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Review

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Recent Developments in Nano-Emulsions' Preparatory Methods and their Applications: A Concise Review

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Abstract

Nano-emulsion is one of the most effective and size-controlled mediums for effective drug delivery systems, the formation of cosmetic, food preservatives, insecticidal and antimicrobial products. Therefore, a durable and sophisticated approach is primely important in preparing effective nano-emulsions. Some of the established fabrication approaches towards nano-emulsion are the high and low-energy methods. Depending upon the required results of formulations, these two methods are further divided into sub-categories such as ultra-sonicators, micro-fluidizers, high-pressure homogenizers, phase inversion temperature, phase inversion composition, etc. This review highlights all the available methods to form nano-emulsion by adopting high-energy and low-energy techniques. In addition, this review also elaborates on the importance of nano-emulsions in various end products, as nano-carriers and patents have also been awarded in this field. Besides, the required improvements in this field have been discussed briefly to establish the most authentic approach toward nano-emulsion formation.

Keywords: Nano-emulsions, High energy methods, Low energy methods, Nanocarriers, Drug delivery, Cosmetics, Antimicrobial agents, Food preservatives.

Introduction

Nano-technology is a multi-faceted technique involving the construction and utilization of various nano-scale systems. Nano-technology is gaining attention in nano-biomedicines, food engineering, pharmacology, entomology, and parasitology. Nano-technology has also demonstrated that it can speed up the function of a medicine delivery system by allowing the drug to reach its precise target and perform a specified action. Nano-medicines provide several advantages, including increased therapeutic efficiency and fewer side effects. Nanoemulsions are proving to be the best alternative for all these purposes. The emulsion refers to a colloid in which both dispersed and dispersion phases are liquids. Surfactant is added to control the division of these two phases. At the point when the arrangement of emulsion comes to the size of 20-200 nm, it is named nanoemulsion. The nano-emulsions (containing nano-sized droplets) tend to boost bioavailability and

optical transparency; hence nano-science is now being employed to treat a variety of infections and disorders [1-4]. Nanoemulsions (also regarded as nano-formulations) can be arranged using the dispersal component of the oil in water or water in oil that additionally permits the settlement of different unique constituents [5]. Nano-emulsion has nano-size; which enhances the surface region, with the goal that the assimilation rate is precisely upgraded and expands the bioavailability of the definition. Nano-emulsions can improve the proficiency of the medication conveyance framework. Nano-emulsions aren't deadly or harmful, so they can undoubtedly be utilized for skin and mucous film. Nano-formulations formulated using essential oils have been acknowledged for human utilization. Nano-emulsions are dynamically and thermodynamically stable items, henceforth, they don't show issues of creaming, flocculation, coalescence, and sedimentation. Because of higher physical stability, nano-emulsions are produced in many forms, such as foams, sprays, liquids, and creams. Nano-emulsions don't damage human and animal cells, so these are human and animal-friendly [6]. Considering the topic's importance, this comprehensive review focuses on various preparatory approaches and applications of nano-emulsions.

Fabrication of Nanoemulsion

The nanoemulsions consist of two phases termed the discontinuous and continuous phases or dispersed phase and dispersion medium, respectively. There are three essential/basic components required for the fabrication of nanoemulsions.

1) Oil phase
2) Aqueous phase
3) Surfactant/ emulsifier
4) Co-surfactants (in some cases)

The surfactants or surface tension reducers are utilized to blend the two phases. Surfactants blend the two phases by reducing the interfacial tension. Protein, polysaccharides, small molecules, and phospholipids are some common examples of emulsifiers [7-9].

Classification of Nanoemulsion

Based on the type of dispersion medium and dispersed phase, emulsions are divided into two essential classes, i.e., i) oil-in-water (O/W) and ii) water-in-oil (W/O). In the oil-in-water class, droplets of oil are distributed to the continuous phase of water, while in the water-in-oil, water drops are distributed in oil [7]. Both types are displayed in Fig. 1.

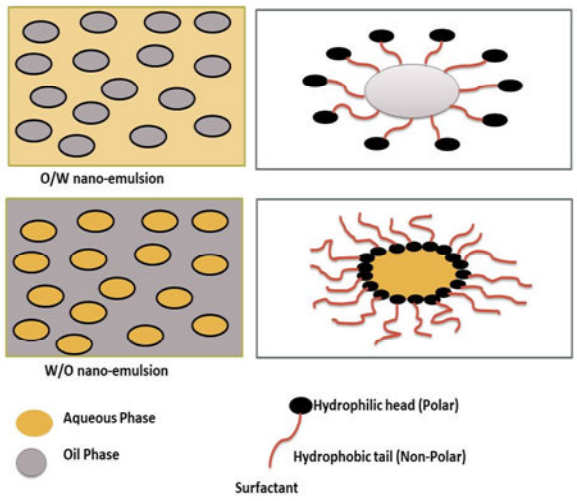

Figure 1. **Types of nanoemulsion [4]**

For the preparation of oil in water nanoemulsion, a surfactant/emulsifier with a high value (8-18) of hydrophilic-lipophilic balance is used, while for the preparation of water in oil nanoemulsion, an emulsifier with low hydrophilic-lipophilic balance (3-6) are preferred [7-10].

Comprehensive detail of preparation methods of both these classes is described in the next points.

Methods of Nanoemulsions Preparation/ Fabrication

Utilizations of nanoemulsions are reliant upon their strength of stability and their capacity to deliver required components to respective points. Nanoemulsions can solubilize non-polar dynamic composites. Numerous nanoemulsions are stable, and a couple might be unstable. Thus, if a nanoemulsion is not too long stable, it is formulated just before to utilized [9].

