# Background, Limitations, and Future Perspectives in Food Grade Microemulsions and Nanoemulsions

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**ABSTRACT**

The interest towards microemulsions and nanoemulsions, the colloidal systems utilized to encapsulate bioactive compounds, is constantly increasing in food industries. Major characteristics of microemulsions and nanoemulsions should be assessed to better differentiate these two systems. Limitations and restrictions being imposed on the development of food-grade microemulsions and nanoemulsions should be addressed to improve their significant role in the food and beverage industry. Food studies should consider practical issues more thoroughly and apply effective inexpensive measures to minimize surfactant losses and improve surfactant recovery to bridge the gap between laboratory experiments and industrial processes.

**KEYWORDS**

Bioactive compound; delivery system; encapsulation; nano-Sized; thermodynamic stability

## Introduction

Dietary habits are major risk factors in chronic diseases, especially cardiovascular diseases, cancers, and diabetes. The key roles of essential nutrients, such as vitamins, antioxidants, and omega-3 fatty acids in preventing these and a broad range of other chronic diseases, have well been documented. Supplementary foods rich in such bioactive compounds can be administered in the form of colloidal dispersions for target delivery and optimal functionality.[1,2]

Colloidal dispersions are heterogeneous systems composed of small particles of any nature dispersed in a continuous phase of a different nature or composition. The functional properties of a dispersion are substantially dependent on its stability. Physical stability or, for short, stability is the period over which dispersed phase particles continue to remain suspended as individual units. The development of extremely small-sized dispersions at nanoscale is of greater importance than dispersions containing larger particles or droplets in terms of stability and functional properties.[3,4]

Microemulsions (MEs) and nanoemulsions (NEs) are the two most common types of colloidal dispersions having tiny droplet sizes of <200, in most cases <100 nm.[4,5] Conventionally, the term "nanoemulsion" is sometimes used for dispersed droplets with a size >100 nm but <200 nm, while "microemulsion" refers to systems with an average droplet diameter <100 nm.[6] These nano-sized colloidal systems are found to be of increasing interest to scientists in recent years for their wide range of potential applications in food and beverages, pharmaceutical, cosmetics, chemicals, oil recovery, biomedical and other interdisciplinary areas.[4,5]

Regardless of differences between MEs and NEs, both are good delivery systems due to their potential to increase loading, solubility, stability, and bioavailability of biologically active substances. Comprising hydrophilic and hydrophobic phases, MEs and NEs can solubilize a broad spectrum of either hydrophilic or lipophilic compounds, which are of interest in the formulation of foods,

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beverages, drugs, and personal care products. Among the other advantages of MEs and NEs are longer shelf life and ease in preparation and administration.[7,8] MEs and NEs scatter light weakly due to their small size of droplets. Therefore, they appear transparent or only slightly turbid, making them attractive for incorporation into consumer products required to be visually clear or only slightly hazy. They can also be developed in such a way to have desired rheological behavior, such as high viscosity, low yield stress or semi-gelled state, which can be exploited in the development of desired beverage and food products. Therefore, the investigation of MEs and NEs has attracted a great deal of recent research attention.[4]

However, despite similarities between MEs and NEs (e.g., size and capability to form water-in-oil or oil-in-water emulsions), there are some significant differences. This impacts the most proper preparation method, the key parameters affecting their overall stability and shelf life, structural, physicochemical, rheological and functional properties, and subsequent applications that have not been deeply investigated yet.[4,9]

The purpose of current review is to clarify and elaborate on (i) fundamental concepts, similarities and differences between MEs and NEs in terms of the terminology used to define them and current fabrication methods, (ii) significant factors impacting the stability of these colloidal dispersions, along with understanding the pseudo-ternary phase diagrams used for their preparation, elucidation of formation regions and selection of appropriate ratios of oil, aqueous, and surfactant phases, and (iii) applications of MEs and NEs in the food industries as encapsulation technique, natural preservatives, extraction method, delivery system for bioactive compounds, reaction media and active food packaging.

## Classification of emulsions

In general, emulsions are colloidal systems composed of two liquids that are ordinarily incapable of being mixed where one liquid is evenly dispersed as tiny droplets (dispersed phase) throughout the other one (continuous phase). Emulsions can be classified according to the nature of the internal (dispersed phase) and external (continuous phase) phases into different types of water-in-oil (W/O), oil-in-water (O/W), water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O). Most of these types of emulsions exist abundantly in either natural or man-made systems, including foods and beverages.[10–12]

Besides, emulsions can also be categorized based on mean droplet size into macroemulsions or coarse emulsions (2–100 μm), miniemulsions (100–1000 nm) and nanoemulsions (1–100 nm). It is noteworthy that there is still no unanimous consensus on the aforementioned values among specialists in this field, and there is overlap amongst these classifications to some extent. Moreover, all of these emulsions, other than the sub-group of NEs, known as ME, are thermodynamically unstable but only kinetically stable systems where their kinetic stability had been improved by means of introducing various surfactants and stabilizers or significant reduction of final droplet sizes and/or optimization of process parameters, transportation and storage. However, in terms of kinetic stability, mini-emulsions have higher stability than coarse emulsions, and NEs are substantially more stable when compared with miniemulsions.[4,10–14] Macroemulsions, MEs and NEs will be discussed more in-depth in the following sub-sections, focusing on two latter ones.

### *Macroemulsion*

Macroemulsions, also known as coarse or conventional emulsions, are temporarily stable colloids formed by combining two liquids that are not ordinarily miscible. They have average droplet sizes larger than 200 nm resulting in a white/milky appearance. Such systems are thermodynamically unstable and ultimately separate into layers of oil and water by coalescence of the dispersed phase from the dispersion medium. The inclusion of a third component, an emulsifier, is required for the stabilization of a macroemulsion.[13] The concept of hydrophilic–lipophilic balance (HLB) can be of

assistance as a pre-screening tool for emulsifying agents. Surfactants with high HLB values are predominantly hydrophilic and are expected to stabilize O/W (direct) emulsions. On the contrary, those with low HLB values are more oil-soluble and tend to form W/O (reverse) emulsion.[15] Nevertheless, there is currently no reliable predictive technique for selecting the appropriate emulsifier-(s), which would result in the development of a kinetically stable macroemulsion.[13] The impact of adsorption rate and the interaction strength would be more significant rather than the amount of available reactive bonds in selecting the appropriate emulsifier for a particular type of emulsion. Moreover, the emulsifying ability can be dependent on processing variations and presence of other substances like salt and sugar.[15] Therefore, review of literatures and design proper experiments to test scientific theories may be applied as the ultimate method in assessing optimal emulsifier(s).

### Microemulsion

Microemulsions are isotropic transparent dispersions of organic and aqueous phases stabilized by a surfactant or a mixture of surfactants and, in many cases, a co-surfactant.[16] In contrast to the kinetic stability of conventional emulsions, MEs are thermodynamically stable. This is because the latter (ME) forms spontaneously on bringing its main components together under a specific condition. However, the stability of ME can be affected by varying the composition and temperature. The outstanding features of a ME are due to high interfacial area and ultralow interfacial tension (<.01 mN/m) attained between the oil and water phases leading to the development of remarkable small droplets <100 nm in size, regardless of the preparation procedure used.[17,18]

Basically, the major components of a ME include an aqueous phase, an oil phase, a primary surfactant and in most cases, a secondary surfactant, also called co-surfactant/co-solvent. Various concentration of these main components would result in the development of different nanostructures.[19] An established classification of ME nanostructures is that of Winsor predicting four basic types of equilibria that can be experimentally verified:

- Winsor I (two-phase system): Oil-in-water (O/W) ME is formed and exists in equilibrium with an excess upper oil phase where the surfactant is only present as a monomer.
- Winsor II (two-phase system): Water-in-oil (W/O) ME is formed and exists in equilibrium with an excess lower water phase.
- Winsor III (three-phase system): A middle-phase (bicontinuous) ME, rich in surfactant, exists in equilibria with both excess oil and water phases.
- Winsor IV (single-phase system): An isotropic (single-phase) mixture is formed in the presence of perfect matching ratios of oil, water, surfactant(s), co-surfactant or some other components (e.g., salt).[19]

When using the term "microemulsion", it is, therefore, essential to precisely identify the kind of nanostructures formed, which basically include direct (oil dispersed in water, O/W), reversed (water dispersed in oil, W/O) and bicontinuous (oil and water interspersed within the system). Graphical representation of the ternary/pseudo-ternary phase diagram is an invaluable tool in ME and NE techniques as multi-component systems. It provides precise data on multi-component systems and is utilized in several formulation processes. This can be used to study the complex series of interactions that potentially take place when different amounts of main emulsion components are mixed. Moreover, ternary/pseudo-ternary phase diagrams are of value in interpreting structure-property–function correlation and investigating phase boundaries of different regions as a function of temperature and composition.[20,21]

In a typical pseudo-ternary phase diagram, the phase behavior of a ME system consisting of water, oil and surfactant/co-surfactant, as well as different nanostructures formed at various compositions of oil, water and surfactant/co-surfactant, can be depicted (Fig. 1). The structural transition along a plotted dilution line occurs through several intermediate structural changes (e.g., from point A to point B,

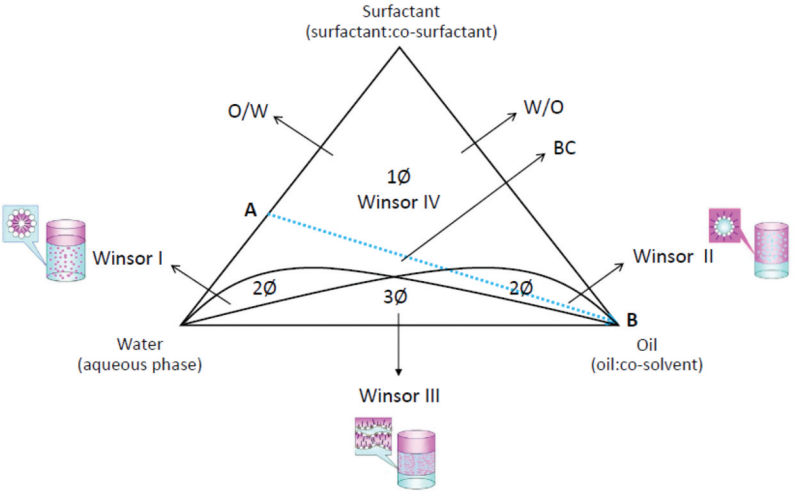


**Figure 1.** Pseudo-Ternary phase diagram representing internal nanostructures in a microemulsion (ME) system including monophasic (1Ø) Winsor IV [oil-in-water (O/W), bicontinuous (BC) and water-in-oil (W/O) MEs], biphasic (2Ø) Winsor I (O/W ME in equilibrium with an excess upper oil phase)/winsor II (W/O ME in equilibrium with an excess lower water phase) and three-phasic (3Ø) Winsor III (BC middle-phase ME in equilibria with both excess oil and water phases).

monophasic O/W ME to monophasic bicontinuous ME to biphasic W/O ME) (Fig. 1). This can occur, for example, upon progressive dilution of a mixture of deionized water and surfactant/co-surfactant with oil.[22–24] Different nanostructures formed at various ratios of the main components of a system may have different visual characteristics, viscosity, and electrical conductivity over different regions of a pseudo-ternary phase diagram. This makes it possible for different nanostructures to be differentiated from each other through the techniques applied to study nanostructures formed within a ME system.[20,25]

However, ternary, and pseudo-ternary phase diagrams only provide information on the composition of system in terms of main constituting components by weight percent (wt%) and represent phase diagrams by outlining the composition regions corresponding to various Winsor types on the plot. It should be noted that triangular phase diagram is not sufficient to represent the complete phase behavior of the system and structural distribution of different phases. In order to have the full-phase behavior, even for such a simple system as water-oil-surfactant, one should plot ternary phase diagrams at different temperatures. Furthermore, the percentage composition of the main components in a ternary or pseudo-ternary system should be calculated precisely and phase behavior of the system during phase titration needs to be monitored accurately. Subsequently, the acquired results can be plotted on a triangular graph to represent the phase boundaries of microemulsion and surrounding regions. Therefore, accuracy of a ternary or a pseudo-ternary phase diagram is highly dependent on the precision and accuracy of input data.

