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Microemulsion Systems: Generalities and Fields of Application

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ABSTRACT

Microemulsions are two-phase oil-aqueous systems stabilized by a surfactant/cosurfactant system, formed from the spontaneous self-assembly of hydrophobic or hydrophilic parts of surfactant molecules, essential at the industrial level for their unique properties, improving processes and reducing costs. Their main uses in the pharmaceutical, cosmetic, and food industries are to improve the biopharmaceutical and pharmacokinetic properties of drugs, the skin penetration properties, and the stability and solubility of different ingredients. They are obtained by techniques such as phase inversion and phase titration. They are characterized by different techniques that allow obtaining information on the dynamic properties, droplet size, structural arrangement and orientation, molecular aggregation, and system interactions, which allow improving the formulations continuously. Due to their proven advantages and utilities, as well as their potential applications, it is essential to study these systems.

Keywords: Bioavailability, formulation, microemulsions, solubility, surfactants.

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I. Introduction

Professor Shulman introduced the concept of microemulsions in 1959. The definition has varied over time, but it is known as oil-aqueous biphasic systems stabilized by a surfactant system, formed from the spontaneous self-assembly of hydrophobic or hydrophilic parts of the surfactant molecules [1]–[4]. Macroscopically they present a transparent appearance and are observed as monophasic and isotropic structures that are impossible to differentiate with the naked eye. While microscopically, they are seen as microheterogeneous dispersions formed by domains of oily and aqueous phases separated by a layer of surfactant [5]–[7]. They also have a dynamic character since they produce continuous exchanges of materials between the different domains and can be reversibly formed or destroyed by variations in temperature and composition [8].

Microemulsions differ from emulsions in appearance, particle size, stability, and energy required for their preparation. Emulsions are biphasic dispersions composed of macromolecules, which present a cloudy or milky appearance, with a particle size that varies within the range of 1 to 100 um, characterized by presenting thermodynamic instability and requiring external energy for their preparation.

On the other hand, microemulsions are observed as single-phase systems composed of micromolecules with transparent or translucent appearance, having a particle size ranging from 5 to 100 nm, characterized by high thermodynamic stability and requiring little energy for their preparation [1], [9].

Microemulsion formulations are generally composed of; 1) an aqueous phase containing the hydrophilic active ingredient and 2) an oily phase, which counteracts the decrease in formulation volume and the delivery of the active ingredient in encapsulated form. 3) a surfactant and cosurfactant system responsible for stabilizing the aqueous and oily phases, although a system composed only of surfactants could also be used [10].

Finding three types of microemulsion systems is possible depending on the nature of the dispersed particles. Water-in-oil (W/O) microemulsions consist of the dispersion of an aqueous solution in a water-immiscible liquid. The aqueous solution is considered the internal or discontinuous phase, and the water-immiscible liquid is the external or continuous phase. On the other hand, oil-in-water (O/W) microemulsions are formed from the dispersion of a water-immiscible liquid in an aqueous solution. In this case, the water-immiscible liquid is the internal or discontinuous phase, while the aqueous solution is the external or continuous phase. Finally, there are bicontinuous microemulsions consisting of an infinite bilayer with multiple curvatures, which connects randomly with itself so that it divides the system into two different solvents and therefore does not present a discontinuous or continuous phase. This type of microemulsion is formed at the inversion temperature when the amount of aqueous solution and water-immiscible liquid is similar. It should be noted that in all cases, the system is stabilized by a suitable surfactant/co-surfactant mixture, which ensures the stability of the microemulsion [1], [11].

The transitions of microemulsion systems from one type to another are generally explained by the Winsor classification, created in 1948. He classifies microemulsions into four types; in Winsor I, the oil-phase nanodroplets (discontinuous phase) are stabilized in the aqueous phase (continuous phase) by the effect of the surfactant/cosurfactant system, and there is an excess of the oil phase. The opposite is true in Winsor II since the aqueous phase nanodroplets (discontinuous phase) are stabilized in the oily phase (continuous phase), and an excess of the aqueous phase is observed. On the other hand, Winsor III is composed of a bicontinuous O/W microemulsion system in equilibrium with part of the oily phase and part of the aqueous phase. Finally, Winsor IV presents only the bicontinuous phase because the oily and aqueous phases are mixed proportionally [12].

Microemulsions can be arranged in different self-assembled structures, such as spherical micelles, cylindrical micelles, vesicles, and flat bicontinuous interfaces. These structures occur in water-in-oil (W/O), oil-in-water (O/W), or bicontinuous systems [13].

As mentioned above, one of the essential components of microemulsions is surfactants. These are amphiphilic compounds with relatively balanced properties; they have a structure with hydrophilic and hydrophobic parts. In addition, they must be able to form micellar aggregates; these show a strong tendency to migrate toward the interfaces directing their hydrophilic group toward the water and its hydrophobic group toward oil [14]. The surfactant molecules are located with the hydrophobic chain towards the inside, in the case of the direct type O/W (the dispersed phase is hydrophilic), creating a non-polar medium. Furthermore, in the indirect type W/O (the dispersed phase is hydrophobic), the polar heads are directed inward, forming a polar center, unilamellar, multilamellar, and tubular vesicles [6], [7].

There are different types of surfactants; anionic surfactants have a negatively charged hydrophilic region; cationic surfactants have a cationic group on their polar region, and amphoteric surfactants can act like anionic ones in alkaline pH or like the cationic ones in acidic pH since the pH of the medium regulates their activity, great care must be taken to control it at all times. Finally, non-ionic surfactants have no charge, and their lipophilic part is composed of a fatty chain of average variable size. Moreover, its hydrophilic part is also highly variable [15]–[17].

On the other hand, cosurfactants are short to medium-chain alcohols (C3-C8) that can reduce interfacial tension and increase interfacial fluidity. These are alcohols, amines, and cholesterol. Moreover, they are used to increase the effectiveness of the surfactant [10].

