Research Progress in Preparation, Stability and Application of Nanoemulsion

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Abstract. Nanoemulsion has excellent functional characteristics, it is widely applied in medicine, food, cosmetics, agriculture and other fields, thus gradually becoming hit issue in scientific research. This article combines various literature reports around the world. In order to provide reference for further research and development of nanoemulsion, we thoroughly analyze the preparation technology, composition, stability mechanism and the factors affecting its stability, and summarize the application of nanoemulsion in the food field.

1. Introduction
Traditional emulsion is composed of oil phase, water phase and emulsifier. The particle size of emulsion ranges from nanoscale to micro scale. Nanoemulsion refers to emulsion system with average particle size of 50-200 nm. To obtain nanoemulsion with small and uniform particle size, nanotechnology could be used to homogenize and refine the emulsion. Compared with normal emulsion, nanoemulsion has a series of advantages. Firstly, its smaller size and ability to disperse evenly lead to good stability and no precipitation. Secondly, nanoemulsion has good permeability, which is good for improving the rate of absorption. Thirdly, transparency of nanoemulsion is quite good, thus leading to the unique properties for improving the quality of cosmetic and personal care products [1]. Nanoemulsion is widely used in many fields, from nutritional agents in food to daily chemical product, to the use of pesticides and coatings for produce. Therefore, it is necessary to discuss and summarize the relevant research of nanoemulsion. This article can provide reference for the further development and research of nanoemulsion.

2. Preparation technology
2.1. High-pressure homogenization
The preparation methods of nanoemulsion include high-energy emulsification and low-energy emulsification [2]. The high-pressure homogenization method is one of the high-energy emulsification methods, which uses special equipment to produce shear, impact, cavitation, turbulence and eddy, etc., to break the droplets [3].

In the process of high pressure homogenization, the working pressure range of traditional high pressure homogenizer is usually 50-100 MPa, and the newly developed homogenizer is up to 350 MPa [4].

The mixture of oil, water and surfactant is pushed through the narrow slit of the 5-10 mm homogenizer under high pressure. Due to the relatively small gap size, as the pressure increased, a tremendous shear force formed, and make the droplet to deform and break into smaller droplets.
In general, the mixture has been treated several times before the droplets become homogenized. During this process, the temperature and pressure also affect the droplet size, which can become small with the increase of temperature and pressure.

Cheng et al. [5] used high-pressure homogenization method to prepare nanoemulsion with polyoxyethylene dehydrated sorbate monolurate (Tween20) as the main emulsifier and mixed with sodium dodecyl sulfate. By optimizing the proportion of emulsifier, the particle radius of the dispersed phase was within 100 nm (Figure 1).

![Figure 1. The particle size distribution of nanoemulsion [2].](image)

2.2. Ultrasonic homogenization
Phacoemulsification is the use of ultrasonic wave to generate strong mechanical vibration and cavitation to break the oil-water interface, which can achieve mixture at molecular level, resulting in smaller emulsion particle size and more uniform particle size distribution. Phacoemulsification can reduce the size of nanoemulsion droplet effectively. Phacoemulsification technology has these following advantages. Compared with other high-energy emulsification methods, it requires less emulsifier and energy, which produces less pollution. Moreover, operation of phacoemulsification is not complicated. In terms of disadvantages, the active substance degrades when the macromolecules are broken by ultrasonic waves. Besides, phacoemulsification is more suitable for small scale production because effective emulsification only occurs near the waveguide radiator and large production will affect the droplet size distribution.

In the work from Hemant and Vimal [1], they consolidated methodology of isothermal low energy and ultrasonication together to form combined energy mixed surfactant nanoemulsion, which could be utilized as a fuel without NOx and particulate matter pollution. This nanoemulsion displayed kinetic stability. Katsouli and Tzia [6] used ultrasonic homogenization to prepare conjugated linoleic acid nanoemulsion. By investigating the microstructure and stability of nanoemulsion, it was found that the nanoemulsion droplets prepared by this method were smaller than 300nm in diameter, and nanoemulsion showed good physical and chemical stability for a long time. Moreover, the degradation rate of conjugated linoleic acid was significantly reduced, which effectively broadened the application range of conjugated linoleic acid in the food field.