According to a literature survey (from 2005 to 2021), high and low-energy methods are two well-known ways to prepare O/W or W/O nanoemulsions. As the word ''high energy'' represents, it is the method in which required large disturbing forces are given by mechanical devices. In contrast, the ''low-energy method'' does not need any external energetic force [6, 8].

All preparation methods have importance as they lead towards the formation of desired droplets size-based nanoemulsion to make our life easy. But, some methods also have adverse limitations. The following sections describe the details of high energy as well as low energy methods and sub-types involved in them, along with the details of instrumentations used.

High energy methods to prepare nanoemulsion

Earlier reports (from 2005 to 2017) show that higher energy stirring and ultrasonic emulsification have remained the most extensively consumed approaches by researchers [9]. In high-energy methods, various mechanical devices (such as ultra-sonicators, micro-fluidizers, high-pressure homogenizers, etc.) are used to provide distracting forces that lead to the production of small-sized droplets. Types of equipment, conditions of production (for example, temperature and time) together with the properties and composition of the sample are the aspects that influence the size of produced droplets [10-12]. A brief overview of the reported high-energy methods is given below.

Advantages of High Energy Methods

High-energy methods are used because they require less time for formation [10-12]. These methods allow the well-controlled size of droplets that make it remarkable. These methods also provide a large selection of integral components [12].

Disadvantages of High Energy Methods

High-energy methods of nanoemulsion production are not cost-effective as they consume more energy and require refined instruments. High-energy methods require huge energy and disruptive force, these methods are not useful to thermo-labile (heat sensitive) and macro-molecules with nucleic acids, enzymes, and proteins [13-15].

High-pressure homogenization

Among different reported high-energy methods, High-pressure homogenization is the most commonly used method to produce nanoemulsion. This method got supreme importance due to its piston homogenizer or high-pressure homogenizer that leads to the production of nanoemulsions up to 1 nm in size. The production of nano-emulsion by this method involves macro-emulsion which is forcefully forwarded by a short orifice at a pressure between 500-5000 psi [13, 14]. Poly-Dispersity Index (PDI) is used to specify the uniformity of the droplets. So, the high-pressure homogenization procedure is/can be repeated again and again till the final product approaches to desired PDI and droplet size [15]. The PDI score is inversely proportional

to droplet size uniformity. In nanoemulsions, a higher PDI suggests less droplet size uniformity. PDI less than 0.08 indicates monodispersed samples, PDI between 0.08 and 0.3 indicates a narrow-sized distribution, and PDI greater than 0.3 indicates a broad-sized distribution [2, 16]. Chenni *et al.* prepared emulsions by adopting this method of the formulation [17]. Muhammadi *et al*. and Liu *et al*. prepared nanoemulsion of essential oil of peppermint/ eucalyptus and cinnamon essential oil by using the method of high-pressure homogenization, respectively [18, 19].

Advantages of High Pressure Homogenization

As numerous forces such as hydraulic shear, severe turbulence, and cavitation work throughout the process, hence extremely small droplet-sized nanoemulsions are achieved, as shown in Fig. 2. The main advantage of the high-pressure homogenization procedure is/can be repeated again and again till the final product approaches to desired PDI and droplet size [19].

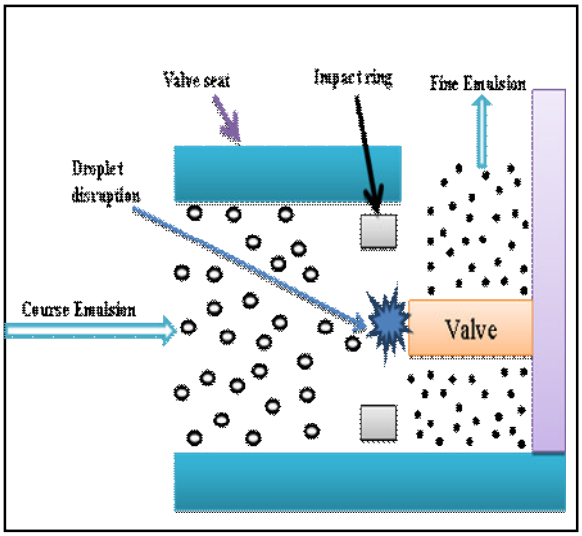

Figure 2. **High pressure valve homogenizer [2, 3]**

Disadvantages of High Pressure Homogenization

Acquiring small droplets in submicron levels requires a lot of energy. During high pressure homogenization, this amount of energy and increasing temperatures process may lead to the denaturing of the components. Proteins, enzymes, and nucleic acids (thermo-labile compounds) may be denatured [2].

High shear stirring

The method of high shear stirring, including high-energy mixers and rotor-stator systems has been used (2005 to 2021) to synthesize nano-emulsions. These have been used because by increasing their mixing intensity, the operator can considerably reduce the size of the internal phase droplet. However, emulsion preparations with an ordinary droplet size of less than 200-300 nm are difficult to obtain. By conventional mixers, the process can be carried out in batches. In recent studies, colloid mills are being used to provide an incessant mode and enhance shear stress at dispersion.