To select a mixture between surfactant and cosurfactant, the compound's solubility to be included, the area of the auto-emulsification region in the phase diagram, and the drop size distribution must be evaluated [18]. Surfactants with hydrocarbon chains of moderate length preferably form O/W microemulsions, surfactants with bulky hydrophobic tails form bicontinuous microemulsions, and surfactants with branched hydrophobic tails form W/O microemulsions [19]. Surfactants can be absorbed in the interface; however, this absorption is not instantaneous; it depends on the kinetics and composition of the liquid, especially on the ionic strength [19]. It should be considered that mixtures of surfactant with HLB (Hydrophilic-Lipophilic Balance) value less than 10 W/O emulsions were obtained, and greater than 10 O/W emulsions were obtained [14].

Since microemulsion formulations are essential at the industry level due to their unique properties, improving processes, and even reducing the cost of products [2], the objective of this article is based on providing an overview of the use of microemulsions in the industry, mainly in the pharmaceutical, cosmetic and food industries, as well as their characterization techniques and production processes.

II. Applications in the Pharmaceutical Industry

Microemulsions have many advantages, including easy preparation and thermal stability. Also, having microdomains of different polarities in the same solution makes it possible to solubilize both hydrophilic and hydrophobic molecules within the same formulation. The literature has observed that the active principle is usually solubilized in the internal phase [20]. These systems have been studied extensively in the pharmaceutical field to improve the bioavailability of poorly soluble active principles. Due to their components, a fluid system can carry drugs through biological barriers such as the skin. They can also be used as prolonged-release systems, thus improving the physicochemical limitations of the drugs [15], [21]. Furthermore, it can help increase or decrease the speed at which the active principles are released and generate

a systemic or local effect through different mechanisms [21]. Microemulsions, being so versatile, can be administered by different routes.

On the other hand, depending on the surfactant used in the formulations, it can enhance bioavailability by improving the dissolution of the drug, increasing intestinal epithelial permeability, increasing permeability in tight junctions, and reducing or inhibiting Glycoprotein P efflux [14],[22]. Medium-chain fatty acids influence the tight junctions of epithelial cells. Long-chain fatty acids stimulate lipoprotein synthesis and subsequent lymphatic absorption; this knowledge should be considered when formulating drugs [18]. As the materials must be biocompatible, the choice of these is complicated. The most widely used surfactants are polysorbates, alkyl polyethers, and sorbitan monoesters. While for the excipients of the formulations, vegetable oils such as those from corn, cotton, orange, mint, eucalyptus, and coconut, triglycerides, and fatty acid esters such as isopropyl myristate, ethyl oleate are used [23]. Another critical factor is the droplet size of the microemulsions, as they result in a larger surface area where the drug can be dispersed and absorbed through the membranes. Hence, dissolution is no longer a limiting factor [24].

In general terms, the main advantages of microemulsions for the formulation of pharmaceutical products are; its high solubility potential for hydrophilic and lipophilic drugs [21], the reduction of the interindividual and intraindividual need in the absorption of orally administered drugs, decrease in the affectation of the drug performance by the presence of food [18], as well as the little difficulty in the preparation and reproduction processes, since it only requires simple and low-cost manufacturing facilities [25].

A. Topical Delivery

Microemulsions applied topically show a significant increase in the skin absorption of drugs. Studies with hydrophilic drugs such as 5-fluorouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate, and lipophilic drugs such as estradiol, finasteride, ketoprofen, meloxicam, felodipine, and triptolide showed improved skin penetration when administered as microemulsions [10], [26].

Depending on the components, the vehicles often act as penetration enhancers [21]. The thermodynamic drug diffusion process through the interfacial surfactant film between the microemulsion phases can increase the distribution and diffusion in the stratum corneum. However, the drug can be retained in the formulation droplets, resulting in a higher surfactant concentration in the dispersed systems, causing a decrease in skin penetration [27].

For topical delivery, it has been shown that vehicles with a positive charge improve the bioavailability of the drug compared to those with negative and neutral charges since those with a positive charge interact strongly with cells, thus improving the permeability of the drug and prolonging the effect exerted by this very [28]. Although neutral and amphoteric surfactants are the least toxic [15]. These formulations should consider the skin-irritating aspect, especially for long-term use [10].

B. Ophthalmic Delivery

Correctly formulated ophthalmic microemulsions provide easy application because they do not need to be as frequent as long-acting applications [29]. In vivo, experiments using different ocular drugs administered as microemulsions such as timolol, levobunolol, pilocarpine nitrate, dexamethasone, pilocarpine hydrochloride, chloramphenicol, pilocarpine hydrochloride, and everolimus, showed a delayed drug effect as well as increased corneal penetration [29].

As described, surfactants are of utmost importance in the manufacture of microemulsions. In this case, they can help the drug penetrate through the cornea due to its low surface tension allowing an ideal mixture to be given with tears, and it is guaranteed to be spread correctly on the ocular surface. The most widely used surfactants are non-ionic, such as polysorbates and polyethylene glycols. Since ionic ones are very toxic to the ocular surface, these agents are the most versatile due to their improved solubilization characteristics, such as non-irritability and ability to prolong the precorneal retention with improved patency make them ideal. It is essential to know that the aqueous phase must always contain additives such as electrolytes, antibacterial compounds, and isotonic agents [29], [30].

C. Oral Delivery

One of microemulsions' most interesting oral administration applications is the administration of peptide and protein drugs. Peptides are difficult to administer orally because they lose much of their activity and have low bioavailability, presenting much intersubject variability. The ability to control drug concentration in vivo is achieved by maximizing bioavailability by formulating the drug in a microemulsion system [31].

Furthermore, it can potentially administer poorly soluble lipophilic drugs with low bioavailability. Microemulsions' advantages for oral use are improving the solubilization and protecting the drug against enzymatic hydrolysis. In addition, it enhances the improved absorption provided by the fluidity induced by the surfactant in the membrane, which generates permeability changes [18], [32]. Lipids found in medium and long-chain formulations are transported in the organism differently; medium-chain lipids are transported directly to the portal vein, passing into the systemic circulation. In contrast, the intestinal lymphatic vessels transport long-chain fatty acids (14 carbons or more). Likewise, monounsaturated and polyunsaturated fatty acids promote lymphatic lipid transport more rapidly and efficiently than equivalent saturated fatty acids. It should be noted that the lymphatic absorption pathway offers the opportunity to improve the bioavailability of highly lipophilic drugs [33], [34].

