINA E 24	5%	1:6	44 ± 11	142.1 ± 20
150218_1; 150218_2; 150218_3	TEGO SML 20	5%	1:6	44 ± 11	142.5 ± 12
150219_1; 150219_2; 150219_3	TEGO SMS 60	5%	1:6	48 ± 11	161.5 ± 9
150219_5; 150219_6; 150219_7	SISTERNA SP 50	5%	1:6	6 ± 23	118.7 ± 10

For a better explanation of how different lipophilic and hydrophilic emulsifiers affect the EE value, results and its corresponding reproducibility (error bars) are representing in Figures 5.3 and 5.4. Furthermore, particle size distribution is also displayed in Figures 5.5 and 5.6. Morphology of capsules made by the different emulsifiers is shown in Appendix II “Morphology of capsules”.

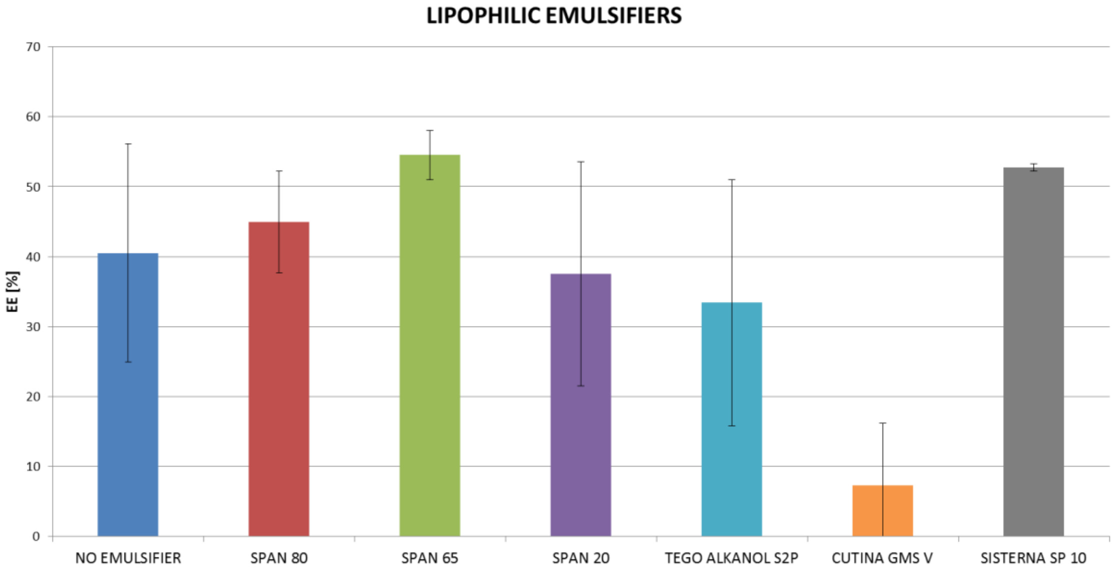


Figure 5. 3 Encapsulation efficiency of capsules stabilized by lipophilic emulsifiers

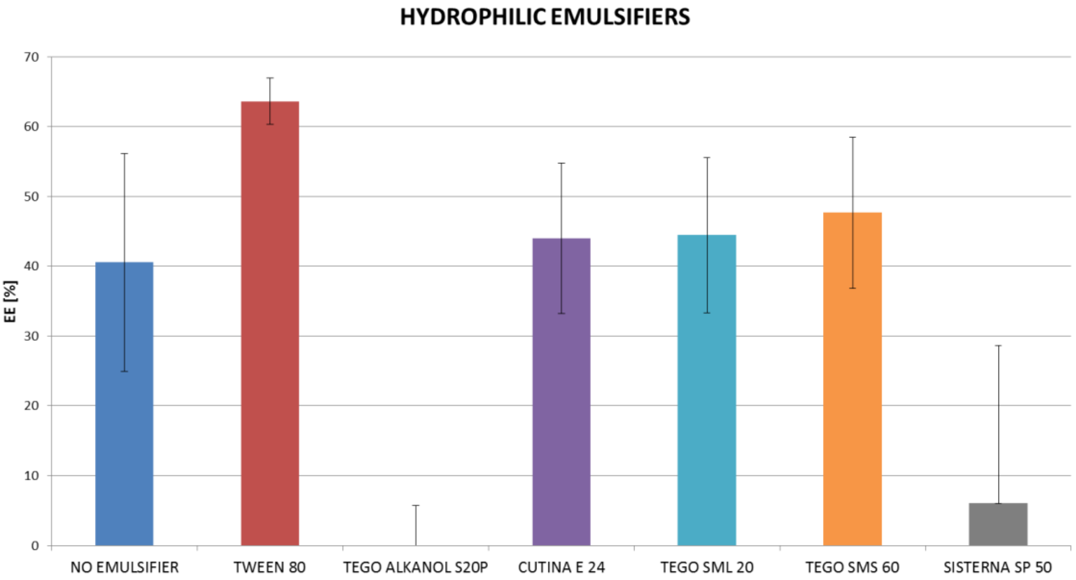


Figure 5. 4 Encapsulation efficiency of capsules stabilized by hydrophilic emulsifiers

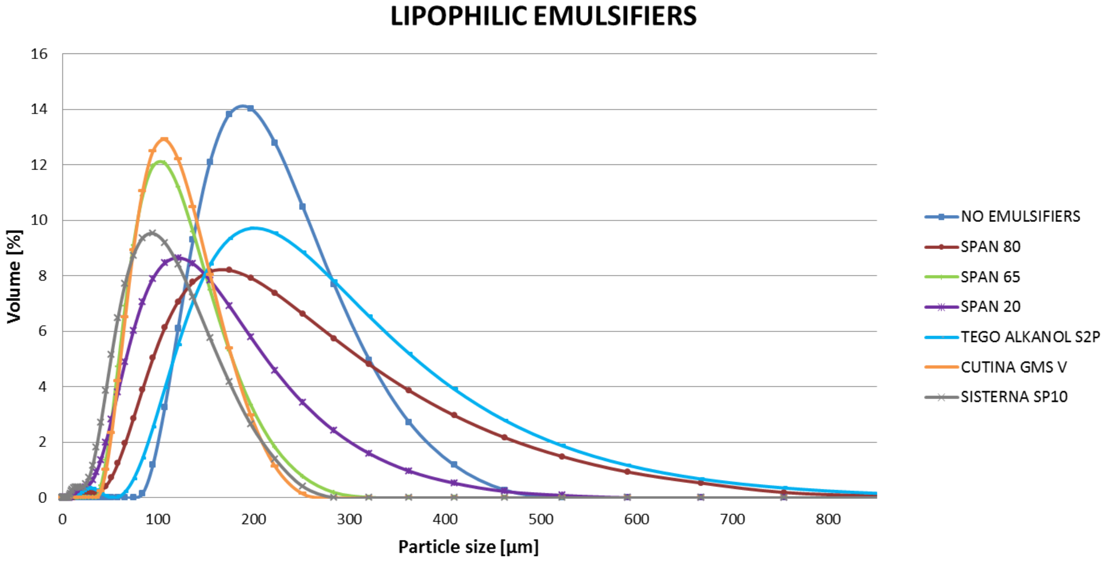


Figure 5. 5 Particle size distribution of capsules stabilized by lipophilic emulsifiers

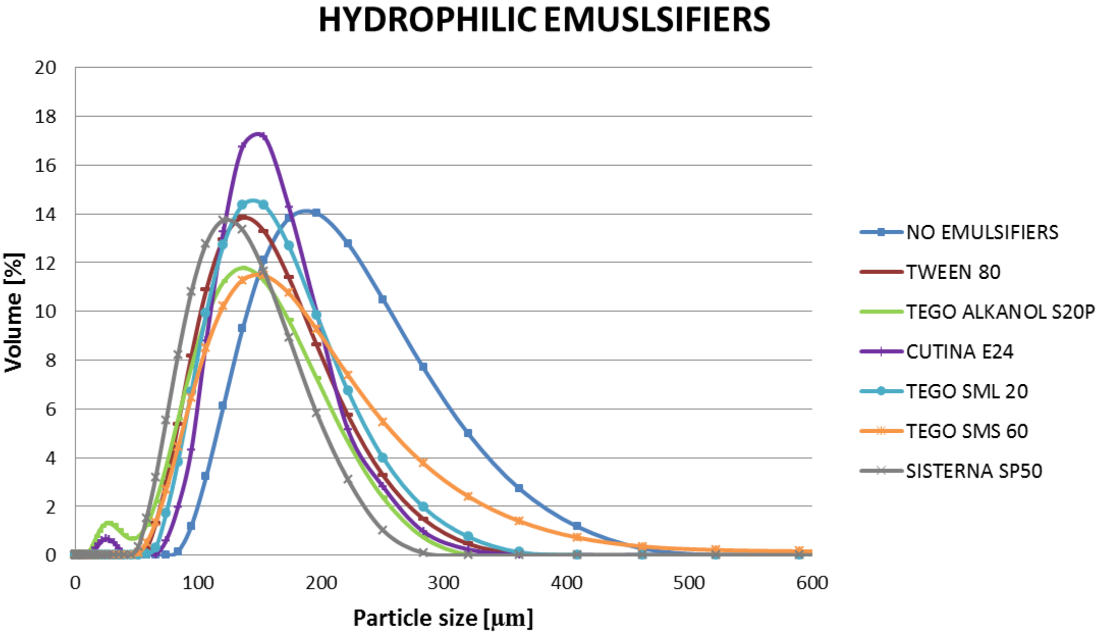


Figure 5. 6 Particle size distribution of capsules stabilized by lipophilic emulsifiers

Looking at particle size distribution of lipophilic emulsifiers (Figure 5.5), it could be said that the worst particle size distribution is given by SPAN 80 and TEGO ALKANOL S2P, with span values of 1.654 and 1.39, respectively, whereas the best are given by SPAN 65 (span=1.14), CUTINA GMS V (span=0.95) and SISTERNA SP 10-C (span=1.23).

