INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Review Article

A Detailed Comprehensive Review of Multiple Nanoemulsions on Pharmaceutical Applications

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ARTICLE INFO

Published: 04 Sept 2025
Keywords:
Multiple nanoemulsions, Drug delivery systems, Controlled release, Nanocarriers, Bioavailability enhancement, Surfactants, Targeted delivery.
DOI:
10.5281/zenodo.17054542

ABSTRACT

Biphasic systems, composed of two immiscible liquid phases such as oil and water, are extensively utilized for the transportation of both hydrophilic and hydrophobic substances. Emulsions, particularly nanoemulsions, have attracted notable interest due to their tiny droplet sizes (≤100nm), improved dispersion characteristics, and enhanced kinetic stability. In contrast to thermodynamically stable microemulsions, nanoemulsions are in a metastable state, which allows for increased formulation possibilities and higher concentration of additives. These attributes render them highly adaptable for application in drug delivery, cosmetics, and material science. Advancing the functionality of nanoemulsions, multiple emulsions, which contain immiscible droplets encapsulated within other droplets (for example, water-in-oil-in-water), present the distinct benefits of being able to co-encapsulate both hydrophilic and lipophilic compounds. Nonetheless, traditional multiple emulsions frequently experience challenges with poor physical stability and low encapsulation efficiency. To address these issues, multiple nanoemulsions have been created, merging the advantages of both multiple emulsions and nanoemulsions. These systems, typically featuring droplet sizes ranging from 20-200nm, demonstrate improved kinetic stability, enhanced encapsulation efficiency, minimized creaming, and favorable controlled release profiles. They are particularly advantageous in pharmaceuticals, neutraceuticals, and cosmetic sectors where high bioavailability and effective performance are essential. This review presents a thorough examination of multiple nanoemulsions, detailing their structural type, mechanism of formation, preparation methods, characterization techniques, and various applications. The progress of these systems signifies a promising path in the fields of drug delivery and multifunctional formulation science.

INTRODUCTION

A biphasic system consists of two immiscible liquid phases, mainly water and oil. These systems

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

are frequently utilized for distributing hydrophilic or hydrophobic substances in an effective and controlled way. Emulsions are a major class of biphasic systems, where it finely distributes one liquid (the dispersed phase) within another (the continuous phase). Emulsions are extremely versatile liquid composites that are widely utilized in variety of fields and applications. For instance, emulsions offer a dispersible platform to solubilize flavoring components and active substance while maintaining their mechanical properties in food, cosmetics and pharmaceutical applications.[1] In chemical industry, emulsion polymerization has been employed as a safe approach for the quick synthesis of high molecular weight polymers [2]. Emulsions offer advantages at larger scales than individual drops; dense emulsions create continuous interfacial networks that can be utilized as templates for inorganic materials, which can be given internal structure and shape by surfactant structure and porous materials. It is possible to polymerize high-internal-phase emulsions into foams with porosity templated by the packed droplet structure if the dispersed-phase volume fractions are high enough for the dispersed droplets to interfere with their neighbors [3].The seif-assembly of thermodynamically stable microemulsions can be used to template materials which include mesoporous materials with ordered structure that emerge from the liquid crystalline morphology in microemulsion mesophases and nanoparticles, whose geometry reflects the size and form of the microemulsion droplets they are generated within These templating schemes- whether forming a structure using the emulsion directly or a material templated from it-take advantage of the structural flexibility and chemical versatility of emulsion, which are absent in other colloids.

Recently, it has been realized that nanoemulsions have special properties that make them more versatile than other emulsion system. Nanoemulsions are made up of immiscible liquid droplets with a diameter of 100nm or less that have been stabilized by surface active materials. However, they resemble microemulsions in size and appearance, but they differ in their stability. Nanoemulsions are transient, kinetically stable structures that gradually change into two macroscopic phases, whereas microemulsions are thermodynamic phases that are indefinitely stable. However, the kinetic stability of nanoemulsion is remarkably great because of their small size [4]. Due to this metastability, nanoemulsions can be functionalized with a wider variety and higher concentration of additives by eliminating the thermodynamic constraints that restrict the composition space of microemulsion. Therefore, it's crucial to consider the prospective uses of nanoemulsions, which range from new material templates to drug delivery vectors [5], where is it important to add a large fraction of functional compounds to disperse the phase of emulsion without compromising its stability or other characteristics. Alongside the rising interest in naoemulsion, recent decades have witnessed substantial progress in the development of multiple emulsions- which can disperse droplets that contains embedded immiscible droplets. Multiple emulsions are highly appealing systems for several reasons, most notably their ability to simultaneously encapsulate both hydrophilic and lipophilic molecules within a single particle, as well as their potential to shield delicate hydrophilic compounds from surrounding continuous phase. They are formed by re-emulsifying a pre-existing emulsion into another continuous phase. Multiple emulsions serve as flexible platforms for encapsulating chemical ingredients with varying polarities or solubilities. They are also useful for templating multiphase materials [6,7] and enabling a range of other applications. Commonly used processing