Among them, the most trendy colloid mill is ‘Silverson flow mixers’ (Fig. 3), wherein rotors and stators possess configurations to obtain extra efficient emulsification. In this mixer, a high rarefaction is formed inside the disintegration head at high rotor speeds, and the emulsion constituents are slurped up by the rotor and stator unit [20]. The emulsion is thrown to the periphery by centrifugal force, resulting in significant dispersion in the space between the rotor and the stator's inner wall. The emulsion is passed through the stator's outer orifice at high speed before exiting the apparatus. The degree of aeration during emulsification should be kept minimum using modern technologies [21]. Because the highest degree of dispersion possible for the system is

typically not obtained in the single-pass regime, the multi-pass regime is frequently applied [8, 9, 14].

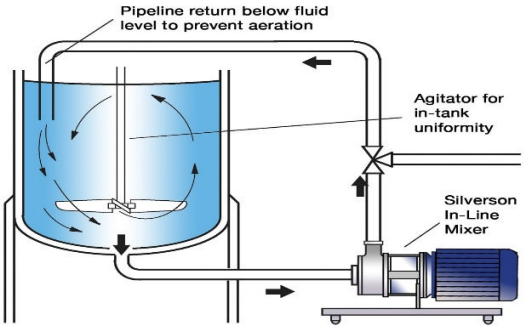

Figure 3. **Silverson flow mixer**

Disadvantages of high shear stirring

For viscous media and emulsions with a high fraction of the internal phase along droplet size of more than 1 mm, the effectiveness of high-shear stirring is traditionally reduced. This process also requires high energy [12].

Ultrasonic emulsion

Ultrasonic emulsification can be done *via* two methods. Firstly, the oil phase can be scattered in the continuous phase as droplets via an acoustic field (that creates interfacial waves). Secondly, ultrasounds incite auditory cavitation that affords the creation and breakdown of micro-bubbles correspondingly due to the pressure vacillation of sound waves. Droplets of the desired size are produced by collapses of micro-emulsions which mess up large droplets into sub-micron or nano size [21, 22], as shown in Fig. 4.

The pre-mixed macro-emulsion is disturbed by a vibrating solid surface at 29 kHz or higher frequencies in the ultrasonic emulsion process. The produced sound field in most ultrasonic systems is not homogenous. As a result, considerable power is required since all droplets must encounter the maximum shear rate and emulsion recirculation. Even at modest concentrations, this method of recirculation (many times) makes the emulsion homogeneous in size. The emulsifier, the amount of emulsifier, and the viscosity of the phases are the most important parameters influencing homogenization efficiency. These variables must be optimized to make nanoemulsions with fine and desirable droplets [23, 24]. Kaur *et al.* prepared aloe vera essential oil-based nanoemulsions by adopting the ultrasonic emulsification method [25].

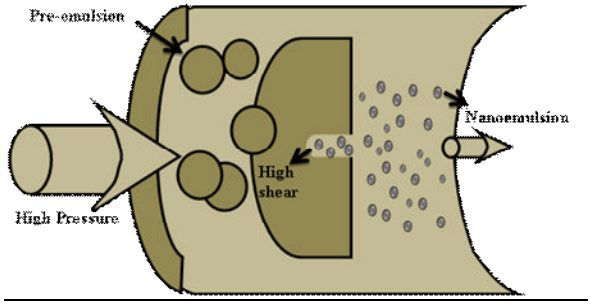

Figure 4. **Working principle of ultrasonicator emulsifier [1]**

Advantages of the Ultrasonication process

Nanoemulsions fabricated by ultrasonication process have excellent loading as well as release efficiency. The ultrasonic emulsion also has good physical properties because of its small droplet size [23].

Disadvantages of the Ultrasonication process

Sonication procedures can cause denaturation of proteins, de-polymerization of polysaccharides, and oxidation of lipids [22].

Micro-fluidization

Micro-fluidizers are commonly used in the pharmaceutical industry to create fine droplet-sized emulsion. A device called a micro-fluidizer is utilized in this procedure, which provides high pressures. The high

pressure causes the macro-emulsion to pass through the interaction chamber during the action, resulting in nano-emulsions with submicron-sized particles. Reiterating the technique numerous times while varying the operating pressure to acquire preferred-sized particles can result in a uniform nano-emulsion [2, 14]. Micro-fluidizer consists of two jets (also called micro-channels) from which crude emulsion is added. From both jets, a crude emulsion is added that combines at the interaction chamber and then undergoes collision, as shown in Fig. 5.

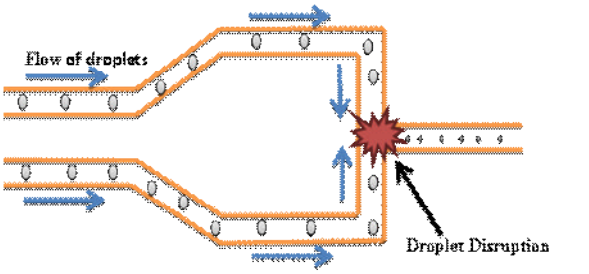

Figure 5. **The working approach of microfluidizer [3]**

A pneumatic power pump (capable of compressing air up to pressures 150-650 MPa) is used to provide mobility to the crude emulsion [14, 24]. This high pressure allows the crude emulsion to travel through micro-channels, and when crude emulsion from opposite channels collides, a massive shearing force is created, which aids in the development of fine emulsion [26]. Patel *et al.* prepared micro-emulsion via the micro-fluidization technique [27].

Advantages of Micro-fluidization

Micro-fluidization could be used to make nanoemulsions containing active substances to produce edible films with various functional and physical qualities [23].

Disadvantages of Micro-fluidization

This process has the following disadvantages. The nanoemulsion temperature increased due to the use of high pressure. As emulsification requires a longer time so droplet size increases due to coalescence [23].