On the other hand, the self-micro emulsifying drug administration system (that has received much attention in an oral delivery drug is the self-micro emulsifying drug administration system (SMEDDS). This is a system of oil-in-water microemulsions obtained when mixed with water under gentle agitation. This system's advantage is that it improves the drug's solubilization, release, and absorption properties due to how the active dissolves in the formulation and the small diameter of the droplets, which gives it a large interfacial surface area. SMEDDS have faster gastric emptying than non-emulsifying systems, and they act independently against bile, suggesting that they will not be digested before the drug is absorbed [23]. This formulation is

usually administered in soft gelatin capsules, which is convenient for patients [33].

Several studies have been conducted with formulations of these self-emulsifying systems. For example, the results of a study evaluating the in vitro release and ex vivo drug penetration of a SMEDDS formulation of docetaxel indicated superior performance compared to the traditional drug suspension. On the other hand, when studying a SMEDDS formulation of lornoxicam, higher AUC, Cmax, and tmax values were found compared to the commercial tablet. Likewise, a SMEDDS formulation of clopidogrel exhibited an increase in AUCR Cmax and tmax compared to the traditional solid formulation. Finally, another study determined that a SMEDDS formulation of dutasteride had a more significant effect and increased AUC and Cmax parameters compared to the commercial product [35].

D. Parenteral Delivery

Parenteral administration can avoid the three critical steps of oral administration: gastrointestinal dissolution, absorption, and hepatic clearance. It is also the preferred route in an emergency, guaranteeing a rapid onset of action. However, designing microemulsions for this route is a complicated task since the number of excipients approved for parenteral administration is limited, having to meet the biocompatible and sterilizable requirements, non-pyrogenic, non-irritating, and non-hemolytic. However, it is possible to design parenteral microemulsions with the appropriate selection of excipients and obtain characteristics such as sustained-release or prolonged blood circulation, improving patient compliance, thus obtaining a better treatment result [36],[37]. These systems exhibit more excellent physical stability in plasma than liposomes and other vehicles and present an internal oil phase more resistant to drug leaching [10].

Non-ionic surfactants such as n-alkyl polyoxyethylene ether are often used to stabilize microemulsions for parenteral use since this compound does not need a cosurfactant. This is an advantage at the pharmaceutical level since many of the cosurfactants used in the industry are not pharmacologically acceptable. Furthermore, it also has another advantage of being infinitely dilutable, which is not the case with non-ionic surfactants, not to mention that non-ionic ones are considered the least toxic [38]. Several poorly soluble drugs have been formulated in o/w microemulsions for parenteral administration, which is beneficial when the administration of suspensions is not required [10].

E. Nasal Delivery

Nasal drug delivery systems for peptide and protein drugs have gained attraction in recent decades due to the relatively high permeability of the nasal epithelium compared to other non-invasive routes. This route avoids first-pass metabolism and promotes improvement of patients regarding their compliance as it offers the possibility of a simple and comfortable drug administration. However, the bioavailability achieved after nasal administration has usually shown poor results for hydrophilic molecules and high molecular weight drugs such as insulin. Different strategies have been explored to improve drug absorption through the nose's mucosa, including chemical penetration enhancers [39], [40].

The diazepam microemulsion based on ethyl laurate, used for the initial treatment of epilepsy, shows a rapid onset of action where a 2 mg/kg dose achieves a maximum plasma concentration in 2-3 minutes [18]. In addition, a microemulsion of nimodipine showed that the AUC ratio in brain tissues and cerebrospinal fluid after a nasal application was significantly higher than those obtained after IV application, leading to improved brain solubility and uptake [24].

III. Applications in the Cosmetic Industry

Microemulsion-based cosmetic formulations have outstanding attributes in addition to being able to penetrate the skin very quickly and can efficiently hydrate. This system's usefulness lies in incorporating many lipophilic cosmetic active ingredients in the oily phase that would otherwise be difficult to formulate. In addition to having hydrophilic and interfacial domains, they allow ingredients with different solubilities to be added to the formulation. In addition, due to their high solubilization capacity, it is possible to incorporate significant amounts of active ingredients into the formulation. On the other hand, due to the ability of surfactants and cosurfactants to reduce the skin's barrier properties, they can be used as permeation promoters in formulations [41], [42]. In the cosmetic field, microemulsions can be formulated as moisturizers, sunscreens, antiperspirants, cleaning, hair care, and aftershave products, mainly intended to be administered topically [41].

For the formulation of cosmetics using microemulsions, the surfactant/cosurfactant system used must have a low potential to sensitize the skin, high biocompatibility, and correct skin penetration since otherwise, it can generate adverse effects such as allergic reactions, irritation, and cytotoxicity, among others. Likewise, during formulation, aspects such as physicochemical properties of the system components, structural descriptors, the molecular weight of the active ingredient, and the incorporation of permeation promoters should be considered since they influence the ability of the cosmetic to penetrate through the skin [42].

Several studies have been carried out using different active ingredients. It was observed that microemulsion formulations for cosmetic purposes have significant benefits in the formulation and delivery of the active ingredient.

A study showed that microemulsion formulations successfully solved formulation problems presented with naringenin for producing anti-aging products. Microemulsion systems could modify naringenin's poor stability and water solubility while obtaining a formulation with optimal bioavailability and low skin irritation. In addition, in vitro and ex vivo studies showed a decrease in aging factors and a positive change in gene expression levels compared to the control group [43].

In another study where O/W and W/O microemulsions containing Aloe vera and celery extract, compounds used in hair growth promotion, were prepared, it was determined that the O/W microemulsion showed more significant hair growth promotion activity, demonstrating that a delivery system with hydrophilic and semi-polar active ingredients in microemulsion form was able to penetrate the pilosebaceous

gland satisfactorily to promote hair growth [44].