techniques- such as microfluidic devices and sequential emulsification offer fine control over the number, size, and composition of the encapsulated droplets. This precise control allows for a wide range of morphological designs in internally structured multiphase droplets and the colloidal particles derived from them. However, most method to create controlled multiple emulsions produce micrometer-scale droplets at low throughput.

Table 1.1 Overview of nanoemulsion and microemulsion

Characteristics	Nanoemulsion	Microemulsions
Droplet size	20-200nm	10-100nm
Visual appearance	Transparent to slightly turbid	Transparent to translucent
Stability type	Kinetically stable	Thermodynamically stable
Energy requirement	High-energy method	Form spontaneously with minimum energy input
Drug delivery potential	Suitable for controlled and target release	Useful for rapid solubilization and delivery

Although multiple emulsions offer considerable potential for encapsulating both hydrophilic and lipophilic substances, their practical application is hindered by several limitations. These systems often exhibit low physical stability, low encapsulation efficiency making them prone to phenomena such as coalescence, phase separation, and the diffusion or leakage of encapsulated compounds; especially water-soluble molecules. Moreover, their complex formulation process, possess significant challenges for scalability and long-term storage. To address these issues, multiple nanoemulsion have emerged as a more stable and efficient alternative. Owing to their nanoscale droplet size, these systems demonstrate enhanced kinetic stability, reduced creaming, and superior encapsulation efficiency. Furthermore, multiple nanoemulsion offer better control over the release profiles of active ingredients and are particularly advantageous in pharmaceutical, cosmetics, and nutraceutical applications, where improved bioavailability and functional performance are essential. As such, the development of multiple nanoemlsions represents a significant advancement in pharmaceutical field.

In this review, we analyse the broad and up-to-date perspective on multiple nanoemulsions, delating their types, mechanism of formation, preparation methods, characterization techniques, applications, and future directions.

1.1 MULTIPLE NANOEMULSION

Multiple nanoemulsions are an emerging class of sophisticated drug delivery system that are distinguished by their nanometric size range usually 20-200nm and intricate droplet-in-droplet structure. A two-step emulsification technique is used to prepare these systems. With this architecture, both hydrophilic and lipophilic drugs can be simultaneously encapsulated in discrete internal phases, providing a multifunctional platform for pharmaceutical applications [8]. In pharmaceutical field, multiple nanoemulsion have demonstrated enormous promise for improving the stability, bioavailability, and controlled release for therapeutic agents. Their tiny droplet size makes it much simpler for drug absorption and perpetration, especially for poorly soluble or poorly permeable drugs. Additionally, this system can protect sensitive active pharmaceutical ingredients (APIs) from degradation, reduce dosing frequency through sustained release, and offers targeted drug delivery, making them ideal for oral, topical, transdermal, and parenteral administration.

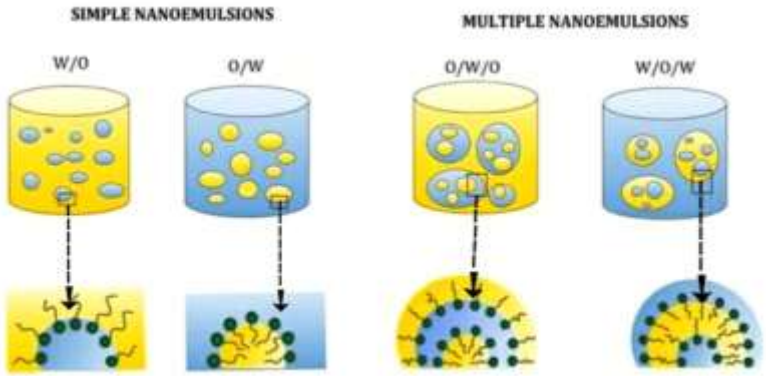

Figure 1.1 Two main categories of nanoemulsions: simple nanoemulsion and multiple nanoemulsions, each with distinct structures.[47]