In the case of hydrophilic emulsifiers (Figure 5.6), all of particle size distributions are quite good. The worst is obtained with TEGO SMS 60, with a span value of 1.165.

In Figure 5.7 is represented the EE of all used emulsifiers according to their HLB value. Different emulsifiers with similar HLB value provide very different values of encapsulation efficiency. It could be said that HLB value is important but is not the only one parameter that has to take into account. Compatibility of emulsifiers with the oil phase can explain some differences between the EE of emulsifiers tested.

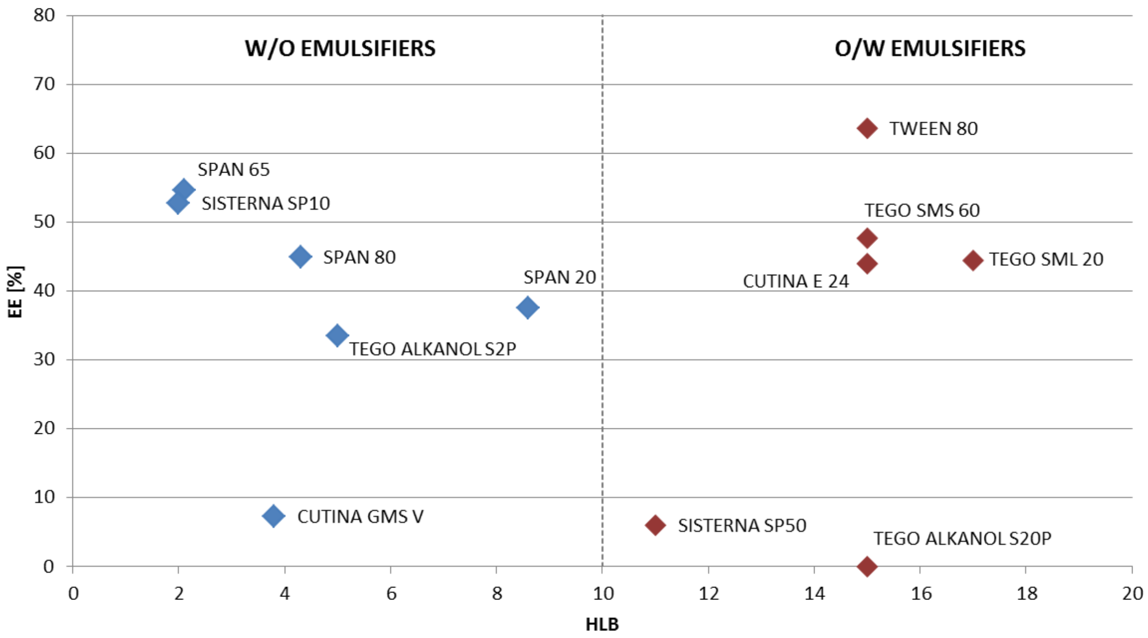


Figure 5. 7 Comparison of EE of lipophilic and hydrophilic emulsifiers according to their HLB value

SPAN 65 and SISTERNA SP 10-C are the lipophilic emulsifiers that present the best results regarding the encapsulation efficiency, the morphology of the capsules and the droplet size. If it is looked at their structures, both of them are very similar, with chains of 18 carbons and at least one ring in their hydrophilic region. As it was said in Chapter 3.1 “Shell Material”, Witepsol W31 is mainly composed by triglycerides obtained by transesterification of natural vegetable fatty acids with glycerol. That means that their structures may be very similar. Unfortunately, it is not known the right structure of the hard fat, consequently further studies have to be done to prove that the similarity between the emulsifiers and the oil phase has quite influence in the encapsulation efficiency. In spite of this, SISTERNA SP 10-C is not permitted in food in Europe [92], which is the aim of this work.

In the same way, TWEEN 80 is the best hydrophilic emulsifier that has been tested. It also has in their structure 3 chains of 2 carbons each and a ring in their hydrophilic region. Therefore, it is possible to apply the same conclusion as SPAN 65 and SISTERNA SP 10-C.

The efficient encapsulation of SPAN 80 can be due to their particular large particles, as it was checked in Table 5.10. TEGO SMS 60 has also a good EE value but it is has to take into account the high value of its conductivity after being mixed and heated with water (See Chapter 5.2.2 “Conductivity of emulsifiers”). It is observed that capsules made by no addition of emulsifier provide a really bad reproducibility as regards EE value.

Emulsifiers that present the worst EE value are CUTINA GMS V, SISTERNA SP50 and TEGO ALKANOL S20P. The low EE value of SISTERNA SP50 could be explained because of their low HLB value, that maybe it is not enough for O/W emulsifier.

It was decided to continue with the stability studies with the emulsifiers SPAN 80 because it is the more used; SPAN 65 because is the best lipophilic emulsifier and it is used in food industry in Europe unlike SISTERNA SP 10-C; and TWEEN 80 as hydrophilic emulsifier because it presents the best EE. These three emulsifiers will be compared to capsules in which was no added any emulsifier.

5.2.4 Indigo Carmine as a tracer

Experiments with Indigo Carmine as a marker were done for these selected emulsifiers (SPAN 80, SPAN 65 and TWEEN 80) and for capsules made by no addition of emulsifiers, to check under the light microscope how the encapsulation takes place. To this aim, 0.5 g of Indigo Carmine was dissolved in 50 g of distilled water to get a concentration of 1%. After this, 1 g of the solution was used as internal water phase (W_1) like in the previous experiments (ϕ=1:6). Capsules were left to dry for one day and after that morphology was observed. Results are shown in Figures 5.9 a), 5.9 b), 5.9 c), 5.9 d).

Capsules made without emulsifiers are quite heterogeneous and most of them are not spherical. Furthermore, the presence of indigo carmine in the inner water phase is very slight (Figure 5.9 a)).

On the other hand, it could be observed that the addition of emulsifier change completely the morphology of capsules. With SPAN 80 the formed capsules are spherical but have very differents size, as it was observed in particle size results in the previous Chapter (Table 5.10) (Figure 5.9 b)). It seems that particles are not so much coloured, what it is consistent with its EE value presented also in Table 5.10. The sphericity of the microcapsules stabilized by emulsifiers can be explain because of their distribution at the interphases (Figure 5.8)[93].

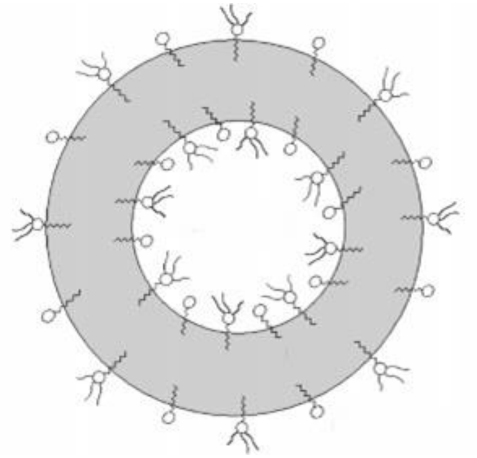


Figure 5. 8 Distribution of emulsifiers at the interphases [93].

Capsules in which used emulsifier were SPAN 65 show the best spherical and homogeneous particle size (Figure 5.9 c)).

TWEEN 80 capsules show the most entrapment of Indigo Carmine (Figure 5.9 d)). However, some particles are not spherical but stretched. That could be explained by the hydro-affinity of TWEEN 80. Water molecules of the inner water phase tend to diffuse through the oil phase, to come in contact with the hydrophilic particles. Once they are in contact, the water is absorbed in the interphase and it can bring to swelling of these particles [91].

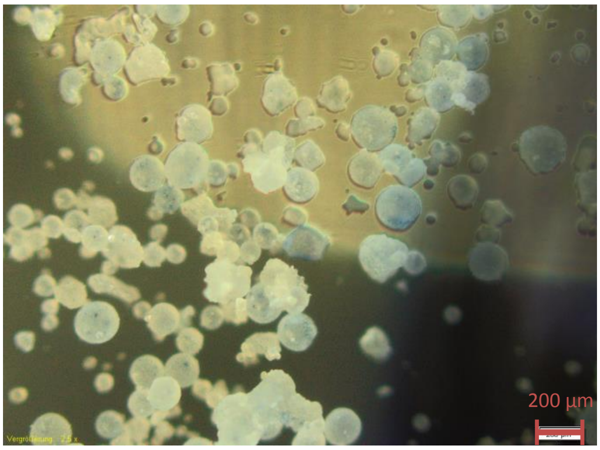


Figure 5. 9 a) Morphology of capsules made by 1% of Indigo Carmine as W1 and without emulsifiers.

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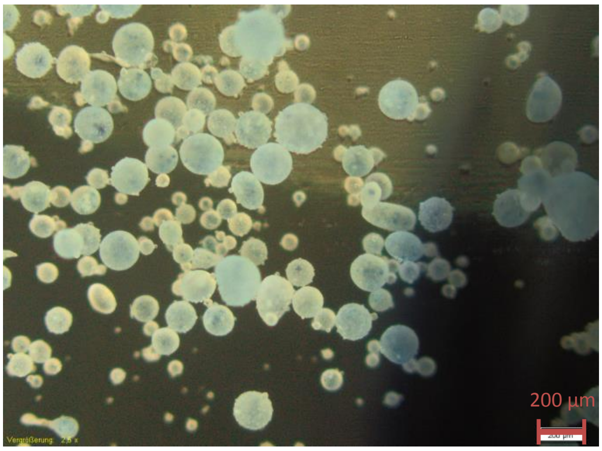

Figure 5. 9 b) Morphology of capsules made by 1% of Indigo Carmine as W1 and 5% of SPAN 80 as lipophilic emulsifier.