## *Nanoemulsion*

Nanoemulsions are defined as metastable colloidal dispersions of nano-sized droplets formed by forcing two immiscible liquids (water and oil) into a uniform homogeneous state by means of an appropriate type and amount of surfactant alongside high shearing external force. NEs are considered kinetically stable as there is higher stability to gravitational separation and droplet aggregation compared to coarse emulsions. This higher stability can be associated with the increased repulsive forces between nano-droplets.[13,26]

NEs can be formed in the presence of a smaller amount of surfactant without the need of co-surfactants allowing their oral administration at a remarkably higher dosage of nearly ten times than that of a ME and lowering the administration volume/frequency.[13,26] Another extraordinary property of a NE is the comparatively high proportion of droplet total volume filled by the hydrophobic phase in an O/W NE. This allows increased loading capacity for a wide variety of bioactive compounds in the development process of food bioactive–loaded nano-carriers.[6]

Nonetheless, like coarse emulsions, NEs are thermodynamically unstable formed in a non-equilibrium state. An NE system tends to reduce the interface area by various destabilizing mechanism due to a comparatively high interfacial tension. This causes the droplets to coalesce, and the phase separation occurs at gravitational force given sufficient time.[27] Contrary to the thermodynamical stability of ME, the stability of NE is not only associated with the environmental and processing conditions (e.g., pH and temperature), but it is also highly related to its free energy level. Furthermore, proper selection of appropriate composition, the order in which its components are added, and preparation technique are of great importance in developing a stable NE, which must be taken into account.[13,27]

The major characteristics of MEs and NEs are briefly compared in Table 1. The primary practical method to distinguish NEs from MEs can be the determination of the free energy level of the system. The free energy depends on the extent of surface tension of the oil–water interface. Ultralow free energy of formation is required for extreme diminution in surface tension accompanied by a large positive entropy. The free energy level of a ME system is lower compared to that of the phase-separated components (water and oil) from which it is prepared whereas a NE has higher free energy than separated phases. In such cases, contrary to NEs, MEs are spontaneously generated by self-assembly and, furthermore, are thermodynamically stable.[8,19,28,29]

**Table 1.** Comparison of the main distinctions between microemulsion (ME) and nanoemulsion (NE).

| Parameter | ME | NE | Remarks |
|---|---|---|---|
| Droplet shape | Spherical or non-spherical | Spherical | ME droplets can be spherical or not spherical [e.g., sponge-like bi-continuous and cylindrical (rod-like) micelles] because of their very low interfacial tension. The spherical shape of NEs is the consequence of a small radius and high interfacial tension. |
| Stability | Thermodynamic | Kinetic | MEs do not break down and have an infinite lifetime provided the storage condition remains unchanged. NEs usually separate into two distinct phases with time since their free energy is higher than their separate components. |
| Optical property | Transparent | Slightly translucent | ME is a transparent colloidal dispersion, while NE can take on a transparent or slightly turbid appearance. |
| Sample history | Path-independent | Path-dependent | Under a particular set of conditions, ME's structure remains unchanged, independent of the way of preparation, whilst the nanostructure of NE depends on its preparation method. |
| Particle size distribution (PSD) | Single narrow peak | Single or multiple peaks (narrow or broad) | The PSD of a ME system usually exhibits only one narrow peak, but that of NE can contain broad peaks or more than one peak. |
| Fabrication methods | Self-emulsification, spontaneous formation | Requires external energy | NEs are non-equilibrated colloidal systems; consequently, high-energy input is required for their formation. |
| Concentration of surfactant | Relatively high | Relatively low | NEs can be formed using relatively low surfactant concentration (up to 10 w/w %) compared to MEs (20 w/w% or even higher depending on oil and water contents). |

However, there is only a limited number of reports on the stability assessments of the developed ME and NE systems, as the main factor in differentiation between these nano-sized systems.[15,30–37] The other important factor neglected in many studies is the particle size distribution (PSD) of the developed systems. Presentation of particle size distribution along with the mean droplet size data can be of great value to differentiate MEs from NEs (Table 1). The PSD of a ME system usually exhibits only one narrow peak, but that of NE can contain more than one peak or broad peaks.[8,19,28,29]

## Fabrication of microemulsions and nanoemulsions

### *Microemulsion*

MEs are developed when interfacial tension between the water and oil phases is brought to ultra-low values (<.01 mN/m). The nature and concentration of the main components of ME significantly impact its formation and flow behavior. Two main methods reported for ME preparation are phase titration and phase inversion methods.[19,38,39] Gibbs defined the phases as any portion of a system that is in homogeneous chemical composition and uniform physical state. The construction of phase diagrams is a critical tool in the investigation and analysis of the phases. Different regions, including oil-in-water (O/W), water-in-oil (W/O) and bicontinuous (BC) regions, can be depicted in a phase diagram.[40,41]

### *Phase titration method*

From a mechanistic viewpoint, in self-emulsification or so-called spontaneous emulsification, the formation of ME occurs due to the expeditious diffusion of surfactant, co-surfactant and/or solvent molecules between the continuous and dispersed phases (interface). Upon diffusion and arrangement of those molecules at the interface, interfacial tension (<.01 mN/m) and free energy (~zero) will reduce and the nano-size droplets or ME will be formed.[42–44] As described, this type of ME can be developed by merely mixing the microemulsion main components. However, proper types of components in appropriate ratios are essential to form a thermodynamically stable and single-phase ME (Fig. 1).

It has to be emphasized that the determination of appropriate compositions and proportions is a significant aspect, where it would result in the formation of different nanostructures at different compositions of microemulsion main components. In this regard, different compositions of the main components are required to be mixed. Subsequently, stability and the type of phase(s) formed over mixing various components at different ratios are monitored by applying a phase diagram.[4,10,44–49]

For MEs consisting of four, five or six different components, the quaternary (four components), quinary (five components) or senary (six components) phase diagrams need to be plotted. However, plotting these kinds of phase diagrams can be complicated and take a long time to interpret. In such cases, the construction of a pseudo-ternary phase diagram is helpful in which each apex of the diagram represents a constant ratio of more than one component, and the recognition of single and multiple phase zones is readily doable. In a pseudo-ternary phase diagram (Fig. 1), each corner represents 100% of a particular component (e.g., surfactant) or 100% of components (e.g., surfactant and co-surfactant) that had been blended at a certain proportion.[10,47]

Plotting ternary/pseudo-ternary phase diagram in this method is through titration with oil or aqueous phase (Fig. 1). By taking a constant ratio of components except for the oil (or water), the mixture can be diluted along a dilution line in order to form ME. A dilution line (see line AB in Fig. 1) is considered as being any line drawn from the oil (or water) vertex to any point on the line opposite to the vertex containing different concentrations of oil (or water) but a constant proportion of other components within a ternary or pseudo-ternary phase diagram. It needs to be emphasized that this type of transitional phase inversion (PIC) occurs in single-phase ME (Winsor IV type MEs) region.[10,40,44]

Phase titration method has been successfully applied for preparation of castor oil MEs that remained stable and transparent up to 10 months of storage. First, Tween 80 (surfactant) and ethanol (co-surfactant) were blended at fixed ratio of 1: 2 (w/w) and added to castor oil. Phosphate buffer (pH 7.4) as aqueous phase was then added dropwise to the mixture at 25°C with continuous stirring, where monophasic, isotropic, and clear solutions were considered as ME. Analysis of surface tension, viscosity and electrical conductivity along dilution lines revealed the nanostructural changes from W/O to bicontinuous and then to O/W ME.[21] Similarly, phase behavior and formation of peppermint oil-loaded ME stabilized with non-ionic surfactants has been investigated by phase titration method. Peppermint oil and sugar esters were mixed at a constant weight ratio and titrated with double distilled water until the cloud point transition (solubilization limit of water) was attained.[46]

### *Phase inversion method*

Another low-energy microemulsification technique is the phase inversion method in which a ME is developed due to changes in temperature, composition, pH and/or ionic strength (salinity). Upon these manipulations, the curvature of the surfactant film at the interface alters, e.g. from normal micelle (positive spontaneous curvature) to the reverse micelle (negative spontaneous curvature) and *vice versa*, usually by crossing a zero-curvature zone (Figs. 2 and 3). There are two forms of phase inversion methods (i) transitional phase inversion (TPI), which includes phase inversion temperature (PIT) and phase inversion composition (PIC) methods, and (ii) catastrophic phase inversion (CPI), which involves emulsion inversion point (EIP). It is worth noting that spontaneous curvature or surfactant affinity is changed during TPI, whereas in CPI, it does not.

In the PIT method, by changing the temperature of a system composed of a constant amount of non-ionic surfactant, oil phase, aqueous phase, and other components, it will convert from an O/W ME, at low temperatures, to a W/O ME at higher temperatures by crossing a point of zero-curvature (bi-continuous phase, BC) (Fig. 2). Again, during cooling, the system will form an O/W ME.[47]

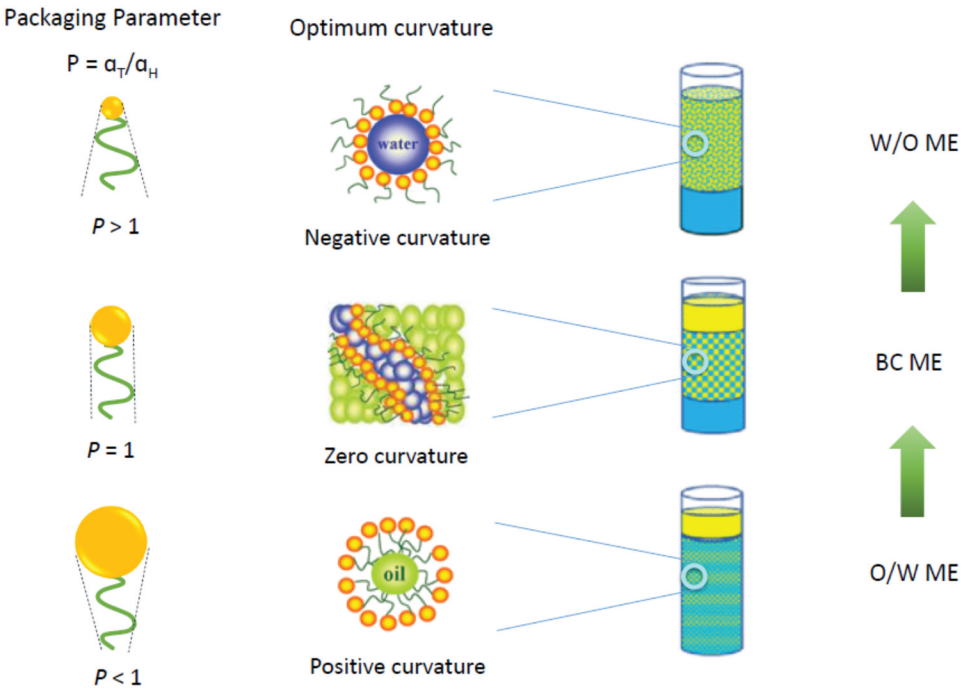


**Figure 2.** Diagram depicting phase inversion from oil-in-water (O/W) to bicontinuous (BC) to water-in-oil (W/O) microemulsion (ME) and *vice versa* in response to changes in temperature (non-ionic surfactants)/ionic strength and pH (ionic surfactant)/composition (in Winsor IV or monophasic ME).

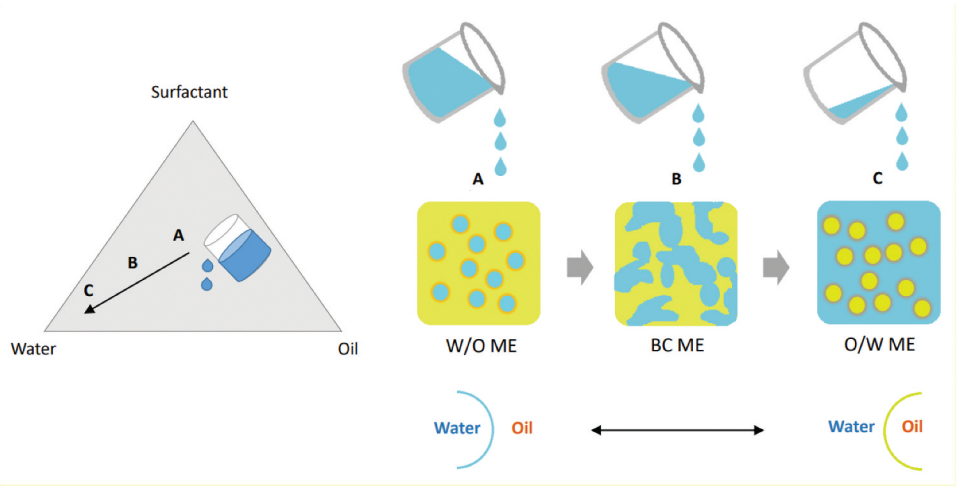


**Figure 3.** Schematic representation of change in affinity (spontaneous curvature) of surfactant layer at the interface and phase transition from water-in-oil microemulsion (W/O ME) to bicontinuous microemulsion (BC ME), and to oil-in-water microemulsion (O/W ME) by successive addition of the dispersed phase.

