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Available encapsulation technologies at industrial level for cosmetic applications.

Tecnologías de encapsulación disponibles a nivel industrial para aplicaciones cosméticas.

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June 2018

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Als meus tutors, per la seva dedicació.
I a Alba, pel seu temps i les seves aportacions, el quals han fet possible aquest projecte.

Report

CONTENTS

SUMMARY

Cosmetics have been used from ancient times, associated to medicine and religious practices or to personal care and beauty.

Cosmetic sector has a noticeable innovator character that force manufactures to look for new cosmetic formulas and ingredients that position their products at the forefront of the competitors. The current trend is based on the incorporation of new technologies in cosmetics formulations in order to enhance their effectiveness and make them more attractive for the customers. Delivery systems have been designed for responding this demand.

Delivery systems are technological vehicles designed to bear, protect or carry an active ingredient and promote their controlled and targeted release. Several kinds of them are available in the market, of which the most popular would be: vesicular systems, polymeric capsules, and polymeric and lipid particles. The last ones can be divided into spheres and sponges according to their physical structure.

The selection of one of them depends on a wide variety of factors, such as active ingredient nature, final application of the product, release mechanism desired, requirements of the production process, materials involved, manufacturing conditions, etc.

All the above-mentioned factors have been studied in order to be able to provide a tool that facilitates the selection of the most suitable delivery system for beginner companies by limiting initial options, thus reducing the number of studies that have to be performed and the cost of the design process.

Once the delivery system has been chosen, the manufacturing company has to guarantee its quality. The main quality parameters provided by manufacturing companies are reported in the project, as well as the most typical techniques used for their determination.

1. INTRODUCTION

Cosmetics have been used from ancient times, associated to medicine and religious practices or to personal care and beauty.

Art.2 of Regulation 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, defines a cosmetic product as "any substance or preparation intended to come in contact with the various surface areas of the body (epidermis, hair, and capillaries, nails, lips, and external genital organs) or with the teeth and buccal mucosa, solely or principally for cleansing, perfuming, or protective purposes in order to maintain them in good condition, modify their appearance, and/or improve body odor, and/or protect or maintain them in good order". [1]

The cosmetics boom has been made evident worldwide in the recent years due to the increased interest of consumers in healthy appearance and skin care. Besides, the cosmetic sector has a noticeable innovator character that force manufactures to look for new cosmetic formulas and ingredients that position their products at the forefront of the competitors. The current trend is based on the increased demand for innovative formulas that provide better results and higher effects through the development of cosmeceuticals (cosmetics with pharmaceutical ingredients) and the new nutracosmetics (nutraceutics in cosmetic delivery systems).

The associated benefits with this kind of new products are mainly influenced for the formulation of active ingredients, their ability to maintain their activity and the vehicle that allows their efficient and target delivery. Depending on the composition, the vehicle used can reach five main types of skin effects: cleaning, discoloration, care, hydration and protection. To achieve this aim, the active ingredient is required to been liberated in the target place at correct concentration, with and optimal delivery speed and in a desirable distribution without any systemic implication.

The most frequently strategy used by cosmetic companies to achieve the above mentioned goal is the use of delivery systems. These systems are in continuous advance and they usually require encapsulation technologies, which are based on the active ingredient protection inside nano- or micrometric structures, from the product until their target site.

In addition, the cosmetic active ingredients release by topical and transdermal path requires a safety and non-toxic medium to achieve its target destination inside the skin. The selection of encapsulation technique and materials depends on the final product application and other factors, such as physical and chemical properties of the active ingredient, their stability, concentration, particle size required, release mechanism and production requirements.

2. OBJECTIVES

The present project lies in studying the available topical delivery systems for their use in cosmetics at industrial level.

Nowadays, there is a wide variety of delivery systems in the market for cosmetic application. In consequence, when a manufacturer company wants to introduce a delivery system in one of its products, the selection process of the most suitable delivery system can become in a very complicated task that may be time-consuming and expensive.

The aim of this study is to provide tool that facilitates the selection of the most suitable delivery system for beginner companies by limiting initial options, thus reducing the number of studies that have to be performed and the cost of the design process.

In order to achieve this purpose, the following secondary aims must be accomplished:

- To know and to classify, trough bibliographic search, the active ingredient delivery systems most used, and to associate them with the most suitable encapsulating materials.
- To study the main reported encapsulation methodologies in articles and patents with the purpose of classify them according to criterion considered most appropriated, as well as to analyze and to discuss their viability and possible advantages and inconveniences at industrial level, in order to provide a tool that helps the correct selection of the methodology based on the materials, available equipment, production scale and required quality of the encapsulated.
- To study the possible release mechanisms of the active ingredients. Through scientific articles, release mechanisms associated with each delivery system will be defined and it will be possible to gauge in which kind of final product they should be included.
- To study the main evaluation methods of the obtained encapsulations. Based on information provided by some suppliers of this kind of products and on scientific bibliography, the parameters which guarantee the quality of the encapsulated and the relation between them and the selected process will be analyze and discuss.

3. DELIVERY SYSTEMS

According to Hibbott, H.W. (1963), cosmetic delivery systems emerged as consequence of the following market needs [2]:

- Achieving a higher exposition time of the skin to the active ingredients.
- Improving the penetration trough deep skin layers of ingredients that for their physical or chemical characteristics would not be able to reach these layers due to the skin barrier, or because they would be degraded during the penetration process.

Delivery systems are technological vehicles designed to bear, protect or carry an active ingredient and promote their controlled and targeted release [3][4]. Human skin acts as a barrier against exogenous molecules and these systems are able to carry these ingredients trough the different skin layers, controlling their concentration, due to their affinity of the layers.

Nevertheless, these are not the only advantages of delivery systems. Numerous authors [4-8] collect in their texts the benefits of this kind of technologies. They also allow:

- To control the release of active ingredients.
- To stabilize and protect active ingredients against reactive or incompatible compounds, as well as against environmental conditions to which they are sensitive (oxygen, light, pH, temperature…).
- To mask unpleasant organoleptic properties and improve feeling of the final product.
- To mix incompatible compounds.
- To facilitate the manipulation of liquid ingredients turning them into solids.
- To improve the final products from a point of view of marketing trough the claim of innovative technology.

Looking over the companies offers of active ingredients and based on the information given by Guevara, N.A. (2008) and Cea. R. (2016), we can conclude that the most interesting ingredients to encapsulate in cosmetics are: nutraceutical ingredients, actives, vitamins, essential oils, pigments, enzymes and perfumes [5][9].

According to Jyothi, S. (2012), the material to encapsulate will be liquid (dissolved or dispersed), and can be accompanied by additives, stabilizers or deliver velocity improvers [10].

In order to be able to classify them, to know the current delivery systems in the market and their characteristics is needed. In this chapter, a general description of the most used or well-established systems in the sector is given.

3.1. Liposomal delivery systems

Liposome-based topical formulations were formulated and launched in cosmetics by Christian Dior (Capture™).

In sixties, Dr. Alec Banham of the Babraham Institute in Cambridge observed that phospholipids have the property of forming bilayers when they are in aqueous medium. This is the principle in which liposomes technology is based on.

Liposomes are spherical vesicles that may be composed of cholesterol and natural non-toxic phospholipids. Their centre consists of an aqueous cavity, which is encapsulated by one or more bimolecular phospholipid sheets, which are relatively impermeable to the entrapped substance. Aqueous layers separate each bilayer from each other. The polar head group, which may be zwittertonic, negatively or positively, forms the interface at both the external and internal surfaces of liposomal bilayers. [11][12]

Consequentially, liposomes can accommodate hydrophobic, hydrophilic or amphiphilic molecules. Hydrophobic molecules are entrapped between the lipid bilayer whereas hydrophilic ingredients can be encapsulated in the core. Amphiphilic molecules are located between these two regions, in the water/lipid surface according to their affinity to the liposome components.

Several authors have reported the advantages and disadvantages of liposomes in contrast to other delivery systems [7,13-15]. Table I. shows some of them:

Advantages	Disadvantages
▪ Biocompatibility ▪ Biodegradability ▪ Low toxicity ▪ Can be made to be target selective ▪ Flexibles ▪ Easy preparation ▪ Possibility of large-scale production ▪ Physicochemical behaviors ▪ Increased efficacy of drugs ▪ Increased stability via encapsulation	▪ Low solubility ▪ Short shelf life and stability ▪ Difficulties in controlling liposome size ▪ Batch-to-batch irreproducibility ▪ High production cost ▪ Concerns about sterilization ▪ Low entrapment coefficient ▪ Short circulation half-life ▪ Poorly water-soluble drugs incorporated are often rapidly released ▪ Leakage and fusion of encapsulated drug/molecules

Table 1. Advantages and disadvantages of liposomal delivery systems

Liposomes can be classified according to their size and structure:

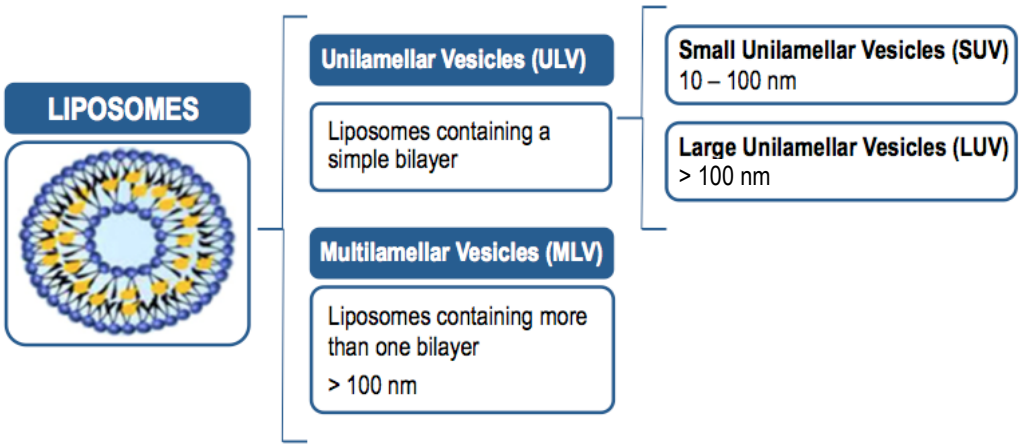

Figure 1. Liposomes classification according to their size and number of bilayers

MLVs are not usually good for the encapsulation of active ingredients.

- For the encapsulation of water-soluble actives, the ratio of encapsulated aqueous phase volume to lipid content is relatively low.
- In the case of lipophilic actives, they should be the better option. However, they have some disadvantages:
- They are not reproducible.
- They will appear like liposomal preparations with a very wide range of size and a variable number of bilayers. Therefore, the content of lipophilic active will be also variable and this fact could be dangerous due to it penetrates quickly in the skin and could be toxic for the cellules.

Nevertheless, some studies have demonstrated the existence of some mechanisms which increase the load capacity of lipophilic actives in MLVs. The use of charged phospholipids or polyethylene glycols with different molecular weight would be an example. These may increase repulsion between the different layers and, as consequence, the internal volume of vesicle. [11][12][14]

It is necessary to state that manufacturing companies and consumers do not appreciate the use of polyethylene glycols in cosmetic products since French companies, sector leaders, started to eliminate them of their products.

For these reasons, MLVs are not considered very useful but they are very used because they are formed spontaneously during manufacturing processes and their transformation into LUVs requires additional production steps.

Regarding ULVs, they are reproducible and have a higher capacity of water-soluble ingredients encapsulation. SUVs have lower charge capacity than LUVs and it is usually that the irregular distribution of lipids in their bilayer involves structural deformations. LUVs are more stables but it is important to bear in mind that bigger liposomes present a lower penetration.

According to Montero, F.J. (2006) and Akbarzadeh, A. (2013), the vesicle size is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount encapsulation in the liposomes. Liposomes between 50 and 250nm and with one or two bilayers are considered the best for topical applications. [14][16].

In cosmetics, vesicles smaller than 100 nm are considered nanomaterial due to the possibility of penetration into the circulatory system exists. Therefore, liposomes between 100 and 250 nm with one or two bilayers would be the most appreciated for cosmetic applications. [1]

3.2. Another delivery systems based on lipid vesicles

After the development of liposomes, other forms of vesicular systems appeared. Most of them can be considered as modified liposomes.

Niosomes

Niosomes were developed and patented by L'Oréal in seventies and eighties [17-18]. They are vesicles composed by self-assembled non-ionic surfactants in aqueous medium and, in some cases, cholesterol or its derivatives, which improve their stability and determine their stiffness and shape.

Their structure is similar to that of liposomes, so they can also deliver hydrophilic, lipophilic, or amphiphilic ingredients and, based on their size and number of bilayers, be divided into unilamellar vesicles, multilamellar vesicles and large unilamellar vesicles. [18][19]

Niosomes present a more effective delivery by topical application because of non-ionic surfactants give higher versatility to the vesicular structures. Besides, phospholipids are more expensive and more unstable than non-ionic surfactants, so niosomes result cheaper and more stable than liposomes [4][19]. Their main drawback is that they do not usually contain GRAS ingredients (Generally Recognized As Safe), so they often are irritant. [8][20]

Marinosomes

Marinosomes are liposomes based on natural marine lipids containing high ratio polyunsaturated fatty acids that are metabolized by skin epidermal enzymes into anti-inflammatory metabolites. Therefore, they are considered as potential candidates for cosmeceutical in view of the prevention and treatment of skin diseases. [8]

Transfersomes

Transfersomes are deformable vesicles composed of phospholipids and an edge activator. The edge activator is usually a single chain surfactant with a high radius of curvature, able to decrease bilayer stiffness. This elastic behavior avoids the vesicle rupture and it is responsible for its penetration into the skin. [4][12][13][21]

Transfersomes usually present a diameter between 200 – 300 nm.