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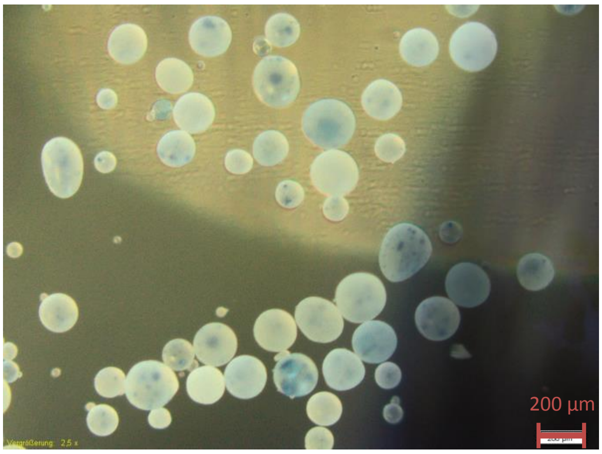

Figure 5. 9 c) Morphology of capsules made by 1% of Indigo Carmine as W1 and 5% of SPAN 65 as lipophilic emulsifier.

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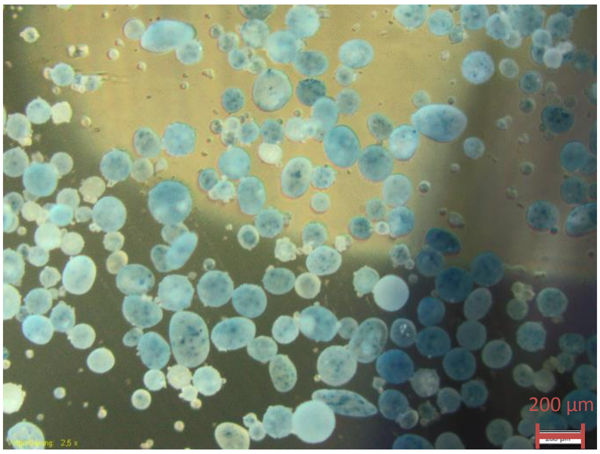

Figure 5.9 d) Morphology of capsules made by 1% of Indigo Carmine as W1 and 5% of TWEEN 80 as hydrophilic emulsifier.

Sample number 150309_4.

5.3 PARTICLE STABILITY TESTING

5.3.1 Parameters and experiments done.

This study was done as it was explained in Chapter 4.2.4 “Particle stability testing”. In each experiment, it was measured the conductivity of 1 g of capsules in 100 g of distilled water after seven different time intervals: 1, 5, 15 and 30 minutes, 1 hour, 2 and 4 hours. As lipophilic emulsifiers, SPAN 80 and SPAN 65 were selected, while as hydrophilic emulsifiers, TWEEN 80. Two concentration of each emulsifier (1 and 5%) was used to check how the amount of emulsifier affects the stability of capsules. Experiments without emulsifiers also were done to see the difference between adding or not an emulsifier.

Table 5. 12 Parameters used in particle stability testing

CAPSULES PARAMETERS	
Concentration of NaCl	10%
Witepsol [g]	5
W_1 [g]	1
W_2 [g]	30
ϕ	1:6
Cold water [g]	80
Temperature [°C]	70
t_1 [min]	5
t_2 [min]	5
Stirring speed [rpm]	750

STABILITY PARAMETERS	
Capsules [g]	1
Water [g]	100
Stirring speed [rpm]	150
Time intervals	1, 5, 15 and 30 minutes 1, 2 and 4 hours

5.3.3 Results and comments

For each emulsifier, 6 experiments were made. Then, according to their EE value measured by the first method (external water phase conductivity), 2 pairs of samples were chosen (i.e. capsules with EE more similar were evaluated together). In such a way, stability in the time was measured two times one day after the preparation of the capsules.

In Figures 5.10 and 5.11 the cumulative release of NaCl is represented in %. It means the NaCl releases in each time interval per NaCl total determined by the second method of encapsulation efficiency calculation (Chapter 4.2.4 “Encapsulation Efficiency testing”, Equation 4.2). All experiments were done at least twice to prove the consistency of them.

Table 5. 13 Cumulative NaCl released in % of capsules stabilized by SPAN 80, SPAN 65, TWEEN 80 and no emulsifiers at a concentration of 1%. Encapsulation efficiency calculated by the two methods, EE1 and EE2

NaCl RELEASED [%]; CONCENTRATION 1%				
TIME [min]	**NO EMULSIFIER**	**SPAN 80**	**SPAN 65**	**TWEEN 80**
1	6% ± 1%	5% ± 0%	6% ± 0%	6% ± 0%
5	7% ± 2%	6% ± 1%	7% ± 0%	7% ± 1%
15	9% ± 2%	7% ± 1%	9% ± %	8% ± 1%
30	9% ± 2%	8% ± 1%	15% ± 1%	16% ± 5%
60	10% ± 3%	10% ± 1%	20% ± 0%	38% ± 14%
120	12% ± 3%	13% ± 1%	40% ± 3%	75% ± 12%
240	15% ± 1%	23% ± 3%	88% ± 6%	88% ± 8%
EE$_1$ [%]	37% ± 24%	57% ± 3%	50% ± 8%	65% ± 1%
EE$_2$ [%]	36% ± 1%	100% ± 1%	59% ± 5%	76% ± 1%

Table 5. 14 Cumulative NaCl released in % of capsules stabilized by SPAN 80, SPAN 65, TWEEN 80 and no emulsifiers at a concentration of 5%. Encapsulation efficiency calculated by the two methods, EE1 and EE2

NaCl RELEASED [%]; CONCENTRATION 5%				
TIME [min]	**NO EMULSIFIER**	**SPAN 80**	**SPAN 65**	**TWEEN 80**
1	6% ± 1%	10% ± 1%	5% ± 1%	11% ± 0%
5	7% ± 2%	12% ± 2%	5% ± 1%	15% ± 2%
15	9% ± 2%	14% ± 2%	5% ± 1%	38% ± 4%
30	9% ± 2%	15% ± 3%	6% ± 1%	64% ± 9%
60	10% ± 3%	16% ± 3%	8% ± 1%	82% ± 15%
120	12% ± 3%	18% ± 3%	17% ± 4%	86% ± 18%
240	15% ± 1%	22% ± 1%	41% ± 6%	92% ± 12%
EE$_1$ [%]	37% ± 24%	21% ± 3%	56% ± 4%	59% ± 9%
EE$_2$ [%]	36% ± 1%	65% ± 3%	87% ± 2%	56% ± 4%

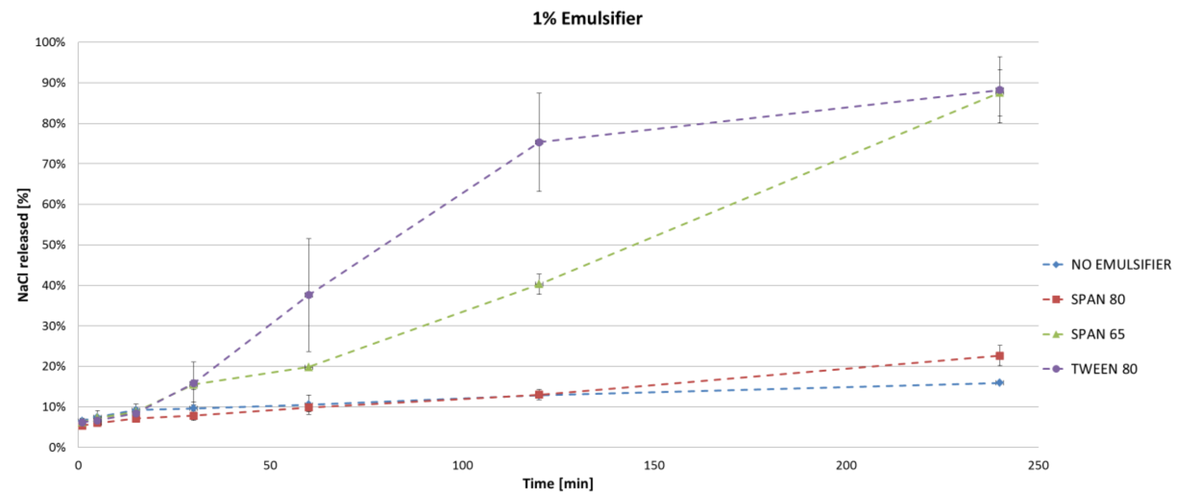

Figure 5. 10 Cumulative NaCl released in % since initial moment till 4 hours later, of capsules stabilized by SPAN 80, SPAN 65, TWEEN 80 and no emulsifiers at a concentration of 1%

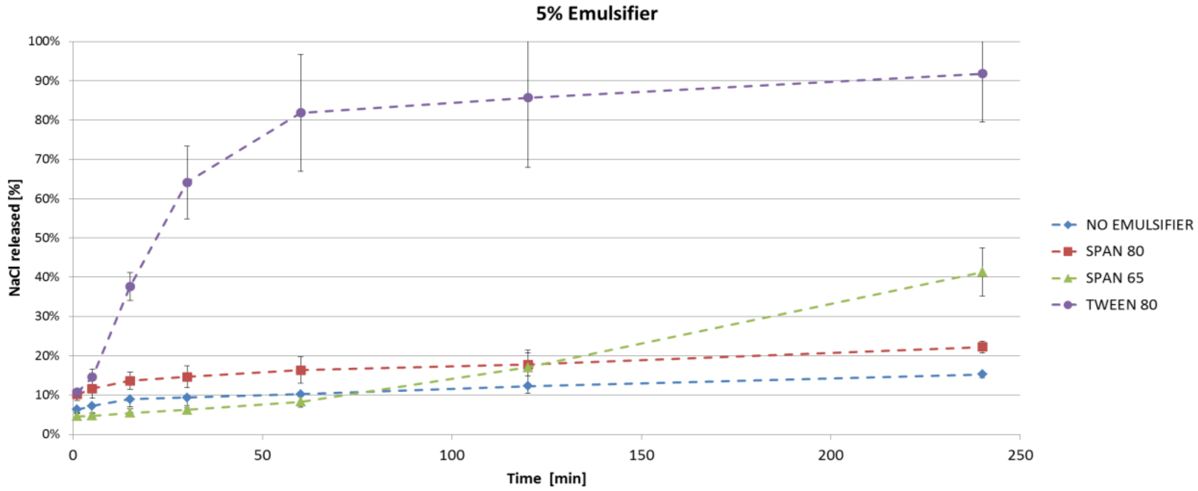

Figure 5. 11 Cumulative NaCl released in % since initial moment till 4 hours later, of capsules stabilized by SPAN 80, SPAN 65, TWEEN 80 and no emulsifiers at a concentration of 5%

Different stabilities can be explained by the particle size and the nature of the interface [2].