Recent studies have shown that microemulsions can contribute to the protection and stability of drugs sensitive to light, pH, and temperature. It was observed that microemulsion for topical administration of trans-resveratrol showed good skin penetration, photosensitivity, and improved solubility. Also, it was found that topical formulations of catechin with microemulsions loaded with Eugenia dysenterica plant extract presented better skin permeation and deposition and improved stability without compromising the antioxidant activity of the substance [42].

On the other hand, a study on ceramide encapsulation using lectin-based microemulsions showed that these systems improved in vitro freeing, and penetration of ceramides compared to other formulations on the market. Also, a study on the permeability of ceramide microemulsions through the stratum corneum showed that low-viscosity microemulsions containing smaller droplets were able to penetrate deeper layers of the skin to a greater extent compared to standard hydrophilic cream containing ceramides [42].

IV. Applications in the Food Industry

Microemulsions present colloidal delivery systems, as they are created from food ingredients using simple processing protocols [45]. Some stages of food production require the solubilization of lipophilic ingredients due to their nutritional and functional importance, such as fat-soluble vitamins, essential fatty acids, carotenoids, low polarity additives, flavorings, colorants, preservatives, and antioxidants, among others [46]–[48]. The industry needs help in incorporating these components in hydrophilic formulations. In addition, the low chemical stability of these compounds poses a problem since many of them are essential substrates in oxidative reactions. Also, there is increasing interest in investigating methods to protect hydrophobic bioactive compounds, such as vitamins, within the food industry. The need for susceptible, low-cost, rapid, and environmentally friendly chemical analytical methods must also be considered. These obstacles drive the application of microemulsion technology to the food field [46],[49],[50].

The application of microemulsions to the food industry is an idea that has been raised recently. The main focus of research has been to improve the solubilization and stability of ingredients, which are everyday problems. Despite this, there are a limited number of studies on this topic. It is known that systems with controlled composition and physicochemical properties are used that often do not match the characteristics of many foods [46],[51].

A. Solubilization and Stability of Nutrients and Bioactive Compounds

Incorporating lipophilic nutrients such as vitamin E, retinol, fish oil, omegas, and beta-carotenes in aqueous systems has been possible thanks to the O/W type microemulsions have proven to be an efficient vehicle due to the more excellent solubility they provide [46], [52], [53].

In some studies of the stability and solubility of bioactive compounds and vitamins using microemulsions, increases in these aspects were observed. For example, lycopene can be solubilized in a concentration up to 10 times higher in microemulsion than in a solely lipophilic system. Its stability increased, showing a percentage of degradation in time from 48% to 65% lower. [46], [54]. Another example could be a carotenoid such as lutein. In non-esterified and esterified forms, its solubility is less than ten ppm. In comparison, the solubility of this carotenoid in a microemulsion reached 1520 ppm (in its esterified form) and 390 ppm (in its non-esterified form) in a hydrophilic phase system of water and glycerol [46], [55].

In a study, 25% of fish oil was solubilized in a microemulsion with controlled-release type O/W. This system is formed with water, ethyl oleate, and Tween® 80 as surfactant. The system increases stability and bioavailability. In addition, it masks the taste of fish oil [56]. W/O type microemulsions composed of fish oil (omega 3), propylene glycol, and Tween®80 showed an increase in water solubility, demonstrating the potential of microemulsions as a system to release bioactive substances in food [48].

B. Release of Flavoring and Preservative Compounds

In addition to improving solubility, an advantage of using microemulsions is the rationing and substitution of solvents in flavoring preparations and the chemical stability of flavorings [57],[58].

The use of citrus oils as flavorings is essential in the beverage industry. For this reason, studies on microemulsions have focused on their solubilization [47]. Few studies on this, but they all suggest fewer solvents, more excellent chemical stability, and lower losses in their solubilization process [59].

The increase in the antimicrobial activity of preservatives when using microemulsions may be related to their excellent solubility and diffusion, thus obtaining more significant contact with microorganisms. Tests were carried out with additives widely used in the food industry to verify this. This increase can be attributed to changes in the permeability of the plasma membrane of the microorganism. This is caused by the surfactant, causing an improvement in the adsorption of the antimicrobial on the cell surface [57]–[59].

C. Microemulsions as Antimicrobial Systems

Recent studies have shown the effectiveness of microemulsions against microorganisms of interest to public health, including bacteria, molds, and yeasts, although these studies are scarce [60], [61].

The antimicrobial activity of O/W microemulsions based on water, ethyl oleate, Tween® 80, and low molecular weight alcohols were tested against a Pseudomonas aeruginosa and Staphylococcus aureus culture. With this, it was discovered that said system was highly effective against these cultures, suggesting that microemulsions are highly effective antimicrobial agents that act on the plasma membrane causing it to rupture. These studies also showed a decrease in biofilm formation by Pseudomonas aeruginosa. The system was also tested against Salmonella spp, Escherichia coli: H7, Staphylococcus aureus, Listeria monocytogenes, and to prevent the formation of biofilms of Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Listeria monocytogenes, which are commonly found in food. After these pathogens were exposed to these systems, there was a 99% reduction in viability, thus confirming the antimicrobial activity. Regarding the formation of the films, the only one that

presented resistance was Listeria monocytogenes [55], [60], [62], [63].

The mechanism of action against fungi is similar to that of bacteria. Despite this, the effect against fungi is minor. Recently, cytoplasmic membrane structure alteration and dysfunction have been proposed as a mechanism of action, which causes changes in the cell wall, cytoplasmic coagulation, interruption of intracellular metabolism, and cell death [60].

D. Microemulsions Applications in Food Chemical Analysis

The use of microemulsions in food analysis is due to the higher sensitivity of analytical methods. Microemulsions can extract the analyte from the sample more efficiently than conventionally used solvents, all without using any procedure under extreme conditions, resulting in less loss of analytes [49], [64].