2. TYPES OF MULTIPLE NANOEMULSIONS

Multiple nanoemulsions are complex dispersion systems where primary emulsions (O/W or W/O) are further emulsified in an external phase, resulting in systems such as [9] :

- O/W/O (oil-in-water-in-oil) nanoemulsion
- W/O/W (water-in-oil-in-water) nanoemulsion

2.1 O/W/O (oil-in-water-in-oil) nanoemulsion : It is a complex system where oil droplets are dispersed within water droplets (aqueous droplets), which are then subsequently encapsulated in another external oil phase. This system is primarly employed for the regulated delivery og hydrophobic drugs or active ingredients in a controlled manner. They provide orotection from environmental deterioration (such as oxidation and hydrolysis). In O/W/O nanoemulsion two distinct emulsifiers are needed for stabilization; hydrophilic surfactant and lipophilic surfactant. Hydrophilic surfactants are used for stabilizing the O/W interface where as the lipophilic surfactants are used for stabilizing the outer W/O interface. This system is mainly used for topical formulations (trabsdermal delivery), used in cosmetics for long-lasting and it is also potential in controlled-release pesticides.

2.2 W/O/W (water-in-oil-in-water) nanoemulsion : It is a double emulsion system in which water droplets are dispersed within oil droplets, which are then encapsulated in another external aqueous phase. This system permits the co-encapsulation of subsatnces that are both hydrophilic and lipophilic. It provides controlled release, taste masking and protection of sensitive compounds in oral delivery. To stabilize the inner and outer interface of W/O/W nanoemulsion the combination of both hydrophilic surfactant (like tween80) and lipophilic surfactant (like span 80) are needed. The main application of this system are oral and parenteral drug delivery, especially for poorly soluble drugs,

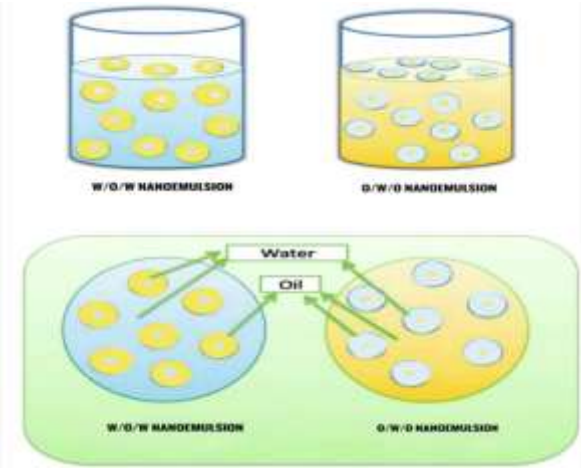

Figure 1.2 Two types of multiple nanoemulsions. [48]

1. MECHANISMS OF FORMATION

There are actually four mechanisms for the formation of an emulsion droplets within each other, specifically sequential mixing, Engulfment, Co- emulsification, and Phase separation.

Sequential mixing involves combining an emulsion with a third fluid that does not mix with the continuous phase of the initial emulsion. Engulfment takes place under high shear conditions, where a small droplet of the continuous phase becomes enclosed by a droplet of the dispersed phase, leading to the creation of multiple emulsions. Co-emulsification takes place by three or more phases of liquid must exist together there by resulting in multiphase droplets whose morphologies are determined by the wetting of the scattered phase. Finally, the phase separation relies on modulating thermodynamic variables, which trigger phase transitions and result in the spontaneous formation of nested droplet's structure. A common challenge of these approaches is that, due to the inherently unstable nature of multiple nanoemulsion formation, the newly created internal and external interfaces are highly susceptible to coarsening and must be promptly stabilized [1]. We now go over these strategies, emphasizing how internal interfaces arise and stabilize.

3.1 Sequential emulsification: Sequential emulsification is a well-established method for the preparation of multiple nanoemulsions, involving the stepwise combination of immiscible fluids to construct hierarchically structured droplet systems. At every stage, the emulsion from the prior step, is combined to the fluid that is immiscible with the emulsion's previous continuous phase to create an extra fluid shell on each droplet. This multistep process typically requires varying surfactants formulations and concentrations at each stage to assure the stabilization of newly formed interfaces [10]. Although at the beginning for the production of multiple emulsions at the microscale, this approach of sequential emulsification is readily adaptable to nanoscale systems. For formulating the multiple nanoemulsion by this method often necessitates the use of high-energy inputs, such as ultrasonication or high-pressure homogenization, to achieve droplet sizes within the nanoscale range. Usually, these sequential nano emulsification processes produce nanodroplets with multicore morphologies, reflecting the complexity of their internal structure. For example, Zambaux et al. fabricated polylactic acid (PLA) nano capsules, a two-step procedure was used to generate a double nanoemulsion: first a water-in-oil (W/O) nanoemulsion was formulated