SPAN 80 shows a really good stability both at 1% of concentration and at 5%. In Chapter 5.2 “hydrophilic and lipophilic emulsifiers”, it was verified that capsules in which it was added SPAN 80 have a bigger d_{50} (and span) than those where other emulsifier were added. In larger particles, the rupture of the inner droplets could take more time which brings to an improvement in stability [90]. That could be the reason why SPAN 80 shows that good stability. In such a way, in SPAN 65 capsules, since one hour the stability decreases sharply. With SPAN 65 the smaller particle size was obtained, and in this case it is easier and faster for inner water phase to pass through the fat phase [90; 93].

Regarding the concentration of emulsifier, it could be seen that capsules with 5% of SPAN 65 are more stable in time than those who have 1% of concentration of this emulsifier. In the same way, 5% SPAN 80 capsules are more stable in the time that 1% SPAN 80 capsules apart from the initial moment. This is may be due to content of emulsifiers as spans have an influence on the structure of the capsules. They have more compact walls when more emulsifiers are around the interface. When the interface between the phases is firm, the rupture of the inner droplets is more difficult. Hence, the stability is increased [90; 93].

As concentration of TWEEN 80 increases, the stability of capsules decreases. This can be explained because of the affinity of TWEEN 80 to water. Molecules of TWEEN 80 are placed in the interphase. Nevertheless, water molecules of the inner water phase tend to diffuse through the fat phase, to come in contact with the hydrophilic particles, as it was said in the previous chapter. This could help the diffusion of inner water phase through the fat phase. The higher the concentration of hydrophilic emulsifier, the faster is the release [91].

About no addition of emulsifier, its stability is quite constant. However, it is remarkable to say that it was not obtained small particles, but they were agglomerated and they formed as single sheets of capsules. It is because of this it is necessary the addition of emulsifiers to get small particles. Encapsulation efficiency for capsules with no emulsifier was also lower than the one obtained by adding emulsifiers. Further investigations have to be done in order to understand whether this could affect the release.

Encapsulation efficiency of capsules was determined using both methods of calculation presented in Chapter 4.2.4 “Encapsulation efficiency testing” (Equations 4.1 and 4.2). The purpose of calculate it by two ways was check the consistency of this method. In Figures 5.12 and 5.13 are represented the encapsulation efficiency of capsules made by adding 1% and 5% of emulsifier, respectively.

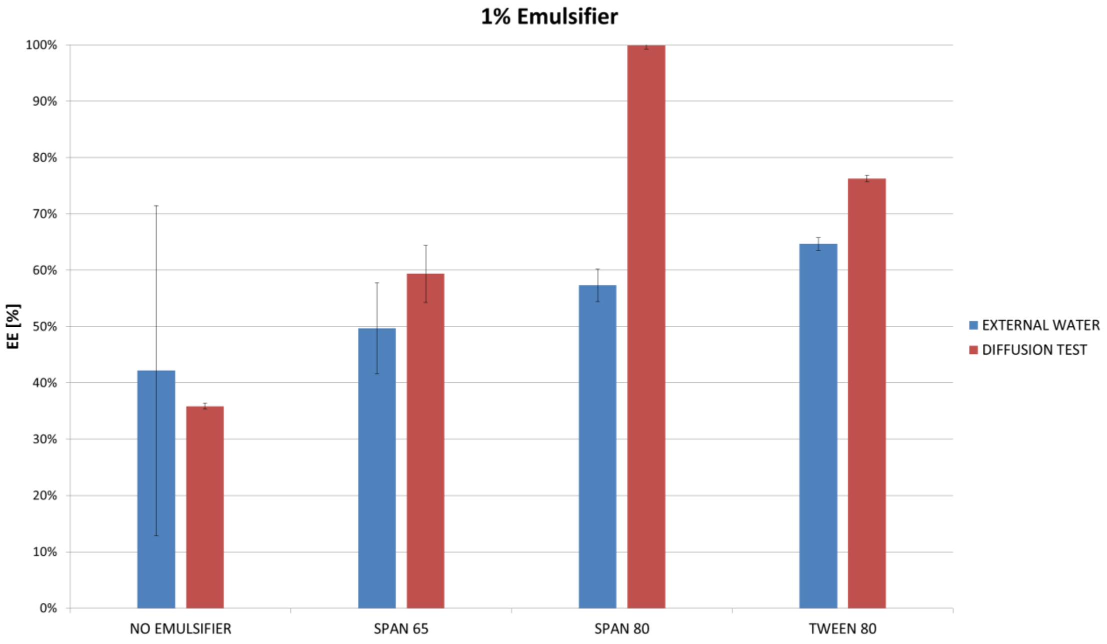

Figure 5. 12 Encapsulation efficiency of capsules in which they are added no emulsifier, SPAN 80, SPAN 65 and TWEEN 80 in a concentration of 5%. It is calculated by method one, external water conductivity measurements, and method 2 diffusion water after melting.

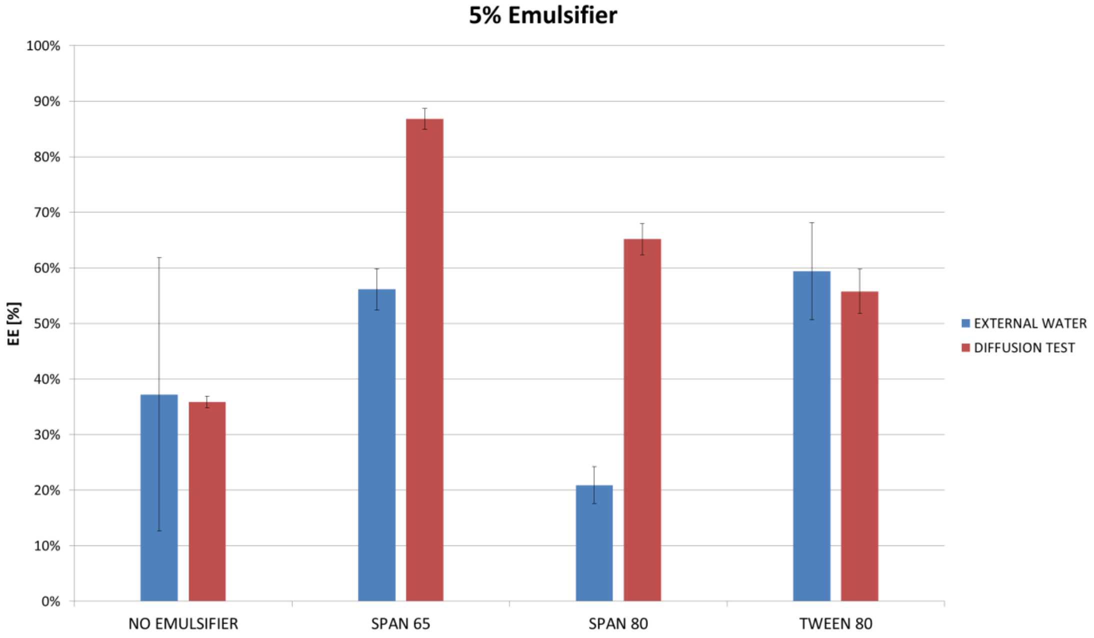

Figure 5. 13 Encapsulation efficiency of capsules in which they are added no emulsifier, SPAN 80, SPAN 65 and TWEEN 80 in a concentration of 5%. It is calculated by method one, external water conductivity measurements, and method 2 diffusion water after melting.

The encapsulation efficiency measured by both methods is similar in all cases except for SPAN 80 and SPAN 65 5%. Further investigations are necessary in order to determine with more precision the EE of capsules using this method.

It is observed that no addition of emulsifier provides the most error as regards the encapsulation efficiency value measured by the first method.

The difference between the results obtained by the two methods can be influenced by the entrapment of NaCl in the pores of the used filters.

6. LARGE-SCALE EXPERIMENTS

The aim of this chapter is to prove if what has been found in small scale can be extended to larger scale. To this end, Ultra-turrax system is used. Experiments at the same ratio $W_1/O/W_2$ and at different stirring speeds and stirring times will be done.