However, when using PIT method, it should be considered that major changes can occur in droplet diameter. For instance, phase inversion from O/W ME to W/O ME occurs through a middle phase, bicontinuous microemulsion, where some droplet coalescence or ripening could happen.[10] The changes in droplet diameter subsequently could drive variations in controlled release behavior of bioactive compounds in both *in vitro* and in *in* vivo as well as final product stability.[50] Therefore, in order to keep the droplet diameter as small as possible, a very rapid change of temperature would be considered as a solution.

In the PIC method, the pH, ionic strength, or the composition (the volume fraction of water or oil) of the system is altered, for instance, water is added to an oil–surfactant mixture, or oil is added to the water–surfactant mixture. In the presence of adequate surfactant and other components, the volume fraction of the added phase will increase, and eventually, the curvature of the interface will change from O/W (or W/O) to the zero-curvature zone (BC), and by any further increase (when the transition composition is exceeded), the curvature will change to W/O (or O/W). Thus, changing the composition, pH, or ionic strength of the system causes phase inversion or simply called the PIC method.[43] Figure 3 depicts PIC method in which by a gradual increase of the mass ratio of water, the W/O system converts to BC and, with a further increase to O/W system. As can be seen, by successive addition of the dispersed phase (i.e., water), transition of W/O ME to intermediate BC ME (zero-curvature zone) and then to O/W ME occurs as a result of change in spontaneous curvature or affinity of surfactant layer at the interface from oil to water (Fig. 3). The main limitation of PIC method is that the composition and solubility of the surfactant film alter upon addition of water with continuous stirring due to gradual solubilization of the co-surfactant.[51] Consequently, some amount of surfactant would be carried with water in the aqueous phase via the leaching resulting in a significant increase in required concentration of surfactant and co-surfactant in the system.

In the EIP method, phase inversion is influenced mainly by the volume fraction of the dispersed phase rather than the type of surfactant, and the surfactant must present in the dispersed phase.[52] As the surfactant predominantly exists in the dispersed phase of the microemulsion, so when it is mixed with continuous phase, the system does not behave as a normal emulsion (i.e., unstable and abnormal emulsion) therefore does not obey Bancroft's rules according to which the emulsifier should be mainly presented in the continuous phase of microemulsion.[53] Then, by increasing the volume fraction of the dispersed phase, which includes a surfactant, the catastrophic phase inversion takes place based on

which the abnormal emulsion converts and forms a more stable normal emulsion.[54] However, it should be noted that EIP method would not be applicable for medium- and long-chain surfactants which cannot build up a flexible surfactant monolayer at the interface of oil and water and then would not be able to form bicontinuous phase at the inversion point.[52]

Phase inversion temperature emulsification method has been previously applied for an emulsion composed of 48.5% water, 48.5% hydrocarbons (oil phase), and 3% non-ionic surfactant (polyoxyethylene alkylphenyl ethers) by continuous mixing of the main components of the emulsion in the vicinity of its critical temperature (phase inversion temperature), and then a sudden cooling process. Emulsification at around 2–4°C lower than phase inversion temperature was reported to result in development of droplets of remarkably lower size compared with any other temperature examined.[51] Phase inversion composition technique has also been satisfactorily applied for fabrication of isopropylmyristate emulsion with mean droplet diameter lower than 200 nm. For this, PEG-60 hydrogenated castor oil, isopropylmyristate, and ethanol were mixed followed by addition of water at a rate of 1 ml/min at constant temperature of 23°C. Change of interfacial spontaneous curvature was induced by varying the weight fraction of ethanol from 0.3 to 7.0 wt% with unchanged weight fractions of PEG-60 hydrogenated castor oil and isopropylmyristate.[9]

By the way, construction of the ternary/pseudo-ternary phase diagram is an useful tool in preparation of ME using any of the two preparation methods (phase titration and phase inversion methods) and investigating phase boundaries of different regions.[20,21] Preparation of samples in order to experimentally map out a phase diagram and investigate ME regions and the various other regions can be performed by two different techniques. In the first technique, a mixture of two components (i.e., surfactant: water or surfactant: oil, over various ratios 0: 100 to 100: 0 but at a constant weight) is titrated with the third component (i.e., oil or water) along a dilution line and under mild stirring to the turbidity point which can visually be observed by a naked eye. For example, a mixture of oil and surfactant is titrated dropwise with deionised water.[20,38] This method only identifies the monophasic ME region.

In the second technique, varying relative concentrations of different components (i.e., surfactant: water or surfactant: oil, over various ratios 0: 100 to100: 0 and at varying weight ratios ranging from 0 to 100) are mixed with the third component (i.e., oil or water at varying weight ratios 100 to 0). Then, the mixtures will stay over a slightly long time (i.e., one or two weeks) at a certain temperature, and their physical stability (turbidity, translucency, gelation, or number of phases) is carefully investigated and monitored. By doing so, the complete phase diagram can be plotted where different regions (e.g., single-phase, two-phase, or three-phase) can be identified. There is also another technique, which is called 'formulation scan', where equal volume fractions of water and oil phases are mixed, and their stability in the presence of tuning parameter (e.g., surfactant, salt, or temperature) is monitored. The latter technique is typically used to identify the solubilizing factor of a surfactant.[24,38]

### *Nanoemulsions*

The following sections outline the main high-energy techniques typically employed for the preparation of NEs. High-energy methods, which are extensively applied in the formulation of NEs, use high mechanical energy to provide strong disruptive forces and mix the water and oil phases thoroughly into a single-phase NE with high kinetic stability. The most techniques widely used to produce disruptive forces include ultrasonication, high-pressure homogenization and microfluidization.[55,56] Figure 4 depicts general principles of high-energy techniques used to prepare a NE in which conventional high-speed mixer is usually applied during pre-emulsification process to form a coarse emulsion. The developed course emulsion then passes through high-energy nanoemulsification process using mechanical devices (i.e., ultrasonication, high-pressure homogenization, and microfluidization). These high-energy techniques are discussed more in detail in the following sub-sections.

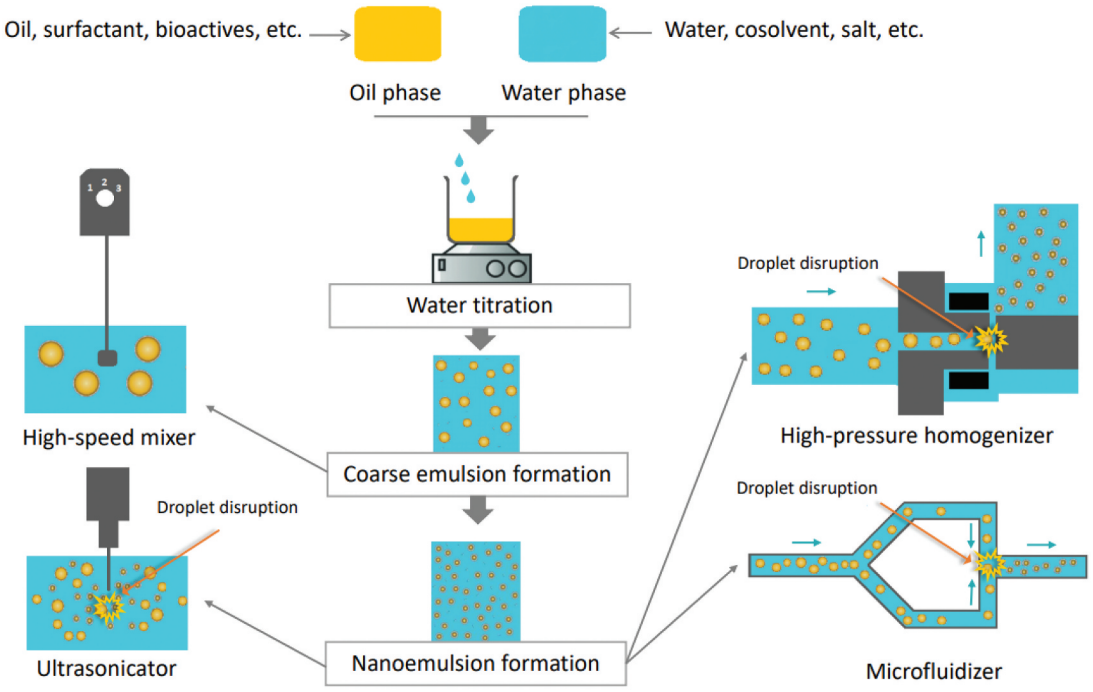


**Figure 4.** Preparation of coarse emulsion using a high-speed mixer followed by nanoemulsion formation applying high-energy techniques (ultrasonication, high-pressure homogenization, and microfluidization).

### *High-Pressure homogenization*

High-pressure homogenizers are the most widely employed devices to prepare food, cosmetics, and pharmaceutical NEs, consisting principally of a high-pressure plunger pump, homogenizing valve, homogenization chamber and interaction chamber. The macroemulsion is sucked with great intensity into the homogenization chamber by means of the suction stroke of the plunger and pushed out via a micrometric orifice at high speed by the very high pressure (10–500 MPa).[57] Several principles such as sudden formation of intense turbulence, cavitation and shearing mechanisms work together during this stage to provide NEs of the desired droplet sizes and characteristics.[58] The predominant mechanism in the formation of nano-droplets is mainly dependent on different valve geometries applied. For instance, in high-pressure radial diffuser valves, droplet breakdown is governed by the inertial forces in the turbulent regime and somehow by the cavitation process. On the other hand, in narrow orifice valves, where flow is usually considered to be laminar, droplets are disrupted primarily by the shear forces.[58,59]

NE formation using the high-pressure homogenization method occurs in two steps. In the first stage of homogenization, large droplets of the dispersed phase are disrupted into nano-sized droplets in the homogenization chamber, which increase the surface area: volume ratio. In the second stage, the newly formed interfaces are coated by sufficient surfactant molecules in the interaction chamber.[59] The mean droplet diameter of a NE produced by high-pressure homogenizers can be controlled by adjusting the sample composition and properties (viscosity and surface tension), cycles applied and operating parameters such as energy input, time, and environmental conditions (pressure and temperature).[54] It is worth noting that in high-pressure homogenization, there would be limitation in terms of volume fraction of the oil dispersed in the continuous aqueous phase (percent dispersion effectiveness) in the oil-in-water nanoemulsions. This is because the ratio of dispersed phase viscosity

to continuous phase viscosity should not exceed a range of 0.05 to 5.[60] In addition, temperature increase during intense homogenization can result in increased droplet diameters and can have a significant deteriorate effect on the bioactive compounds.[60]

Kenaf seed oil NEs with remarkably high encapsulation efficiency for vitamin E (83% after 8 weeks storage at 4°C) and phytosterols (65% after 8 weeks storage at 4 °C) have been formulated applying high-pressure homogenization. First, coarse emulsions were prepared by dissolving surfactants (sodium caseinate, $\beta$-cyclodextrin, and Tween 20) in water as aqueous phase followed by titration of kenaf seed oil as organic phase into the aqueous phase. After high-shear mixing of developed emulsion at 8,600 rpm, nanoemulsification was performed by passing the coarse emulsion through high-pressure homogenizer at $1.93 \times 10^8$ Pa for up to 4 passes.[31] In the same way, coarse emulsion of pepper extract has been developed by adding a mixture of pepper extract and Span 80 (oil phase containing the surfactant of lower HLB value) to a mixture of water and Tween 80 (water phase containing the surfactant of higher HLB value) before mixing by a high-speed homogenizer at different speeds 7,000 to 25,000 rpm. Nanoemulsification was then carried out by passing the coarse emulsion through homogenizer running at varying operation pressures between $6.9 \times 10^7$ and $13.8 \times 10^7$ Pa for 5 to 20 passes resulting in nano-droplets of 132–145 nm in diameter.[32]