by sonicating the monomer and the aqueous phase in the presence of a lipophilic surfactant, followed by further sonication to produce a stable water-in-oil-in-water (W/O/W) nanoemulsion [11]. The application of high energy facilitates the production of nanoscale droplets, while the distinct surfactant properties at each interface determined the final structure of the emulsion. Similar techniques using a variety of oils, aqueous phases, and surfactants have been also successfully reported to create both W/O/W and O/W/O multiple nanoemulsions [12]. Sequential emulsification offers many advantages by its simplicity of use and its ability to tailor the composition of each phase introduce during the emulsification process. The primary advantage is that each succeeding phase's composition can be chosen separately, with the only condition being that the alternating phases are immiscible [13]. It is also one of the only methods that preferentially forms multicore-droplet morphologies, thereby allowing the encapsulation of discrete internal droplets with varied chemical compositions within a single capsule. However, there are also limitations, because the size or quantity of internalized droplets cannot be controlled beyond what the formulation supports, high-energy emulsification approaches only affect a bulk droplet dispersion. Therefore, the capability to formulate emulsions with a consistent internal structure has not yet been demonstrated. Furthermore, emulsification energy is imparted uniformly across all fluid interfaces during each processing steps, previously formed nanoemulsion layers can be disrupted. This may lead to rupture of the internal phase, ultimately destroying the structural integrity of the emulsion. To overcome these challenges, the emulsification conditions and stabilizing surfactants must be optimized in order to control the nested emulsion structure's morphology and encapsulation efficiency. Sequential emulsification using microfluidic devices has been broadly successful for generating multiple emulsions of larger size, typically > 10µm in diameter [1].

3.2 Engulfment: Engulfment works on the principle which is similar to high-energy emulsification methods, relying on significant deformation of the emulsion interface by fluid-induced stresses. In this mechanism which involves the deformation of an emulsion-droplet interface to the point whereby a droplet of the continuous phase is internalized as the interface closes around it. This internalization may occur when a single droplet coalesces with itself or when many droplets coalesce with a tiny entrained patch of the continuous phase between them. In both cases, the internalized droplets tend to rapidly merge with the external continuous phase or undergo coarsening. Engulfment typically yields core-shell-droplet morphologies [14]. Due to the length scale between the internal core and the outer continuous phase is small as well as the curvature of the oil-water interface in the inner and outer surface are opposing to each other, these structures are unstable and there may be a rapid occurring of destabilization. Therefore, formulation must be outlined with a focus on stabilization of the internalized droplets.

The main advantage of the engulfment for formulating multiple nanoemulsion is that in single step we can generate complex droplet structures. This generally simplifies the emulsification workflow compared to conventional methods by reducing the surfactants amount and processing conditions that must be optimized. Additionally, by changing the surfactant ratio and their molecular asymmetry, grands some degree of control over the resulting droplet morphologies can be achieved [14]. However, the ease of processing comes with limitations in formulation flexibility, as the

production of multiphase droplets is very sensitive to both the fraction of dispersed phase and the surfactants used in the formulation. Additionally, this approach restricts the internal droplet phase to be same as the dispersed phase. This constraint reduces the complexity of the final emulsion that can be formulated, as it is currently impossible to encapsulate mutually miscible droplets containing different solutes due to the emulsion being created in a single step. Once the multiple nanoemulsion is generated, the dispersed phase composition can be altered through post formation modification-such as filtration, dialysis, or repeated washing. But these are secondary steps beyond the initial emulsification.