6.1 PARAMETERS AND EXPERIMENTS DONE

For large-scale experiments, it was used Ultra-turrax system as it was explaned in Chapter 4.1.2 “Ultra-turrax”. The ratios between the internal water phase (W_1), the oil phase (O) and the second water phase (W_2) is the same as in the small-scale experiments but the amount used is eleven times more. Concentration of NaCl as a tracer in W_1 is also 10 % and concentration of emuslifiers used is 5%. The first W_1/O emulsion was from a height of 20 mm from the bottom of the container till the rotor-stator of the Ultra-turrax, whereas the second $W_1/O/W_2$ emulsion was done from a height of 35 mm. These parameters are summarized in Table 6.1.

Table 6. 15 Parameters used in Ultra-turrax experiments

PARAMETERS	
Concentration of NaCl	10%
Witepsol [g]	55
W_1 [g]	11
W_2 [g]	330
ϕ	1:6
Cold water [g]	880
Temperature [°C]	70
Height W_1/O emulsion [mm]	20
Height $W_1/O/W_2$ emulsion [mm]	35
Emulsifier [%]	5 %

Experiments have been done for different stirring times and stirring speeds. SPAN 80, SPAN 65 and TWEEN 80 were used as emulsifiers to stabilize the microcapsules. Experiments without using any emulsifier have been done to test the influence of emulsifiers in microcapsules. The purpose of this is to check how these parameters affect the emulsion and if there is relation between experiments at small-scale and large-scale. Four different tests with different conditions of stirring times and stirring speeds were performed. In Table 6.2 all experiments carried out are shown.

Table 6. 16 Experiments done in Ultra-turrax system

TEST	SAMPLE	EMULSIFIER	W_1/O EMULSION		$W_1/O/W_2$ EMULSION	
			t_1 (min)	rpm_1	t_2 (min)	rpm_2
1	150312_1	NO EMULSIFIER	5	6400	5	4000
	150312_2	SPAN 80	5	6400	5	4000
	150312_3	SPAN 65	5	6400	5	4000
	150312_4	TWEEN 80	5	6400	5	4000
2	150316_1	NO EMULSIFIER	2	6400	3	4000
	150316_2	SPAN 80	2	6400	3	4000
	150316_3	SPAN 65	2	6400	3	4000
	150316_4	TWEEN 80	2	6400	3	4000
3	150317_1	NO EMULSIFIER	2	4000	3	4000
	150317_2	SPAN 80	2	4000	3	4000
	150317_3	SPAN 65	2	4000	3	4000
	150317_4	TWEEN 80	2	4000	3	4000
4	150319_1	NO EMULSIFIER	1	6400	2	4000
	150319_2	SPAN 80	1	6400	2	4000
	150319_3	SPAN 65	1	6400	2	4000
	150319_4	TWEEN 80	1	6400	2	4000

6.2 RESULTS AND COMMENTS

Encapsulation efficiency was calculated according to the second method of calculation (Chapter 4.2.3 “Encapsulation efficiency testing” Equation 4.2). The particle size was evaluated in Mastersizer 2000 as it is explained in Chapter 4.2.1 “Particle size measurements”. The time of the project was not enough and each experiment was done once, so there is no possibility to probe the consistency of results. Anyway, they are useful to get an idea about the particle size and the encapsulation efficiency value of the obtained microcapsules. In Table 6.3 the experiments and their results are summarized.

Table 6. 17 Results of experiments done in Ultra-turrax system

TEST	SAMPLE	EMULSIFIER	W/O EMULSION		W/O/W EMULSION		EE (%)	d_{50} (µm)
			t_1 (min)	rpm_1	t_2 (min)	rpm_2		
1	150312_1	NO EMULSIFIER	5	6400	5	4000	4	22.6
	150312_2	SPAN 80	5	6400	5	4000	4	33.4
	150312_3	SPAN 65	5	6400	5	4000	3	16.1
	150312_4	TWEEN 80	5	6400	5	4000	16	12.9
2	150316_1	NO EMULSIFIER	2	6400	3	4000	6	29.1
	150316_2	SPAN 80	2	6400	3	4000	10	39.2
	150316_3	SPAN 65	2	6400	3	4000	6	16
	150316_4	TWEEN 80	2	6400	3	4000	12	17.6
3	150317_1	NO EMULSIFIER	2	4000	3	4000	4	21.3
	150317_2	SPAN 80	2	4000	3	4000	9	34.9
	150317_3	SPAN 65	2	4000	3	4000	2	13.9
	150317_4	TWEEN 80	2	4000	3	4000	6	8.8
4	150319_1	NO EMULSIFIER	1	6400	2	4000	4	24
	150319_2	SPAN 80	1	6400	2	4000	6	108.9
	150319_3	SPAN 65	1	6400	2	4000	3	13.5
	150319_4	TWEEN 80	1	6400	2	4000	18	12.1

To compare in a better way the obtained results, values of EE are represented in Figure 6.1 according to the emulsifier and the stirring conditions. The encapsulation efficiency in all cases is very low (the maximum value of EE is obtained by TWEEN 80 microcapsules in the fourth test, but is less than 20%). This could be due to the high force used to form the second emulsion that has an influence on the first formed droplets, causing their disruption. Another possible explanation could be that the amount of emulsifier was not enough to cover all the formed interphases. This affect the stability of the emulsion. In all cases, further should be performed in order to compare EE values carried out in stirring plates and in Ultra-turrax systems.

Regarding to the particle size, it is not possible to notice any relation between the stirring time, the stirring speed and the d_{50} value. With each emulsifier, the particle size of the microcapsules is approximately constant in all experiments carried out: For no emulsifiers, around 24 µm; for SPAN 80, 54 µm; for SPAN 65, 15 µm; and for TWEEN 80, 13 µm. These sizes are too much smaller than those obtained by stirring plates, which is in accordance with the literature: the greater energy input, the smaller dispersed phase droplet size [1].

Due to the small particle size, it was not possible to separate and stabilize the microcapsules from the water. Pores of filters were not small enough and the smallest particles passed through them.

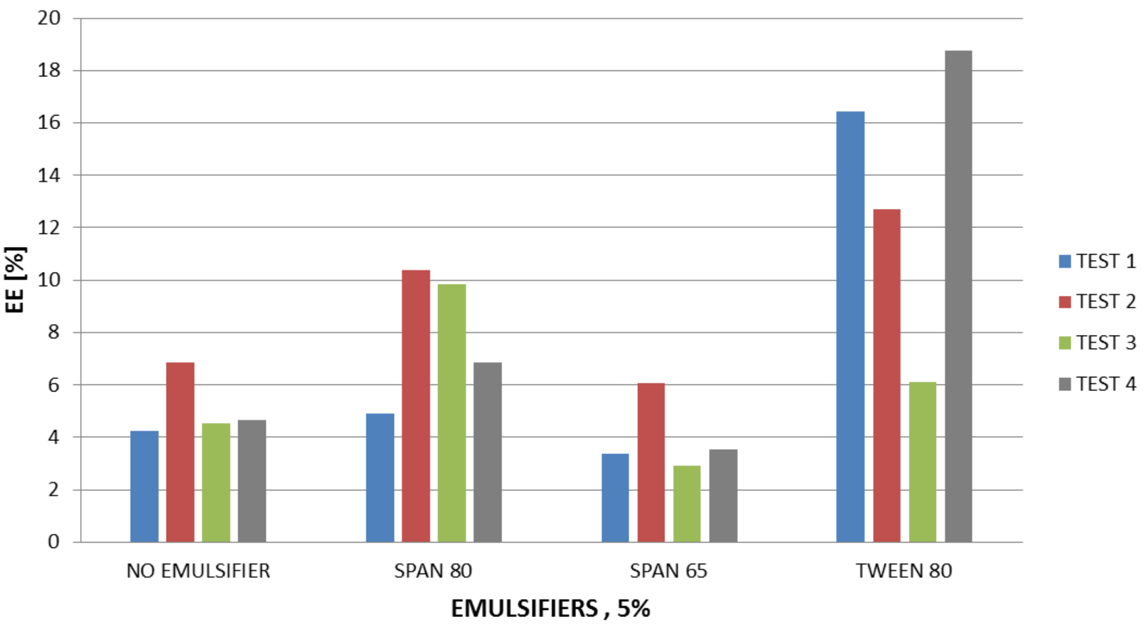

Figure 6. 1 Encapsulation efficiency of microcapsules made in Ultra-turrax at different speeds and times of stirring

It could be said that the best encapsulation efficiency is obtained by the conditions of the second test, excepting for TWEEN 80 experiments. As an example, particle size distribution of these microcapsules is represented in Figure 6.2, while the others can be found in Appendix III “Particle size of capsules”. In all cases, distributions of SPAN 65 and TWEEN 80 are quite good with d_{50} values less than 20 µm; whereas SPAN 80 and NO EMULSIFIERS distributions have two peaks, one around 50 µm and the other one around 150 µm. That may be explained by agglomerations of capsules while the measurement was carrying out. In any case, the EE value of SPAN 80 capsules could be due to their higher particle size regarding to the small particle size of SPAN 65 and TWEEN 80.