Ethosomes

Ethosomes are phospholipid vesicles with high concentrations of ethanol (20 - 50%), which acts as permeation enhancer, since it affects the bilayer structure of the *stratum corneum*.

Compared to liposomes, ethosomes are able to deliver the entrapped ingredient more deeply in the skin and typically they exhibit a smaller size, higher entrapment efficiency and improved stability. As opposed to transfersomes, ethosomes are able to improve the skin delivery under occlusive and non-occlusive conditions. The main drawbacks of this delivery system are its instability due to oxidative degradation and the lack of purity of natural phospholipids that affects it public acceptance. [4][12][21]

There are other liposomal systems, such as ultrasomes, photosomes, aquasomes, etc., but they are less consolidated in the market.

3.3. Cyclodextrin complexes

Cyclodextrins were described for the first time in 1891. They are cyclic oligosaccharides composed of D-glucopyranoside units linked by (1→4) glycosidic bonds. Their main property is the ability to modify the physicochemical and biological characteristics of low-soluble drugs through the formation of inclusion complexes.

There are three kinds of natural cyclodextrins according to the number of units forming the rings: α -cyclodextrin (6), β-cyclodextrin (7) and γ-cyclodextrin (8).

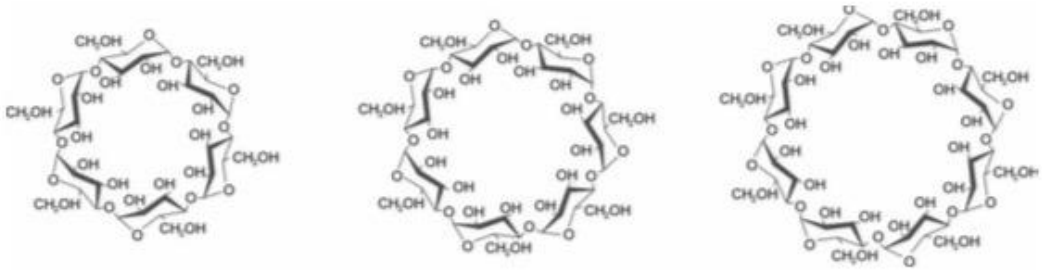

Figure 2. Chemical structure of α-,β- and γ-cyclodextrins, respectively [22]

Cyclodextrins with less than 6 units of glucose do not exist for stoichiometric reasons and those with more than 8 units offer low complexing yields. [22]

Cyclodextrins display a truncated cone structure due to the chain conformation of glucose units. Because of their apolar inner cavity and to their hydrophilic outer surface; they can form inclusion complexes with hydrophobic molecules by entrapping them in their internal cavity.

Figure 3. Cyclodextrin complexes structure [23]

It would be to expect that the higher the number of units, larger the compound amount that could be incorporated. Nevertheless, guest molecules need to adjust within the cavity, which complicates the inclusion and involves that the use of cyclodextrins is limited (Valle, 2004). The following table presents some properties of the inner cavity of cyclodextrins.

Property	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
External diameter (Å)	14.6	15.4	17.5
Internal diameter (Å)	4.7 – 5.3	6.0 6.5	7.5 – 8.3
Height (Å)	7.9	7.9	7.9

Table 2. α-, β- and γ-cyclodextrins dimensions

A strategy often used to enhance complexation between active ingredients and cyclodextrins is the addition of water-soluble polymers to the system, which increases solubility efficiency.

Despite their hydrophilic exterior, cyclodextrin complexes are able to penetrate into the skin in significant amounts because of they can be dispersed in the lipophilic areas of *stratum corneum* and in the hydrophilic areas of epidermal layer.

Concerning release, inclusion complexes dissociate easily and they deliver the entrapped molecules. The dissociation kinetics will be inversely proportional to the strength of the bond between the cyclodextrin and the active ingredient molecules. The slower the dissociation kinetics, the stronger bond. Even in this situation, the dissociation speed of the complexes is considered to be practically instantaneous [24].

3.4. Microsponges

Microsponges are polymeric delivery systems consisting of porous microspheres, each one containing an enormous amount of interconnecting voids within a non-collapsible solid structure with large surface. They vary in diameter from 5 to 300 μm and their highly compartmentalized nature lends them a very high internal surface area and high-level payload. These porous polymers consist of a polymeric membrane that holds together the solid particles, which compose the core of the sphere. The outer membrane is interrupted by a multitude of pores that allow entrapped active to keep its activity and to flow out of the system over longer periods of time. [12]

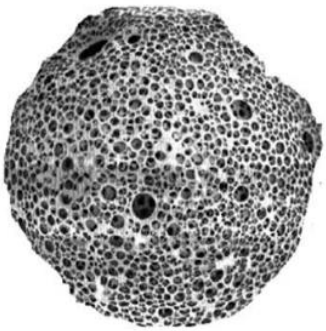

Figure 4. Microsponge [25]

As has been said previously, they have high payload capacity. They can hold up to 50-60% of solid, semisolid or liquid material, which can comprise hydrophilic as well as hydrophobic materials.

According to Bugaj, A.M. (2015), the unique advantage of this delivery system is its large entrapment capacity, up to 3 times the weight of the polymer alone, while its major disadvantage is the needed of using of harsh processing conditions for forming the particles, thus limiting their application to very stable active ingredients which can resist these conditions. [21]

3.5. Micro- and nanocapsules

Encapsulation is a process in which coating materials are deposited around solid particles or droplets of liquid containing an active ingredient, permanently or temporarily. As a result, solid particles, generally spherical, are obtained.

Figure 5. Capsules [23]

If the particle is larger than 1 μm, the result of the encapsulation process is denominated as microparticle, whereas it is lower than 1 μm, it is denominated as nanoparticle. The small size of these capsules provides a large surface area that is available for sites of adsorption and desorption, chemical reactions, light scattering, etc.

The shell can be composed of natural polymers (albumin and gelatin), synthetic compounds (polylactic acids or polyglycols), or natural modified products (wax, gums or proteins) [26]. So, it can be permeable, semi-permeable or impermeable.

As mentioned, in order to obtain capsules, polymeric material has to been deposited around an oily phase. Aqueous phases have not enough consistency to allow the deposition of the polymer around them. So, only capsules with oily core are possible and, in consequence, these delivery systems are only suitable for lipophilic actives encapsulation. Otherwise, a hydrophilic active can be entrapped in a polymeric capsule if it form part of a multiple emulsion, but this is quite unusual because of its complexity and low load capacity.

A suitable mix of polymers with different degree of solubility and surfactants presence may allow the incorporation of water-soluble active ingredients in the shell. Nevertheless, this situation would not correspond to a really encapsulation process since the assets would be located around the capsule, in the surface.

The morphology of the internal structure of capsules depends largely on the shell materials selected and encapsulation method employed [10]. According to this, they can be classified as:

- Mononuclear: Capsules containing the shell around the core.
- Polynuclear: Capsules having many cores enclosed within the shell.
- Matrix: Core material is distributed homogeneously into the shell material.

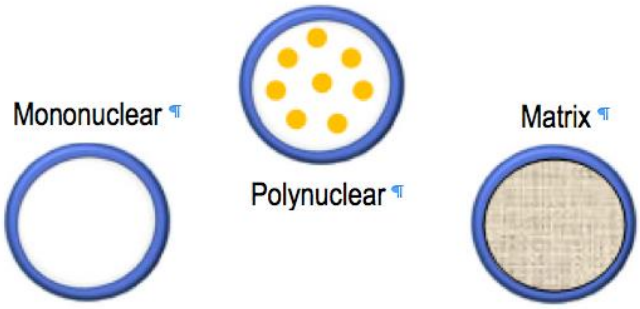

Figure 6. Internal morphology capsules [23]

In addition to these three basic morphologies, capsules can also be mononuclear with multiples shells, or they may form clusters of capsules.

3.6. Micro- and nanospheres

Spheres consist of a dispersion of an active ingredient in a polymeric porous or solid matrix. However, the active ingredient can also be incorporated by adsorption at the sphere surface. In this case, the active release is biphasic with an initial burst phase followed by sustained release.

They are generally used for lipophilic actives encapsulation. Nevertheless, the composition of the polymeric matrix defines the system affinity by the active ingredient. Consequently, coating materials with a high ratio of low hydrophobicity polymers are able to encapsulate a certain amount of hydrophilic compounds. [23]

Spheres results a suitable option for encapsulating fragrances, since their high cationic charge density and their high surface area allow them to remain on the skin long after application, even after washing. [9]

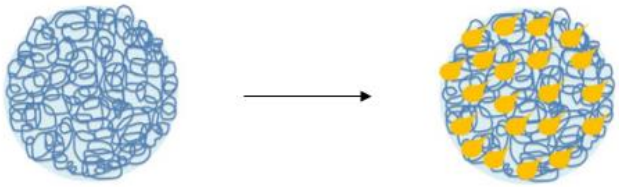

Figure 7. Internal morphology capsules [23]

3.7. Lipid nanoparticles

Lipid nanoparticles [12,16,23,26,27] with solid particle matrix are derived from O/W emulsions by replacing the liquid lipid by a solid lipid, in case of Solid Lipid Nanoparticles (SLNs), or a blend of solid and liquid lipids, for the formation of Nanostructured Lipid Carriers (NLCs).

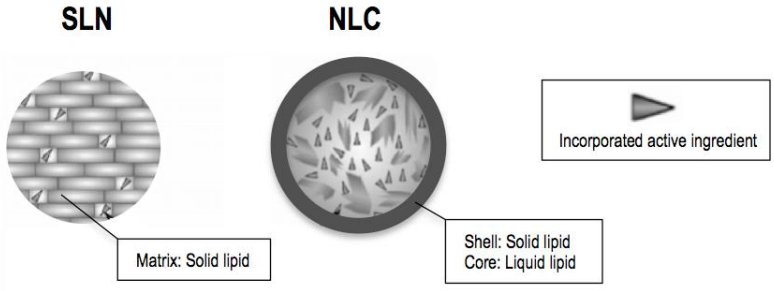

Figure 8. SLNs and NLCs structures [27]

These lipids are usually GRAS, therefore no problems of biocompatibility or toxicity are associated. In addition, compared to liposomes, lipid nanoparticles have slower degradation ratio in vivo, which provides a better protection and controlled release of the encapsulated molecules, apart from a more cost-effective production on large scale.

Some of the main drawbacks of lipid nanoparticles are the general tendency for aggregation when combined with some ingredients, and gelation behavior associated with SLNs. However, NLCs present an improved physical stability in suspension comparing to SLNs.

3.7.1. SLNs

SLNs were first developed, in the early nineties. They consist in a dispersion of solid spherical particles, with a mean size between 40 and 100 nm, consisting of hydrophobic core of triglycerides or fatty acid derivatives surrounded by a layer of phospholipids. Because of their structure, they are suitable for lipophilic actives entrapment although the presence of phospholipids covering the lipid particle may allow the incorporation of hydrophilic actives but they would not be encapsulated.

Some of the advantages of this delivery system are:

- The absence of harmful additives required for polymerization and biodegradability of physiological lipids.
- Compared with liposomes, SLNs have better stability against coalescence because of the solid nature and reduced mobility of incorporated active molecules, preventing the active leakage from the carrier.
- High encapsulation efficiency.
- Possibility of large-scale production.
- Flexibility in controlled release.
- High ability to reach the target site.

The main drawback of SLNs is that solid lipids forming the particle can suffer polymorphic changes or crystallization along the time, which may involve the lost of the entrapped active ingredient.

3.7.2 NLCs

NLCs are characterized by being composed of matrices formed by solid and liquid lipids at room temperature. As consequence, the matrix adopts a less ordered structure, whose conformation does not change along the time. This avoids the uncontrolled release of the active ingredients from the matrix.

According to their structure, three different types of NLCs can be formed: imperfect, amorphous and multiple. The imperfect type structure results from using different molecules that lead to the formation of imperfections, which in turn allow the accommodation of the active ingredient. The amorphous type structure results from avoiding the crystallization process leading to an amorphous state. Finally the multiple type structure corresponds to an oil-solid lipid-water dispersion were oil compartments are inside a solid lipid matrix.

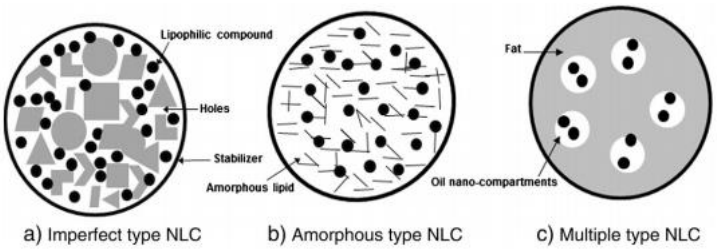

Figure 9. Types of NLCs [28]

NLCs are able to incorporate higher amounts of the active ingredient, since it can be located between the fatty acids chains, between the lipid layers and also in the lipid matrix imperfections. Additionally, the higher order degree of the structure also leads to a faster expulsion of the active ingredient.

3.1.7. Multiwalled Delivery Systems

Multi-walled Delivery Systems (MDS) are vesicles made up of non-phospholipid amphiphilic molecules (oleic acid, derivatives of polyglycerols or amino acid residues) that contain five to seven bilayer walls. This structure is analogous to that of lipid membrane found in the intracellular matrix. [12][21]

These delivery systems increase the liposomes stability, delay the release of the encapsulated active ingredient and moisturize the skin.

3.8. Silicone vesicles and matrices

The permeability of silicones makes them suitable for controlled release applications and for this reason they are used widely in transdermal delivery systems.