3.3 Co-emulsification: Multiple nanoemulsion can also be formulated by combining two or more emulsions that share the same continuous phase, but they contain immiscible dispersed phases. The resulting mixture contains multiple distinct phases, characterized by a continuous phase and dispersed droplets pf particular wetting immiscible liquids. Different droplet morphologies develop depending on how much wetting occur between the droplet stages. If one phase gets incompletely wet on other, droplets are formed, where each of this liquid in the droplets extends into the continuous phase, which is demonstrated at the microscale using microfluidic approach. In contrast, complete wetting of one phase in other leads to the formation of multiple emulsion, where one droplet becomes completely encapsulated within another. By increasing the number of immiscible phases in the dispersed droplets it enables the generation of higher-order structures. Core-shell or lens shaped droplets are typically produced by co-emulsification based on the relative wettability of the dispersed phases. Co-emulsification allows greater control over the final morphology of multiple nanoemulsion than other available methods because each miscible phase partitions into a single domain in the droplets. Dispersing immiscible phase by high interfacial tension may leads to the coalescence of internal droplets of the same composition into a single domain, and the shape of which depends on the relative volume ratios of constituent oils, as well as wetting between the neighboring phases. This approach can be used to generate anisotropic droplets, which is similar to the use of dewetting to synthesize polymer, metal and metal-oxide dumbbell-shaped nanoparticles [1].

3.4 Phase separation: Thermodynamic phase instability can be habitually driven to generate multiple nanoemulsion by triggering liquid-liquid phase separation, either in continuous phase of an emulsion or inside the single phase nanodroplets. The phase separation can occur during the emulsification process or after the emulsification process performed. In both cases, a thermodynamic phase boundary is crossed through a change in a state variable or composition to drive phase separation. When phase separation occurs in the continuous phase, the objective is to facilitate partial phase inversion; for instance, arresting the transition from water-in-oil (W/O) to oil-in-water (O/W) emulsion in an O/W/O state. This technique has been demonstrated to produce multiple emulsions at micrometer scales, and recent studies indicates its potential applicability for the formation of multiple nanoemulsion [15,16]. In each case both low HLB and high HLB co-surfactants are employed to stabilize interfaces of both positive and negative curvature. Internal phase separation can also drive the formation of multiple nanoemulsion. Internal phase separation offers an alternative mechanism for generating multiple emulsions from a single oil-in-water (O/W) or water-in-oil (W/O) within a single nanoemulsion system. In this method, the dispersed phase is composed of two or more partially miscible substances-either immiscible liquids or solute-

solvent mixtures. A change in thermodynamic conditions, such as variation in temperature, pressure, or chemical environment, or the occurrence of a chemical reaction, can disrupt the miscibility balance and trigger spontaneous demixing. As phase separation proceeds without the presence of interfacial stabilizers for a newly formed phases, the components reorganize based on their interfacial tension and relative wettability. This often leads to the spontaneous formation of structured droplets, such as core-shell configurations. However, if internal phase separation is unstable rather than metastable, it could proceed through spinodal decomposition to produce co-continuous morphologies. Phase separation provides a predictable strategy for producing multiphase droplets with control over the internal structure. By adjusting formulation parameters, it is possible to tailor the droplet morphology ranging from single-core to multicore structure. However, due to the small changes in the composition, chemistry or viscosity can be changed the final emulsion or prevent the multiple nanoemulsion altogether, this adds complexity to the optimization of nanoemulsification processes. Furthermore, using phase separation process final products requires returning to unfavorable conditions for several emulsions, which has the power to demolish their buildings. However, this structural inversion might be viewed as benefit rather than a drawback in some applications, such as triggered delivery [1].

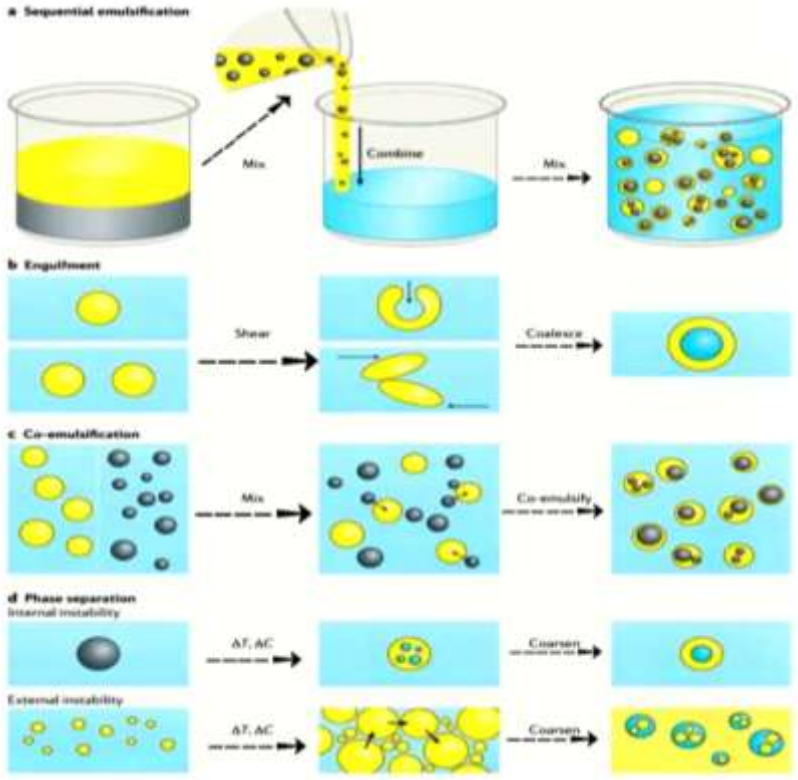

Figure 1.3 Mechanism of formation of multiple nanoemulsions. [49]