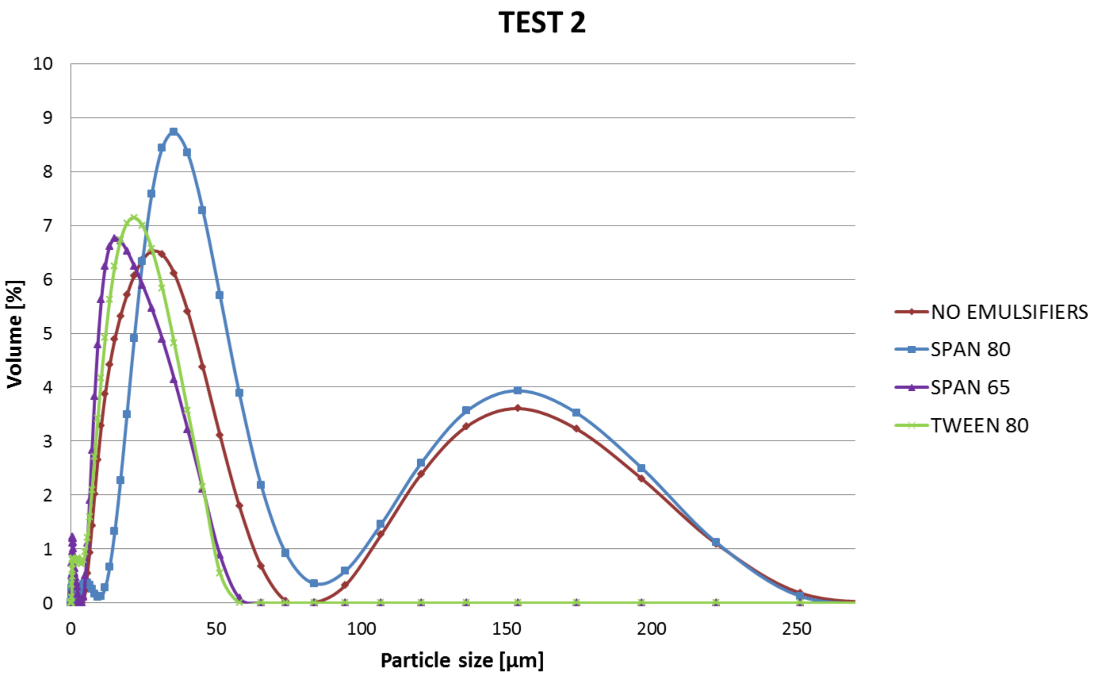

Figure 6. 2 Particle size distribution of microcapsules stabilized by SPAN 80, SPAN 65, TWEEN 80 and without emulsifiers in test 2 conditions.

Due to the low EE and the inability to separate capsules from water phase, it was decided not continue with Ultra-turrax system. Further investigations have to be done to find the stirring speed and stirring time conditions that give better results of EE as well as other method to stabilize the microcapsules obtained by using this procedure.

7-. CONCLUSIONS AND OUTLOOK

Capsules obtained by multiple $W_1/O/W_2$ emulsions method were prepared in stirring plates (small-scale experiments) and in Ultra-turrax system (large-scale experiments). The aim of this work was to evaluate some parameters that have influence in the encapsulation efficiency and the stability of microcapsules, as kind of emulsifier, dispersed phase volume fraction or stirring conditions. Furthermore, characteristics as particle size distribution and morphology of capsules were studied.

The encapsulation efficiency of microcapsules was determined by conductivity measurements, using a 10% NaCl solution in the inner water phase as a tracer. Two methods of EE calculation were studied. In the first one, conductivity of external water phase was measured after the preparation and filtration of microcapsules. In the other one, a selected amount of microcapsules was molten and stirred for a while. The conductivity was checked after that. Characteristic of microcapsules as particle size distribution, morphology and stability were also studied.

First of all, influence of the dispersed phase volume fraction and different concentration of emulsifiers in the encapsulation efficiency was studied. It is observed that EE decrease as dispersed phase volume fraction increase, obtaining EE values less than 20% in all cases for ϕ=4:6. Capsules stabilized by emulsifiers in a concentration of 10% show the best EE in almost all the experiments and not with the highest concentration tested, which can indicate that 10% of concentration could give the maximum effectiveness. Nonetheless, it is a high concentration to be used in food industry, and all the next experiments were done at a concentration of emulsifier of 5% or less than it. Regarding to the particle size, there appears to be no relation between the particle size, concentration of emulsifier and dispersed phase volume fraction.

The effectiveness of the surfactant is related to its chemical structure and its “orientation at the interphase”. Some lipophilic and hydrophilic emulsifiers were studied with the purpose of evaluating their influence in the encapsulation efficiency. The best values of encapsulation efficiency were obtained by SPAN 65 as lipophilic emulsifier (EE=55%) and TWEEN 80 as hydrophilic emulsifier (EE=64%) at a 5% concentration. In addition it is concluded that HLB value of emulsifiers is important but it also has to be taken into account the compatibility between they and the oil phase, as well as their concentration. With SPAN 65 quite spherical capsules where obtained whereas with TWEEN 80 some particles are stretched, maybe due to the water that it is absorbed in the interphase and it causes swelling. All particles obtained have a particle size higher than 100 µm so they cannot be considered as microcapsules.

Stability of microcapsules stabilized by SPAN 80, SPAN 65 and TWEEN 80 at concentrations of 1 and 5% was evaluated along with those who have not been stabilized by emulsifiers, in time intervals until four hours. To this aim, capsules prepared in these conditions were dissolved in SPAN 80 proved to be the most stable at the two concentrations, with only a 23% of NaCl released after 4 hours. There is a quite difference between capsules stabilized by SPAN 65, at 1% of concentration the stability is so much worse than at 5%. It can be due to the particle size: with SPAN 65 the microcapsules are smaller, what means there are more interphases and it is necessary a higher amount of emulsifier to cover all of them. TWEEN 80 shows the worst stability in water (90% of NaCl released after four hours), which can be explained in order to the affinity of water molecules to be in contact with the hydrophilic region of the emulsifier, promoting the release.

To finish off, parameters as stirring speed and stirring times were studied in Ultra-turrax system for microcapsules stabilize by SPAN 80, SPAN 65, TWEEN 80 and without emulsifiers. Four combinations of stirring speeds and stirring times were tested. The encapsulation efficiency in all cases is very low the maximum value of EE was less than 20% obtained by TWEEN 80 microcapsules. Main particle size was so small than size of capsules obtained by stirring plates confirming that the greater energy input, the smaller dispersed phase droplet size in the same conditions.

Regarding to the stability of microcapsules, more experiments should be done and for more time to observed if there is a relation between the characteristics of the emulsifier and the release of the inner water phase. In addition, more studies are necessary to prove the consistency of EE calculation by the two methods presented in this work.

Further investigations have to be done to get a better encapsulation efficiency of microcapsules obtained by Ultra-turrax system. Increasing the amount of emulsifier could be an option, to make sure that all the interphases are cover by the emulsifier and avoid coalescence between the inner droplets. In the same way, another method to separate the microcapsules from the water must be investigated.

Another method could be by using Particles from Gas Saturated Solution process (PGSS) after the production of the first emulsion. By this method, it is used a saturated gas to decrease the melting temperature and later obtain microcapsules instead of cold water. This could possibly avoid the possibility of disruption the inner droplets due to the high mechanical agitation. The PGSS would make much easier the handling of the microcapsules, making possible to skip the separation stage.

Likewise and to sum up, high pressure homogenizer experiments could be done to scale up the production and check the influence of pressure input in the second emulsion, as it was made in literature [3].

8-. LITERATURE

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APPENDIX

APPENDIX I

Group contributions in HLB system

Table A: anionic hydrophilic group contributions [15]

Hydrophilic group	HLB contribution	hydrophilic group	HLB contribution
– COO- Na+	19.1	– SO3- Na+	20.7
– O - SO3- Na+		20.8	

Table B: cationic and betaine hydrophilic group contributions [15]

Hydrophilic group HLB contribution		Hydrophilic group HLB contribution	
– NH3+ Cl-	20.0	– NH2+- CH2 - CH2 - CH2 – NH3+ 2 Cl- 38.6	
>NH2+ Cl-	20.0		
CH3 | – N+- CH3 Cl- | CH3	22.0	CH3 / > N+ Cl- \\ CH3	22.5
CH3 / > N+ Cl- \\ CH2 - Ø	19.7	CH3 | – N+- CH2 Ø Cl- | CH3	19.2
CH2 - CH2 – OH | – N+– H Cl- | CH2 - CH2 – OH	21.9	CH2 - CH2 – OH | – N+- CH3 Cl- | CH2 - CH2 – OH	24.9
CH3 CH3 | | – N+- CH2 - CH2 - CH2 - N+- CH3 2 Cl- | | CH3 CH3 43.5		CH2 – CH2 – OH / – NH+- CH2 - CH2 - CH2 – NH+ 2 Cl- | \\ CH2 - CH2 –- OH CH2 - CH2 – OH 41.4	
CH3 I – N → O I CH3	21.0 (at pH = 3)	CH2 - CH2 – OH I – N → O L CH2 - CH2 – OH	2 3.8 (at pH = 3)
CH3 | – N+- CH2 - COOH Cl- | CH3	24.1	CH3 | – N+- CH2 - COO- | CH3	38.0

Table C: cationic and nonionic hydrophilic group contributions [15]

hydrophilic group	HLB contribution	hydrophilic group	HLB contribution
– NH2	9.4	– NH - CH2 - CH2 – CH2 – NH2	17.4
> NH	9.4		
CH3 / – N \ CH3	8.5	CH2 - CH2 – OH / – N \ CH2 - CH2 – OH	11.3
CH2 - CH2 – OH / – N - CH2 - CH2 - CH2 - N \ CH2 - CH2 – OH CH2 - CH2 – OH	20.2	O ‖ – C - NH2	9.6
– COOH	2.1	– O –	1.3
– OH	1.9	– CH2 - CH2 –O –	0.33
		CH2 - CH2 – OH	0.95

Table D: hydrophobic group contributions [15]

hydrophobic group	HLB contribution	hydrophobic group	HLB contribution
– CH3	0.475	– CH = (unsaturated)	0.475
– CH2 -	0.475	– CH2 - Ø	1.66
– CH - (branched)	0.475	– CH2 – CH – O – CH3	0.15