Silicone vesicles consist in silicone surfactants assembly that rearrange spontaneously in aqueous medium to form bilayers. This structure makes them suitable for the incorporation of hydrophilic and lipophilic active ingredients in the core and in the bilayer respectively. [29][30]

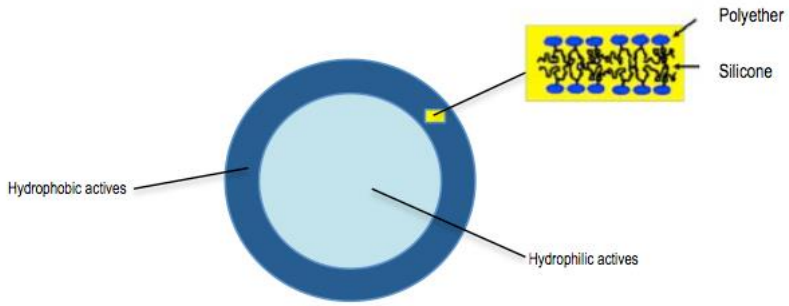

Figure 10. Silicone vesicles structure [31]

Generally, silicone vesicles present a diameter from 0.05 to 1 μm and a membrane thickness about 3 to 4 nm. In addition, just like liposomes, they can be classified in three groups according to their size and bilayers number: [29][30]

- Small unilamellar vesicles (20 - 50 nm)
- Large unilamellar vesicles (200 - 500 nm)
- Multilamellar vesicles (200 - 1000 nm)

In addition to the advantages associated to liposomes, silicone vesicles are stable up to a high temperature, approximately 90ºC, and their bilayer is more flexible.

Regarding to silicone matrices, they are formed by cross-linked silicone elastomers. The interconnections between polymer chains make the elastomers solid material. Due to this, an active ingredient can be trapped in the matrix and will not separate even if it is not soluble in the elastomer matrix. 31]

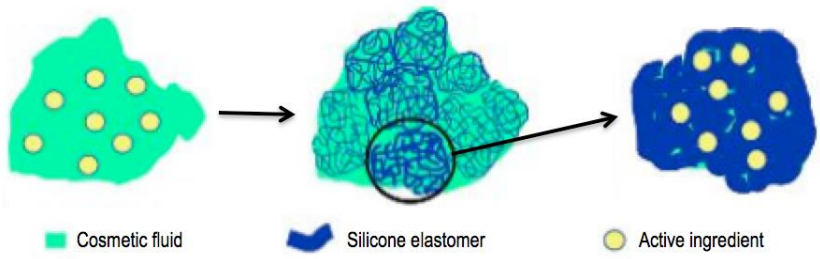

Figure 11. Silicone matrices structure [31]

3.9. Classification

Several authors have classified delivery systems. In this chapter, a review of some of these categorizations has been performed in order to be able to carry out our own classification.

The most generic classification is the one performed by authors like Montero, F.J. (2006) [16]. They divide the delivery systems in the following families:

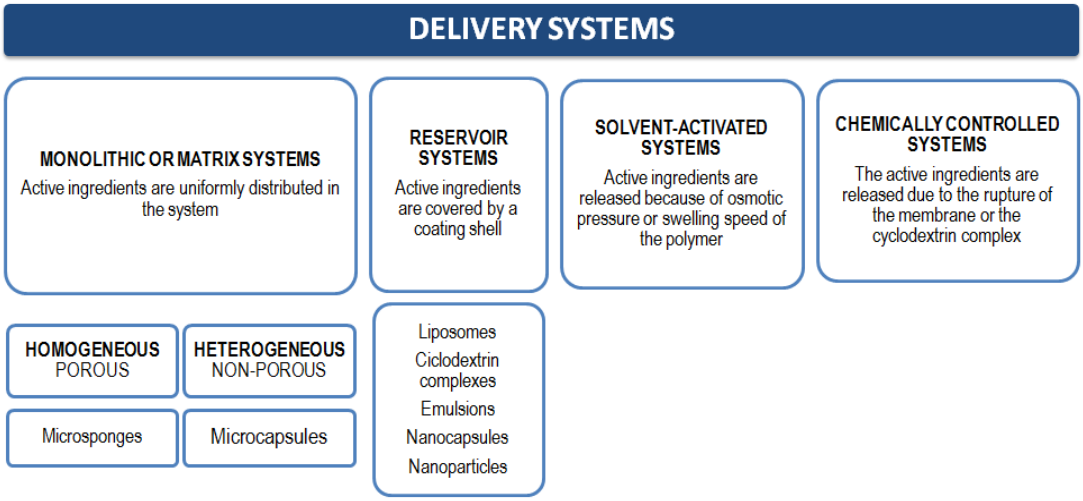

Figure 12. Classification of delivery systems (I)

This classification is not the most suitable according to the aim of the project due to:

- The classification is not based on a uniform criterion. The first two types of systems differ in the distribution of the active ingredient in the global system whereas the second two differ in the release mechanism involved.
- Solvent-activated systems are little or no used.
- Most of the encapsulation systems would be included in the reservoir systems group, so that no classification would be taking place.

This distinction, with a few modifications, has been used in chapter 8 in order to make a most intelligible description of release mechanisms, but it is not the most suitable for the study of other characteristics of delivery systems.

Other alternative divisions have been proposed. The most extended is the one proposed by Costa, R. et al. (2017) [4]. According to them, delivery system may be divided into vesicular systems (liposomes, niosomes, transfersomes), emulsions (microemulsions and nanoemulsions), and particulate systems (microparticles, nanoparticles).

Other authors, being based on the same classification, give a more complete version including other delivery systems in these families.

For example, Hougeir, F.G. et al. (2012) [20] include the marinosomes, multi-walled delivery systems and silicones into vesicular systems group. They also extend the emulsions family, incorporating into it the Pickering emulsions (emulsions stabilized by solid particles), and the particulate systems family, incorporating the porous polymeric systems and cyclodextrin complexes.

Patravale, V.B. et al. (2008) [12] also mention the liquid crystals and the multiple emulsions like a part of emulsions family.

Finally, Bugaj, A.M. (2015) [21] distinguishes different kinds of micro- and nanoparticles:

- Microparticles: Microspheres, microcapsules and microsponges
- Nanoparticles: Nanospheres, nanocapsules, SLNs and NLCs.

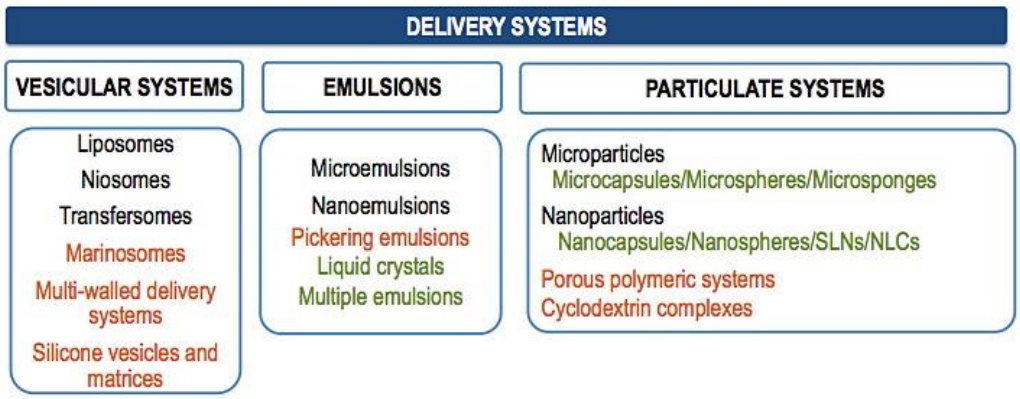

Figure 13. Classification of delivery systems (II)

Some drawbacks have also been found to this division:

- As stated above, delivery systems are technological vehicles designed to bear, protect or carry an active ingredient and promote their controlled and targeted release. The group of emulsions involves systems that are not able to provide a controlled or targeted delivery. In any case, they would be a previous step to the preparation of one of these systems. Therefore, they should not be considered delivery systems.
- Regarding vesicular systems, silicone vesicles and matrices do not present the same structure.
- The same occurs with cyclodextrin complexes. The other systems classified as particulate systems entrap the active ingredient inside a solid shell or mass. Instead, cyclodextrin complexes entrap the active ingredients inside the molecules due to their spatial structure.
- Capsules, spheres and sponges are made of polymeric materials, while lipids compose SLNs and NLCs.

Díez, O. et al. (2012) [26] make a very different categorization. Their proposal consists in divide the available delivery systems in base on their structure. According to this, three different families can be distinguished:

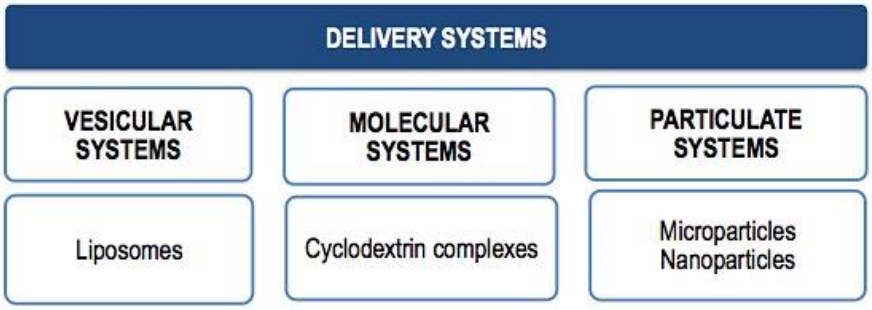

Figure 14. Classification of delivery systems (III)

This last categorization is very basically and it does not contemplate the most of the above-mentioned delivery technologies. In addition, it seems that the classification is based on the structure of the systems; nevertheless, it does not make a distinction between the different kinds of particles.

Based on all this information, it has been pretended to perform a new proposal of categorization of these technologies. Due to the aim of the project is to provide a tool to facilitate the selection of the most suitable encapsulation technique according to the needs of the user, it has been considered that the most adequate classification must be based on the structure of the systems. It has been considered that they may be divided into: vesicular systems, particles and capsules. Details of systems that compose each group can be found below.

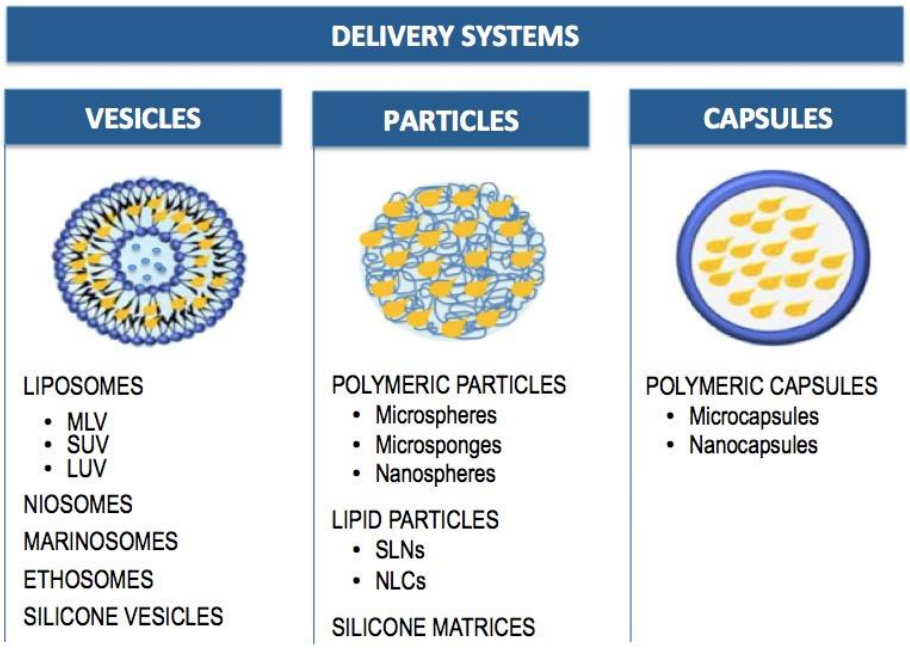

Figure 15. Proposal of classification of delivery systems

Vesicular systems family includes systems based on molecules that contain hydrophilic and hydrophobic groups that confer them the capability of forming bilayers that surround and entrap the active ingredients.

Particles family includes the systems in which the active ingredients are entrapped due to they are scattered in a solid, polymeric or lipid, matrix.

Finally, the group of capsules includes the systems in which the active ingredients, dissolved or dispersed in a liquid phase, are covered by a shell composed of a polymeric coating material.

Due to silicone-based systems are no appreciated in cosmetics market, they will not be considered studied during this project. Nevertheless, they must be included in this classification because they accomplish with the delivery systems definition.

Cyclodextrin complexes have not been included in this classification. They should be included in molecular inclusion systems group, since they entrap the active ingredients due to the spatial structure of the molecules forming them; active ingredients get caught within interstices formed by the molecular complexes, not by a layer covering them. Nevertheless, although they enhance solubility and penetration of included molecules in their inner cavity, they do not allow control of release (they deliver instantaneously the included material) and only allow the entrapment of actives at molecular level. Defining them as carrier systems instead of as delivery systems would be more appropriate.

Multi-walled delivery systems are only a tool for delay the release, but they are not which entrap the active ingredients. Thus, they have not been considered as delivery system.

4. ENCAPSULATING MATERIALS

Currently, a wide variety of materials used for encapsulation exists, including both coating materials and encapsulated active ingredients.

The active agents are those compounds or ingredients that are protected by encapsulation. Based on the offer of encapsulated products and different publications of cosmetic journals, those ingredients whose encapsulation is of interest in the sector are: nutraceutical compounds, antimicrobials, vitamins, antioxidants, pigments, enzymes, essential oils and aromas.[5]

The encapsulated material may be liquid or solid. In case of liquid cores, the active ingredient may be located dissolved or dispersed in the system and other additives, such as diluents, stabilizers or release speed enhancers, may also accompany it.