Jet disperser

Jet disperser may have two or more jets, each from opposing bores, for introducing crude emulsion. Typically 0.3-0.5 mm is the diameter range of bores in jet dispersers. To coordinate the energy dispersion of the emulsion jet an orifice plate (also known as a homogenizing nozzle) is used. In front of the bores, due to laminar elongation flow droplets are disrupted predominantly, as shown in Fig. 6. Because orifice plates have no moving parts, jet dispersers can be employed at high pressures (300-400 MPa) [28, 29].

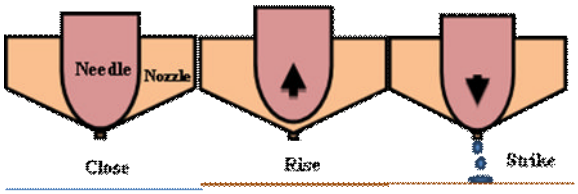

Figure 6. **Jet disperser**

Advantages of jet disperser

In the Jet disperser method, by driving the circulation stream via microchannels at high pressure toward the impregnated area, a great shearing action is created, resulting in an extraordinarily thin emulsion [28].

Disadvantages of jet disperser

The main disadvantage of this method is that if the emulsions have high viscosity, then droplet size gets disturbed due to laminar elongation flow and forces in the turbulent flow [28].

Phase inversion Temperature

In this method, a high temperature is given to micro-emulsion to bring about

change in its phase and production of nanoemulsion [8, 15]. A detailed description of this method is provided in the following points.

Membrane Method

Two types of membranes, hydrophilic and hydrophobic, are used for emulsion formation. In the membrane methods, fluid discharge via numerous pores or micro-channels within the membrane leads to the production of fine droplets. When the internal phase is expelled by the membrane, droplets are produced within the membrane/continuous phase interface. Membrane emulsification is used after preliminary dispersion to produce emulsions with smaller internal phase droplets as shown in Fig. 7.

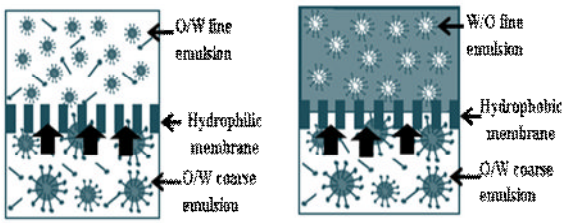

Figure 7. **Working approach of membranes**

The hydrophilic membrane causes the crude emulsion to be pushed through the membrane, resulting in smaller droplets [30, 31]. Due to this pumping, an inversion of phase took place, which resulted in the production of fine emulsion. When a hydrophobic membrane is used, it leads to the formation of the reverse emulsion. When the membrane is static, quick detachment of droplets from the surface re-circulation or stirring of the produced emulsion is done. There are two membrane types: fixed and vibrating [31]. Recirculation or stirring is done to detach formed emulsion droplets from devices with fixed membranes. Devices with rotating or vibrating membranes are moved fast to detach the droplets, but this considerably depends upon the designs of the gadgets [9, 23].

Advantages of the Membrane Method

Membrane emulsification has several advantages, including 1) uniform particle production, 2) droplet size control via appropriate membrane pore size selection, 3) low shear stress, 4) reduced energy requirements, 5) high plant flexibility, 6) precise, flexible and selective manufacturing of a variety of particles such as simple and multiple emulsions, liposomes and microspheres [30].

Disadvantages of the Membrane Method

Membrane pore size and distribution are critical aspects of membrane emulsification [30].

Low Energy Methods to prepare nanoemulsion

Low-energy methods are trendy nowadays because low-energy approaches rely on the chemical potential of the components to provide energy to nanoemulsions. By gently mingling the components, nanoemulsions develop spontaneously at the oil-water phase interface [23]. Low-energy methods are mainly categorized as phase inversion temperature and phase inversion of components which uses physiochemical properties of the system to provide the fine size of droplets [31]. But the spontaneous nanoemulsion formulation method also includes in these methods.

Advantages of the low-energy methods

These methods are easy, non-destructive, and do not cause any damage to encapsulated molecules [32]. Nanoemulsion

(having fine droplet size) can be attained via low-energy methods without any adverse effects [33, 34].

Disadvantages of the low-energy methods

Although low-energy methods are generally more efficient in obtaining small-sized droplets than high-energy methods, there are some limitations to low-energy methods related to the usage of some types of oils and emulsifiers (possessing proteins and polysaccharides). To avoid this drawback, higher-level concentrations of synthetic surfactant are added to get nanoemulsions in low-energy techniques, but this addition minimizes their application area, especially for various food processes [3].

Spontaneous Nanoemulsion

The spontaneous nanoemulsion method is considered of supreme importance due to releasing chemical energy within the continuous phase while diluting at a constant temperature without any transition of phase during the emulsification procedure [8, 14, 35]. By low-energy methods, at room temperature without requiring any special devices, nanoemulsions can be obtained depending upon the interfacial tension, viscosity of interfacial and bulk, phase transition region, surfactant structure, and surfactant concentration [36, 37]. When oil, water, and surfactant (depending upon the choice of oil) are mixed nanoemulsion is formed spontaneously, as shown in Fig. 8.

This method depends upon the handling conditions (speed of stirring, the addition rate of material, etc.) [3]. B. Sundararajan *et al.* prepared a nanoemulsion of *Ocimum basilicum L.* through a spontaneous low-energy method [4].