4. PSEUDOTERNARY PHASE DIAGRAM OF MULTIPLE NANOEMULSION

A pseudo-ternary phase diagram illustrates the phase behavior of systems that comprise oil, water, surfactant, and a co-surfactant blend (Smix). When dealing with various nanoemulsions, the system tends to be more intricate, typically necessitating a two-step emulsification procedure to form W/O/W or O/W/O configurations. [9,31]

Step-by-step procedure

Step 1: Selection of components

1. **Oil phase:**
- Choose based on solubility and target application
- For O/W/O, select internal and external oils.
- For W/O/W, select internal and external aqueous phases.
2. **Aqueous phase:**
- Usually distilled water, buffer, or drug solution.
- For W/O/W, inner and outer water phases may differ
3. **Surfactant and Co-surfactant:**
- Choose based on HLB value and compatibility.
- Common surfactants: Span 20, Span 80, Tween 80, lecithin, poloxamers etc...
- Co-surfactants: ethanol, propylene glycol, PEG 400, etc.

Step 2: Preparation of surfactant Mixtures (Smix)

- Combine the surfactant and co-surfactants at various weight ratios (for instance, 1:1, 2:1, 3:1, 3:1, 2:1, 4:1 etc...)
- Mark them appropriately (Smix 1:1, 2:1, 3:1, etc...)

Step 3: Construction of primary nanoemulsion (O/W or W/O)

Use water titration method

- Create mixtures with different oil: Smix ratios from 1:9 to 9:1
- Slowly titrate with water for water-in-oil (W/O) or with oil for oil-in-water (O/W).
- Following each addition, mix well and make observations.

Observation criteria:

- Visual clarity (transparent, translucent, turbid).
- Phase separation
- Flow behavior (viscous, fluid, gel-like)
- Record these observations to plot boundaries between: (nanoemulsion zone, emulsion zone, gel/mesophase, phase separation)

Step 4: Constructing the pseudoternary diagram (primary emulsion)

Use software like Chemix, Origin, ternary plot, GraphPad Prism, etc...

- Corners: oil, water, Smix
- Plot compositions and mark different regions
- Identify and highlight nanoemulsion region.

Step 5: Preparation of multiple nanoemulsions:

- Utilize the enhanced primary nanoemulsion as the internal component.
- Blend it into the secondary phase (either oil or water) using a different Smix, potentially incorporating another surfactant system.

For O/W/O

- Primary: Oil-in-water (O/W) nanoemulsion
- Secondary: Emulsifying into an external oil phase.

For W/O/W

- Primary: Water-in-oi (W/O) nanoemulsion
- Secondary: Disperse into external aqueous phase with hydrophilic surfactant.

Step 7: Pseudoternary phase diagram of multiple nanoemulsion

Create new ternary diagrams were

- One corner: Primary nanoemulsion
- Another corner: External phase (oil or water)
- A third corner: Smix 2 (second surfactant system)
- Conduct titration, visual examination, and plotting as previously done
- Locate the stable region for multiple nanoemulsions

Creating pseudoternary diagrams is an essential step in the informed design of various nanoemulsions, including W/O/W and O/W/O systems. By methodically altering the ratios of oil, water, and surfactant combinations, regions of stable nanoemulsions can be distinguished and refined for optimal size, stability, and encapsulation efficiency. These diagrams serve not only to direct formulation development but also to improve the reproducibility and scalability of multiple nanoemulsions for use in pharmaceuticals, cosmetics, and food products.