As a method of separating the analyte, the microemulsions dispense with any pretreatment for the sample, which gives it fewer sources of error. Therefore, the analysis is more straightforward and faster and does not require organic solvents. With this, a lower environmental impact since they are generally low cost, biodegradable, non-toxic, and less flammable [49], [65].

The preparation of samples in the form of microemulsion has increased the sensitivity, optimization, and reproducibility of the analysis of traces of metals and additives in vegetable oils, metals in chocolates, and contaminants, additives, and bioactive compounds in beverages [49], [65].

V. Preparation Methods

Concerning the formulation of microemulsion substrates, there are two main preparation methods; phase titration and phase inversion. These formulation procedures are simple, inexpensive and can be carried out at room temperature [1].

In the phase titration method, the oil phase must be added dropwise to the aqueous phase with constant stirring for reverse-phase microemulsions. The oily phase is a mixture of oil and surfactant in a determined weight ratio. When the oily phase is added, the sample becomes cloudy and continues to be added until the turbidity disappears; at that point, it becomes a microemulsion. Once this process is finished, the exact percentages by weight are determined and represented in the phase diagram. All the formulation components are in the mixture in proportions ranging from 0 to 100% [19], [29]. Similarly, to obtain a microemulsion of the opposite sign, this procedure can be performed by adding dropwise the aqueous phase to a previously prepared mixture composed of the oily phase and surfactant system [1].

On the other hand, the phase inversion method allows obtaining microemulsions by modifying experimental conditions such as the proportion of salts, temperature, and volume fractions, which generates that the dispersed phase of a microemulsion obtained initially becomes the continuous phase and vice versa because the surfactant arrangements at the interface undergo a spontaneous change [66].

It should be noted that it is necessary to apply and adapt the preparation methods according to the active ingredients used, the ingredients of the aqueous and oily phase and their concentration, the surfactant/cosurfactant system used, as well as the type of microemulsion to be prepared. In addition, factors affecting solubility and stability, pH, salinity, and zeta potential, among others, must be controlled [1].

VI. Characterization

In order to obtain a characterization of the microemulsions, it is necessary to carry out a set of techniques since the data obtained from just one needs to be more representative to characterize a microemulsion system safely. Through these characterization techniques, it is possible to obtain information about the dynamic properties, droplet size, structural arrangement and orientation, molecular aggregation, and system interactions, among others [67]. Among the most prominent techniques are:

A. Pseudoternary Phase Diagrams

Phase diagrams study the effects of different proportions of surfactant/cosurfactant systems in the region of stable microemulsion formation to determine the appropriate concentrations of each component to establish a balance between the phases of the microemulsion [15], [68].

B. Viscosity

Rheological measurements provide information on the aggregate shapes of microemulsion systems and are related to structural effects. Generally, these systems have low viscosities. However, depending on the intended application of the formulation, it is necessary to make changes in the viscosity by reducing or increasing the shapes of the aggregates [7].

C. Electrical Conductivity

Electrical conductivity determines if the microemulsions are continuous in oil or water. The O/W microemulsions have high conductivity, while the W/O have low conductivity [7], [14]. They also allow determining the transformation of drop-shaped structures to bicontinuous through a percolation threshold. This threshold refers to the critical fraction of the volume of water in which the isolated drops form infinite groups; this is associated with bicontinuous structures [69].

D. Nuclear Magnetic Resonance (NMR)

It allows for determining the diffusion coefficient of molecules, which provides information on the localization of molecules, the mobility of the different components of a microemulsion, and the predominant interactions in microemulsion systems. It also provides information on the internal connectivity of the structure and makes it possible to follow the transition from single particles to interconnected, bicontinuous structures [70], [71].

E. Static and Dynamic Light Scatterring (SLS and DLS)

These techniques contribute to the deduction of particle size from molecular weight and hydrodynamic radius. They also provide information about the shape of the microemulsion aggregates, the interactions between the droplets, and the transition of the microemulsion droplets to a bicontinuous phase [71].

F. Microscopy

The microscopy techniques currently used to characterize microemulsions include transmission electron microscopy (TEM) and freeze-fracture electron microscopy (FFEM). These allow the evaluation of microemulsions' morphology and confirm the droplet size information obtained by light scattering techniques. It also allows evaluation of the isotropy properties presented by the microemulsions [67].

G. Fluorescense

Fluorescence techniques allow for obtaining information about the diffusion of the microemulsion particles, as well as the mobility of different compounds or additives present in the microemulsion system. It also helps to deduce the microemulsion's size and droplet size distribution to determine the number of aggregates in the system and the polar character within the microemulsion domains [71].

H. Interfacial Tension (IFT)

The measurement of interfacial tension makes it possible to measure and optimize the solubilization capacity of an emulsion, a fundamental property of microemulsions. In these cases, a lower interfacial tension increases the solubilization capacity of the surfactant system, which increases the microemulsion's structural units. Consequently, the fundamental properties of the system are controlled by controlling the interfacial tension through parameters such as temperature, salinity, and the addition of surfactants, among others [71].

VII. Conclusions

Microemulsions are helpful in several areas of the industry since they facilitate and improve the manufacturing processes of a great variety of products, reducing production costs and generating new alternatives that satisfy the users' needs. These systems are mainly used in the pharmaceutical field to improve the biopharmaceutical and pharmacokinetic properties of a wide range of drugs with different routes of administration. They are also valuable for the cosmetics industry, offering improved skin penetration properties and stability. Likewise, in the food industry, they provide advantages by improving the solubility, stability, and antimicrobial activity of different ingredients. On the other hand, the methods for obtaining microemulsions are considered simple and low-cost techniques, which, complemented with adequate characterization methodologies, make possible the creation of new and improved systems to meet the emerging needs of today's market. Due to the above, it is essential to continue research on the formulation of microemulsion systems and their different applications in the industry.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

References

[1] Talianu MT, Dinu-Pîrvu CE, Ghica MV, Anuţa V, Jinga V, Popa L. Foray into Concepts of Design and Evaluation of Microemulsions as a Modern Approach for Topical Applications in Acne Pathology. *Nanomaterials.* 2020 Nov 19;10(11):2292. doi: 10.3390/nano10112292.