As for the encapsulating agents, they must be compatible and inert with respect to the material to be encapsulated, stabilizers thereof, economical and be able to provide a controlled release under specific conditions. [9][32]

Several authors, like Lakkis (2007) [9] make a review of the most used encapsulating materials. They can be classified in the following categories:

- Waxes and lipids: vegetable waxes, phospholipids, paraffin, natural fats, etc.
- Proteins, both in their native and modified form: gelatines, soy proteins, gluten, etc. Proteins present excellent functional properties, such as their solubility, viscosity and emulsifying capability. They have the capacity of film forming due to their various chemical groups, their amphoteric properties, their association and interaction capacity with different kind of substances, their high molecular weight and the flexibility of their molecular chains.
- Carbohydrates: polysaccharides, starches, maltodextrins, alginates, carrageenan, etc. Carbohydrates are widely used due to their low viscosity at high concentrations, low cost, good solubility and because they are available in a wide range of sizes.
- Natural or synthetic polymers: they can be classified according to their chemicals properties. [33]
    - Water-soluble polymers: polyethylene glycol, methylcellulose, carboxymethylcellulose.
    - Biodegradable polymers: poly-(lactic acid), poly-(glycolic acid).
    - Non-biodegradable polymers: polyethylene, polyurethane.

- Cyclodextrins: α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin. They are special carrier agents because they are the only materials that protect the active component based on molecular selectivity.
- Silicones
- Organic compounds: silicates, polyphosphates, etc.

To know the properties of involved materials in encapsulation process, taking into account the utilized production technique and the purpose of the product, is very important in order to obtain a good-quality product with optimal characteristics, which allow achieving the desired aim. Frequently, due to the broad range of available carrier systems and their properties, combination of several materials in order to obtain synergistic effects and better quality products, is usually advantageous.

5. ENCAPSULATION TECHNIQUES

A study on the encapsulation techniques available in the market for the manufacture of release systems has been carried out. Throughout this chapter, the most relevant information will be presented, in order to be able to discern which are the most appropriate methods according to the stated objective.

To facilitate the processing of the information, the techniques studied have been divided in groups based on the material that will constitute the coating in each case.

5.1. Lipid-based vesicles manufacturing techniques

Techniques for lipid-based vesicles manufacturing can be classified in conventional methods, non-conventional methods and post-formation treatments.

Generally, regardless of manufacturing technique used:

- When ionic strength decreases, repulsion between hydrophilic groups decreases too and the vesicles obtained are larger.
- When phospholipids used are stiff, the vesicles obtained are smaller.
- When stirring speed increases, the obtained product is more homogeneous with respect to size and lamellarity.
- When aqueous phase availability is low, the vesicles obtained are smaller.
- Methods in which lipid phase is injected produce larger vesicles and more homogeneous products when injection speed is high.

5.1.1. Conventional methods

Conventional methods for vesicle production consist of a production step, during which vesicles are formed as dispersion in an aqueous phase, and posterior steps, during which the desired structural characteristics and purity are achieved.

Generally, they are easy preparation, especially at the laboratory scale, but they usually result complex when scale-up to industrial level.

5.1.1.1. Thin film hydration method

This technique, also known as classical method of Bangham, allows the production of large vesicles with a heterogeneous size distribution and lamellarity (MLVs). It consists in:

1. Phospholipids are dissolved in an organic solvent together with the active ingredient (if it is hydrophobic).
2. Organic solvent is removed by low-pressure evaporation and a phospholipids film is obtained at the bottom of the vessel.
3. Film obtained is hydrated by means of aqueous phase addition, which will contain the active ingredient, if it is hydrophilic, causing spontaneously formation of the vesicles.

All the process is carried out under stirring.

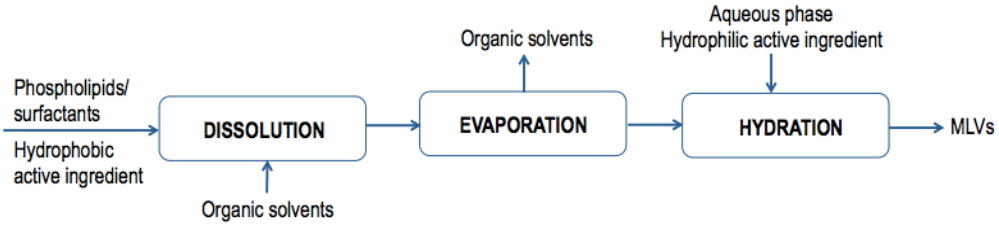

Figure 16. Thin film hydration method

It can be concluded that this method is not carried out at industrial level due to it requires the use of very bulky equipment in relation to the size batch produced. However, it has been considered interesting commenting it because of it is a simple process about which some patents [34] have been published but it is important to know that any information about its application in a production process has not been found.

5.1.1.2. Ethanol injection

Firstly, surfactants, phospholipids and the asset (if it is lipophilic) are dissolved in an organic solvent. Subsequently, dissolution obtained is added by injection over an aqueous phase containing the active ingredient (if it is hydrophilic). Finally, organic solvent is removed from the mixture by evaporation at low pressure or moderate temperature. As a result of this process, SUVs kind vesicles are obtained.

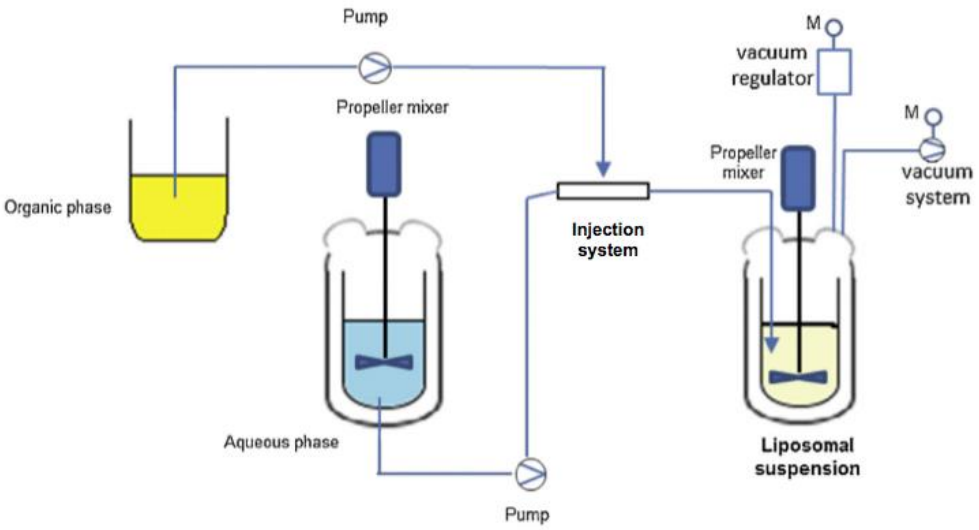

Figure 17. Ethanol injection process [35]

5.1.1.3. Reverse-phase evaporation

Reverse-phase evaporation can be described as following:

1. Inverse micelles formation by sonication, it means, small water-droplets stabilized by a phospholipid monolayer and dispersed in an excess of organic solvent. Aqueous phase contains the active ingredient that will be encapsulated.
2. Organic solvent removal by evaporation causing the transformation of the inverse micelles into a viscous gel, which collapses and provokes some micelles disintegration.
3. Vesicles are formed due to the contribution of the excess of phospholipids.

5.1.1.4. Detergent removal methods

Several authors reported detergent removal methods, like dialysis, in seventies. However, in the last few years there have not been important publications about them.

It seems that the trend of the sector is getting away from this kind of techniques. Consequently, some references about them are given, but they are not considered an alternative for companies that are interested in starting in liposomal systems manufacturing.

Alpes, H., Allmann, K., Plattner, H., Reichert, J.,Rick, R., Schulz, S., 1986. Formation of large unilamellar vesicles using alkyl maltoside detergents. BBA – Biomembranes 862, 294 – 302.

Milsmann, M.H.W., Schwendener, R.A, Weder, H., 1978. The preparation of large single bilayer liposomes by a fast and controlled dialysis. Biochiimica et Biophysica Acta (BBA) – Biomembranes 512, 147 – 155.

5.1.2. Non-conventional methods

Over the last few years, microfluidic technologies have received increasing interest as novel methods for the production of vesicle-based delivery systems.

5.1.2.1. Microfluidization

Microfluidization consists of introducing a lipid solution through a central inlet channel of a microfluidic device, and focusing this central stream by the flow of an aqueous solution through two or more side channels. Organic solvent is removed of the solution because of its diffusion into the aqueous phase. As a consequence, water replaces organic solvent around lipid molecules, thus changing their environment from the one in which they are soluble to the one in which they are not and vesicles are formed spontaneously. [36]

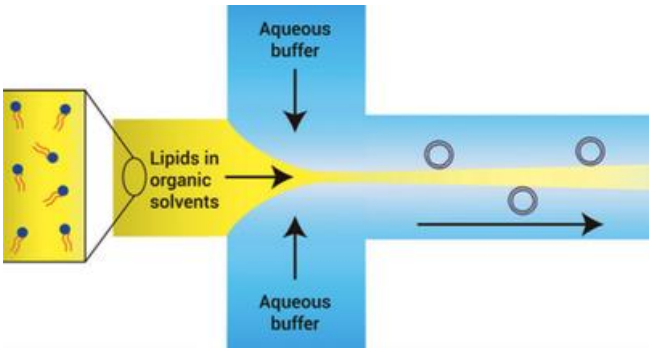

Figure 18. Microfluidization scheme [37]

5.1.2.2. Modified Rapid Expansion of Supercritical Solution technique

Common RESS technique is not feasible for vesicles production, due to phospholipids only are able to self-assemble in presence of an aqueous phase. Therefore, for its use some modifications are needed.

The procedure is the following:

1. Previously to the critical fluid addition, phospholipids are dissolved in ethanol.
2. Supercritical fluid is added to the mixture, which is sealed in a vessel, via syringe pump. After a certain time, at specific conditions of temperature and pressure that will depend on the system that composes the mixture, all the components will be dissolved in the supercritical fluid.
3. Mixture is dispersed into the aqueous phase and it is sprayed into a collector. Spraying allows the fast removal of ethanol and supercritical fluid and vesicles formation.

5.1.2.3. Particles from Gas Saturated Solution (PGSS)-Drying process

Normally, this method is used to incorporate essential oils in polymeric particles, and only have been able to find some studies of Varona et al. (2011) [38] about its use for encapsulations in liposomal systems. For this reason, it has been considered that this technique is not consolidated in the sector for the manufacturing of this kind of delivery systems and it has been discarded as an alternative.

Lévai, G., Martín, Á., Moro, A., Matias, A. A., Gonçalves, V.S., Bronze, M., & ... Cocero, M. J. (2017. Production of encapsulated quercetin particles using supercritical fluid technologies. Powder Technology, 317, 142 – 153.

Martín, Á., & Weidner, E. (2010). PGSS-drying: Mechanisms and modeling. The Journal Of Supercritical Fluids, 55, 271-281.

Machado, L. Pelegati, V., & Oiliveira, A. (2016). Study of simple microparticles formation of limonene in modified starch using PGSS-Parciles from gas-saturated suspensions. The Journal of Supercritical Fluids, 107, 260-269.

5.1.3. Post-formation treatments

Some of the above-mentioned techniques give rise to the formation of large vesicles of different lamellarity (MLVs). As consequence, to treat the obtained vesicles by means of additional steps is required in order to transform them into SUVs or LUVs and to obtain more homogeneous product. The most commonly used methods are sonication, extrusion, homogenization and freeze– thawing.

5.1.3.1. Sonication

The application of ultrasonic waves provides enough energy to cause the transformation of MLVs into SUVs. [39]

Although this technique is presented like a simple process of easy application, the truth is that it can provoke the degradation of phospholipids or other substances. In addition, the obtainment of vesicles by the disruption of other previously formed can involve the release of the entrapped material and the formation of new vesicles with low encapsulation efficiency.

5.1.3.2. Extrusion

Extrusion method consists in forcing the pass of MLVs through a membrane made of polycarbonate provided with porous of different diameter by means of pressure.

Size of obtained vesicles will depend on diameter of the porous, pressure applied and number of cycles.

This method is easily reproducible and very used at industrial level. Nevertheless, it is important to take into account that the membrane clogging may cause important product losses.

5.1.3.3. Homogenization

Homogenization is extensively used for reduction of liposome size and lamellarity for batches prepared at the industrial scale.

During homogenization, the liposome suspension is continuously pumped through an orifice and collides with a stainless steel wall in the homogenizer system at very high pressures. As a result, smaller vesicles are obtained.

5.1.3.4. Freeze-thawing

It consists of subjecting the obtained product to several cycles of freezing and thawing.

According to the studies of Moghimipour et al., (2012) [40], this process is useful for obtaining LUVs with high encapsulation efficiency when the active to be encapsulated is hydrophobic, given that during freeze and thaw cycles an increase is achieved of the interactions between the lipid film and the active principle. According to these same studies, the shorter the freezing process, the smaller the LUVs obtained.

5.2. Lipid particles manufacturing techniques

SLNs and NLCs can be produced by the same manufacturing techniques. There are multiple methods reported for its collection [41], among which are:

- High-pressure homogenization
  - Hot high-pressure homogenization
  - Cold high-pressure homogenization
- Emulsification by evaporation of the solvent
- Microemulsification with high speed agitation or ultrasound
- Emulsification method by contact membrane

Other methods have been reported for the production of lipid particles, such as solvent dispersion, high-temperature emulsion evaporation, low temperature curing or melt emulsification method, but they are not applicable at industrial scale and therefore they are not the object of this study.

5.2.1. General considerations for the elaboration of SLNs and NLCs

In general terms, the processes for SLNs and NLCs obtainment include the following steps:

Preparation of the oily phase by fusion or dissolution of the fatties

According to Wissin S.A., et al. (2004) [41], it is advisable that the select lipids would be analyzed by Differential Scanning Calorimetry (DSC), in order to know the possible decrease in melt point that lipids may suffer and the polymorphic transformations that may occur in the matrix.