### *Microfluidization*

Microfluidization is a high-pressure homogenization technique that utilizes a special microfluidizer having typical dimensions between 50 and 300 μm. Microfluidizer uses high pressure (up to 275 MPa) to force the fluid stream to pass through the microchannels resulting in a very high shearing force that rupture the emulsion into exceptionally fine droplets. The principle behind microfluidization is forcing the coarse emulsion through a fine orifice to a fixed geometry interaction chamber by a pneumatically powered pump, which leads to the formation of NE under a mixture of laminar and turbulent flow and cavitation shear forces.[61] Droplet size distribution of NEs, produced by microfluidization, depends on the composition and process parameters, such as interaction chamber used, the number of passes through a microfluidiser and residence time in the interaction chamber.[61]

The microfluidizer offers many advantages over traditional homogenization methods because the distributions of produced droplet sizes appear to be narrower and centered around smaller sizes compared to those produced by high-pressure homogenizers, providing an enhancement in dissolution rate and bioavailability of different bioactive compounds. Moreover, highly stable NEs can be obtained at a low surfactant: oil ratio using the microfluidization technique.[54,61] However, microfluidization is the most expensive technique among high-energy methods and would not be considered as a proper technique for the preparation of nanoemulsions in industrial process.[60]

Docosahexaenoic acid-loaded NE with increased physical, oxidative, and fatty acid profile stability over 20 days storage at 4°C and 28°C has been previously fabricated using microfluidization. Oil-in-water emulsion was first formulated by dissolving surfactants Tween 40, sodium caseinate, and soya lecithin in water (aqueous phase) to which docosahexaenoic acid was added as oil phase. After mixing by high-speed homogenizer at 1,000 rpm for 10 min, the developed coarse emulsion passed for 5 to 7 cycles through microfluidizer at different pressures ($9 \times 10^7$ to $10 \times 10^7$ Pa) leading to nano-droplets formation.[33] Similarly, encapsulation of vitamin E in dispersed oil droplets of a NE has been successfully carried out by a dual-channel microfluidization method resulting in rather desirable bioaccessibility (53.9%) for vitamin E. Oil phase composed of corn oil and vitamin E at various weight ratios was dispersed into aqueous phase containing quillaja saponin by using stirrer for 2 h. Nanoemulsification was then carried out using a dual-channel microfluidizer at pressure of about $9.6 \times 10^7$ Pa.[34]

### *Ultrasonication*

Ultrasonication is more efficient than other high-energy methods in developing finer droplet sizes, less polydispersity, higher stability, and lower surfactant concentration required. This process also offers higher energy efficiency (lower heat loss), lower operating costs, easier cleaning methods, the potential for aseptic operation and more adaptability and scalability.[62] In ultrasonication, high-intensity ultrasonic waves provide cavitation forces that form microbubbles or voids. Consequently, microbubbles oscillate rapidly within the medium and eventually collapse violently, thereby exerting intense shear forces that break the macroemulsion to NE. Desired droplet size and stability of the NE can be attained by adjusting ultrasonic process parameters, such as power (i.e., amplitude and size of sonotrode), frequency and sonication time or changing the nature of the surfactant.[54]

Threshold of ultrasound frequencies to commence the cavitation proved to be between 20 and 24 kHz, while lower frequencies are required to generate acoustic waves with high intensity. Increasing the time of ultrasonication usually decreases the mean droplet size and makes the droplet size distribution curve narrower though a U type correlation that can be seen in some cases.[62] In the case of short sonication times, the mean size of NE droplets is mainly influenced by the specific energy input.[63] Nevertheless, it is important to minimize further transformation of droplet sizes due to surface or shear induced coalescence when increasing sonication frequency, power, and time.[60]

Fabrication and characterization of d-limonene NE with mean droplet diameter ranging from 150 to 700 nm has been carried out using ultrasonication. Coarse emulsion of d-limonene (oil phase) and solution of modified starch, maltodextrin, and whey protein concentrate (aqueous phase) was formed by using a high-speed blender. Then nanoemulsification was induced by applying a sonicator running at 24 kHz. Extension of sonication time from 20 to 60 s led to a significant decrease in droplets diameter and polydispersity index where nano-droplets with mean diameter of 243 nm were achieved after 20 s sonication.[63] Ultrasonication has been also used to prepare soybean oil NEs stabilized by phosphatidylcholine (as surfactant), and sodium palmitate and sucrose palmitate (as co-surfactants) without any pre-emulsification process. Dispersion of the components was subjected to ultrasonic probe for a period of 60 min at 55°C. Even though ME with mean droplet diameter of 50 nm could be fabricated with no pre-emulsification process, the sonication time was extremely long (60 min), and coalescence of nano-droplets occurred with time.[62]

Applying high-energy techniques leads to higher flexibility on particle size, surfactant, and internal structure, and subsequently, more controls on rheology and stability of developed NE compared to low energy methods. However, high-energy techniques are expensive and cannot be applied for thermolabile bioactive compounds, like vitamin C and bioflavonoids. Moreover, application of these methods in industrial production of nanoemulsions is still far away from being well-explored and faces technological challenges that need to be dealt with in terms of the suitable processing operations and cost efficiency.[55,56,60,62]

## Stability of microemulsions and nanoemulsions

MEs are thermodynamically stable formulations independent from the preparation method and therefore have prolonged shelf life, whereas NEs are kinetically stable but are thermodynamically unstable.[8] The thermodynamic stability is related to the level of Gibbs free energy changes ($\Delta G$). The free energy of a ME is lower compared to that of the phase-separated components (water and oil), from which it is prepared (Fig. 5). This justifies its spontaneous formation and its thermodynamic stability. On the contrary, a NE has higher free energy than separated phases. Thus, a NE is a thermodynamically unstable system, and phases will separate ultimately.[4]

Two main mechanisms contributing to the stability of MEs and NEs formulations prepared using any technique are electrostatic repulsion and steric hindrance. High electrostatic repulsion between the dispersed phase droplets leads to improved stability of the system over a long period. The electrostatic repulsion between the droplets surpasses the depletion and van der Waals attractive

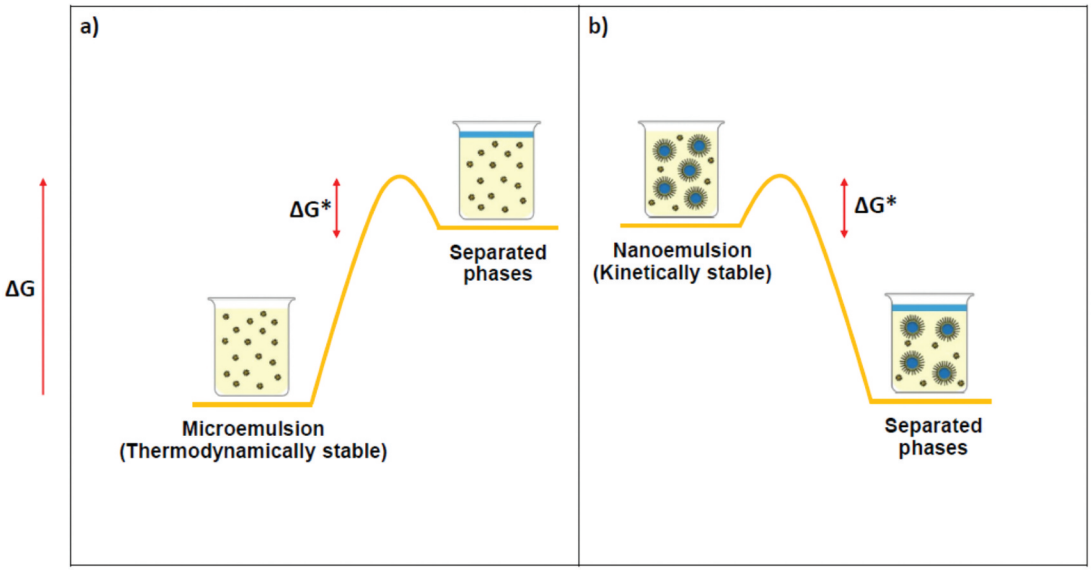


**Figure 5.** Comparison of differences in the level of free energy of a) microemulsion and b) nanoemulsion and their corresponding separated phases state.

forces increasing the overall stability and impacts on physicochemical properties. Effective electrostatic repulsion between dispersed phase droplets is due to highly electrically charged surfaces of the droplets, which prevent droplet coalescence and Ostwald ripening. Thus, an emulsion can be stabilized for an extended period.[64] On the other hand, droplets having low surface potentials (neutral or weakly charged) aggregate more easily and decrease the emulsion lifetime. The stability of an emulsion can be improved via enhancing electrostatic repulsion by mean of ionic emulsifiers, which reduce the interfacial tension between dispersed and continuous phases.[64,65]

The stability of MEs and NEs can be estimated through zeta potential measurements that signifies the electric potential of the interfacial layer surrounding the dispersed droplets in an emulsion. The literature considers emulsions with zeta potentials values of higher than either +30 mV or −30 mV as stable. On the other hand, droplets with low zeta potential values (negative or positive) generally show poor stability.[64–66] It is worth noting that zeta potential is highly influenced by temperature and properties of the aqueous solution, such as composition, ionic strength, and pH. Hence, the average of absolute differences of zeta potential between the values should not be less than 10 mV to enable accurate comparison.[67]

Steric hindrance is another mechanism known to affect emulsion stability. The adsorption of polymeric species (e.g., protein, polysaccharide, and surfactant) chains onto the surface of newly created droplets in an emulsion induces steric forces between the droplets. Steric force is steric bulk preventing the geometrical arrangement of droplets necessary for droplets aggregation. As droplets approach very closely, surface-active interfacial layers adsorbed on dispersed droplet molecules start to interact with each other and make approaching droplets move from their original positions to avoid overlaps. This would result in repulsive forces between droplets and prevent them from coagulation.[65] Stabilization of ME or NE through steric effect seems to be more effective when the interfacial layer is developed by polysaccharide and/or protein rather than those formed by surfactant aggregates. This is due to the relatively large dimensions, dynamic nature and conformational flexibility of the proteins and polysaccharides.[68] Contrary to electrostatic repulsive forces, steric hindrance is irrespective of the surface charge of droplets and external environmental conditions such as pH and electrolyte conditions. Such a feature can

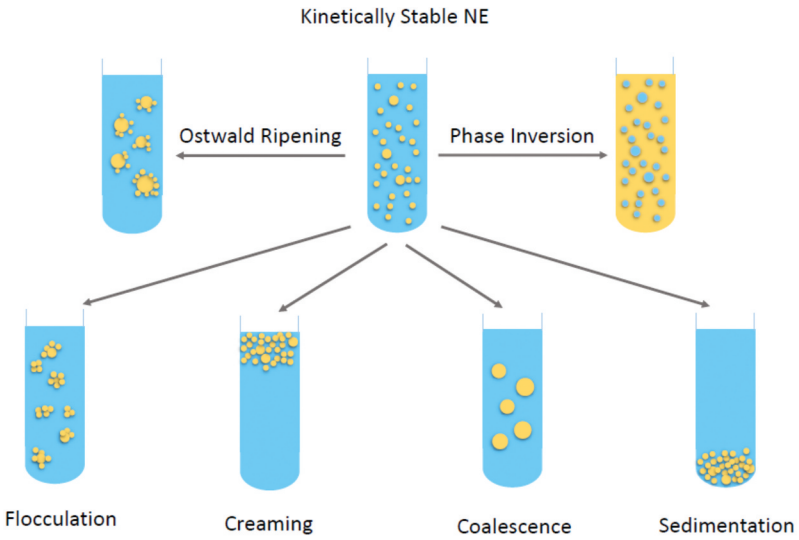


**Figure 6.** Schematic diagram representing destabilization mechanisms of a nanoemulsion (NE).

be exploited for stabilizing MEs and NEs composed of protein and/or polysaccharide as an emulsifier, especially O/W MEs and NEs in which water as a continuous phase has an extremely low electrical conductivity.[69,70]

Regarding NEs, the nano-size droplets have caused their further stability against unfavorable environmental conditions in addition to relatively high kinetic energy of the system. In NE systems, Ostwald ripening is the primary and main instability mechanism. Other destabilization phenomena of less importance are sedimentation, creaming, flocculation, coalescent and phase inversion which are depicted in Fig. 6.[27,65]

However, composition, molecular interactions, and homogenization conditions in formation process of a nanoemulsion are of great importance and can later contribute to destabilizing mechanisms. The optimum composition and processing conditions to prepare a stable nanoemulsion is the one which minimize droplets diameter and its distribution. For instance, nanoemulsions formulated using low viscosity oils and stabilized by small-molecules surfactants may lead to the formation of smaller, more stable nano-droplets. In addition, fast cross-linking and constant reassembly of surfactant molecules may occur in the vicinity of oil-water interface in the preliminary prepared coarse emulsion droplets before passing through homogenization. These rearrangements would result in decreased efficiency of high-energy techniques (high-pressure homogenization, microfluidization and ultrasonication) in terms of droplet disruption. Then, preparation of nanoemulsion containing well-distributed nano-droplets and keeping nanoemulsion in the right environmental conditions may result in systems which are stable for relatively long time upon storage.[4,8,27]

## Applications

Food-grade MEs and NEs have high potential to serve as encapsulation techniques, natural preservatives, extraction methods, delivery systems for functional ingredients, nano-reactors, and active food packaging. Major applications of MEs and NEs in the food industry are presented in the following subsections, with particular focus on the most recent studies and publications. However, limitations and restrictions being imposed on the development of food grade MEs and NEs in industrial scale should be considered and surmounted to improve their important role in the future food industry.