2.3. Dynamic high pressure microfluidization
Dynamic high pressure microfluidization (DHPM) is a method that emulsifies and homogenizes the emulsion under a pressure of more than 100 MPa. The emulsions pass through the microchannel
interaction chamber under high pressure and the two emulsions accelerate and collide with each other from the two opposite channels at a high speed, generating huge shear force.

Cheng and McClements [5] discovered that the increase of homogenization pressure and treating times could prompt a more modest molecule size of nanoemulsion. The particle size is not only simply identified with the homogenization pressure and the treating numbers, yet additionally relying upon the viscosity ratio of the oil phase and the water phase engaged with homogenization [7].

Because there is no pre-emulsification step, there is no need for a homogenizer, which is the advantage of microfluidization technology over high-pressure homogenization. In addition, the microfluidization method can prepare emulsion with uniform and controllable particle size through channels. As for other advantages, the processing time is short and the operation is safe and particle size distribution is uniform. In terms of disadvantages, the equipment is expensive and the application is not as common as other methods.

Li et al. [8] successfully prepared camellia seeds oil nanoemulsions using high pressure microfluidization method. They used spherical droplets to produce stable nanoemulsions and they displayed anti-bacterial activity and promising effect for treating LPS-induced sepsis.

2.4. Low energy emulsification

Low energy emulsification is a method to prepare nanoemulsion by using the internal energy of the system. Small droplets can be produced without consuming large quantity of energy based on the system with specific characteristics. The technique can be divided into self-emulsification and phase transition emulsification according to whether the spontaneous curvature of the emulsifier molecule changes. The latter method is also categorized into Phase Inversion Temperature, Phase Inversion Composition, Emulsification Inversion Point and D-Phase Emulsification. The self-emulsification method is that the oil phase is added into the water phase at a slow speed under constant agitation, and it relies on the emulsifier molecules to rapidly diffuse from the oil phase to the continuous phase, leading to the formation of small emulsion droplets without changing the spontaneous curvature of the surfactant. Phase-transition emulsification involves changes in the spontaneous curvature and solubility of non-ionic surfactants due to changes in system temperature or system composition.

Compared with high-energy emulsification, low-energy emulsification requires less equipment and energy but requires a large amount of surfactants, which may bring safety and odor problems. In addition, the low-energy emulsification is driven by the internal thermodynamics of the system and is easily affected by the external thermodynamic parameters such as temperature, pressure and concentration, the current preparation methods of food-grade nanoemulsion are dominated by the high-energy method. For oil phase with high carbon number and high viscosity, it is difficult to emulsify it into nanoemulsion through low-energy emulsification method, but emulsification of such oil phase is relatively simple by high-energy emulsification method. At present, the preparation of nanoemulsion by low-energy emulsification method is carried out under laboratory conditions and the scale is small. Zhang et al. [9] used the emulsion Phase Inversion method to prepared DHA and EPA nanoemulsions and both nanoemulsions were stable during storage for 20 days at different temperature and pH. As a result, this method is a promising approach to incorporate DHA and EPA into water-based systems to enhance their food fortification and large-scale production.

2.5. Phase transition emulsification method

The phase transition emulsification method is used to prepare nanoemulsion by changing the curvature of the surfactant layer at the interface, which can be divided into phase inversion composition (PIC) method and phase inversion temperature (PIT). PIC is a method to prepare nanoemulsion by changing the proportion of each component to promote reverse rotation of the system. The curvature radius of the surfactant layer at the interface is altered as the ratio of each component in the system changing. When the ratio of each component shifts above a certain
value, the phase transition will occur. PIT method is based on the change of molecular geometry characteristics of non-ionic surfactants with temperature (Figure 2). In a mixture containing water, oil, and surfactant, the affinity of the surfactant for water and oil reaches equilibrium when the temperature rises to the phase transition point, and then the mixture is cooled (heated) instantaneously, resulting in the formation of nanoemulsion.