Lipids forming the matrix of lipid particles should be selected taking into account their capability of dissolve the active ingredient. Due to it will rest dispersed or dissolved in the oily phase, its concentration in the matrix can affect the size and structure of the obtained particles. If during the cooling stage the lipid crystallizes before the active ingredient, a phase separation may take place.

Preparation of the aqueous phase

Generally, it consists in the dissolution of a high concentration of surfactants, combined with co-emulsifiers or stabilizer polymers.

Surfactants have a high impact in the lipid particles quality, since they are absorbed into the surface of the fatty droplets.

Pre-emulsion formation, by mixing both phases at high or low temperatures, by means of vigorous stirring or ultrasounds

Disaggregation of lipid droplets requires enough energy, thus high speed or pressure homogenization has to continue during several cycles.

Concentration of the aqueous lipid particles dispersion

It is carried out by evaporation, centrifugation, ultrafiltration, drying or lyophilization processes. Due to normally, for cosmetic applications, these kinds of encapsulations are commercialized in a suspension form for easily manipulation, this step has not been considered in this study.

Regardless manufacturing technique, the form of addition of the surfactants places an important role in the characteristics of SLNs and NLCs.

When they are added into lipid phase, particle size is significantly lower than when they are added into the aqueous phase. This would be due to the greater speed of fat disaggregation and

the speed with which the droplets are coated with the surfactant, which must compete with the speed of coalescence of the uncoated lipid particles. The dispersion of the surfactants in the lipid matrix facilitates the formation of the protective film. This process is less efficient when the surfactants are dissolved in water, since they would be in lower concentration with respect to the lipid, a fact that would not favor the adsorption in the new surfaces formed during the homogenization, giving rise to larger particles.

The presence of surfactant in the water could decrease the efficiency of the encapsulation of highly lipophilic components, causing the distribution of the active in both phases and favoring its deposition on the surface of the particles.

Concerning the temperature of the process, its influence is usually in any case lower in the NLCs than in the SLNs, since its loading capacity is determined by the concentration of liquid lipid.

5.2.2. High-pressure homogenization

Homogenization refers to a physical process of subdividing large polydispersed particles into a relatively large number of smaller particles of narrow size range. The most common type of homogenizers consists of a high-pressure pump and a disruption valve. [42]

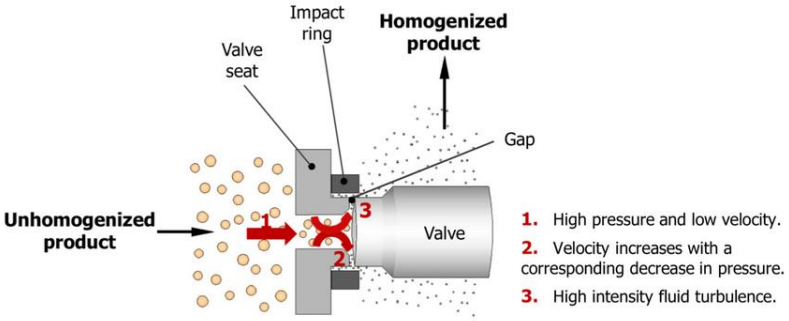

Figure 19. Disruption valve scheme [43]

It is the most used method, since it can be carried out at high or room temperature.

In general, the formation of fine particles is favored by increasing the temperature, the pressure, and / or the number of homogenization cycles. Extremely high pressures could result in particles too small that could easily flocculate and precipitate during storage.

5.2.2.1. Hot high-pressure homogenization

It consists of heating the mixture containing lipids and active ingredients at temperature 5 or 10ºC above lipids melting point. Subsequently, it is added into an aqueous dissolution of surfactant at the same temperature, under vigorous stirring or ultrasounds.

Pre-emulsion obtained is processed at high-pressure homogenizer (300 to 500 bars) during 3 to 5 cycles, at controlled temperature. When the emulsion cools down to room temperature, it crystallizes forming SLNs or NLCs, normally less than 500 nm in size. [44]

5.2.2.2. Cold high pressure homogenization

Cold high-pressure homogenization consists of melting the mixture of lipid components and dispersing it at high speed to form microparticles that are subsequently mixed with a cold aqueous solution of surfactant. The homogenization step is carried out at room temperature (or lower), generally at 500 bars for 5 cycles. [44]

5.2.3. Emulsification by evaporation of the solvent

Lipophilic active ingredients and lipid components are dissolved in a water-immiscible organic solvent previously saturated. Resultant organic phase is dispersed in an aqueous phase, which contains surfactants, using a high-speed homogenizer in order to form a pre-emulsion. Upon solvent low-pressure evaporation, nanodispersion is formed by precipitation of lipid material in the aqueous medium. Solidified nanodispersion is then filtered to remove lipid and drug agglomerates. [45]

Regarding influencing factors in the characteristics of the products obtained through this method, it is worth highlighting:

- An increase in the stirring speed increases the dispersion of the solvent and could decrease the particle size.
- An increase in the solvent volume increases the particle size.
- Assets of low solubility in water and high solubility in the organic solvent used tend to increase the carrying capacity of the lipid particles due to the low diffusing capacity of the active ingredient during the evaporation of the solvent.
- Low temperatures generally increase the efficiency of encapsulation.

5.2.4. Microemulsification with high speed agitation or ultrasound

It is an easily scalable technique without the need for specialized equipment, with low energy consumption and which results in particles with high load efficiency, an average size lower than 200 nm and a polydispersity index lower than 0.6.

However, the emulsions obtained in the first stages by this method contain high concentrations of surfactants and co-surfactants, so its use in humans is subject to sanitary regulations. As a consequence, cosmetic products obtained by this procedure become unattractive for the customers.

5.2.5. Emulsification method by contact membrane

The oil phase contained in a pressurized vessel with a nitrogen atmosphere, at a temperature regulated above the melting point, is fed to the membrane module through the pores. At one end, the aqueous phase is fed also at a controlled temperature, which flows tangentially in the interior, establishing contact with the lipids and releasing the small droplets. The outflow is cooled with stirring to room temperature.

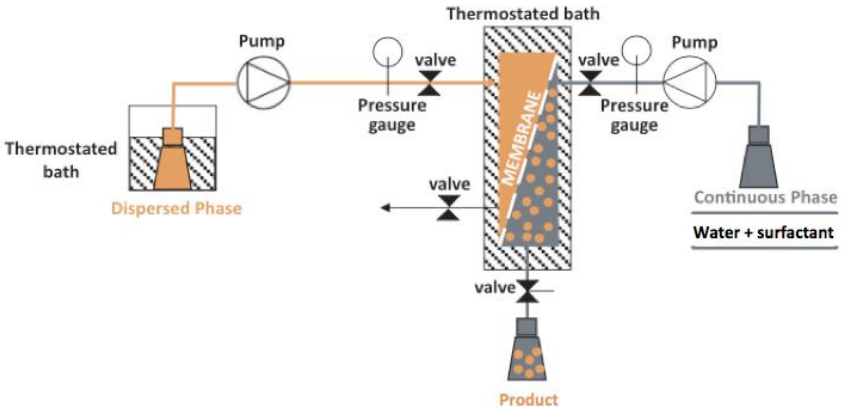

Figure 20. Emulsification method by membrane contact scheme [46]

The most influential factor in the product obtained by this method is the amount of lipid phase fed, whose increase saturates the pores, decreasing the flow and increasing the particle size. The size of the pores of the membrane is not an influencing factor, because when they are larger, the flow increases, without an impact on the size due to the greater influence of surface tension.

5.3. Polymeric particles manufacturing processes

5.3.1. Spray drying

Spray drying process is the most used technique for the production of polymeric capsules/particles at industrial scale. It consists of the following steps:

1. Raw materials are fed to a vessel where the emulsion/dispersion takes place.
2. If capsules are desired, the emulsion has to contain an oily phase, which is the dispersed phase and contains the dissolved active ingredient, and a continuous phase, with which the polymer is more compatible.
3. If spheres are desired, polymer must be more compatible with the dispersed phase than with continuous phase.
4. The emulsion/dispersion, optionally after a filtration step, is atomized into a drying chamber through an atomizer. Atomizers are generally classified as rotary atomizers, pressure nozzles, pneumatic nozzles and sonic nozzles based on the type of energy that acts upon the fluid.

5. Into drying chamber, the formed small droplets are exposed to a warm gas that causes their drying. In most cases, drying gas consists of heated and filtered atmospheric air. Sometimes, nitrogen or other inert gases are used if the active ingredient is instability, or sensitive to oxygen.
6. The dried particles fall towards the bottom of the drying chamber or travel along with the outgoing air for their separation in a cyclone or bag filter.

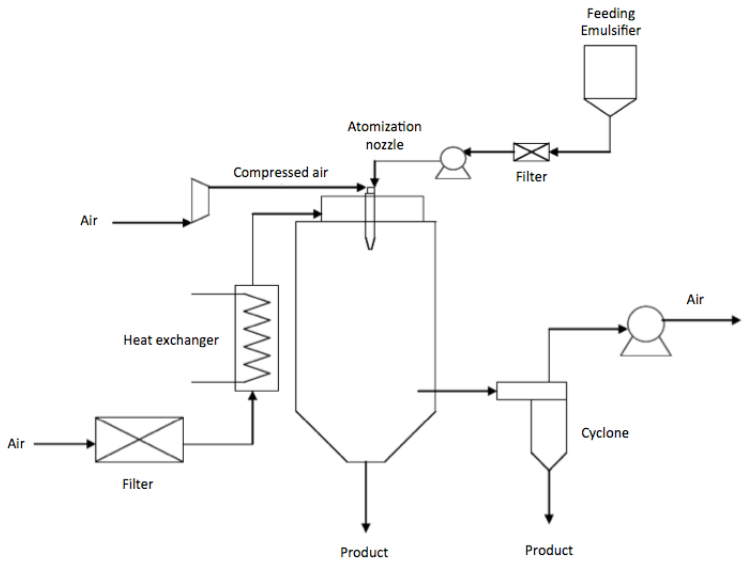

Figure 21. Spray drying process scheme [46]

According to Murugesan, R. et al (2012) [47] and Kostov, G. (2016) [48], the physicochemical properties of the final product mainly depend on preparation of the emulsion/dispersion, feed flow rate, inlet and outlet temperatures, pressure and type of atomizer. Ogrodowska, D. et al. (2017) [49] performed a study about the influence of the inlet temperature and emulsion/dispersion preparation in final product properties. Their results indicate that:

- The higher inlet temperature, the lower encapsulation efficiency and moisture content. Higher temperatures also may cause the production of porous structures.
- Low inlet temperatures decrease sphericity of the obtained particles.
- If the emulsion/dispersion is previously homogenized, particle size and heterogeneity decrease.
- Higher feed atomization pressure decreases particle size but it does not affect encapsulation efficiency.

5.3.2. Nanoprecipitation or solvent displacement technique

During the process, polymer and active ingredient are dissolved in a polar solvent. This solution is added, under stirring, on a water-miscible solvent, in which the polymer is not soluble. During the addition, the insolubility of the polymer is caused, so it precipitates. Polymeric particles are spontaneously formed due to the fast diffusion of the solvent to the aqueous phase, which is later removed from suspension by means of reduced pressure. [50]

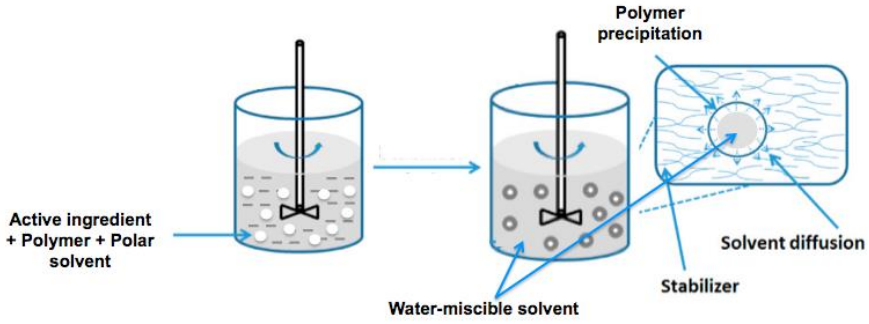

Figure 22. Nanoprecipitation process scheme [51]

Several authors summarized data obtained in different studies about the influencing factors in nanoprecipitation process. [49][51] Reviewing them can be concluded that:

- Amount of active ingredient, polymer molecular weight, amount of surfactant and organic solvent evaporation rate do not influence properties of the final product.
- An increase of polymer amount increases particles size and load capacity of particles.
- A decrease in oil/water phase ratio decreases load capacity of particles.
- An increase of organic phase addition rate decreases the particle size.
- An increase in stirring rate decreases particle size.

5.3.3. Coacervation

Coacervation is defined as two liquid phases separation in a colloidal solution and the subsequent deposition of the polymeric material around the active ingredient suspended or emulsified in the same media. The first phase, denominated coacervate, is rich in polymer and the second one, which does not contain polymer, is denominated equilibrium solution.

Phase separation can be induced by changing pH or temperature or by addition of an ionic salt, an incompatible polymer or a non-solvent.

Coacervation may take place in the aqueous phase, for non water-soluble actives encapsulation, or in the organic phase, for hydrophilic actives encapsulation. It is known that complex coacervation is used mainly for encapsulation of hydrophobic compounds, but if a double emulsion at the beginning of process is prepared, encapsulation of hydrophilic compounds is possible (Eghbal, N. et al., 2017) [52][53].

This technique may either be simple or complex if one or two polymers are used, respectively.

According to El Asbahani, A. et al. (2015) [6], simple coacervation is based on the addition of a poor solvent to a hydrophilic colloidal solution which results in the formation of two phases: one is rich in colloid molecules (coacervate), and the other is almost coacervate free. Instead, in case of complex coacervation, phase separation takes place spontaneously as consequence of electrostatic attraction between two opposite charge polymers.

The last step consists in wall hardening by addition of a crosslinking agent in order to obtain hard capsules.