### Encapsulation and carrier system

In recent years, a growing number of research studies have addressed the benefits of applications of MEs and NEs in foods and beverages as carrying systems for desired lipophilic bioactive ingredients. Most flavoring and coloring agents and bioactive compounds (e.g., vitamins, antioxidants, and nutraceuticals), especially the lipophilic ones used in the food industry, are vulnerable to oxidative and photochemical reactions, rendering their incorporation in food products and beverages quite challenging and complex.[71] It is well documented that once a food compound is encapsulated and further dispersed within a continuous phase of the emulsion, it can benefit in terms of increased stability, enhanced protection under food processing, preventing their interactions with food components and masking their potential unpleasant smell or taste. Encapsulation assures long-term stability of bioactive ingredients in the final products through entrapping them within a carrier such as a biopolymer matrix or coating and consequently their protection from adverse environmental influences such as oxidation, hydrolysis, and heat degradation.[30,71]

MEs and NEs have been extensively applied in the encapsulation of fat-soluble vitamins such as vitamins A, D, E and K, as well as carotenoids like $\beta$-carotene and lycopene, which play a crucial role in human metabolic processes. Vitamin A (retinol) is necessary for good vision, wound healing, proper bone growth and a healthy immune system. Vitamin D is best known for bone mineralization, calcium regulation, cancer prevention and its inhibitory role in inflammatory responses.[71] An adequate intake of vitamin K has been shown to be essential for blood clotting and hemostasis as well as bone and cardiovascular health. Vitamin E and carotenoids are known to be the most active biological antioxidants capable of protecting against damage through scavenging reactive oxygen species generated in tissues and cell during photo-oxidative stress. However, all of these vitamins and carotenoids are fat-soluble and exhibit poor solubility in water; therefore, their incorporation in aqueous media is very challenging.[38,71]

Similarly, food additives such as flavors, coloring agents and antioxidants used in the food industry have multiple aliphatic esters, aldehydes, or ketones as functional groups, making them insoluble in water-based media and prone to oxidative and thermal alterations during storage, heating, and thawing. Application of MEs and NEs for encapsulation of food additives can improve their solubility in both aqueous and nonpolar media and prevent deleterious changes during processing and storage, leading to their enhanced stability.[72]

A summary of the main recent studies on the application of MEs and NEs as encapsulation matrix and carrier for food bioactive compounds is presented in Table 2. In this regard, water-soluble and fat-soluble food bioactive compounds have successfully solubilized and encapsulated in W/O, O/W and BC MEs and NEs with the intent of addressing issues of solubility and stability of food bioactive compounds.

Most recent studies in this regard have analyzed the ME and NE composition and process factors influencing the physicochemical stability of the bioactive compounds just in the developed nano-sized dispersions. However, incorporating ME and NE containing food bioactive compounds in water-based and oil-based food systems and investigating their performance in food matrices to study their solubility, encapsulation efficiency, and stability with varied pH and under different process conditions can provide more practical advice to the food industry. Further studies are also likely to consider other features like organoleptic properties of the developed formulations and concurrent encapsulation of nutraceuticals to take advantage of potential synergistic interactions between them followed by digestion/absorption simulation.

In addition, particular attempts should be made to reduce the gap between laboratory findings and industrial applications regarding proper carriers used for encapsulation and processing conditions. Encapsulation material and process parameters should be selected in a way that not only meet the encapsulation efficiency and stability criteria for a bioactive ingredient of interest but also improve the process yield rates.

**Table 2.** Major recent advances of the applications of microemulsions (MEs) and nanoemulsions (NEs) as nanoencapsulator and carrier systems for edible bioactive compounds.

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Beetroot Extract | Spontaneous emulsification (ME) Ultrasonication (NE) | W/O and O/W ME/NE | Whey protein concentrate (WPC) and Sunflower oil | NE showed higher efficiency than ME in encapsulation, sustained release and improving the thermal and pH stability of beet extract. | [73] |
| Tocopherol and tocotrienol | Spontaneous emulsification | O/W ME | Span 60 and Palm oil | Palm oil-loaded MEs (7% Span 60 and oil: water ratios of 50: 50 and 30: 70), as a carrier for delivery of vitamin E and other hydrophobic nutrients, demonstrated the highest stability (28 days at 8, 25 and 45 °C). The lowest stability was also perceived at 7% of Span 60 and oil: water ratio of 70: 30. | [74] |
| Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) | Emulsion phase inversion | O/W NE | Tween 20, 40, 80, 85/Span 20, 80 Polyoxyethylene and Castor oil/ Polyoxyethylene hydrogenated castor oil/Oleoyl polyoxyl-6 glyceride/ Soybean oil/Isopropyl myristate/Ethyl oleate/Triacetin | Physical stability and the retention rate of DHA/EPA enhanced through optimization of NE composition and incorporation of tea polyphenols as antioxidants. | [36] |
| Coenzyme Q10 (CoQ10) | Spontaneous emulsification | O/W ME | Polysorbate 80 and Caprylic/Capric triglycerides (TG)/Rice glycosphingolipids (RGSLs) | Solubility of CoQ10 in freeze-dried particles and its oral absorption, when administered as ME, remarkably improved. | [75] |
| Rosemary (*R. officinalis L.*) essential oil | Microfluidization | O/W NE | Tween 80/Span 80 and Rosemary essential oil | HLB values, surfactant/oil ratio and rosemary oil concentration were optimized using response surface methodology and physically stable NE containing rosemary essential oil was developed. | [76] |
| "*Dedo-de-moça*" pepper extract | High-pressure homogenization | O/W NE | Tween 80/Span 80 and Soybean oil | The ideal composition of pepper extract NEs together with optimum processing conditions led to NEs, which were stable under almost all environmental stresses at 4°C and room temperature for over 120 days. | [32] |
| Curcumin | High-pressure homogenization | O/W multilayer NE | SDS/Alginate/Chitosan (coating agents) and Medium-chain triglycerides (MCTs) | Curcumin NEs showed increased bio-accessibility and antioxidant capacity. Multilayer NEs had better control on *in vitro* digestion of lipids than non-coated NEs. | [77] |
| Peppermint essential oil | Spontaneous emulsification | ME and NE | Tween 20/Tween 40/Tween 80 and Peppermint essential oil/MCTs | Non-ionic surfactants showed proper ability in spontaneous emulsification together with vegetable oil and MCT. MEs were formed at SORs higher than 150%, whereas NEs were formed at all SORs. | [78] |

(*Continued*)

**Table 2.** (Continued).

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Kenaf seed oil | High-pressure homogenization | O/W NE | Sodium caseinate (SC)/Beta-cyclodextrin (*β*-CD)/Tween 20 | NEs showed efficacy in terms of antioxidant activity and stability of bioactive ingredients with 90% retention of vitamin E and 65% retention of phytosterols after 8 weeks of storage under refrigerated condition. | [31] |
| Astaxanthin | Spontaneous emulsification | O/W NE | Phospholipids/Chitosan/Carrageenan/ Acetone and Ethane | All developed NEs were stable at 4°C for at least 18 days and provided protection of astaxanthin from photodegradation. | [79] |
| *α*-tocopherol | Spontaneous emulsification | O/W ME | Tween 20/Glycerol Isoamyl acetate/ Isopropyl myristate and Lemon oil | The antioxidant capacity of *α*-tocopherol encapsulated within ME slightly increased with time, indicating a slow and controlled release of entrapped *α*-tocopherol from the dispersed droplets of ME. | [80] |
| Vitamin D ($D_3$: cholecalciferol) | High-pressure homogenization | O/W NE | Tween 20/Soybean lecithin and Soybean oil/Cocoa butter | Whole-fat milk fortified using vitamin D-loaded NE demonstrated high stability to aggregation, creaming, sedimentation and phase separation for more than ten days. | [81] |
| Citral | High-pressure homogenization | O/W NE | Tween 20/Gelatin and Medium-chain triacylglycerol | NEs showed high efficiency to protect citral from degradation and increased its stability under acidic conditions. | [82] |
| Lemon oil | Phase inversion temperature (PIT) | O/W NE | Tween 20/Sodium caseinate (NaCas) and Lemon oil | Combination of NaCas and Tween 20 applied successfully in preparation of lemon oil-loaded NEs using phase inversion temperature method where NaCas could partially replace Tween 20. | [83] |
| Tea seed oil (*Camellia oleifera* Abel.) | Spontaneous emulsification | W/O, BC, and O/W ME | Tween 20/Ethanol and Oleic acid | W/O MEs containing tea seed oil were successfully developed. Dilution of the dispersed phase of tea seed oil-loaded ME resulted in phase inversion from W/O to BC and finally to the O/W phase without any phase separation. | [84] |
| Lycopene | High-speed homogenization/ Microfluidization | O/W ME | High-methylester-pectin (HMP)/Whey protein concentrate (WPC) and Corn oil | Concentrations of HMP and WPC, as well as their interactions, remarkably affected the stability of the developed two-layer ME encapsulating lycopene against thermal degradation and pH changes. The two-layer MEs were found to be more stable under environmental stress than the one-layer one. | [35] |

(*Continued*)

**Table 2.** (Continued).

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| *β*-carotene | High-pressure microfluidization | O/W NE | *β*-lactoglobulin/Tween 20 and Orange oil | *β*-carotene was significantly more stable in NEs stabilized using *β*-lactoglobulin compared with those stabilized using Tween 20. *β*-carotene degradation accelerated at high temperatures (55 °C) and acidic conditions (pH 3) while its stability was independent of ionic strength. | [85] |
| Citral | Hot, high-pressure homogenization at 45 °C | O/W NE | Lecithin and Palm kernel fat | NE containing different natural antioxidants remarkably improved the chemical stability of citral and decreased the formation of many off-flavor and off-odor volatile substances over storage. | [86] |
| *α*-Tocopherol, naringin, catechin, chlorogenic acid, quercetin and baicalin | Spontaneous emulsification | O/W ME | Tween-20/*n*-Butanol Methyl linoleate/ Styrene | Developed MEs provided a synergistic effect between water-insoluble and water-soluble antioxidants. Results revealed the highest (44.8%) synergistic activities in the case of chlorogenic acid. | [87] |
| Lutein | Spontaneous emulsification | W/O, BC, and O/W ME | Tween 80/Glycerol/Ethanol and R-(+)-Limonene | Maximum solubilization of lutein was achieved within the BC region. Alcohol and glycerol improved lutein solubilization, whereas vegetable oils decreased its solubilization. | [88] |
| Lycopene | Spontaneous emulsification | W/O, BC, and O/W ME | Tween 60/Ethoxylated mono-diglyceride /Sucrose ester (O-1570)/Triglycerol monooleate (3G1O)/Ethanol/Propylene glycol and (R)-(+)-Limonene | Solubilization capacity of the developed ME for lycopene was 10 times higher than (*R*)-(+)-limonene and other edible oils. The use of mixed surfactants and surfactants with lower HLB enhanced the lycopene solubilization capacity. | [89] |

### Natural preservative

Food safety is still an issue of concern for consumers and regulatory agencies as foodborne diseases cause substantial health and economic burden to people and manufacturing sectors. Increasing demand for natural organic ingredients and fewer chemical additives in food products have simulated current strong interest in using natural preservatives to replace chemical compounds in the food industry.[90,91] Previous studies have well established the use of natural food preservatives to protect processed foods from spoilage due to exposure to pathogenic and spoilage microorganisms.[90–93] However, major obstacles in the application of many natural food preservative agents are their low solubility in water-based formulations and their intense taste and smell, which can cause sensory changes and consumer rejection.[90]

These technical issues can be resolved by encapsulating the natural food preservatives using ME and NE techniques to improve their solubility and minimize potential organoleptic changes caused by them. An essential oil-containing ME or NE can be considered as a potential alternative to conventional antimicrobial compounds for the treatment against pathogens with the ability to develop robust biofilms and evolve resistance to antibiotics.[94–96]

Main recent studies on the development of nano-sized emulsions containing natural food preservatives are listed in Table 3. Essential oils and plant extracts as potential natural substitutes for conventional preservatives mostly have been incorporated into a lipid phase of O/W NEs. Subsequently, the effect of essential oil-loaded NEs on values of biochemical parameters and growth rate of target bacteria, yeast and mold has been investigated.