APPENDIX II

Morphology of capsules

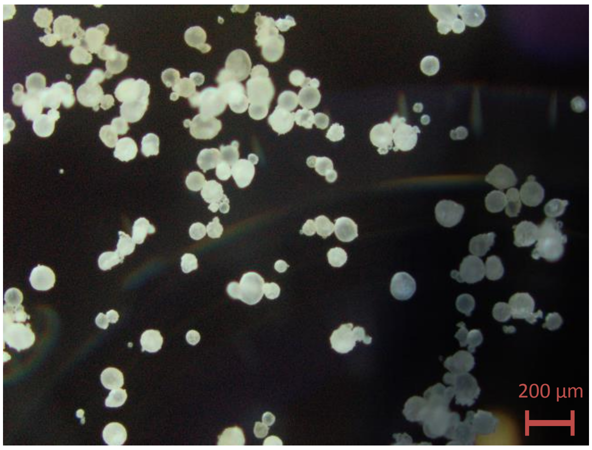


Figure A Microcapsules stabilized by SPAN 80

SAMPLE 150203_1

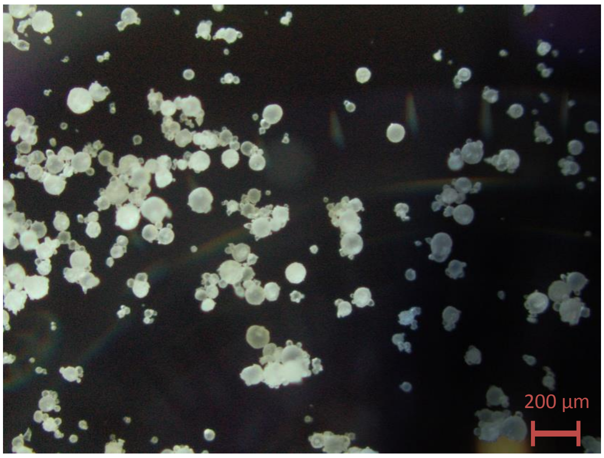


Figure B Microcapsules stabilized by SPAN 20

SAMPLE 150203_4

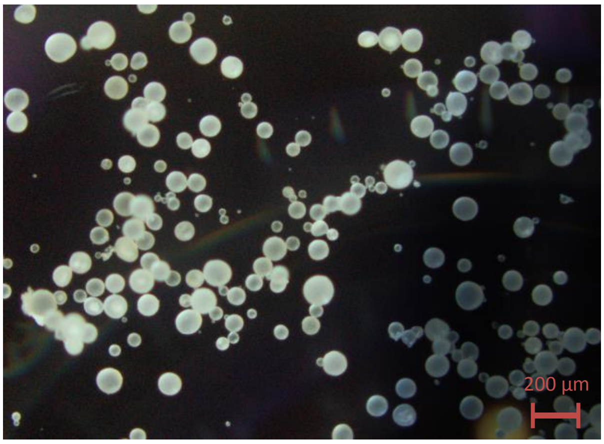


Figure C Microcapsules stabilized by SPAN 65

SAMPLE 150203_7

Figure D Microcapsules stabilized by TEGO ALKANOL S2P

SAMPLE 150130_4

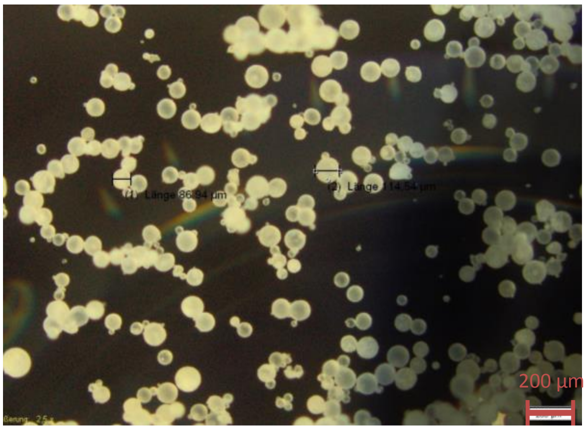


Figure E Microcapsules stabilized by CUTINA GMS V

SAMPLE 150129_1

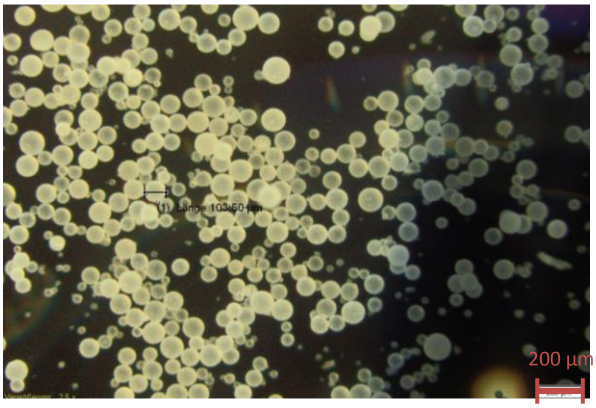


Figure E Microcapsules stabilized by SISTERNA SP10

SAMPLE 150128_1

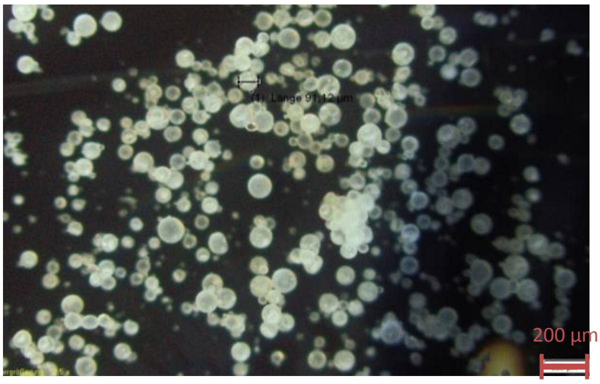
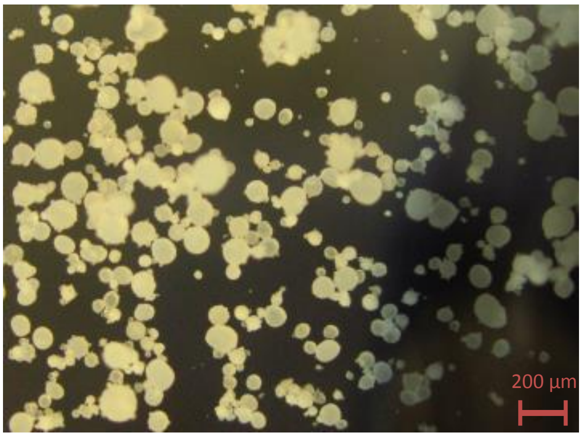