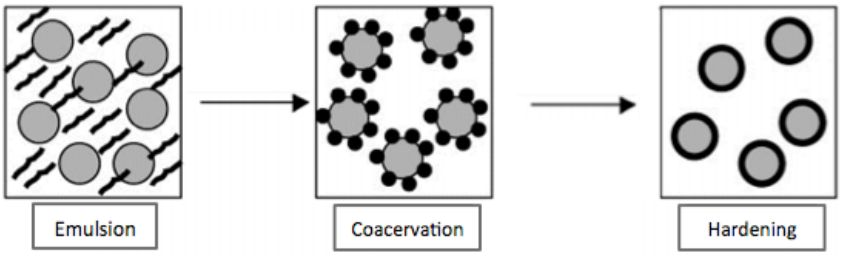

Figure 23. Coacervation process scheme [46]

Particle size may be controlled by temperature, stirring speed, viscosity, polymer concentration or surfactant type (if it is used). The effect of these parameters depends on polymer-active-solvent system.

5.3.4. Rapid Expansion of supercritical fluids (RESS)

In polymeric systems production via RESS process, active ingredient and coating polymer are disolved in a supercritical fluid at high pressures. Subsequently, solution is expanded forcing its pass through a nozzle and the pressure is reduced until atmospheric pressure. At low pressures, solubility of the solutes decreases and they precipitates giving rise to the product.

Among the variety of supercritical fluids, supercritical CO_2 is widely used due to its environmentally benign nature, low cost and its low critical temperature (31.1ºC). [54][55]

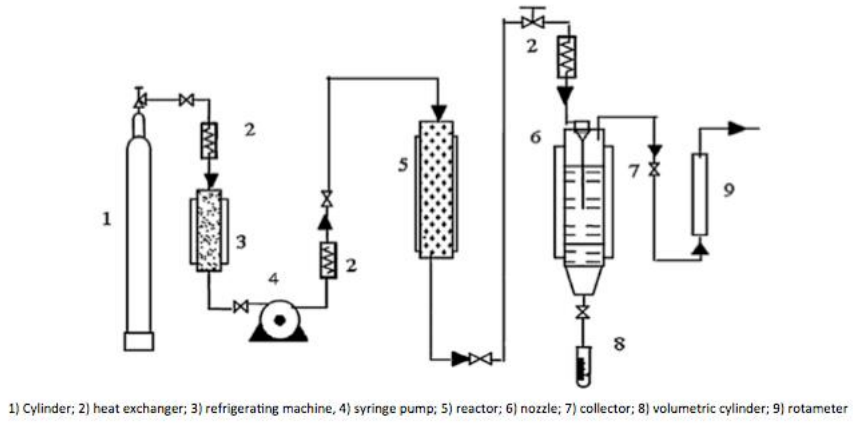

Figure 24. RESS process scheme [46]

5.3.5. Interfacial polymerization

First, an O / W emulsion is prepared; the dispersed phase will contain the material to be encapsulated and an oil-soluble reactive monomer, which is usually isocyanate. The polymerization process takes place at the interface between both phases of the emulsion by the addition of an activator or a monomer with which the one already present in the emulsion reacts rapidly, giving rise to the formation of polymeric capsules. [56]

5.3.6. Ionic gelation

Alginate is the most characteristic coating material used for polymeric systems production via ionic gelation procedure.

The active ingredient whose encapsulation is desired is dissolved in a solution containing alginate. Then, this solution passes through an extruder device, which controls the size and drip speed, and droplets formed fall into solution containing a divalent ion, generally calcium ion, that induces the gelation. [57][58][59]

5.3.7. Evaporation solvent

It is based on the same principle that other methods, like nanoprecipitation or coacervation, but in this case the deposition of the polymer is caused by the evaporation of the organic solvent in which it is dissolved.

An O/W emulsion is prepared in which external phase it contains a surfactant. The coating material and the substance to be encapsulated (if it is lipophilic) compose the internal phase; if not, the preparation of a W/O/W emulsion is needed. Polymeric material is deposited over the drops when the solvent is extracted or evaporated. [57][58]

6. CONTROLLED RELEASE

As mentioned thought the document, one of the main objectives of the encapsulation of active ingredients is allowing the controlled release of them.

Along this chapter the main profiles and release mechanisms will be described, how they are associated with the delivery systems studied and the mathematical models that describes the most relevant phenomena involved.

6.1. Delivery profiles

Controlled release is based on providing the optimal amount of active ingredient in the properly moment and location. It means that the delivery can be designed to controlling its action:

- Along the time (temporary control): to release the active ingredient during a defined period of time is desired [59].
    - Burst release: fast release of the most of the active ingredient in a short period of time.
    - Sustained release: extended release of the active ingredient to a relatively constant release rate.

- In the place of action (distribution or local control): the objective is releasing the active when the system are located in the properly place (target site) of the organism [60]. This kind of delivery is also known as delayed release.
    - Triggered release: the release of the active ingredient takes place due to a specific environmental characterizes, as pH, temperature, ionic force, enzymatic activity or mechanic forces.
    - Targeted release: the release of the active ingredient takes place in a specific site. It is usually be achieved though the union of a specific ligand to the surface of the delivery system.

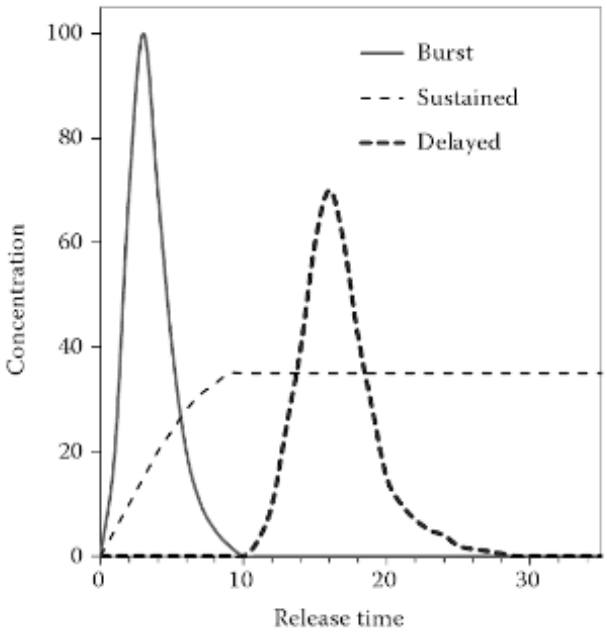

Figure 25. Delivery profiles [44]

Although the concept of controlled release may be explained in a simple and easy way, designing devices, formulations or systems that can reproduce the desired behavior can be extremely complicated.

6.2. Release mechanisms

The mechanism involved in the release of active ingredients depends on the kind of delivery system selected and the materials used. It is mandatory to make a distinction between liposomal systems, based on lipid bilayers, and the rest of delivery systems. Liposomal lipid bilayer and cellular membrane have some common components; this fact promotes the interaction between the liposomal systems and the cells, allowing actives release. However, the other delivery systems release the active ingredient due to the interactions between it and the coating material.

6.2.1. Lipid-based vesicles

Santos y Guerrero (1994), as many other authors, has defined four different possible kinds of interaction between liposomal vesicles and cells, some of which can occur at the same time [61]:

- **Fusion:** Firstly, a specific recognition of the liposome by the cell occurs, through the membrane proteins that are able to interact with those of the liposome. Once the interaction place is recognized, liposomal lipids fusion with the cellular membrane takes place, which allows the release of the ingredients entrapped in the vesicles.
- **Adsorption:** the liposome is absorbed in the surface of the target cell allowing the diffusion of the active ingredient through the cellular membrane.

- **Endocytosis:** during this process, liposomes are invaginated by the cellular membrane resulting in a vesicle named endosome that go through the cell membrane. Once inside the cell, the endosome is degraded by the enzymatic action of lisosomes and the inner material is released.
- **Lipid exchange:** this is the mechanism through which the liposome is able to release the hydrophobic ingredients contained within lipid bilayer. Due to the similar composition of the layers, the cell is not able to differentiate between the endogenous and exogenous layer of the liposome. Liposomes are aggregated in a specific area of the cellular membrane and are able to spread the ingredients entrapped in their bilayers from one to another until reaching de cellular membrane.

6.2.2. Other delivery systems

In the rest of the delivery systems, the release mechanism involved is controlled mainly on one of the following factors or processes [62]:

- Control by diffusion
- Control by contact with a solvent
    - Swelling
    - Dissolution
- Erosion or degradation
- External forces

6.2.2.1. Systems controlled by diffusion

Diffusion phenomenon is the main and more common for release of active ingredients. The active ingredient leaves the matrix by molecular diffusion, it means, it is displace due to a gradient of concentration.

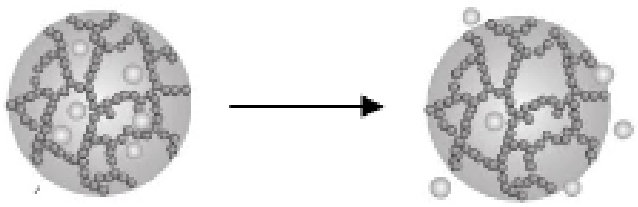

Figure 26. Diffusion [44]

When diffusion is the main release mechanism involved, two types of systems can be defined according to their structure: reservoir systems and monolithic systems.

Reservoir systems

They are composed by an inert membrane surrounding the ingredient has to be delivered. This last one will spread through the membrane at a constant rate during the most part of the useful life of the device.

Two system categories can be distinguished:

- **Type I:** Inner reservoir contains a saturated solution of active ingredient. The established equilibrium in the interface of the membrane induces a constant gradient of concentration and, therefore, the release presents an order zero kinetics. In general, these devices contain a saturated solution and an excess of active ingredient in solid state in order to keep the saturation for more time.
- **Type II:** Inner solution is not saturated. Concentration of active ingredient decreases exponentially over time (first-order kinetics independent of geometry).
  Some devices containing saturated solutions can produce a first-order release because of the extern solvent gets into the system and dissolves the excess of active ingredient altering the equilibrium.

Monolithic systems

The active ingredient is uniformly distributed in the matrix, so the charge or concentration of the entrapped agent, the nature of the components and the devices geometry determine the rate of release. These systems are characterized by a constant decrease of the release rate.

There are two main types:

- Monolithic solutions: active ingredient, generally a liquid, is found dissolved in the coating material.
- Monolithic dispersions: active ingredient is poorly soluble in the coating material and it is found dispersed in the matrix.

6.2.2.2. Systems controlled by contact with solvents

Mathematical models describing release rate controlled by diffusion assume that the carrier material does not undergo changes. Nevertheless, in some cases, encapsulating material is able to absorb part of solvent of the media and swelling, dissolving or slowly degrading, which can affect delivery.

Swelling

Active ingredients are released from the system when it absorbs solvent molecules and swells.

An active ingredient could be initially trapped within a particle with a pore size sufficiently small to prevent its movement; swelling can involve an increased of the internal pore size or the solvent entered into the system can dissolve the coated material causing its diffusion through the matrix.

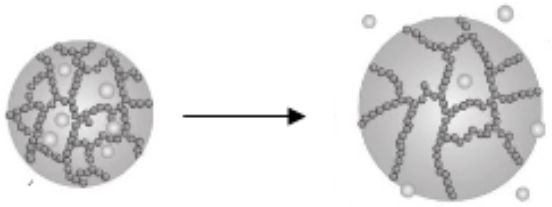

Figure 27. Swellling [44]

Therefore, it can be concluded that release rate will depend on absorption speed of the solvent by the system and on the diffusion of the encapsulated material.

Dissolution or erosion and degradation

Both terms refer to loss process of encapsulating material in order to provide a sustained delivery of the coated material.

Dissolution mechanism is based on the dissolution of the encapsulation system in the surrounding medium to allow the availability of the active ingredient. Generally, it is assumed that the process begins in the surface of the particle and advances inland.

Thus, coating dissolution rate is the main controlling factor of release rate. That means that the release rate can be controlled by means of composition and particle structure, as well as through magnitude and duration of the environmental factor responsible for the dissolution.

By contrast, erosion and degradation occur by chemical, physicochemical or enzymatic degradation of the encapsulating material. It can occur in the whole system simultaneously or only in the surface, depending on the speed at which the medium comes into the system. When entry is faster than the degradation process, general erosion takes place. In contrast, surface erosion occurs when the average invasion is slow or when degradation is faster. [35]

6.2.2.3. External forces

The main mechanism involved is fragmentation.

Particles breaks due to a physical disruption and the encapsulated material can leave the fragments by diffusion, dissolution or erosion processes. In these cases, the release occurs faster due to the increase of the surface area with respect to the original particle.

Release rate will depend on tension applied when the fracture occurs, as well as size and form of the formed fragments.

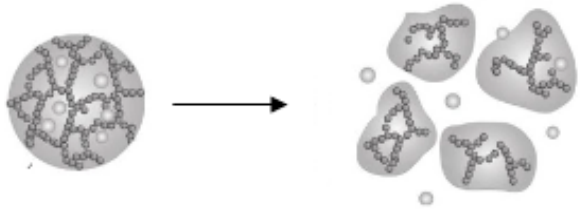

Figure 28. Fragmentation [44]

6.3. Mathematical models

Mathematical modelling of the liberation processes is an important tool to better understand the phenomena involved in our system and to determine which factors influence them, thus reducing the number of experimental studies that have to be carried out. [63]

As mentioned above, the limiting stage of the release processes is usually the diffusion, which is the most modeled mechanism. Three kinetic models are commonly used in these cases: zero order, first-order and Higuchi model. [64]

Zero order		First-order		Higuchi's equation (1961)	
$\frac{M_t}{M_\infty} = k \cdot t$	(Eq. 1)	$\frac{M_t}{M_\infty} = 1 - e^{-k(t-t_0)}$	(Eq. 2)	$M = \sqrt{D \cdot t \cdot (2 \cdot C - C_s) \cdot C_s}$	(Eq. 3)

Table 3. Kinetic models

Where:

M_t is the mass of active ingredient released in a period of time t

M_o is the total mass of active ingredient released

k is a constant related to the structural and geometric characteristics of the system, which n the cases of zero order and first-order kinetics can be approach to:

$$k = \frac{4 \cdot \pi \cdot D \cdot K \cdot C_s \cdot r_e \cdot r_i}{r_e - r_i} \qquad \text{(Eq. 4)}$$

Where:

K is the distribution coefficient

D is the diffusion coefficient

Cs is the solubility of the active ingredient in the solvent (saturation concentration).