However, investigation of antimicrobial activity of NEs encapsulating natural food preservatives in more realistic environments, such as food matrices, followed by optimization of process parameters, is a neglected but still important area in most studies. The antimicrobial potency of natural ingredients decreases during food processing as it is greatly influenced by process conditions (e.g., pH, temperature and aeration) and the extent of microbial contamination of various food.[102,103] Therefore, data extrapolation from *in vitro* studies to food systems is challenging and may cause crucial misinterpretations.

Furthermore, despite the documented potential of natural food preservatives in the developed NEs (Table 3), high concentrations are required to efficiently delay or prevent the growth of microorganisms in food products, thereby limiting their application as preservatives in the food sector.

Although essential oils are the natural concentrates of various plants, there are regulatory limitations set by international organizations regarding the accepted daily intake (ADI) of essential oils, which should not be exceeded.[102] Possible solutions to overcome this issue can be achieved by introducing natural preservatives into active packaging rather than food formulations or exploiting the potential synergies among essential oils components. In addition, future studies can be conducted to further improve the antimicrobial activity of the developed NEs by introducing additional antimicrobial agents, such as plant-derived substances (e.g., isothiocyanates, polyphenols), chelating agents (e.g., citric acid, EDTA), enzymes (e.g., lysozyme), and peptides (e.g., bacteriocins, lactoferrin).

### Active food packaging

The ever-growing interest of the present health-conscious society for healthy and nutritious foods with clean label features and lower environmental impact, but still guaranteed to be hygienic and safe for consumption, have guided food scientists and the food industry toward the use of more eco-friendly alternatives to packaging materials for food applications. Various publications have documented the potent antibacterial activity of essential oils obtained from different medicinal and aromatic plants, namely cinnamon, garlic, sage, clove, pimento, and rosemary, against a wide range of bacterial species. However, essential oils tend to be lipophilic, chemically unstable and highly volatile, which lead to a reduction in efficiency and their limited applications in food packaging.[90–94,96]

**Table 3.** Major recent advances of the applications of microemulsions (MEs) and nanoemulsions (NEs) as natural food preservatives.

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Curcumin | Emulsion inversion point method | O/W NE | Tween 80 and Cinnamon essential oil/Garlic essential oil/ Sunflower oil | Curcumin-cinnamon essential oil NE showed the highest antimicrobial activity against psychrophilic bacteria, yeast and mould growth and reduced thiobarbituric acid (TBA) and total volatile nitrogen (TVN) values in refrigerated chicken fillets. | [97] |
| Oleoresin capsicum | High-pressure homogenization and ultrasonication | O/W NE | Tween 80 and Glycerol | Oleoresin capsicum NEs reduced *E. coli* and *S. aureus* count up to 2.79 and 5.89 log, respectively. NEs prepared using high-pressure homogenization showed higher antimicrobial activity than those manufactured by ultrasonication. | [98] |
| Oregano essential oil | PIT (phase inversion temperature) method | O/W NE | PEG-40 hydroxylated castor oil (*Ricinus communis*)/ Polyoxyethylene 4-laurylether (Brij 30)/Sorbitan monooleate (Span 80) and Sunflower oil | Encapsulated oregano essential oil in NE exhibited inhibitory activity against all the genera of fungi (*Fusarium* sp., *Cladosporium* sp. And *Penicillium* sp.) isolated from Minas Padrão cheese samples. The observed inhibitory effect was remarkably dependent on the concentration of NE, immersion time into NE, as well as storage temperature and water activity. | [93] |
| Zataria multiflora Boiss. essential oil | Ultrasonication | O/W NE | Tween 80 and Sunflower oil | NE containing different concentrations of *Zataria multiflora* Boiss. essential oil prevented lipid oxidation and increased shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets during cold storage for at least 15 days. Results of the sensory analysis revealed higher acceptability for odor, taste, and texture of rainbow trout fillets treated with the NE compared to the control. | [99] |
| Cinnamon leaf oil | Spontaneous emulsification, microfluidization and ultrasonication | O/W NE | Tween 80 and Coconut oil | Cinnamon oil NEs showed high physical stability over 30 days of storage, where the cinnamon oil: coconut oil ratio of 6: 4 was the most appropriate in terms of the NE stability. Developed NEs presented antimicrobial activity against *E. coli* to some extent, but complete inhibition was not achieved. | [100] |
| Essential oils of rosemary, laurel, thyme and sage | Ultrasonication | O/W NE | Tween 80/Ethanol and Essential oils of rosemary, laurel, thyme and sage | NEs containing the herb oils prolonged the shelf life and improved organoleptic characteristics of rainbow trout fillets after immersing the fillets in the developed NEs for 3 min. The highest antibacterial activity was identified in samples treated with rosemary NE followed by thyme NE. | [101] |
| Anise oil | High pressure homogenization | O/W NE | Soy lecithin and Medium-chain triacylglycerol | NEs and coarse emulsions of anise oil were found to have long-term physicochemical stability and were more effective than bulk anise oil in reducing *Listeria monocytogenes* and *Escherichia coli* O157:H7 population. Developed anise oil-loaded NEs allowed the gradual and sustained release of anise oil. | [50] |
| Cetylpyridinium chloride (CPC) | Microfluidization | O/W NE | Triton X-100/Tween 60/Tween 80/ Soy sterol and Soybean oil/Ethyl oleate/Octanol | All NE preparations containing cetylpyridinium chloride (CPC) showed antimicrobial activities against four *Acinetobacter baumannii* strains, among which NE containing 10% (vol/vol) Triton X-100, 25% (vol/vol) soybean oil, and 1% (wt/vol) CPC with a droplet mean diameter of 213.9 nm, was found the most efficient in affecting *A. baumannii* in both its biofilm and planktonic states. | [96] |

**Table 4.** Major recent advances of the applications of microemulsions (MEs) and nanoemulsions (NEs) in active food packaging.

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Thymol | Spontaneous emulsification, ultrasonication and a combination of both methods | O/W NE | Tween 80 and Miglyol 812 | The best results in terms of size, polydispersity and encapsulation efficiency of thymol were obtained for NEs prepared by spontaneous emulsification method. The quinoa protein/chitosan film activated with thymol NE showed porous and heterogeneous characteristics. The ultimate tensile strength of the developed film demonstrated a meaningful decrease, whereas mean elongation at break and water vapor permeability did not show any significant difference compared to control film. Tomatoes coated with the developed film showed a remarkable reduction in the growth of fungal *B. cinerea* during 7 days storage at 5 °C. | [105] |
| Rutin | Microfluidization | O/W NE | Span 80/Tween 80 and Soybean oil | All the gelatin films prepared using rutin NEs offered potent antioxidative activities determined using free radical DPPH scavenging assay and reducing power assay. Microstructure of all the developed film characterized as compact and homogeneous. The incorporation of rutin NEs enhanced the mechanical properties of gelatin films and remarkably improved film resistance to mechanical deformations. Gelatin films prepared using rutin NEs exhibited greater tensile properties and increased elongation at break than the control film prepared just by gelatin. | [106] |
| Zataria multiflora essential oil | Ultrasonication | O/W NE | Tween 80 and essential oil | The developed film in which *Zataria multiflora* essential oil-loaded NEs incorporated in the basil seed gum films presented antimicrobial activity against potentially pathogenic microorganisms of *Bacillus cereus* and *Escherichia coli*. Antibacterial properties of *Zataria multiflora* essential oil increased with a decrease in the mean droplet diameter of the NEs. *Zataria multiflora* essential oil-loaded NEs performed as a nano-sized filler within the film matrix, where mechanical and physical properties of the activated film improved with the rise in NE concentration. | [107] |

(*Continued*)

**Table 4.** (Continued).

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Ginger essential oil (GEO) | Microfluidization | O/W NE | Tween 20/Span 80 and Canola oil | Gelatin-based films incorporated with ginger essential oil NE and montmorillonite nano-clay exhibited antioxidant activity but did not present antimicrobial properties against *Staphylococcus aureus*, *Salmonella enteritidis, Escherichia coli* and *Pseudomonas aeruginosa*. The developed active packaging film demonstrated increased thickness, elongation at break, puncture strength and puncture deformation over control. In addition, moisture content, water solubility and superficial hydrophobicity were significantly lower in the active packaging compared to control. | [108] |
| Oregano (*Origanum vulgare*) and Clove bud (*Syzygium aromaticum*) essential oils | Ultrasonication | O/W NE and coarse emulsion | Sorbitan monooleate (Tween 80) and Essential oils | NEs of clove oregano (*Origanum vulgare*) and bud (*Syzygium aromaticum*) essential oils with mean droplet diameters of 180–250 nm were more effective in improving the extensibility and decreasing the rigidity of the methylcellulose films compared with coarse emulsions having mean droplet diameters of 1.3–1.9 μm. Reduction of moulds and yeasts contaminants in sliced bread was more pronounced in films activated by NEs than those activated by coarse emulsions within 15 days, showing higher antimicrobial activity of essential oils in NEs than coarse emulsions. | [109] |

A new trend in antimicrobial food packaging, applying nanotechnology, is the use of MEs and NEs incorporating essential oils and plant extracts having antimicrobial and antifungal activities. This kind of packaging take advantages of unique features like tuneable rheology and optically transparent appearance.[104]

The main recent advances in applications of MEs and NEs for developing antimicrobial food packaging materials are summarized in Table 4. In this regard, nano-sized emulsions containing essential oils or other bioactive components have been developed and subsequently incorporated into film-forming suspensions of polymers. Improvement of antibacterial and antifungal activities of essential oils simply by nanoemulsification and providing an average droplet size <200 nm has been reported as findings of a few studies in this field. These findings have practical implications for food preservation, showing the possibility of having a distinct antimicrobial effect with lower concentrations of preservatives just by their incorporation as nano-particulates in the formulation.

Previous studies on food packaging activated by essential oils and plant extracts are usually oriented to analyze the developed film's antimicrobial properties compared to control. However, consumer attitudes and behavior towards foods packaged using novel antimicrobial films are mainly influenced by the product's sensory characteristics. Active antimicrobial ingredients are mostly essential oils that can cause adverse organoleptic (i.e., smell, taste, and texture) attributes exceeding the acceptable threshold to food consumers.[90–92,94,104] Hence, sensory evaluation of food products packaged using films containing natural antimicrobial ingredients seems crucial to be carried out by an adequately trained panel but overlooked in most studies in the field. Future research should thus assess the proposed active food packaging films regarding sensory characteristics of food products to ascertain their suitability and the overall acceptance of the product by consumers.