Figure F Microcapsules stabilized by TWEEN 80

SAMPLE 150216_1



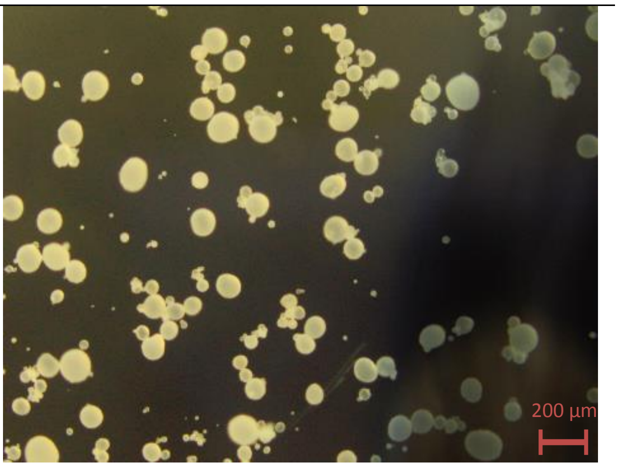


Figure G Microcapsules stabilized by TEGO ALKANOL S20P

SAMPLE 150216_4

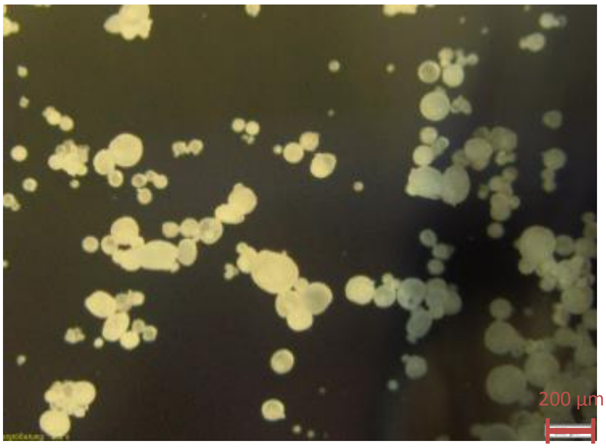


Figure H Microcapsules stabilized by CUTINA E 24

SAMPLE 150216_7

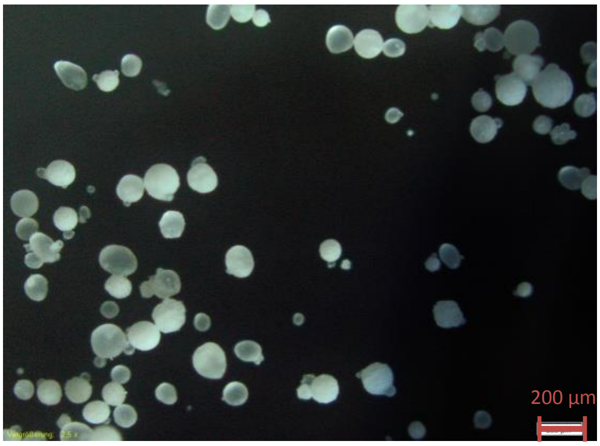


Figure I Microcapsules stabilized by TEGO SML 20

SAMPLE 150218_1

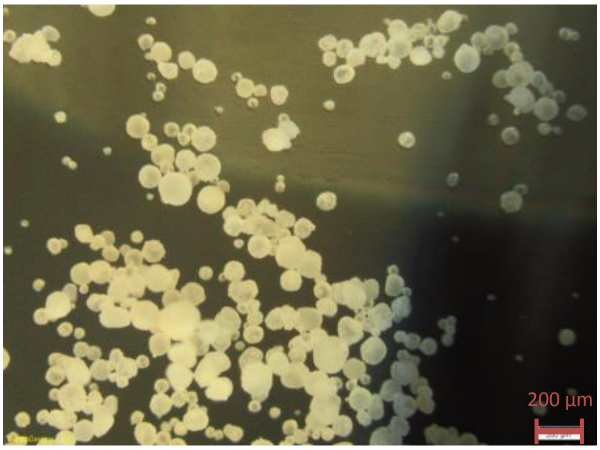
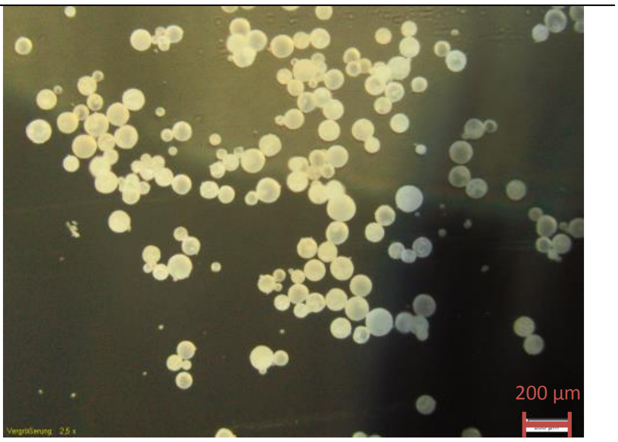


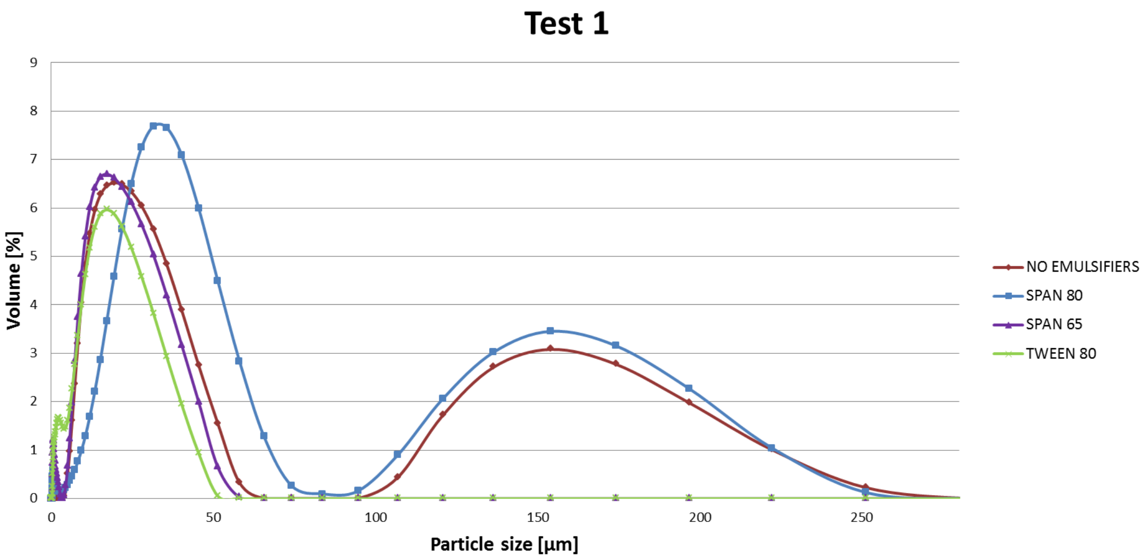
Figure J Microcapsules stabilized by TEGO SMS 60

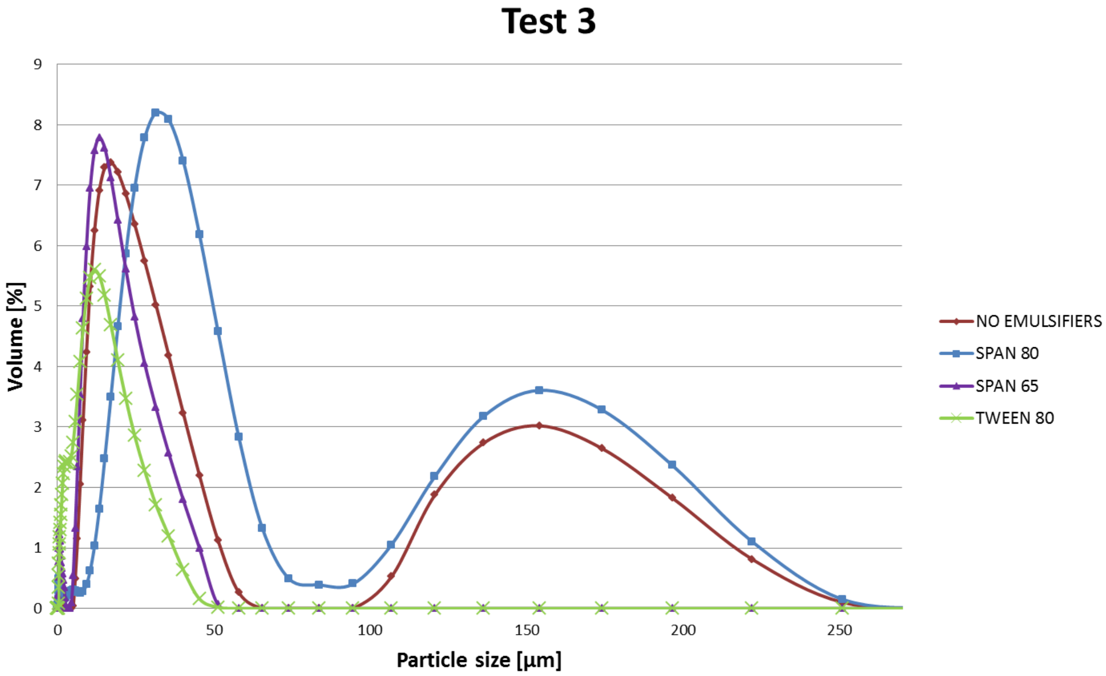
SAMPLE 150219_1

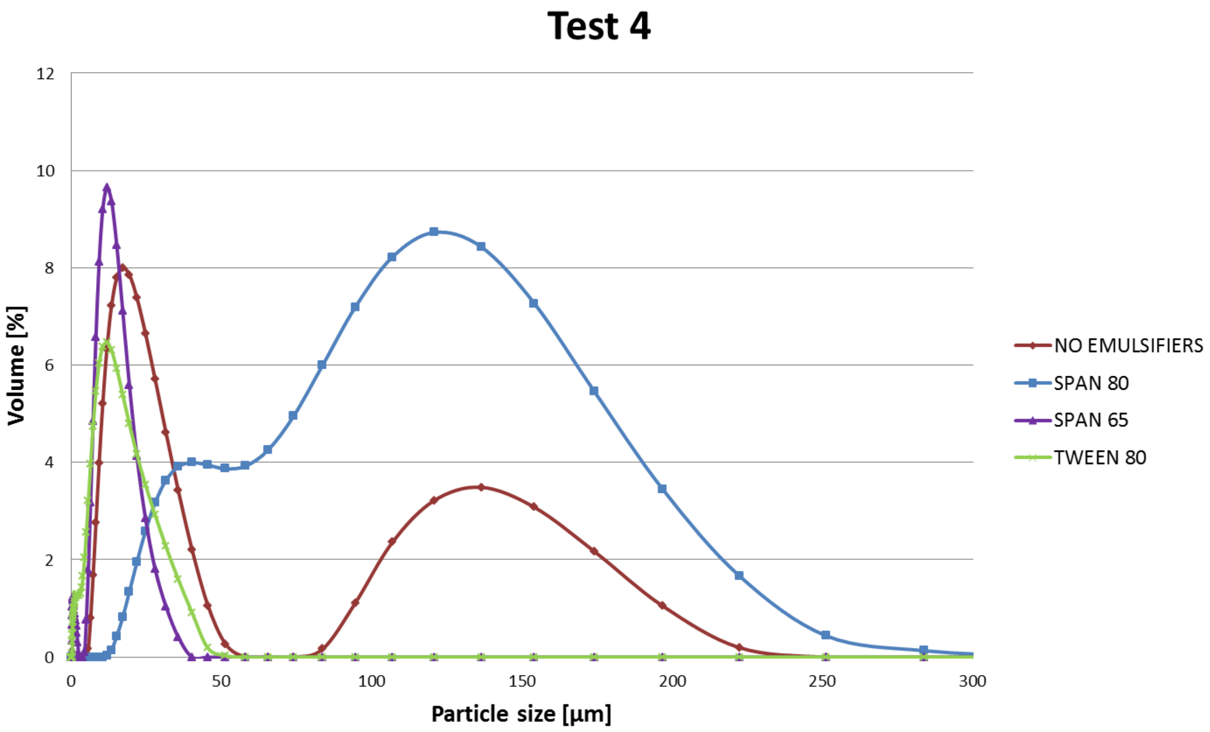
Figure K Microcapsules stabilized by SISTERNA SP50 *SAMPLE 150219_5*	

APPENDIX III

Particle size distribution of capsules. Ultraturrax experiments






Test 4
Volume [%]
0
2
4
6
8
10
12
Particle size [µm]
0
50
100
150
200
250
300
NO EMULSIFIERS
SPAN 80
SPAN 65
TWEEN 80