A is the total surface area of the device

r_e and r_i are the external and internal capsule radius

Later, Higuchi adapted his model to the liberation from homogenous and granular matrices.

When it comes to non-porous matrices (homogeneous), non-inflatable and in which the compound is uniformly dispersed, it is assumed that the active ingredient dissolves in the matrix and then diffuses from there to the surface. As the compound is released, the diffusion distance becomes greater so that the variation of the concentration increases. On the other hand, if the matrix is porous, dissolution medium must first enter through them into the system, so it will be necessary to introduce a tortuosity factor into the equation. Assuming that the concentration of the compound in the matrix is less than its solubility and that its release occurs slowly and after diffusion through the pores, the equation can be expressed as:

$$M = \sqrt{\frac{D \cdot \varepsilon}{\tau} \cdot (2 \cdot C - \varepsilon \cdot C_s) \cdot C_s \cdot t} \qquad \text{(Eq. 5)}$$

Where:

ε is the matrix porosity

τ is the system tortuosity factor

This equation assumes that the system is no coated and that the matrix geometry does not change during release. In general, it is possible to combine the equations of the Higuchi's model in an easy one:

$$M = k' \cdot \sqrt{t} \qquad \text{(Eq. 6)}$$

Where:

k' is a constant

The use of Higuchi's equations is limited to the adjustment of the kinetics until release of the 60% of the active ingredient, it means, for $M_t/M_\infty<0.6$.

In some cases, the release mechanisms deviate from the Fick equation, following an anomalous (non-Fickian) behavior. For these cases, Peppas used a n value in order to characterize the transporting of four different molecules through a matrix. [23]

Korsmeyer-Peppas' equation: $$\frac{M_t}{M_\infty} = k \cdot t^n \qquad \text{(Eq. 7)}$$

Where:

k is a kinetic constant related to the structural and geometric characteristics of the system.

n is the kinetic exponent from which the release kinetics can be interpreted. For spheres:

n = 0.43 → Fickian diffusion mechanism

n = 0.85 → Anomalous diffusion mechanism or non-Fickian, known as "Case II transport" or swellable matrix.

0.43 < n < 0.85 → Intermediate transport mechanism.

n < 0.43 → Pseudo-Fickian diffusion mechanism. Release curves are similar to those obtained for a Fickian diffusion but with a slower rhythm towards equilibrium.

n > 0.85 → Known as Super-Case II, it is characterized by a fast release.

Peppas stated that its use is only suitable for thin films and it only can provide an approximate adjustment in the case of spherical or cylindrical shapes. For this reason, subsequent studies were performed with the aim of developing other models that took account the dependence of the kinetic exponent with respect to the geometry of the system. From the Korsmeyer-Peppas equation, another modified semi-empirical equation is proposed with the incorporation of the so-called delay time introduced by Kim and Fassihi (1997), according to the expression:

$$\frac{M_t}{M_\infty} = k \cdot (t - t_d)^n + b \qquad \text{(Eq. 8)}$$

Where:

k is a constant.

b is the total fraction of compound that have been released by the burst effect.

t_d is the delay time defined as the period of time in which a significant amount of compound released once the burst effect occurs again.

Peppas and Sahlin developed another semi-empiric model that takes into account, besides de diffusion of active ingredient, the anomalous transport contribution due to the swelling of the matrix. This model can be express as:

$$\frac{M_t}{M_\infty} = k_1 \cdot t^m + k_2 \cdot t^{2m} \qquad \text{(Eq. 9)}$$

Where:

m is the Fickian diffusion exponent valid for any system geometry.

k1 is the diffusion constant.

k2 is a constant related to relaxation of polymeric chains.

The first term represents the contribution by Fick diffusion and the second one is referred to Case II or to the contribution due to the relaxation of polymeric chains, since it is assumed that they are additives. [63]

6.4. Discussion

Based on the release profiles described above, it could be said that liposomes are delayed release systems, as they do not release the encapsulate ingredient until it reaches the target site in the *stratum corneum* cells. Nevertheless, the fact that release is sudden or sustained depends on the release mechanism involved.

Although the consulted authors consider that four mechanisms as feasible, in our opinion, this point is debatable:

- Endocytosis process is high selective, as it requires high phagocytosis capability on the part of the cells, as that of hepatic cells. That is why is unlikely that this release mechanism occurs with topical application products.
- Lipid exchange and adsorption are diffusion methods that involve that the active ingredient (hydrophilic or hydrophobic) spreads through the lipid bilayers of liposome and cell, needing to go through low or high polarity zones (hydrophobic chains or hydrophilic heads, respectively). Therefore, they are mechanisms that will occur infrequently, probably when a merger is not possible, and very slowly.

Liposomes fusion with the cell membrane triggers the sudden release of the liposome nucleus. Therefore, if a sustained release is desired, phospholipids compounding the bilayer should have a high melt point in order to avoid fusion and to force the release by diffusion.

Regarding to the other release systems, it is well known that diffusion will be the mainly release mechanism involved. However, depending on the material type used and the system structure, there are other feasible possibilities:

- In polymeric particles (spheres or sponges), the main release mechanism will be the diffusion. In order to release a substance from a matrix, it is needed that this spreads through it; for it, polymeric chains that form the device should move cooperatively to allow the transport of the substance. However:
    - Water-soluble polymers will allow the control of the short-term release, since it is expected that the release will be controlled by the dissolution of the polymer.
    - Biodegradable polymers require a chemical reaction, as hydrolysis, in order to be dissolved or degrade and release the active ingredient. Thus, the release rate will depend on the quantity and type of labile links and its accessibility.
    - Non-biodegradable polymers are basically inert to their environment and provide a sustained release. The release rate will be controlled by diffusion mechanisms. Small active ingredient quantities dissolved in the polymer will spread through the membrane by diffusion mechanism. When the amount of dissolved active principle is higher, particles near the surface of the particles will first diffuse leaving cavities in the system that will favor the faster release of the active. If the amount of active ingredient is very high, as would be the case of sponges, the cavities resulting from the diffusion of the active principle will be interconnected with others forming an exit channel from the inside, favoring the process even more.
- Concerning polymeric capsules, that mostly encapsulate hydrophobic phases, the diffusion mechanism will be less viable due to the poor solubility of the capsule core in the surrounding solvent. Thus, in these cases the main release mechanisms involved will be dissolution, erosion or degradation.
- With respect to SLNs and NLCs, in which both encapsulation system and encapsulated ingredient are lipophilic, the active ingredient diffusion or coating material dissolution is not viable. In these cases, erosion will be prevailing as release mechanism.

There are not widespread mathematical models for the swelling, dissolution and erosion/degradation mechanisms. Therefore, experimental test should be carried out in order to determine the release profile. It can be assumed that these delivery systems allow the sustained release of the active ingredients, being faster in particles than in capsules and in polymeric than in lipid systems.

7. QUALITY PARAMETERS OF ENCAPSULATED PRODUCTS

When it is wanted to encapsulate an asset in a delivery system, a lot of studies have to be performed in order to guarantee that the kind of system and the manufacturing conditions selected have been the correct ones. This conclusion is based on the quality of the product obtained.

Numerous parameters that provide information about this quality exist. Some of them are based on encapsulation process yield and others on the physical and/or chemical characteristics of the delivery system. Below is a summary of the most relevant parameters, as well as some of the most used techniques for its determination, based on the information provided by several authors and by some companies that manufacture this type of systems. [65]

7.1. Performance indices

Firstly, it is important to remark that the calculation of some of the following performance indices requires knowledge of the mass of active ingredient successfully encapsulated in the system. Determination method of this parameter will depend on which kind of delivery system is used.

Generally, when vesicles are used as encapsulation system, the previous removal of the no-encapsulated active ingredient is required. In order to achieve it, a centrifugation is carried out by means of which the vesicles are separated. Hereafter they are washed, usually with PBS, and vesicles are disrupted by addition of isopropyl alcohol. Finally, concentration of active ingredient is determined by means of UV spectrophotometry or High Performance Liquid Chromatography (HPLC). [66]

Both for the particles and capsules, the extraction of the active ingredient is usually carried out by means of a suitable solvent for its good solubility.

Loading capacity (LC):
Measurement of the mass of encapsulated active ingredient per unit of mass of the encapsulation material. $$LC\ [\%] = \frac{Mass\ of\ encapsulated\ active\ ingredient}{Mass\ of\ encapsulation\ material} \cdot 100$$ (Eq. 10)
Encapsulation efficiency (EE):
Measurement of the ability of the delivery system to retain the encapsulated active ingredient. The EE is defined as the percentage of the entrapped active ingredient related to its initial concentration. $$EE\ [\%] = \frac{Mass\ of\ encapsulated\ active\ ingredient}{Total\ mass\ of\ active\ ingredient\ added} \cdot 100$$ (Eq. 11)
Percentage yield (PY):
Measurement of the ability of the elaboration process to produce active ingredient loaded units. $$PY\ [\%] = \frac{Mass\ of\ loaded\ encapsulation\ system}{Mass\ of\ active\ ingredient\ encapsulation\ material\ utilized} \cdot 100$$ (Eq. 12)

Table 4. Performance indices

7.2. Physical and/or chemical characterization

Size and Polydispersity Index (PI)

Both parameters may be determined by various methods. When structures whose size has to be measured show micrometric size, they can be inspected by Optical Microscopy; but when their size increases, the use of Scanning Electron Microscopy (SEM) techniques are needed. This technique presents a good resolution, but the restriction of working at low-pressure conditions does not allow analyzing the samples in their native medium. That is the reason why these parameters are usually determined by Dynamic Light Scattering (DLS) techniques.

DLS method is based on the diffusion of a light beam through a sample of the dispersion in order to determine the size and the homogeneity of the encapsulation system. The dispersed intensity of the laser light is detected by a photomultiplier and targeted to a digital optical correlator, which characterizes the intensity of the signal and translate it to a particle size value. [67]

This technique results very useful for the measurement of particle sizes included in the range 1 nm to 5 μm.

For the measurement of cyclodextrin complexes size, the laser light diffraction equipment should be adjusted because of their no-spherical morphology. In these cases, the particle size is expressed as the average diameter of a sphere with the same volume as the complex.

Regarding Polidispersity Index (PI), it refers to homogeneity of the analyzed samples with respect to their dimensions. The higher the IP is, the larger will be the difference between the size average of the analyzed population and, therefore, the higher will be the heterogeneity of the system. Thus, a PI value closer to the unity involves a heterogeneous sample, while a PI value closer than zero will indicate a high homogeneity in the sample.

Morphology

The determination of the morphology of the particles is studied by high-resolution surface characterization techniques Scanning Electron Microscopy, Transmission Electron Microscopy, and the family of Proximal Probe Microscopies, among which is included and highlights the Atomic Force Microscopy (AFM). [13]

One of the main advantages of Proximal Probe Microscopies techniques over SEM or TEM is that these are not destructive and they can operate in any virtual environment. In addition, in the most of cases a special treatment of the samples is not needed to performing analysis.

AFM is based on the use of a probe whose tip keeps in touch with the surface of the sample while it moves. The tip collects the topography of the encapsulations by means of laser measurement of the deflection it suffers while it is on movement.

Instead, for the encapsulations internal structure determination, fluorescence techniques are usually used.

Zeta Potential

The zeta potential measures the charge that surrounds the particles in suspension and allows determining colloidal characteristics, which constitute an important indicator of the physical stability of the dispersion. According to DLVO theory, a system will be stable in a simple electrolytic solution when repulsion forces between two particles are larger than Van der Waals attraction forces.

In order to determine the surface charge of encapsulated systems, a sample dilution is introduced into *Zetasizer* equipment. This one provides the Zeta Potential value by measuring of the electrophoretic mobility, or degree of movement of colloids in dispersion, under the effect of an electric potential applied through the sample and expressing the net effective charge over the surface of encapsulations.

Osmotic shock

Osmotic shock parameter is specially important for the design of vesicles-based delivery systems, due to it allow determining changes in the size of vesicles when they are incubated in hypotonic, hypertonic and/or isotonic medium.

Delivery systems based on matrices or capsules, composed by polymeric or lipid materials, are less sensitive to fracture due to this phenomena.

Residual solvents content

Organic solvents are involved in many manufacturing process for the production of delivery systems. Because of their toxicity, manufacturer companies should guarantee that the amount of residual organic solvents in the final product is not harmful for the user. The quantification is performed by gas chromatography.

Stability study

Multiple factors may affect the stability of the product because of they have the capability of accelerate its degradation. Stability studies consist in exposing various samples of the same product batch to these conditions for a certain time and analyzing the quality parameters at several times along this period. The observed changes allow making an estimation of the long-term variations that product characteristics may suffer and fixing its shelf life.

8. SELECTION METHODOLOGY

The first thing has been to take into account is the capability of the different kinds of delivery systems for support the active ingredient. Based on nature of last one, some options may be discarded. In the following table is reported the capability of each delivery system studied for transport the active ingredient according to this aspect.