Generally, PIT method is used in the emulsion system containing polyoxyethylene ether non-ionic surfactants. Since it is insensitive to temperature change, PIT method is not suitable for ionic surfactants. Rao et al. [10] used PIT method to add sodium dodecyl sulfate (SDS) to the prepared nanoemulsion and it turned out that the electrification of the droplet interface membrane changed and electrostatic repulsion between the drops increased, thus improving the stability of the nanoemulsion.

Figure 2. Schematic mechanism diagram for nanoemulsion formation using PIT method [11].

3. Stability mechanism

3.1. Oil phase

Nanoemulsion is a colloidal dispersion system composed of water phase, oil phase and surfactant. Compared with oil phase, the composition of water phase is simple. Oil consists of triglyceride and glycerol, which could be further divided into long, medium, and short chain fatty acids. Fatty acids can also be categorized into saturated fatty acids and unsaturated fatty acids, saturated fatty acids usually exist in animal fat, while unsaturated fatty acids usually exist in vegetable oil. The animal fat are solid at room temperature and they not easy to be mixed evenly with water, so vegetable oil are more often used for nanoemulsion preparation.

Essential oil could be applied in the preparation of nanoemulsion. Compared with normal essential oil, essential oil nanoemulsion has more uniform texture and long-term stability. Increase in the diameter of the dispersed phase and decline in ostwald ripening rate is the stabilization mechanism of this nanoemulsion. Besides, as a carrier, fat provides an environment for aroma compounds to spread slowly, which could further prolong the retention time of aroma compounds in nanomaterials. Curcumin has different solubility in different oil phases, the longer the triglyceride chain is, the less the polar groups contained in unit mass, thus reducing the interaction between curcumin and dipolar groups and improving the solubility of curcumin in triglycerides.

3.2. Emulsifier

An emulsifier has the ability that mixes two immiscible liquids (oil and water) to form a homogeneous dispersion. It plays a significant role in the food industry and it could prevent phase separation. The emulsifying characteristics rely on the hydrophile-lipophile balance (HLB) value of the emulsifier, the greater the HLB value, the more hydrophilic the emulsifier is, on the contrary, the stronger lipophilic it
is. Nowadays, there are plenty of food emulsifiers commonly used in China. Emulsifiers could be classified into ionic and non-ionic surfactants based on whether there is a hydrophilic base in the emulsifier. They can also be divided into natural surfactants (such as lecithin, certain proteins, etc.) and synthetic surfactants (such as polyacrylamide, polyglycerides, etc.) due to different sources.

In the phase transition component method, when the surfactant layer curvature on the interface is negative, the type W/O emulsion is formed; when the surfactant layer curvature is near zero, the double continuous phase and layered liquid crystal equivalent phase; when the surfactant layer curvature is positive, the system is transformed into type O/W nanoemulsion.

3.3. Co-emulsifier
Nanoemulsion consists of water, oil, emulsifier and co-emulsifier. The co-emulsifier can assist the emulsifier to reduce interfacial tension and charge repulsion, and adjust the hydrophile-lipophile balance (HLB) value. The most common co-emulsifiers are short chain alcohol, organic ammonia, etc. Alcohols can increase the solubility of the drug. The amount of emulsifier, the ratio of emulsifier to co-emulsifier, and the amount of drug have a significant impact on the size of nanoemulsion.

As food processing system is becoming more and more diversified, a single emulsifier can no longer meet the requirements of emulsion in modern food processing system. Nowadays proper use of a variety of additives to achieve the comprehensive emulsion becomes the trend of modern food processing. In the study of Schantz and Rohm [12], addition of phospholipid and PGPR in a certain proportion and content significantly improve the viscosity and yield values of the cocoa butter chocolate system.