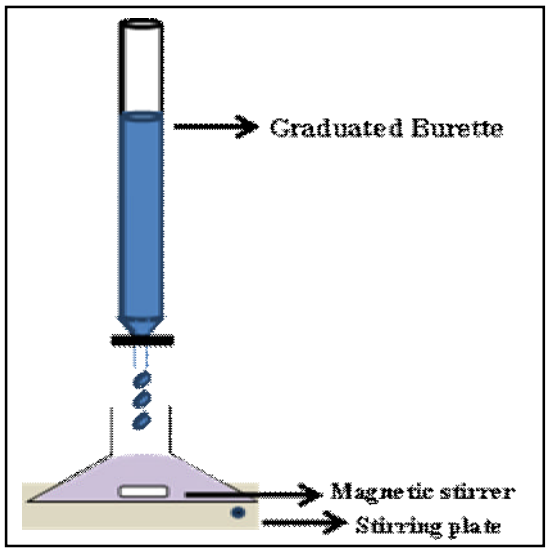

Figure 8. **Production of emulsion via spontaneous method [38]**

Advantages of spontaneous Nanoemulsion

The spontaneous nanoemulsion method is considered of supreme importance due to releasing chemical energy within the continuous phase while diluting at a constant temperature without any phase transition during the emulsification procedure [36].

Disadvantages of spontaneous Nanoemulsion

The lack of an oil phase and the presence of a solvent are the method's limitations [9].

Phase Inversion Method

When the phase undergoes transition, chemical energy is released by the system that leads toward the formation of fine nanoemulsion [38-39]. The required phase transitions can be produced by switching the composition at a constant temperature or altering the temperature at a constant composition [23, 40].

Phase Inversion Temperature

The temperature is changed in this method while the composition remains constant. When dealing with the phase inversion temperature approach of nano-formation, non-ionic surfactants with temperature-dependent solubilities (such as poly-ethoxylated surfactants) are used. Adjusting the affinities of surfactants for water and oil as a function of temperature produces a fine nanoemulsion [41, 42]. Polyethoxylated surfactants become lipophilic when heated because the polyoxyethylene groups dehydrate. To make nano-emulsions using the phase inversion temperature method, the temperature of the sample must be brought to the phase inversion temperature level, also known as the hydrophile-lipophile balance (HLB) level [39]. At hydrophile lipophile-balance (HLB) temperature, interfacial tensions become extremely low, and this is how the method helps to produce fine nanoemulsions. However, this method's emulsion production is very fast and spontaneous, but obtained emulsions are very unstable [43]. It has been observed that instant cooling of the emulsion at the temperature of phase inversion can produce stable and fine emulsion droplets [44, 45]. Safaya *et al.*, prepared nanoemulsion of neem oil by adopting this method [42].

Advantages of phase inversion temperature method

The advantages of this method are that in the bicontinuous phase of microemulsion it provides complete solubilization of the oil regardless of initial phase equilibrium [29].

Disadvantages of phase inversion temperature method

Complexity, accuracy requirements, and the usage of synthetic surfactants are among the limitations of this method [12].

Phase Inversion Composition

Emulsion Inversion Point (EIP) is another name for this approach. At a steady temperature, the phase inversion composition process undergoes configuration alternation [46]. Fine nano-emulsions can be made by gradually adding water or oil to an oil-surfactant or water-surfactant mixture. Because adding one component to an emulsion is much easier than bringing about fast temperature variation, the phase inversion composition approach is more reliable in large-scale production than the phase inversion temperature method [6, 8, 23].

Advantages of phase inversion composition

This method is inexpensive, does not require the use of organic solvents, and has a high degree of thermodynamic stability [23].

Disadvantages of phase inversion composition

This approach did not work well with label-friendly surfactants, including whey protein, quillaja saponin, sucrose monoesters, and casein in nanoemulsions [23].

Solvent Displacement Method

At room temperature, nanoemulsions can be made by mixing an organic phase (including dissolved oil in a solvent such as ethanol, acetone, etc.) with an aqueous phase containing surfactants. Emulsification occurs naturally due to the diffusion of organic solvent. To make fine-sized droplets, a high solvent-to-oil ratio is necessary [47]. The volume viscosities and phases of the emulsion, the types and concentrations of surfactants, the temperature, size, and size distribution of the droplets in the disperse phase, and the temperature, size, and size distribution of the droplets in the disperse phase are all factors that influence the selection of emulsifying tool

[30, 48]. Some parameters, such as interface density, temperature, flow rate, pressure, time of emulsification, and rotation speed, also influence the emulsion's preparatory process. These all parameters should be under consideration while trying to obtain fine nanoemulsions [14, 23, 49].

Advantages of solvent displacement method

The advantages of this technology are freedom in the choice of the internal structure and surfactant, as well as the potential to form nanoemulsions in a short time [12].

Disadvantages of solvent displacement method

Sometimes, it's very difficult to manage all the required parameters for the production of nanoemulsion by adopting the solvent displacement method [14, 23, 49].

Applications of nanoemulsions

Nanoemulsions are getting higher importance throughout the world because of their bountiful applications [19]. Based on the size, specificity and precision of active medicinal components and other products, nanoemulsions could be used in composite and crystal formulations using low-energy techniques.

Nanoemulsions are viewed as a proficient device to treat tumors. Such nano-carriers settle the water-based solvency issues as well as help to defeat multi-drug resistant micro-organisms because of their targeting nature. Ligands of various qualities can be utilized to adjust the focusing nature of nano-emulsions [50]. There are different sorts of diseases, so multifunctional nano-emulsions are getting attention from analysts to treat various types of malignancy. The past examinations additionally have uncovered that nano-emulsions are viably benefited by the cells of cancer, which prompts diminishing the development of growth, eliminating venomousness to sound cells, and dropping off the departure of carcinogenic cells to different organs [51, 52]. In the same way, nano-formulations are also being used for vaccines and syrup. Antigens are loaded into nanocarriers and injected into the human body as a vaccine [53].