DELIVERY SYSTEM		Hydrophilic active ingredients	Lipophilic active ingredients	Hydrophobic active ingredients
Lipid vesicles	Core	✓	✗	✗
	Bilayer	✗	✓	✓
Polymeric capsules		✗	✓	✗
Polymeric spheres		✓	✓	✓
Polymeric sponges		✓	✓	✓
SLNs		✗	✓	✓
NLCs		✗	✓	✗

Table 5. Compatibility between assets and delivery systems

For hydrophilic actives

- Lipid vesicles: the encapsulation of the active ingredient takes place in solution form in the core, so they have a good load capacity.
- Spheres and sponges: contain the active ingredient dispersed into the matrix, so they present a lower load capacity. Between them, sponges have higher load capacity than spheres, due to their porosity.
- Polymeric capsules, SLNs and NLCs: can transport hydrophilic active ingredients in the surface of the system because of surfactants presence. However, this situation would not correspond to a really encapsulation process.

For lipophilic active ingredients

- SLNs and NLCs: are the most compatible system due to the nature of their compounds. NLCs present a higher load capacity than SLNs because of their structure.
- Polymeric capsules: allow the encapsulation of lipophilic active ingredient in their core. Thus, they have a high load capacity. Nevertheless, since polymers forming the system usually present certain hydrophilic character, they would not be the most suitable system.
- Polymeric spheres and sponges: like in capsules case, they are not the most compatible system due to the nature of the compounds forming the structure. They have a lower

load capacity, since they entrap the active ingredient dispersed into the matrix. Again, sponges can entrap a higher amount of active ingredient than spheres because of their structure.

- Lipid vesicles: Although they can entrap this kind of actives, their load capacity is very low due to they would be dissolved in a liquid vehicle and entrapped into the lipid bilayer. In addition, it is important to know that certain lipophilic compounds, like essential oils, may provoke phospholipids degradation.

For hydrophobic active ingredients

- Polymeric spheres and sponges: as has been commented above, polymeric systems are not composed by a single polymer, but rather a combination of them in order to adjust the hydrophily/hydrophobicity degree. Due to this, spheres and sponges would be the best option to hydrophobic substances encapsulation, by affinity.
- SLNs: would be able to contain this type of actives, but due to their nature, they would be the less suitable ones.

If load capacity was the only selection criteria, this would be the order that delivery systems should be tested, from the most suitable option to the most unlikely one.

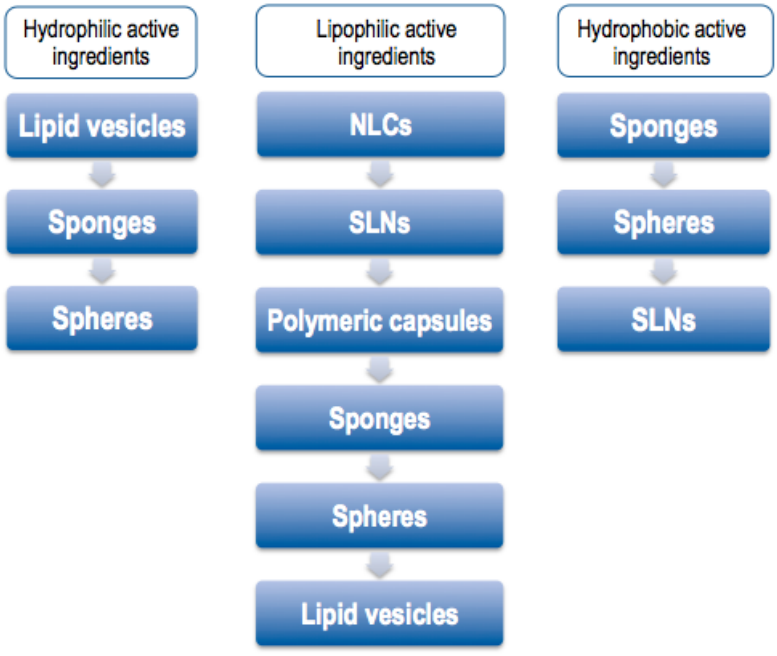

Figure 28. Priorities according load capacity

However, loading capacity is not the only criterion to be taken into account when choosing the most appropriate release system for a purpose, but it is also sought that the encapsulated ingredient is released in the desired place at the dose correct Consequently, not always the release system with more load capacity of an active ingredient is the system chosen for transport.

The release of the encapsulated material will be produced by one mechanism or another based on the material of which the system is composed. Therefore, the selection of materials is an important task, since it affects the final result obtained.

Below is a table with the release mechanisms associated with each type of system depending on its structure and the materials that constitute it:

Lipid vesicles	High melt point phospholipids	Fusion
	Low melt point phospholipids	Adsorption + diffusion
Polymeric spheres and sponges	Water-soluble polymers	Dissolution
	Biodegradable polymers	Dissolution Erosion
	Non-biodegradable polymers	Swelling + diffusion
Polymeric capsules	Dissolution Erosion	
SLNs and NLCs	Erosion External forces	

Table 6. Release mechanisms and coating materials relation

It is not possible to provide a comparison of the release rate achieved by each mechanism, since the phenomena involved in the release are intrinsically linked to the combination of encapsulated material/encapsulating material/system structure.

Nor is it possible to provide a tool that provides us with a unique solution to the problem of selection, since both the selection criteria and the influencing factors are too specific and can not be generalized based on the delivery system alone. For example, vitamin E is a lipophilic active that can be encapsulated in multiple delivery systems. However, it is generally marketed encapsulated in lipid particles. The selection of this alternative is not based on criteria of release speed, protection against its toxicity, ability to penetrate the skin, etc. it is based on its sensitivity to oxidation. In the interior of particles or lipid capsules, thanks to its structure and the robustness of the materials that make up the system, vitamin E is better protected against oxidizing agents. In other cases, such as hydrophilic ingredients that can be toxic to the body at high doses, although lipid vesicles would be the best option based on load capacity, these are usually encapsulated in polymeric systems. The selection is based on the resistance of the system; lipid vesicles are more compatible with the human body, but are flexible and can break causing rapid release of the encapsulated active. Consequently, the companies that require the encapsulation of this type of assets use polymeric systems, more rigid and resistant, which guarantee the sustained release of the ingredient.

Regarding the technique of encapsulation to carry out the manufacture of the product, it will also depend on multiple factors, such as the properties of the product that is intended to be obtained, if the compound to be encapsulated is sensitive to certain operating conditions, if the process requires specific equipment, etc. As a consequence, it is not possible to provide a priority order to choose the technique to implement, but it is possible to give some information about their advantages or drawbacks in order to facilitate the discarding of the options that are less appropriate to the situation at hand.

Lipid vesicles	Thin film hydration method	It is a simple, reproducible and easily scalable process. It does not require specialized equipment. In addition, excessively high temperatures are not used so it is valid to encapsulate temperature sensitive assets.
		This technique implies very bulky equipment and a large amount of organic solvents compared to batch size produced. Hydration does not occur simultaneously throughout the volume, so vesicles obtained will not be homogeneous and should apply subsequent homogenization procedures that will increase the price of the process and also the production time requested.
	Ethanol injection method	It is a simple, reproducible and easily scalable process. High temperatures are not required, so it can be used for temperature sensitive assets encapsulation. It does not require a posterior treatment of the vesicles obtained.
		It requires de use of organic solvents. A lot of studies must be performed in order to determine the most suitable operating conditions; certain combinations of different factors (temperature, injection pressure, concentrations, etc.) can provoke the obtainment of really small vesicles (< 100 nm).
	Reverse-phase evaporation	It is a simple, reproducible and easily scalable process. It does not require specialized equipment High temperatures are not required, so it can be used for temperature sensitive assets encapsulation. The formation of the lipid vesicles occurs simultaneously throughout the volume, giving as a result a homogeneous product.
		It requires the use of organic solvents. It also involves a sonication step for inverse micelles formation. Sonication processes are not very popular at industrial scale for cosmetic products manufacturing, due to its complexity, cost and the high temperatures that the product can achieve during the process.
	Microfluidization	The process is carried out at very high pressures and provides a very homogeneous product.
		Depending on the pressures applied, the concentrations and materials used, very small vesicles can be obtained. Therefore, studies will be required to select the manufacturing conditions.
	Modified RESS	It is a simple, reproducible and easily scalable process. It does not require the use of organic solvents nor additional treatment of the product obtained. Thanks to the supercritical fluids utilization, the global process results environmental friendly.
		The use of this technique is very limited due to it is only suitable for soluble in supercritical fluids active ingredients.

Polymeric capsules/spheres/sponges	Spray drying	This process is simple, easily scalable and flexible; it is suitable for a wide range of feeds (solutions, dispersions, melts, slurries, etc.) and product specifications. It has an affordable cost and allows continuous production.
		Limited availability and high cost of the encapsulating materials and the equipment. Low thermal efficiency; the process requires high warm air volumes crossing the drying chamber without contacting directly with the particles, so they do not contribute to drying. Normally this technique can be used for labile compounds encapsulation due to its contact with high temperatures is very short and the rapid evaporation process keeps the droplets temperature relatively low, but ingredients such as peptides or proteins may be damaged or inactivated.
	Nanoprecipitation	It is a simple, reproducible and fast process. It can be carried out with low energy consumption due to it only requires a slight stirring.
		It is limited to water-soluble solvents and soluble actives in this kind of solvents, so the selection of involved materials can be very difficult. In addition, this technique requires the use of large water amounts and long stirring time.
	Coacervation	High quality products are obtained by this method. They present high encapsulation efficiency and high stability against oxidation. The selection of the solvent is not as complicated as in other cases, since it does not have to meet so many restrictions.
		High variability between lots.. It is more expensive than other methods due to the high amount of solvents needed. The most used organic solvents are dichloromethane, ethyl acetate, acetonitrile and toluene, which are restricted by cosmetic regulation and bad-looked by consumers.
	RESS	It is a simple, reproducible and easily scalable process. It does not require the use of organic solvents or additional treatment of the product obtained. Thanks to the supercritical fluids utilization, the global process results environmental friendly.
		The use of this technique is very limited due to it is only suitable for soluble in supercritical fluids active ingredients.
	Interfacial polymerization	The encapsulation step is often easier than in other chemical processes due to the active ingredient is dissolved in the encapsulated phase.
		Usually, the reactive monomer involved is isocyanate, a precursor of polyurethane which is not well-looked by the consumers.
	Ionic gelation	This technique also consists in a chemical process, but in this case the coating material (alginate) is more accepted by the consumers.
		Encapsulation is more difficult than in other chemical processes due to the asset is not soluble in the same phase that the coating material.
	Evaporation solvent	The encapsulation step is often easier than in other chemical encapsulation processes due to the active ingredient is dissolved in the encapsulated phase. In addition, it allows the production of particles with different size with high encapsulation efficiency.
		It requires the use of organic solvents and their selection can be very complicated because of the restriction they have to accomplish.

SLNs & NLCs	Hot-high pressure homogenization	It is an easily scalable process. Processing time is short and the particles obtained present a low polydispersity index.
		The process is carried out at high temperatures, so it can cause the degradation of the lipid material or the encapsulated compound.
	Cold-high pressure homogenization	It is an easily scalable process. Processing time is short and the particles obtained present a low polydispersity index.
		The process is carried out at lower temperatures than hot-high pressure homogenization, but anyways the high temperatures are not completely avoid, due to the melt of the solid lipids stills being necessary. However, it is more suitable for labile compounds.
		Normally, the particles obtained by this method are more heterogeneous.
	Emulsification by evaporation of the solvent	The process is carried out in a single stage, without the need for special equipment and in benign temperature conditions.
		The process requires the use of organic solvents. In addition, given the low solubility of lipids in this type of compounds, it is usual to obtain a much diluted product that contains traces of these solvents. In addition, the particles obtained are very small, sometimes less than 100 nm.
	Microemulsification with high speed agitation or ultrasound	It is an easily scalable technique without the need for specialized equipment, with low energy consumption and which results in particles with high load efficiency and low polydispersity index.
		The product obtained contains high concentrations of surfactants and co-surfactants, so its use in humans is subject to sanitary regulations.
	Emulsification method by contact membrane	It is a simple, reproducible and easily scalable process. The contact membrane is easy to use, ensures the control of the particle size by means of selecting the operating conditions and can be regenerated until to recover a permeability of more than 80% before being used again.
		The sealing of the pores of the membrane can produce a malfunction of the system and imply greater cost and consumption of time.

Table 7. Advantages and disadvantages of encapsulation techniques

9. CONCLUSIONS

Based on the studied performed about the available delivery systems in the market, it has been concluded that the main and more consolidated ones are: lipid vesicles, polymeric spheres, sponges and capsules, and lipid particles.

They have been classified in three categories based on their physical structure: vesicles (liposomes, marinosomes, ethosomes and silicone vesicles), particles (polymeric and lipid particles and silicone matrices) and polymeric capsules.

Silicone-based systems are not well-looked by the consumers, so many manufacturers are erasing them from their formulations.

Multi-walled delivery systems and cyclodextrins complexes have not been recognized as delivery systems in this project.

- Multi-walled delivery systems are only a tool for delay the release, but they are not which entrap the active ingredients.
- Cyclodextrins complexes are used in cosmetic as enhancers of assets penetration in the skin, but not for control the release. As a consequence, they can be considered carrier systems, but not delivery one. In addition, they only allow the entrapment at molecular level.

The release mechanisms associated to each delivery system, taking into account the coating material involved, has also been studied and it has been concluded which mechanism would be involved in each case.

Available encapsulation techniques at industrial level in cosmetic market have been reported, as well as their main advantages and drawbacks and some influencing factors in the characteristics of the final product obtained.

Based on all this information, it has been possible the development of a tool that facilitates the selection of the most suitable delivery system for beginner companies by limiting initial options, thus reducing the number of studies that have to be performed and the cost of the design process.

Once a delivery system is selected, some tests have to be performed in order to check that the product complies with the quality requested and certify that the selection has been correct. Based on the information provided by some manufacturing companies and bibliographic research, the main parameters measured and the methods used have been also determined.

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