[2] Lee KL. *Applications and Use of Microemulsions*. 2010;6.

[3] Salicio MM, Moreno MD. Colloids and interfaces. [Coloides e interfases]. 1ª ed. España: Universidad de Salamanca; 2005. 480 p.

[4] Flanagan J, Singh H. Microemulsions: A Potential Delivery System for Bioactives in Food. *Crit Rev Food Sci Nutr*. 2006 Apr 1;46(3):221–37. doi: 10.1080/10408690590956710.

[5] Galán JJ. Influence of temperature on the micellar properties of quaternary ammonium salts in aqueous solution. [Influencia de la temperatura en las propiedades micelares de sales cuaternarias de amonio en disolución acuosa]. Universidad de Santiago de Compostela; 2008.

[6] Madrigal GL. Aplicación de la información de patentes para el desarrollo de sistemas coloidales farmacéuticos. *Rev CENIC Ciencias Químicas*. 2015;46:195-205.

[7] Martínez CM. Trabajo Fin De Grado Título: Desarrollo De Micro Y Nanoemulsiones De Liberación Sostenida. [Thesis]. Universidad Complutense; 2016.

[8] Cano AM. Preparación de materiales moleculares nanoparticulados y dispersos -vesículas y microemulsiones- empleando fluidos comprimidos [Ph.D. Thesis]. Barcelona: Universidad Autónoma de Barcelona; 2009.

[9] Paul B, Mitra RK, Moulik S. Microemulsions: Percolation of conductance and thermodynamics of droplet clustering. *Encycl Surf Colloid Sci*. 2006 Jan 1.

[10] Sujatha B, Himabindu E, Bttu S, Abbulu K. Microemulsions – A review. *Journal of Pharmaceutical Sciences and Research.* 2020;12(6):750-753.

[11] Suhail N, Alzahrani AK, Basha WJ, Kizilbash N, Zaidi A, Ambreen J, et al. Microemulsions: Unique Properties, Pharmacological Applications, and Targeted Drug Delivery. *Front Nanotechnol*. 2021;3:1-6. doi: 10.3389/fnano.2021.754889.

[12] El Seoud OA, Keppeler N, Malek NI, Galgano PD. Ionic Liquid-Based Surfactants: Recent Advances in Their Syntheses, Solution Properties, and Applications. *Polymers*. 2021 Jan;13(7):1100. doi: https://doi.org/10.3390/polym13071100.

[13] Mahboob A, Kalam S, Kamal MS, Hussain SM, Solling T. EOR Perspective of microemulsions: A review. *J Pet Sci Eng*. 2022 Jan 1;208:109312. doi: https://doi.org/10.1016/j.petrol.2021.109312.

[14] Fernández A. Preparación, caracterización y estabilidad de emulsiones y microemulsiones O/W. [Thesis]. Universidad de Granada;2006.

[15] Moreno EE, Sampedro MN. Diseño de una nueva suspensión farmacéutica utilizando microemulsiones como vehículo para la incorporación de ibuprofeno. [Thesis]. Ecuador: Universidad de Guayaquil; 2019 [cited 2023 Jun 8].

[16] Martini MC, Chivot M, Peyrefitte G. Dermocosmetica y Estética: cosmetología. Barcelona: Elsevier España; 1997. 154p.

[17] Lafuente VL, Azcárate MIB, Benito BA. Introduction to organic chemistry. [Introducción a la química orgánica]. ed 4. Publicacions de la Universitat Jaume I; 1997. 260 p.

[18] Ghosh PK, Murthy SR. Microemulsions: a potential drug delivery system. *Curr Drug Deliv*. 2006 Apr;3(2):167–180. doi: 10.2174/156720106776359168.

[19] Muñoz M, Ochoa JR, Fernández C. Formación de microemulsiones inversas de acrilamida. [Thesis]. *Rev de Ciencia, Tecnología y Medio Ambiente*. 2005; 3:29.

[20] Rodriguez C. Las Microemulsiones como Vehículos para Administración de Drogas. *Acta Farm Bonaer*. 2004; 23:8.

[21] Flores J. Evaluación *in vitro* del efecto promotor de microemulsiones de clorhidrato de naltrexona sobre la permeabilidad en la piel [Thesis]. México: Universidad Autónoma del Estado de Morelos; 2018.

[22] Lüllmann H, Mohr K, Hein L. Pharmacology: text and atlas. [Farmacología: texto y atlas]. 6 ed. España: Editorial Médica Panamericana; 2010. 412 p.

[23] Cirri M, Mura P, Mora PC. Liquid spray formulations of xibornol by using self-microemulsifying drug delivery systems. *Int J Pharm*. 2007 Aug 1;340(1–2):84–91. doi: 10.1016/j.ijpharm.2007.03.021.

[24] Gupta S, Moulik SP. Biocompatible microemulsions and their prospective uses in drug delivery. *J Pharm Sci*. 2008 Jan;97(1):22–45. doi: 10.1002/jps.21177.

[25] Pather SI, Gupte SV, Khankari RK, Hontz J, Kumbale R. Microemulsions as solid dosage forms for oral administration. [Microemulsiones como formas farmacéuticas sólidas para la administracion oral]. Madrid; 2341510, 2001. p13.

[26] Fialho SL, da Silva-Cunha A. New vehicle based on a microemulsion for topical ocular administration of dexamethasone. *Clin Experiment Ophthalmol*. 2004 Dec;32(6):626–632. doi: 10.1111/j.1442-9071.2004.00914.x.

[27] Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. *Adv Colloid Interface Sci*. 2006 Nov 16;123–126:369–85. doi: 10.1016/j.cis.2006.05.014.

[28] Peira E, Chirio D, Carlotti ME, Spagnolo R, Trotta M. Formulation studies of microemulsions for topical applications of acyclovir. *J Drug Deliv Sci Technol*. 2009 Jan 1;19(3):191–196. https://doi.org/10.1016/S1773-2247(09)50035-4.