Nanoemulsions are likewise a significant tool of the food industries to create smart food having ingredients that exhibit low direct solubility to water [53, 54]. Nano-emulsions are significant builders of different complex materials because of their small size and accessibility to enormous surface regions [54]. Because of their large surface area and small droplets, nanoemulsions have proven to be very useful in improving bioavailability, bioactivity, digestibility, stability, safety, quality, and sensory enhancements of food components and natural extracts, such as lycopene-solubilized and β-carotene-based nanoemulsions [53]. Beverage emulsions are the simplest form of O/W type of emulsions used in food industries. Myonise, drinks, margarine, fatty spreads, and homogenized milk are examples of emulsions used in food industries [55].

Nano-emulsions are also being used in cosmetic industries to make various cosmetic products (such as primers, liquid lipsticks, liquid foundations, etc.) [53]. Ultraviolet radiation coming from the sun can damage skin and produce skin cancer. Nano-formulations having TiO_2 and ZnO are used to protect skin from the effects of ultraviolet radiation. With the passage of time, physical work, and exposure to sunlight, human skin becomes wrinkled, pigmented, and aged due to the degradation of skin collagen. To avoid the degradation of skin collagen, various antioxidants such as vitamins, polyphenols,

and flavonoids are added to cosmetic creams. Vitamin A (retinol) is regarded as the best ingredient in creams that avoid the degradation of skin collagen. Various nano-technology-based anti-wrinkle creams enrich in Vitamin A (retinol) are available on the market. One of the best products is Revitalift from L'Oreal Paris. This product has nanosomes with Vitamin A (retinol). Nanosomes can easily and rapidly penetrate human skin via the dermal route and show anti-wrinkle effects [56].

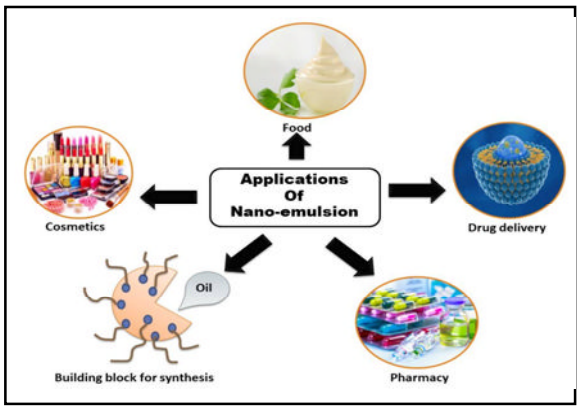

Figure 9. **Applications of nanoemulsions [54]**

Table 1. **Importance of nano-emulsions.**

Method	Protocol	Application	Ref.
Spontaneous method	Sundararajan *et al.* formulated nano-emulsion via "low-energy technique" by utilizing 90% (w/v) of water, 5% (w/v) of essential oil, and 5% (w/v) of Polysorbate-80 at an absolute mass of 50 g. For thirty minutes, the combination of essential oil (of *Ocimum basilicum L.*) and Polysorbate-80 were mixed via a magnetic stirrer at 800 rpm. Then, water (at a 3.5 mL flow rate per minute) was added through drop-wise addition. After the addition of water, the entire blend drove towards mixing at 800 rpm for 60 minutes.	The formulated nano-emulsion showed prominent anti-bacterial, anti-oxidant, and anti-larvicidal applications	[4]
High performance dispersion	Chenni *et al.* formulated emulsion by utilizing water (50% w/w), maltodextrins, and acacia gum (by proportion 1:1) as a carrier (45%) and aroma (5%). The emulsion was ready by dissolving maltodextrins and acacia gum (inside proportion 1:1) in refined water (by 50% w/w), leading to heating at 60°C for 45 min. The mixture was appropriately covered and permitted to stand for 24 hours. After that, the solutions were blended by utilizing Ultra-Turrax T-25 at the rate of 13,500 rpm for five minutes.	Drug delivery system	[17]
High-pressure homogenization method	Muhammadi *et al.* formulated nano-emulsion of essential oil of peppermint/eucalyptus by utilizing a high pressing factor homogenization technique. In a measuring glass, 13 ml (21%) polyethylene glycol was taken and homogenized by utilizing a homogenizer at 11,000 rpm. Then, at that point, 10 ml (16%) polysorbate-80 was included in the measuring glass having polyethylene glycol that drove towards homogenization at 11,000 rpm for five minutes. Then, at that point, 5 ml (8%) oil of *Sesamum indicuml L.* (as a transporter and synergist oil) was poured into the above blend. Then, at that point, 30ml (half) unadulterated fundamental oil of peppermint or eucalyptus was poured into the above blend. The entire blend was left under the homogenizer till all of the constituents were blended appropriately. Following 10 minutes; for appropriate disintegration of constituents; 2ml (5%) butanol was added to the combination and again homogenized at 11,000 rpm at room temperature for five minutes.	Biological applications (mosquitoes repellency)	[18]
Ultrasonication	Ghosh *et al.* prepared nanoemulsion of basil essential oil, Tween 20 (Hydrophile Lipophile Balance (HLB) esteem -16.7), and H_2O. Course emulsion was ready by blending oil, surfactant, and water. Then, at that point, course emulsion was changed over into nanoemulsion by sonication strategy having high recurrence (20kHz) and 750W yield.	Larvicidal activity	[57]
Ultrasonication	Roy *et al.* formulated O/W based course emulsion of betel leaf essential oil by using Tween 20, essential oil, and refined water that was changed into nanoemulsion by sonication strategy by utilizing sonication test (13 mm in width), 20 kHz recurrence, and 750W force yield. Given energy created problematic powers and henceforth, nanoemulsion was acquired.	This formulated emulsion was used against food pathogens	[58]
Ultrasonication	Nirmala *et al.* prepared an emulsion of the drug by dissolving the wanted drug in cinnamon oil. After dissolving the desired drug in cinnamon oil, the combination was permitted to stand for the time being and then, at that point, centrifuged to affirm the solvency. Then, at that point, surfactant (Tween 80) and water were included in the above arrangement, thus course emulsion was formulated. Then, at that point, the course emulsion was sonicated, and the coarse emulsion was changed into nano-emulsion.	Drug delivery system	[59]