5. COMPONENTS OF MULTIPLE NANOEMULSIONS.

5.1 Oils/ Lipids: The main role of oil phase in nanoemulsion is that it acts as the internal phase and external phase depending upon the type of emulsions type. The main impact of oil phase is on the droplet size, the stability, drug solubility, release kinetics. The main advantage is that it has high solubilizing capacity for lipophilic drugs also it has the ability to form fine droplets. Generally multiple nanoemulsions contain 5-20% oil/lipids droplets, though sometimes it may be significantly larger (up to 70%). Medium chain triglycerides like caprylic/ capric triglycerides (eg,miglyol 812) which have high loading capacity and good spreadability. Long chain triglycerides like soyabean oil, olive oil, sunflower oil, sesame oil,[17] cotton seed oil,[18] coconut oil,[19] are used to enhance the solubility and bioavailability of lipophilic drugs or compounds. Also, it can improve the encapsulation efficacy further it can improve the stability, permeation and controlled release of the bioactive agents. Essential oils such as eucalyptus oil, clove oil, tea tree oil are also used as they have dual nature, as it acts not only as oil phase but it shows antimicrobial or therapeutic activity. Volatile oils like isopropyl myristate, isopropyl palmitate are used to enhance the permeation of the skin. Also, fatty acid esters like ethyl oleate, oleic acid are used to improve the penetration and stability [20].

5.2 Surfactants and Co-surfactants: Multiple nanoemulsion is a colloidal system, where careful selection of surfactants to stabilize both the primary and secondary phase are requires. The selection of surfactants and co-surfactants is crucial for droplet size, stability, release behavior, and bioavailability. Surfactants are amphiphilic in nature; they contain hydrophilic as well as lipophilic charterers in a single chemical moiety. This moiety is distributed at the interface of two immiscible liquids and reduces the surface tension. The interfacial adsorption facilitates steric, electrostatic, or a combination of electrosteric stabilization mechanisms. Emulsifying agents can be classified based on their solubilities, such as either water soluble or oil soluble, using the hydrophilic-lipophilic balance (HLB) scale. In the HLB scale the surfactants are classified on an imaginary scale, as the value ranges from 0-20, which are based on the relative proportion of polar-to-nonpolar groups in a nonionic surfactant molecule. For the emulsification agents, for oil-soluble surfactant the HLB value indicates 2-6 and for water soluble surfactants the HLB value ranges from 12-15. This scale has been extended to ionic surfactants too, where it has higher HLB values. Based on the relative nature and type of the emulsion that have to be prepared, a suitable emulsifying agent, either single (for

nanoemulsion) or in combination can be selected based on the HLB classification. If oil is the continuous phase, then the surfactant with an HLB value of 2-6 are more suitable. And if the continuous phase is water, then the surfactant with an HLB value 12-15 is more suitable. In multiple nanoemulsions, the combination of surfactants can be used to achieve the desired stability. When a single surfactant is unable to produce the desired HLB value, then addition of another surfactant with the previous one can be chosen to obtain the accurate HLB value to produce a stable emulsion. Commonly used surfactants are span 80 (W/O/W), span 60 (W/O), tween 80 (O/W/O), tween 20 (O/W), span 20 (O/W/O), tween 40, span 40, lecithin. Other common surfactants used for nanoemulsions are poloxomer 407, sodium dodecyl sulfate, amphiphilic proteins like casiens, and polysaccharides (gum, starch derivatives). Some co-surfactants are used to compliment surfactants as they fit suitably in between structurally weaker areas, fortifying the interfacial film. Co-surfactants they used to penetrate into the monolayer of surfactants thereby providing extra fluidity to interfacial film and thus they distract the liquid crystalline phases which are formed when surfactant film is too rigid. Usually with high HLB scale surfactants, low HLB scale co-surfactants are used to modify the overall HLB of the system. Co-surfactants that are commonly used are ethanol, propylene glycol. Polyethylene glycol (PEG400), transcutol p (diethylene glycol monoethyl ether), glycerol, isopropanol, ethylene glycol [20,9,21]

List of surfactants with their HLB values:

Table 1.2 List of surfactants and co-surfactants with their HLB values used for preparation of multiple nanoemulsion.[9]