[29] Hegde RR, Verma A, Ghosh A. Microemulsion: new insights into the ocular drug delivery. *ISRN Pharm*. 2013;2013:826798. doi: 10.1155/2013/826798. Print 2013.

[30] Molina I. Nano- y micro-sistemas farmacéuticos en la administración ocular de medicamentos. *Anales de la Real Academia de Doctores de España*. 2018;3:394-412.

[31] Sharma VK, Koka A, Yadav J, Sharma AK, Keservani RK. Self-Micro Emulsifying Drug Delivery Systems: a Strategy to Improve Oral Bioavailability. *Ars Pharm Internet*. 2016;57(3):97-109.

[32] Constantinides P, Scalart JP, Lancaster C, Marcelo J, Marks G, Ellens H, et al Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. *Pharm Res*. 1994 Oct;11(10):6.

[33] Gibaud S, Attivi D. Microemulsions for oral administration and their therapeutic applications. *Expert Opin Drug Deliv*. 2012 Aug;9(8):937–951. doi: 10.1517/17425247.2012.694865.

[34] He CX, He ZG, Gao JQ. Microemulsions as drug delivery systems to improve the solubility and the bioavailability of poorly water-soluble drugs. *Expert Opin Drug Deliv*. 2010 Apr;7(4):445–460. doi: 10.1517/17425241003596337.

[35] Rajpoot K, Tekade M, Pandey V, Nagaraja S, Youngren SR, Tekade RK. Chapter 9 - Self-microemulsifying drug-delivery system: ongoing challenges and future ahead. In: Tekade RK, editor. Drug Delivery Systems [Internet]. Academic Press; 2020. p. 393–454. doi:https://doi.org/10.1016/B978-0-12-814487-9.00009-0.

[36] Date AA, Nagarsenker MS. Parenteral microemulsions: an overview. *Int J Pharm*. 2008 May 1;355(1–2):19–30. doi: 10.1016/j.ijpharm.2008.01.004.

[37] Dubey R. Controlled-release injectable microemulsions: recent advances and potential opportunities. *Expert Opin Drug Deliv*. 2014 Feb;11(2):159–173. doi: 10.1517/17425247.2014.870151.

[38] Malcolmson C, Satra C, Kantaria S, Sidhu A, Lawrence MJ. Effect of oil on the level of solubilization of testosterone propionate into nonionic oil-in-water microemulsions. *J Pharm Sci*. 1998 Jan;87(1):109–116. doi: 10.1021/js9700863.

[39] Wang J, Tabata Y, Morimoto K. Aminated gelatin microspheres as a nasal delivery system for peptide drugs: evaluation of in vitro release and in vivo insulin absorption in rats. *J Control Release Soc*. 2006 Jun 12;113(1):31–37. doi: 10.1016/j.jconrel.2006.03.011.

[40] Karasulu HY. Microemulsions as novel drug carriers: the formation, stability, applications and toxicity. *Expert Opin Drug Deliv*. 2008 Jan;5(1):119–35. doi: 10.1517/17425247.5.1.119.

[41] Azeem A, Rizwan M, Ahmad FJ, Khan ZI, Khar RK, Aqil M, et al. Emerging role of microemulsions in cosmetics. *Recent Pat Drug Deliv Formul*. 2008;2(3):275–289. doi: 10.2174/187221108786241624.

[42] Szumała P, Macierzanka A. Topical delivery of pharmaceutical and cosmetic macromolecules using microemulsion systems. *Int J Pharm*. 2022 Mar 5;615:121488. https://doi.org/10.1016/j.ijpharm.2022.121488.

[43] Şahin D, Çağlar EŞ, Boran T, Karadağ AE, Özhan G, Üstündağ Okur N. Development, characterization of naringenin-loaded promising microemulsion formulations, and demonstration of anti-aging efficacy by in vitro enzyme activity and gene expression. *J Drug Deliv Sci Technol*. 2023 Jun 1;84:104422. https://doi.org/10.1016/j.jddst.2023.104422.

[44] Pamudji JS. Microemulsion formulation of aloe vera gel and apium graveolens ethanol extract for optimizing hair growth promotion. *Asian J Pharm Clin Res*. 2015 Jul 1;8(4):319–323.

[45] Aboudzadeh MA, Mehravar E, Fernandez M, Lezama L, Tomovska R. Low-Energy Encapsulation of α-Tocopherol Using Fully Food Grade Oil-in-Water Microemulsions. *ACS Omega*. 2018 Sep 11;3(9):10999–1008. doi: 10.1021/acsomega.8b01272.

[46] Silva JDF, Silva YP, Piatnicki CMS, Böckel WJ, Mendonça CRB. Microemulsions: components, characteristics, potentialities in food chemistry and other applications. *Quím Nova*. 2015:38(9):1196-1206. doi:10.5935/0100-4042.20150135.

[47] Ziani K, Fang Y, McClements DJ. Encapsulation of functional lipophilic components in surfactant-based colloidal delivery systems: vitamin E, vitamin D, and lemon oil. *Food Chem*. 2012;134(2):1106-1112.

[48] Deutch-Kolevzon R, Aserin A, Garti N. Synergistic cosolubilization of omega-3 fatty acid esters and CoQ10 in dilutable microemulsions. *Chem Phys Lipids*. 2011 Oct 1;164(7):654–663. doi: 10.1016/j.chemphyslip.2011.06.010.

[49] Burguera JL, Burguera M. Analytical applications of emulsions and microemulsions. *Talanta*. 2012 Jul 15;96:11–20.

[50] Rao J, McClements DJ. Food-grade microemulsions, nanoemulsions and emulsions: Fabrication from sucrose monopalmitate & lemon oil. *Food Hydrocoll.* 2011 Aug 1;25(6):1413–1423. doi:10.1016/j.foodhyd.2011.02.004.

[51] Edris A, Rawlinson-Malone C. Formulation of banana aroma impact ester in water-based microemulsion nano-delivery system for flavoring applications using sucrose laurate surfactant. *Procedia Food Sci*. 2011 Dec 31;1:1821–1827. DOI:10.1016/j.profoo.2011.09.267.