Current trends in nanoemulsions applications and formulation

Several Non-polar active compounds can easily be dissolved in nanoemulsions; hence nanoemulsions are used to supply required nutrients. Nanoemulsion-based drugs are coming into the markets, and they possess very small droplet sizes that reduce the toxic effects of drugs [60]. Nanoemulsion as lipophilic nanocarriers is used to deliver lipophilic and amphiphilic medications to the blood-brain barrier with remarkable penetration, and their delivery to the brain is a viable approach. The nanoemulsions are getting trendy as antifungal, antibacterial, antiparasitic, and mosquito-repelling agents. Conjugation and physical adsorption methods are being used to load antigens to nanoemulsions. Nanoemulsions are being used against cancerous cells. Sunblock nanoemulsions also protect skin from skin cancer [8, 61, 62].

In preclinical research, many preclinical nanoemulsions containing encapsulated contrast and chemotherapeutic medicines are demonstrated to function specifically on the tumor microenvironment for diagnostic and therapeutic purposes. Gel forms of nanoemulsion are also used before conducting an ultrasound image of the patient. Iron-oxide nano-crystals and Cy7 fluorescent dye are placed in the oil core with hydrophobic glucocorticoid for medicinal applications in a distinct strategy, including MRI and NIRF imaging [51, 63]. Nutraceuticals (nutrients), color, and flavoring are all being delivered via this method in the food sector. Food quality, functional characteristics, nutritional value, and shelf life are all important considerations, and nanoemulsion plays a beneficial role in this regard [47, 55].

Essential oil-based nanoemulsions are regarded as the best food preservers. A nanoemulsion covering with small droplets of lemongrass essential oil shows more success in preserving grapefruit and enhancing microbial safety against *Salmonella* than a coating with large droplets. In the same way, varying the species of essential oils, different fruits can be preserved from microbial attack [64]. To improve the environment, biodegradable coatings and packaging films are being developed [14].

As collectively there are various methods to formulate nanoemulsions, but the spontaneous method is the most trendy nowadays. The spontaneous method is also regarded as the titration method. This method is easy to follow as nanoemulsion is formed by mixing all ingredients in the right proportion with continuous agitation [65]. The spontaneous method currently prepares all forms of nanoemulsion, such as gel and liquid having high flow rates, etc. Nanoemulsion has applications in different industries or fields such as drug delivery systems, food industry, oral products industry, cosmetics, perfumery industry, and anti-microbial agents, etc., formulated by spontaneous method [66-70].

Patents of nanoemulsion

Numerous distinctive nanoemulsion formulation based patents have been issued in recent years in the fields of cosmetics and drug delivery. Table 2 lists a few of these patents now held by different companies (from the year 2012 to 2022). It is evident from the growing number of patents that nanoemulsions, in various fields especially in cosmetics and drug delivery systems, has become more and more well-liked. Customers favour nano-based cosmetics and pharmaceutical products over conventional ones as they become more aware of their advantages.

***Table 2*. Patents on nanoemulsion [71-77].**

Nanoemulsions used in cosmetics			
S. No.	***Formulation***	***Company***	***Patent Number***
1	Sugar fatty ethers and their uses in the cosmetics, dermatological, and/or ophthalmological fields	L'Oreal (Paris, FR)	US 6,689,371
2	Fluid non-ionic amphiphilic lipids and use in cosmetics or dermopharmaceuticals	L'Oreal (Paris, FR)	US 5,753,241
3	Nanoemulsion is based on glycerol fatty esters, and its uses in the cosmetics, dermatological, and/or ophthalmological fields	L'Oreal (Paris, FR)	6,541,018
4	Nanoemulsion is based on oxyethylenated or non oxyethylenated sorbitan fatty esters, and its uses in the cosmetics, dermatological, and/or ophthalmological fields	L'Oreal (Paris, FR)	6,335,022
5	Nanoemulsion is based on ethylene oxide and propylene oxide block copolymers and its uses in the cosmetics, dermatological, and/or ophthalmological fields.	L'Oreal (Paris, FR)	6,464,990
6	Nanoemulsion is based on phosphoric acid fatty acid esters and its uses in cosmetics, dermatological, pharmaceutical, and/or ophthalmological fields	L'Oreal (Paris, FR)	6,274,150
7	Oil-in-water type emulsion sunscreen cosmetic composition	Shiseido Co Ltd.	20130011348A1
8	Nanodiamond UV protectant formulations	International Technology Center	US 20090220556A1
9	Cosmetic composition containing retinol stabilized by porous polymer beads and nanoemulsion	ACT Co Ltd.	US 20130095157A1
10	Cosmetic composition containing retinol stabilized by porous polymer beads and nanoemulsion	ACT Co Ltd.	EP 2583665A2
11	Cosmetic pigment composition containing gold or silver nanoparticles	Korea Research Institute of Bioscience and Biotechnology	US 20090022765A1
12	Skin whitening methods and compositions based on zeolite–active oxygen donor complexes	BIODERM Research	US20070166339A1
13	Nanoemulsion comprising metabolites of ginseng saponin and a skin-care composition for anti-aging containing the same	Pacific Corp	EP 1327434A1