### *Separation technique*

Over the past few years, MEs have become one of the most interesting areas of application in separation technique due to environmental, regulatory, economic, health and safety issues associated with solvent-based extraction techniques.[110–112] Oil-in-water MEs and NEs can be considered as very efficient techniques for extraction of vegetable oils and lipophilic compounds from oil-bearing materials and aqueous solutions on the back of their high solubilization capacity, concurrent extraction and solubilization of the desired compound, nano-sized structures, ultralow interfacial tension, and large interfacial area.[113] From a technological point of view, using ME to extract vegetable oils and lipophilic bioactive compounds offer intrinsic advantages like safe operation and low energy consumption while avoiding extensive use of hazardous solvents which are incompatible with food applications.[38,112,114]

Novel advances in the extraction of vegetable oil and some lipophilic bioactive compounds using the ME technique are provided in Table 5. ME extraction technique has taken advantages of concurrent solubilization and extraction of target solutes where the premix of ME (a mixture of known quantities of water, surfactant, and co-surfactant) mostly has added to oil-bearing matrices within which lipophilic bioactive compound or vegetable oil are trapped. In most reports in this regard, the extracted components have presented in the final form of a ME (Table 5). Since many regularly consumed food products are not emulsion-based, it seems necessary to investigate the required treatments to break the ME and separate its main components following phase separation. This can be achieved, for instance, through a change in temperature and/or main components concentration or the introduction of new components, such as sodium chloride, acetone, and isopropanol. Subsequently, the target components can be recovered by applying centrifugation at appropriate gravity force, temperature, and lengths of time.

The high concentration of surfactants and co-surfactants required for the ME-based extraction technique is another serious concern for food applications of developed systems. Furthermore, in almost all cases, the MEs have shown relatively lower extraction efficiency even at optimum

**Table 5.** Major recent advances of the applications of microemulsions (MEs) and nanoemulsions (NEs) as a separation technique.

| Extracted compounds | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Lutein | Spontaneous emulsification | W/O, BC, and O/W ME | Saponin/Lecithin/Rhamnolipid/ Sucrose monopalmitate (SMP)/Span 20/Tween 20/Tween 80/Sodium dodecyl sulfate (SDS)/Ethanol/1-Propanol and Lutein | Extraction of lutein from marigold petals using ME resulted in maximum extraction efficiency of 85% and higher chemical stability against heat treatment, particularly UV radiation, compared with the solvent extract. Sonication pre-treatment could efficiently decrease droplet diameters (12–163 nm), enhance thermodynamic stability and eliminate the need for co-surfactant in ME development. | [111] |
| Canola oil | Spontaneous emulsification | O/W ME | Lecithin/1-Propanol and Canola oil | A maximum extraction yield of 82.6% was achieved following optimization of process temperature as the most significant factor impacting solubilization and extraction of canola oil. Moreover, demulsification of ME and separation of canola oil from ME premix was easily accomplished with temperature reduction. | [112] |
| Lycopene | Spontaneous emulsification | W/O, BC, and O/W ME | Lecithin/1-Propanol and Olive oil | Four extraction cycles applying 1 g tomato pomace and 5 g olive oil ME at the optimum ratios of ME components (lecithin: 1-propanol: olive oil: water 53.33: 26.67: 10: 10 wt%) led to the highest (88%) extraction yield of lycopene. Developed ME containing lycopene showed good solubility in both polar and nonpolar environments. | [115] |
| Bovine serum albumin (BSA) and cytochrome c | Spontaneous emulsification | BC ME | Sodium dodecyl sulfate (SDS)/ Dodecane/1-Pentanol | Bovine serum albumin and cytochrome c successfully extracted to the BC phase of ME with extraction yield of 64% and 81%, respectively. BC phase of ME after extraction was a nanostructured fluid possessing high protein concentration (32 g $L^{-1}$ bovine serum albumin and 44 g $L^{-1}$ cytochrome c). | [116] |

(*Continued*)

**Table 5.** (Continued).

| Extracted compounds | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Lipids and $\beta$-carotene | Spontaneous emulsification | W/O, BC, and O/W ME and single-phase MEs | Lecithin/Sorbitan monooleate/PEG6-caprylic glycerides and Ethyl caprate | ME extraction of lipids and $\beta$-carotene from lyophilized microalgae showed higher efficiency of Winsor types I and IV MEs in lipids extraction than typically used solvent of hexane and ethyl caprate. All MEs exhibited greater ability in the extraction of $\beta$-carotene from the lyophilized microalgae than hexane and ethyl caprate, while type IV ME presented the most significant extraction yield. | [117] |
| Oil, proteins, and glucosinolates | Spontaneous emulsification | O/W ME | Cetyltrimethylammonium bromide (CTAB)/Triton X-100/Tween 85/ CTAB/AOT/*n*-Butanol/Isopropyl alcohol and Hexane/Isooctane/Cyclohexane | Developed Winsor type II ME could simultaneously extract more than 90% of soluble proteins and glucosinolates (GLs) from cruciferous oilseeds meals. The proposed extraction method could also extract nearly the same amount of oil extracted using volatile organic solvents in conventional solvent-based extraction methods. | [118] |

conditions than conventional organic solvent techniques rendering this technique commercially less favorable. Pre-treatment of the matrix containing the target compounds using appropriate techniques, such as enzymatic and ultrasonic processes, along with the application of co-surfactants could be employed for further increase in the extraction yield. Such pre-treatments also can decrease the surfactant concentration required for the extraction or eliminate the need for the use of co-surfactant(s). Furthermore, the success of extraction techniques can also be assessed by retaining the initial integrity of extracted compounds without compromising their quality, largely overlooked in the existing literature.

### Delivery of bioactive compounds

The application of delivery systems for bioactive compounds has been mainly focused on drugs in the pharmaceutical industry but recently extended to the nutraceutical and functional food sector.[71] ME and NE techniques represent efficient approaches to carry health-promoting food ingredients and to protect chemically labile bioactive compounds against oxidation and degradation or interaction with other ingredients. Digestibility, delivery, and controlled release of poorly water-soluble ingredients can be improved through solubilization in mixed micelles of MEs and NEs. In this regard, a nano-sized emulsion (i.e., ME or NE) is more efficient in retaining and delivering bioactive compounds through the gastrointestinal tract than a coarse emulsion.[71,85]

Relatively low bioavailability of naturally occurring carotenoids present in most plant foods has been associated with the fact that they exist as crystals or bound in protein complexes in cell wall structures and cannot fully liberate themselves from the surrounding environment during digestion in the gastrointestinal tract. Micro- and nano-emulsification of such carotenoids and similar nutraceuticals would be applied as an efficient method to improve their digestibility and delivery and consequently their nutritional value.[85]

Recent applications of MEs as delivery systems for bioactive compounds are listed in Table 6. Studies in this regard have mainly focused on *in vitro* digestion and stability and release behavior of encapsulated bioactive compounds under simulated gastrointestinal fluid conditions. However, understanding the biochemical processes (such as structural deformation, compositional transformation, and release behavior) occurring during digestion has not been sufficiently taken into account but seems of crucial importance to enable examining of the real functionality of developed delivery systems.

The future line of research would be applying new measurement methods and multidisciplinary techniques used in clinical, pharmaceutical, and biological research. Moreover, further research should explore the correspondence between *in vitro* and *in vivo* results using a well-developed model for food systems to enhance the predictive accuracy of existing *in vitro* testing systems simulating different parts of the gastrointestinal tract. Furthermore, *in vivo* investigations are of paramount importance to advance the understanding of the functionality of the bioactive compounds under a realistic gastrointestinal environment.

### Reaction media (nano-reactor)

The use of MEs as nano-reactors for organic and enzymatic reactions in the food industry is a proper solution to overcome reactant incompatibility. However, it is still in the early phases of evolution, and a lot needs to be done before it will be used in industrial-scale processes. MEs are very attractive reaction media due to simple experimental operations, lower energy requirements, mild reaction conditions, and large interfacial area providing sufficient phase contact of reagents. They also exhibit a high capability to solubilize both hydrophilic and lipophilic compounds in a single isotropic system and provide a higher reaction rate than water.[124,125]

**Table 6.** Major recent advances of the applications of microemulsions (MEs) and nanoemulsions (NEs) as food delivery systems.

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| *α*-Linolenic acid | Spontaneous emulsification | W/O/W ME | Polyoxyethylene castor oil EL (CrEL)/Ethanol and Isoamyl acetate | ME containing *α*-linolenic acid (ALA) was exceptionally resistant to the digestive processes and presented improved bioavailability for the ALA mostly in gastric and intestinal phases during *in vitro* digestion. Results of *in vivo* experiments showed that the developed W/O/W MEs were entirely assimilated after 12 h and excreted in both the urine and faeces. | [119] |
| Mangostin extracts | Ultrasonication | O/W NE | Tween20/Span20/Propylene glycol (PG) and Virgin coconut oil | All NEs, prepared using blends of surfactants having varying HLB values (10.2–15.1), were stable during three freeze-thaw processes. Antioxidant capacity and antibacterial activities (against *E. coli* and *S. aureus*) of mangostin extracts in developed NEs were higher compared to crude mangostin extract. Nanoemulgels created through embedding the NE in a hydrogel network demonstrated considerably increased release (87–92%) for mangostin extracts compared to control emulgels (74–78%). | [120] |
| Lycopene | High-pressure homogenization | O/W NE | Lactoferrin and Walnut oil/ Linseed oil/Sesame oil | The stability of lycopene-loaded NEs significantly increased with increasing oil density but decreased by increasing viscosity and overall unsaturation of the oil phase. Bioaccessibility investigations by using *in vitro* digestion process showed that lycopene NE prepared by sesame oil offered better stability, increased retention and slower degradation rate of lycopene than the other NEs. | [37] |
| *β*-carotene and green tea polyphenols | High-pressure homogenization | O/W NE | Tea polyphenols and Corn oil | Incorporating green tea polyphenols into *β*-carotene NE could increase the stability of *β*-carotene and its retention rate over storage. *In vitro* oral and stomach bioaccessibility of *β*-carotene in the presence of tea polyphenols remarkably increased. Results of *in vivo* studies showed that NEs containing green tea polyphenols enhanced the conversion efficiency of *β*-carotene into retinol. | [121] |
| Plant-based vitamin E | Microfluidization | O/W NE | *Quillaja* saponin and Corn oil | Increasing vitamin content in the formulation led to an increase in droplet size of the dispersed phase, decrease in storage stability of the NE, reduced bioaccessibility and reduction of degree and rate of lipid hydrolysis in the small intestine. The optimum formulation of the NE as the delivery system for vitamin E contributed to a rather high bioaccessibility of 53.9% for vitamin E. | [34] |

(*Continued*)

**Table 6.** (Continued).

| Bioactive component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Docosahexaenoic acid (DHA) | Microfluidization | O/W NE | Tween-40/Sodium caseinate/ Soya lecithin and DHA | Nanoemulsification of docosahexaenoic acid using Tween 40 as emulsifier resulted in the smallest mean droplet diameter (148 nm) when compared to NEs prepared using soy lecithin (760 nm) and sodium caseinate (206 nm). NEs prepared using Tween 40 also exhibited lower peroxidation, higher physical stability and greater lipid digestibility and bioavailability than other formulations. | [33] |
| $\beta$-carotene | Spontaneous emulsification | O/W NE and ME | Tween 80 and Long chain triglycerides (LCT)/Medium-chain triglycerides (MCT)/ Orange oil | Chemical stability and overall bioaccessibility of $\beta$-carotene in the simulated gastrointestinal tract were strongly dependent on the composition of the lipid phase, where long-chain triglycerides were found to increase its bioavailability owing to higher solubilization in mixed micelles. However, flavor oil (orange oil) and medium-chain triglycerides could improve loading capacities for long-chain triglycerides and result in smaller droplet sizes and a more stable NE. | [122] |
| Curcumin and resveratrol | High-pressure homogenization | O/W and W/O NE | Soy lecithin/Sugar ester/Tween 20/Glycerol monooleate/ Ethanol and Peanut oil/Palm oil/Sunflower oil | Developed NEs were found to be efficient in the encapsulation of curcumin and resveratrol and protecting them from any degradation over time. Delivery of the curcumin and resveratrol was assessed based on the antioxidative potential of the encapsulated ingredients and showed retained antioxidant properties, which was an indication of their delivery. | [123] |

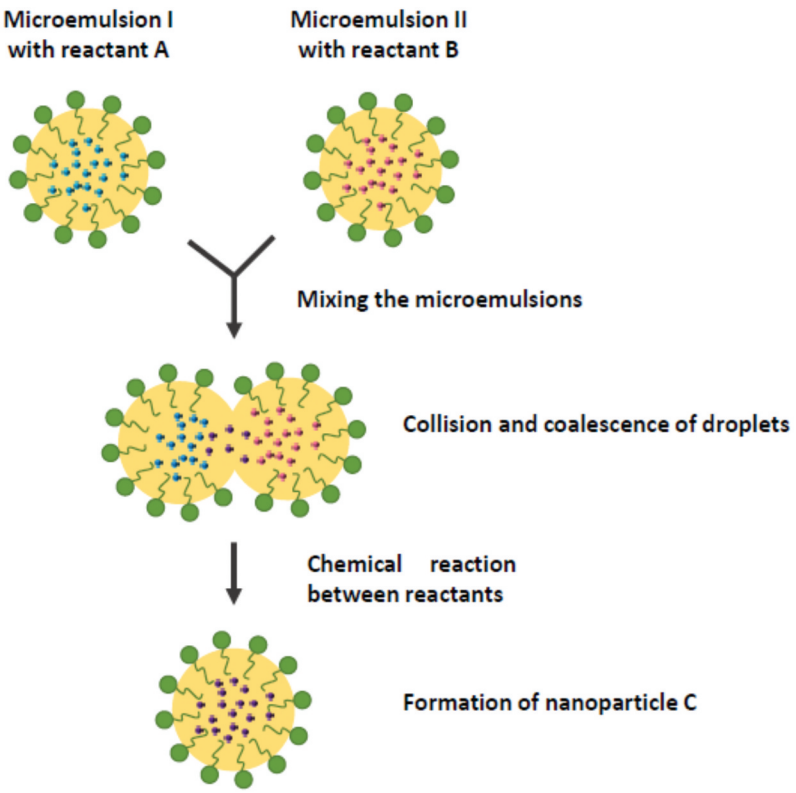


**Figure 7.** Schematic representation of the reaction for the synthesis of a nanoparticle in microemulsion as a reaction medium.