4. Factors affecting its stability
As time goes by, the dispersed droplets in emulsions corrupt, thus leading to the increase in their size and phase separation occurs eventually (Figure 3). During storage and transportation, nanoemulsion will have a series of problems such as settlement and condensation. In terms of droplets settlement, the main driving force is gravity force. As lighter oil phase goes up and heavier water phase subsides, stratification of nanoemulsion occurs. The reason for the droplet coarsening is droplet coalescence, in which two droplets fuse to form a single one. Another factor that affects the stability of nanoemulsions is Ostwald ripening, which leads to a growth in the mean droplet size over time [13] [14]. That is to say, in nanoemulsion polymerization, oil droplets of nanometric range are polymerized without monomer transfer through the continuous aqueous phase. Consequently, it is crucial to preserve the initial droplet size distribution and the initial composition of the droplets. In the miniemulsion polymerization, costabilizers have been effectively utilized to acquire a stable miniemulsion. The retardation of diffusional degradation of nanoemulsions has also been achieved in other areas [15].
5. Nanoemulsion analysis

The evaluation and measurement of nanoemulsion include particle size, electric potential, morphology, and physical stability. The stability of the nanoemulsion is affected by the droplet size, which is determined by mean droplet size (Z-diameter) and polydispersity index (PDI). There are two kinds of methods to analyze nano-emulsion particle size: static light scattering and dynamic light scattering. A static light scattering instrument (Mastersizer 2000, Malvern Instruments, Malvern, UK) could measure the particle size distribution and mean particle diameter of diluted nanoemulsions [17]. The measuring range of dynamic light scattering is 3-5 μm, while that of static light scattering is 0.1-1000.0 μm [1]. To identify the shape and morphology of the freshly prepared nanoemulsion, transmission electron microscope equipped with 40–120 kV operating voltage could be used [18].

Electrostatic repulsion is the main mechanism of stable emulsion and the electric potential value is determined according to the rate and direction at which the emulsion droplet moves under the applied electric field. The stability of the emulsion can be preliminarily analyzed based on the potential value.

Commonly used methods for physical stability include centrifugation, direct storage observation and stability analyzer. The stability analyzer is used to quickly evaluate the emulsion stability and it is simulated by high-speed centrifugation, thus accelerating stratification of the emulsion. Then the analyzer records changes in the transmitted light intensity relative to the time and position, reflects the migration process of emulsion droplets.

6. Application of nanoemulsion in the food field

Numerous nutrients found in foods are highly hydrophobic substances with low oral bioavailability such as carotenoids and flavonoids. Due to the capacity to solubilize bioactive compounds, nanoemulsion-based delivery systems have been developed to encapsulate lipophilic bioactive components. The advantage of nanoemulsions in delivering vitamin plays an essential role in...
pharmacy and biotechnology field. That is to say, nanoemulsions could protect them from degradation and preserving the antioxidant activity.

Tea tree oil (TTO) is used to treat skin diseases. However, its poor water solubility and stability substantially restrict its broad application. To solve this issue, TTO nanoemulsion could be prepared through mechanical ultrasonic methods. Research result showed that nanoemulsion with TTO had no phase separation under a centrifugation test and different storage temperatures. These data suggest that nanoemulsion with TTO can be considered a potential antimicrobial agent by oral administration due to its inhibitory effect on bacteria and relatively lower toxicity [19].

Nanoemulsion could act as the delivery system of antimicrobial agents to prolong the shelf life of fresh produce. After treating citrus fruits with cinnamaldehyde, eugenol and carvacrol nanoemulsion, P. digitatum spore germination was significantly inhibited. Besides, the soluble solids, vitamin C, and titratable acid contents degradation was delayed and antioxidant enzyme activities also increased and maintained during postharvest storage [20].

EPA and DHA supplements are beneficial to human body health such as brain development and cardiovascular health. To increase rate of absorption of EPA and DHA, they can be incorporated with sunflower into nanoemulsions as the carrier oil. In the study, these nanoemulsions were well preserved when they were emulsified with Tween 85:isopropanol (S mix) and physical and oxidative stability of nanoemulsions improved when stored at 4 °C and 25 °C for 28 days without addition of exogenous antioxidants [21].

7. Conclusion
Nanoemulsion is a new type of emulsion system, its remarkable characteristic is the smaller and more uniform dispersed phase droplet size. Preparation technology, oil phase composition and environmental factors all have impacts on nanoemulsion. Nanoemulsion is of crucial importance in food, daily chemical products, biological medicine and agriculture. In the future, with the development and progress of science and technology, the preparation technology and stability of nanoemulsion will be greatly improved and it will be used widely in various fields.

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