[52] Amar I, Aserin A, Garti N. Solubilization Patterns of Lutein and Lutein Esters in Food Grade Nonionic Microemulsions. *J Agric Food Chem*. 2003 Jul 1;51(16):4775–4781. https://doi.org/10.1021/jf026222t.

[53] Spernath A, Yaghmur A, Aserin A, Hoffman RE, Garti N. Food-grade microemulsions based on nonionic emulsifiers: media to enhance lycopene solubilization. *J Agric Food Chem*. 2002 Nov 6;50(23):6917–6922. doi: 10.1021/jf025762n.

[54] Digout L, Bren K, Palepu R, Moulik S. Interfacial composition, structural parameters and thermodynamic properties of water-in-oil microemulsions. *Colloid Polym Sci*. 2001 Jul 1;279:655–663.

[55] Garti N, Yaghmur A, Aserin A, Spernath A, Elfakess R, Ezrahi S. Solubilization of active molecules in microemulsions for improved environmental protection. *Colloids Surf Physicochem Eng Asp*. 2003 Dec 10;230(1):183–190. https://doi.org/10.1016/j.colsurfa.2003.09.020.

[56] Zheng MY, Liu F, Wang ZW, Baoyindugurong JH. Formation and characterization of self-assembling fish oil microemulsions. *Colloid J*. 2011 Jun 1;73(3):319–26. DOI:10.1134/S1061933X11030197.

[57] Fu X, Feng F, Huang B. Physicochemical characterization and evaluation of a microemulsion system for antimicrobial activity of glycerol monolaurate. *Int J Pharm*. 2006 Sep 14;321(1):171–175. doi: 10.1016/j.ijpharm.2006.05.019.

[58] Gaysinsky S, Davidson PM, McClements DJ, Weiss J. Formulation and Characterization of Phytophenol-Carrying Antimicrobial Microemulsions. *Food Biophys*. 2008 Mar 1;3(1):54–65.

[59] Ferreira JP, Alves D, Neves O, Silva J, Gibbs PA, Teixeira PC. Effects of the components of two antimicrobial emulsions on food-borne pathogens. *Food Control*. 2010 Mar 1;21(3):227–230. https://doi.org/10.1016/j.foodcont.2009.05.018

[60] Zhang H, Shen Y, Weng P, Zhao G, Feng F, Zheng X. Antimicrobial activity of a food-grade fully dilutable microemulsion against Escherichia coli and Staphylococcus aureus. *Int J Food Microbiol*. 2009 Nov 15;135(3):211–215. doi: 10.1016/j.ijpharm.2010.05.022.

[61] Teixeira PC, Leite GM, Domingues RJ, Silva J, Gibbs PA, Ferreira JP. Antimicrobial effects of a microemulsion and a nanoemulsion on enteric and other pathogens and biofilms. *Int J Food Microbiol*. 2007 Aug 15;118(1):15–19. doi: 10.1016/j.ijfoodmicro.2007.05.008.

[62] Zhang H, Cui Y, Zhu S, Feng F, Zheng X. Characterization and antimicrobial activity of a pharmaceutical microemulsion. *Int J Pharm*. 2010 Aug 16;395(1):154–160. doi: 10.1016/j.ijpharm.2010.05.022.

[63] Al-Adham ISI, Al-Hmoud ND, Khalil E, Kierans M, Collier PJ. Microemulsions are highly effective anti-biofilm agents. *Lett Appl Microbiol*. 2003;36(2):97–100. doi: 10.1046/j.1472-765x.2003.01266.x.

[64] Nunes LS, Barbosa JTP, Fernandes AP, Lemos VA, dos Santos WNL, Korn MGA, et al. Multi-element determination of Cu, Fe, Ni and Zn content in vegetable oils samples by high-resolution continuum source atomic absorption spectrometry and microemulsion sample preparation. *Food Chem*. 2011 Jul 15;127(2):780–3.

[65] Romero L, Keunchkarian S, Reta M. Extraction of biogenic amines and their dansyl derivatives with reverse microemulsions of bis [2-ethylhexyl] sulphosuccinate (AOT) prior to high-performance liquid chromatographic determination. *Anal Chim Acta*. 2006 Apr 21;565(2):136–144.

[66] Sharma AK, Garg T, Goyal AK, Rath G. Role of microemuslsions in advanced drug delivery. *Artif Cells Nanomedicine Biotechnol*. 2016 May 18;44(4):1177–1785. https://doi.org/10.3109/21691401.2015.1012261.

[67] Egito EST, Amaral L, Alencar EN, Oliveira AG. Microemulsion systems: from the design and architecture to the building of a new delivery system for multiple-route drug delivery. *Drug Deliv Transl Res*. 2021 Oct 1;11(5):2108–2133. https://doi-org.ezproxy.sibdi.ucr.ac.cr/10.1007/s13346-020-00872-8.

[68] Abruzzo A, Parolin C, Rossi M, Vitali B, Cappadone C, Bigucci F. Development and Characterization of Azithromycin-Loaded Microemulsions: A Promising Tool for the Treatment of Bacterial Skin Infections. *Antibiotics*. 2022 Aug 2;11(8):1040. doi: 10.3390/antibiotics11081040.

[69] Ita K. Progress in the use of microemulsions for transdermal and dermal drug delivery. *Pharm Dev Technol*. 2017 May 19;22(4):467–475. doi: 10.3390/antibiotics11081040.

[70] Hathout RM, Woodman TJ. Applications of NMR in the characterization of pharmaceutical microemulsions. *J Controlled Release*. 2012 Jul 10;161(1):62–72. doi: 10.1016/j.jconrel.2012.04.032.

[71] Gradzielski M, Duvail M, de Molina PM, Simon M, Talmon Y, Zemb T. Using Microemulsions: Formulation Based on Knowledge of Their Mesostructure. *Chem Rev*. 2021;121(10):5671-740. https://doi-org.ezproxy.sibdi.ucr.ac.cr/10.1021/acs.chemrev.0c00812.