Nanoemulsion used in drug delivery			
S. No.	***Formulation***	***Company***	***Patent Number***
1	Oil-in-water type terazosin nanoemulsion antihypertensive drug	Zhang Hongli	CN105997873A
2	A kind of compound apigenin nanoemulsion antihypertensive drug	Zhang Hongli	CN106137958A
3	A kind of compound atenolol nanoemulsion antihypertensive drug	Zhang Hongli	CN106176997A
4	Antihypertensive drug of quinapril hydrochloride and rose oil nanoemulsion	Ouyang Wuqing, Sun Jianhong, Zhang Xiaohua	CN102698245A

5	Compound spirolactone nanoemulsion drug	Ouyang Wuqing, Sun Jianhong, Cao Tong	CN102697900A
	A kind of oil-in-water type celiprolol nanoemulsion antihypertensive drug	Zhang Hongli	CN106137961A
6	Nanoemulsion	Biofrontera Bioscience GMBH	US-20090324727-A1
7	DHA Ester Emulsions	Martek Biosciences Corporation	US20110200644A1
8	DHA Free Fatty Acid Emulsions	Martek Biosciences Corporation	US20110200645A1
9	DHA Triglyceride Emulsions	Martek Biosciences Corporation	US20110206741A1
10	Colloidal carrier system with penetrating properties for inclusion of lipophilic drugs and oils for topical application	Gabriele Blume	DE102010056192A1
11	Vesicular formulations	Henk-Andre Kroon	US20120232034A1
12	Cancer heat therapy-enhancing agent	Sbi Pharmaceuticals Co., Ltd	US20130158293A1
13	Topical pharmaceutical compositions containing nanodroplets for the treatment of psoriasis	Cadila Healthcare Limited	US20130273172A1
14	Methods for forming mini emulsions and use thereof for delivering bioactive agents	Ns Technologies Pty Ltd.	US20140322330A1
15	Vesicular Formulations, Kits, and Uses	Sequessome Technology Holdings Ltd.	US20150132349A1
16	Preparation of nanoemulsions	Affinsci Inc.	WO2016182926A1
17	Topical pharmaceutical compositions	Glaxosmithkline Intellectual Property Development Limited	US20160338973A1
18	Methods for photodynamic therapy	Dusa Pharmaceuticals, Inc.	US20190216927A1
19	Adjustable illuminators and methods for photodynamic therapy and diagnosis	Dusa Pharmaceuticals, Inc.	US10603508B2
20	Methods for photodynamic therapy	Dusa Pharmaceuticals, Inc.	US20190216927A1
21	Calcium phosphate core particles / Intraocular delivery compositions and methods	BioSante Pharmaceuticals Inc	US 6,355,271 B
22	Oil-in-water type emulsion (cetalkonium chloride, tyloxapol, and poloxamer) with an average particle size of about 300 nm and positive zeta potential	Santen SAS	US 8,298,568 B2
23	Emulsion eye drop for alleviation of dry eye-related symptoms in dry eye patients and/or contact lens wearers	Saint Regis Mohawk Tribe	US 5,981,607

Future Trends of nanoemulsions

Due to their potential benefits over conventional emulsions in food, cosmetics and drug delivery applications, such as clear formulation, improved bioavailability, longer shelf lives, and superior physical stability, nanoemulsions will be the subject of in-depth study and development. Numerous pieces of research have been conducted in recent years to determine the benefits of encapsulating lipophilic and functional chemicals in nanoemulsions.

However, there are still a lot of obstacles to be cleared before nanoemulsions may be applied more widely. The first requirement is that the right components be used for creating the right nanoemulsions. Second, there is still much work to be done before large-scale commercial applications can be made. As a result, choosing the right processing techniques is necessary to generate nanoemulsions on an industrial scale and at a reasonable cost [78].

The growing number of patents in this field reflects that these nano-products are

gaining the attention of renowned industries worldwide in different fields of applications. Hence it can be predicted that the future is of nanoemulsion-based industries and products [79].

Conclusion

Nanoemulsions offer improved functional qualities in contrast to traditional emulsions. For the encapsulation of different bioactive components, the composition and structure of the nanoemulsions can be adjusted. Among methods of nanoemulsion preparation, high and low-energy methods are subdivided into various types to formulate nanoemulsion. Although each method has its advantages and disadvantages, it can be concluded from the current review that among different methods to formulate nanoemulsions, low-energy methods are best as compared to high-energy methods. This is because they don't require high input devices, higher pressure, and temperature. The importance of these methods can be appraised from the fact that nanoemulsions have applications in numerous fields like the food industry, cosmetics, medicine, pharmaceutical industry, etc. Nanoemulsions are also regarded as nanocarriers. So, according to the demand of the hour, such nanocarriers (that should be cost-effective and task efficient) are needed in the future. If future research fulfills the current gaps, the nanoemulsion system can also be upgraded for various new industries.

Conflict of Interest

The authors declare no conflict of interest.

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