There is a significant relationship between rate constants of chemical reactions with composition, the polarity of the interfacial film and the nanostructure of ME.[126] W/O and O/W MEs can be considered as reaction media for water-soluble and oil-soluble reactants, respectively. By the way, BC MEs provide a large oil-water interfacial area and would enable contact between incompatible (i.e., water-soluble, and oil-soluble) reactants. This owes to the continuously fluctuating surfactant interface and bending interface in MEs at the optimum formulation.[127]

Figure 7 describes the reaction scheme that can happen between oil-soluble reactants in ME droplets to synthesize nanomaterials in an O/W ME.[128] First, ME I, containing reactant A, and ME II, containing reactant B, were prepared. Then, MEs I and II were mixed, leading to collision and coalescence between ME droplets. This would result in a reaction taking place at a nanoscale between different reactants, which are exchanged among the ME droplet cores (Fig. 7). Instant coalescence of the droplets changes the shape of the surfactant film. However, immediately after coalescence, droplets separate into the initial state due to the high free energy of surfactant film. During this continued collision and separation, the reactants would be exchanged between the cores, and the reaction would occur.[129]

Few recent studies in terms of the application of MEs as reaction media are presented in Table 7. Moreover, the application of lipase-containing MEs for the esterification of fatty acids with glycerol has been the focus of a few studies.[130–132] The activity of lipase in developed MEs was reported to be very low. This may be a consequence of applying low concentrations of surfactants as an aim of the project in some studies, such as the one performed by Svensson.[132] In addition, observed low hydrolysis and esterification activity of lipases can be due to enzyme dormancy induced by very low hydration level of applied low water systems, defined by water activity and/or water content of W/O MEs.

**Table 7.** Major recent advances of the food applications of microemulsions (MEs) as reaction media.

| Reaction type/Target component | Preparation technique | Type of emulsion | Surfactant and oil phase | Major findings | References |
|---|---|---|---|---|---|
| Digestion of proteins and lipids | Spontaneous emulsification | W/O ME | Lecithin/Propanol and Virgin olive oil | Virgin olive oil MEs, developed as an *in vitro* model for digestion simulation of proteins and lipids occurring in the small intestine, provided a better understanding of digestion of food lipids and proteins in the presence and absence of food antioxidants in intestinal colloidal phases. *In vitro* efficiency of developed ME as a digestion model was verified based on the catalytic activity of trypsin and alkaline phosphatase as well as stability of the ME and all other additives. | [133] |
| 2-Furfurylthiol (FFT) | Spontaneous emulsification | W/O, and O/W ME | Sucrose stearate/Sucrose laurate/Medium-chain alcohols ($C_2$OH to $C_6$OH) and Dodecane | Formulated ME reported to be a great reaction medium for Maillard reaction and synthesis of FFT regarding fast conversion rates, favorable selectivity and decreased unwanted side reactions. An increase in temperature (30–60 °C) resulted in a higher reaction rate. A decline in rate of reaction observed with lowering water content of ME and increasing the chain length of alcohol. The reaction rate also decreased with the increase of pH in the range of 4–8 in ME containing butanol as a co-surfactant. | [134] |
| Octyl decanoate | Microemulsification facilitated by brief sonication | W/O ME | Lecithin (phosphatidylcholine from egg yolk and soybean) and *n*-Heptane | Experimental results revealed that the primary rate of reaction was directly proportional to the amount of lipase enzyme used, whereas it was not influenced by the surfactant content. MEs composed of soybean lecithin (containing 40–48% phosphatidylcholine) showed a higher reaction rate than those composed of pure phosphatidylcholine obtained from egg yolk. However, the overall yield of esterification was reported to be very low and disappointing. | [132] |
| Intermediate fat blend | Spontaneous emulsification | W/O ME | Canola lecithin and Hexane | Enzymatic interesterification of tripalmitin with triolein catalyzed by *Rhizopus arrhizus* lipase in developed ME led to an intermediate fat blend with potentially desirable rheological characteristics. After 48 h reaction at 47°C the resultant modified fat composed of tripalmitin (29% w/w), triolein (52.1% w/w), 1-oleyl-2,3-dipalmitin (7.5% w/w), 1-palmitoyl-2,3-diolein (7.2% w/w), 1,2-dipalmitoyldiglyceride (1.6% w/w) and 1,2-dioleyldiglyceride (2.4% w/w). | [131] |
| Monoglycerides | Spontaneous emulsification | W/O ME | Sodium bis(2- ethylhexyl)sulfosuccinate (AOT)/Triethylene glycol monododecyl ether (C 12-EO 3)/Nonaethylene glycol monodinonylphenyl ether (DNP-EO 9) and *n*-Hexane/*n*-Octane/Isooctane and Palm oil | Enzymatic hydrolysis of triglycerides to monoglycerides using 1,3-specific lipase carried out in oil-rich MEs prepared without co-surfactant led to an efficient yield of 80%. However, intramolecular acyl migration gradually accelerated converting 2-monoglyceride into 1-monoglyceride, which subsequently went through the last step of lipolysis and was broken down into fatty acids and glycerol. Optimization of hydrolysis process resulted in optimum reaction time of 3 h at 35 °C. | [135] |

Future studies will be required to further characterize developed MEs as reaction media and optimize reaction conditions. Furthermore, in terms of enzymatic reactions, separation of the enzyme from the reaction site and its contact with the aqueous phase, where synthetic ionic surfactants like cetyltrimethylammonium bromide (CTAB) or sodium dodecyl sulphate (SDS) accumulate there, could result in enzyme inactivation.[136] Therefore, the concentration of main components and stability of ME used as reaction media seems crucial in retaining enzymes at the site of reaction.

The use of MEs as reaction medium in food systems has its own inherent limitations resulting from the need to use large quantities of surfactants required to form a single-phase ME, contributing to potential health concerns.[136] Therefore, in order to enhance large-scale process productivity and improve safety, proper non-destructive techniques must be developed to improve surfactant recovery and minimize surfactant losses.

### *Current commercial applications*

There are only a few practical applications of MEs and NEs in the large-scale production of food products.[13] NutraLease, a start-up company specialized in nanoscale delivery of nutraceuticals, has developed beverages containing encapsulated fat-soluble vitamins A, E and $D_3$, omega-3 fatty acids and antioxidants such as lycopene, lutein, $\beta$-carotene and isoflavones. The technology applied on the commercial scale has been self-assembled NEs, which is believed to increase the bioavailability of nutraceuticals.[137]

Another player operating in the commercial market of NE is AQUANOVA AG. It used NovaSOL technology to produce functional beverages fortified with bioactive compounds (e.g., astaxanthin, hop extract, curcuminoids, hydroxytyrosol, trans-resveratrol and vitamins C, A, D, K and E) and natural colorants and additives (e.g., green tea extract, rosemary extract, tocopherol, $\beta$-carotene, apocarotenal and carmine).[138]

As one of the world's largest food companies, Unilever has applied NEs in ice cream formulation with the main aim of lowering fat percentages in the final product. The fat content of the ice cream was reduced from 16% to 1% where the quality and the taste remained unchanged.[139] Nestlé, the world's leading food and beverage company, has developed W/O nanoemulsions using polysorbates and other micelle-forming ingredients. The NEs were successfully applied for more rapid, easier and uniform defrosting of frozen foods in the microwave.[5]

The other food companies currently working on MEs and NEs are the Kraft Heinz Company, WILD Flavors and Specialty Ingredients, Shemen Industries Ltd., Keystone Foods, Jamba, Frutarom Group and DuPont.[140]

One of the major restrictions being imposed on the development of MEs and NEs for food applications is the nature and dosage of surfactant suitable for use in foods. Only a few surfactants are met the safety standards of foods and are permitted to be used in the food industry, while their application still can cause toxicity and irritation if used at high levels.[12,141,142] Therefore, scientists should carefully consider surfactants that are safe for human consumption and have been designated as GRAS (generally recognized as safe), label-friendly, and economically viable for developing food-grade MEs and NEs.

Another limitation in preparing food-grade MEs and NEs is the low solubility of high-molecular-weight triglycerides (HMWTs) found in animal and vegetable oils (e.g., fish oil and soybean oil). HMWTs form undesirable phases such as sponge phases and liquid crystals during formulations. This phenomenon is due to the fact that it is challenging for the interfacial film of surfactant molecules at the water-oil interface to penetrate between the long fatty acid chains of high molecular weight triglycerides and generate proper surface curvature required for spontaneous development of MEs and NEs.[142–145]

Incorporation of medium-chain triglycerides (MCTs) containing fatty acids with an alkyl chain length of 6–12 carbon atoms such as capric or caprylic triglycerides or medium-chain esters like isopropyl myristate and isopropyl palmitate in the formulation could be considered as one solution for low solubility of HMWTs.[142,143] This issue can also be overcome by applying short-chain alcohols and glycols with chain length C2–C10, usually used as the co-surfactant demonstrating solubility in

both the water and oil phases. Co-surfactant reduces migration times for the oil phase by partitioning between hydrophobic tails of oriented and tightly packed surfactants at the oil-water interfaces allowing the hydrophobic parts of the surfactant molecules to move freely along the interface. This would result in an increase of interface film flexibility and fluidity, which generate desired interfacial film curvature required for ME formation.[38,142,146]

## Concluding remarks

The primary practical method to distinguish NEs from MEs can be the determination of the free energy level of the system. The free energy level of a ME system is lower than separated phases, i.e. separated oil and water whereas a NE has higher free energy than separated phases. In such cases, contrary to NEs, MEs are spontaneously formed systems, furthermore, are thermodynamically stable.

Despite extensive investigations regarding the immense potential of MEs and NEs in many areas due to their spectacular properties, these nano-sized systems are far from being fully realized and characterized. With continued research and comprehensive insight, MEs and NEs are likely to play a much more critical role in our future industries and daily lives driven by the increasing importance of energy, environmental concerns, demand for natural food additives and preservatives and strict provisions, regulations, and standards for the approval of any artificial food ingredients.

Incorporation of MEs and NEs loaded with bioactive compounds in more realistic environments, such as food matrices and evaluation of their behavior under different process conditions can give some more practical advice to the food and beverage sector. Further research is also likely to address other features like concurrent encapsulation of food bioactive compounds, digestion/absorption simulation and organoleptic characteristics of the developed formulations to assess the overall acceptance of the product by consumers. Moreover, *in vivo* studies are of great importance to advance the knowledge of the functionality of the bioactive compounds under a realistic gastrointestinal condition. Future investigations should also consider practical issues more seriously to bridge the gap between laboratory findings and industrial applications in terms of proper main components and process parameters. In this way, food research studies can be conducted to apply medium- and long-chain triglycerides as the edible oil phase, short-chain alcohols, and glycols as co-surfactant, and surfactants permissible for food applications, such as lecithin, saponin, rhamnolipid, sucrose esters, monoglycerides and glycolipids. In addition to improve efficiency and safety of large-scale process, proper non-destructive procedures must be implemented to improve surfactant recovery and minimize surfactant losses.

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