NAME	HLB
Ethylene glycol distearate	1.5
Sorbitan tristearate	2.1
Propylene glycol monostearate	3.4
Sorbitan sesquioleate	3.7
Glyceryl monostearate, nonself-emulsifying	3.8
Propylene glycol monolaurate	4.5
Sorbitan monostearate	4.7
Diethylene glycol monostearate	4.7
Glyceryl monostearate, self-emulsifying	5.5
Diethylene glycol monolaurate	6.1
Sorbitan monopalmitate	6.7
Sucrose dioleate	7.1
Propylene glycol (200) monooleate	8.0
Sorbitan monolaurate	8.6
Polyethylene (4) lauryl ether	9.5
Polyoxyethylene (4) sorbitan monostearate	9.6
Polyoxethylene (6) cetyl ether	10.3
Polyoxyethylene (20) sorbitan tristearate	10.5
Polyoxyethylene glycol (400) monooleate	11.4
Polyoxyethylene glycol (400) monostearate	11.6
Polyoxyethylene (9) nonyl phenol	13.0
Propylene glycol (400) monolaurate	13.1
Polyoxyethylene (4) sorbitan monolaurate	13.3
Polyoxyethylene (20) sorbitan monolaurate	15.0
Polyoxyethylene (20) oleyl ether	15.4
Polyoxyethylene (20) cetyl ether	15.7
Polyoxyethylene (40) stearate	16.9
Polyoxyethylene (100) stearate	18.8
Sodium lauryl sulfate	Approx. 40

6. METHOD OF PREPARATION OF MULTIPLE NANOEMULSION

Multiple nanoemulsion have very small droplet sizes in nanometer range typically 20-500nm. Various methods have been refined to prepare multiple nanoemulsion, which is broadly categorized into high energy methods and low-energy methods; where high-energy methods such as high-pressure homogenization, ultrasonication, and microfluidization, and low-energy methods which includes phase inversion technique, spontaneous emulsification, solvent evaporation

technique, and hydrogel method. For laboratory and industrial scale preparation high-pressure homogenization and microfluidization are most commonly involved techniques.[21]

6.1 High-energy methods

By using high energy methods, it utilizes the external mechanical forces to break coarse emulsions into fine droplets, thereby resulting in nano-sized emulsions. It involves applying large fluid stresses to a biphasic liquid mixture. High-pressure hominization, microfluidization, ultrasonication all these provide mechanical energy necessary to overcome the significant interfacial energy barrier for producing dispersion of nanoscale droplets. For laboratory and industrial-scale productions, these methods are widely employed due to their reproducibility and the ability to produce a nano-sized distribution, as well as enhance stability. [21,22,23]

6.1.1 High pressure homogenization (HPH)

This method employs a high-pressure homogenizer or piston homogenizer, resulting in nanoemulsions with very small particle size (down to 1nm). By subjecting these two liquids to extreme pressure through a narrow inlet (ranging from 500 to 5000psi), the product undergoes a significant dispersion, creating harsh pressure conditions. The hydraulic shear and turbulence generated produce exceptionally fine fragments of the emulsion. The liquid lipophilic core of the particles formed is encased in a monomolecular layer of phospholipids, ensuring that they remain separate from the adjacent aqueous phase. Although this approach is highly effective, it does have drawbacks, namely substantial energy consumption and an increase in the temperature. This procedure results in emulsion.

- Influence of homogenization pressure: The optimized process parameters range from 100 to 150 bars. The size of the particles produced, for instance with RMRP 22, diminishes as the size increases.
- Number of homogenization cycles: The particle size achieved decreases as the number of homogenization cycles increases. Options include completing 3,4, or 10 cycles. The poly dispersity index of the medicine examines the number of cycles after each iteration.

6.1.2 Ultrasonication

Various research studies have reported on the creation of nanoemulsion, using ultrasonic sound frequencies to reduce the droplet size. Using a constant amplitude sosnotrode at system pressures higher than the ambient value is an additional strategy. An increase in the external pressure is known to raise the cavitation threshold in an ultrasonic field, thereby minimizing the development of bubbles. However, by raising the external pressure there will be also raises the cavitation bubbles collapse pressure. This indicates that when cavitation takes place, the bubbles collapse more quickly and rapidly than when the pressure is at atmospheric levels. These variations in navigational intensity can be directly linked to fluctuations in the power density because cavitation is the primary mechanism of power dissipation in a low frequency ultrasonic system. To maintain the ideal temperature, the system additionally makes use of a water jacket. [21,22]

6.1.3 Microfluidization

Microfluidization is a mixing approach, which makes a use of device called microfluidizer. This device uses a positively distributed pump (500 to 20,000 pressure) to force the object through the interaction chamber. The microscopic channels that make up this are called “microchannels”.

Submicron sized particles are created when the materials pass through small openings and strikes a surface. To make a course emulsion, aqueous and viscous phases are combined and then processed in an internal homogenizer. A coarse emulsion can be treated to produce a permanent nanoemulsion in a microfluidizer. Until the required particle size is achieved, the coarse emulsion is repeatedly passed through the microfluidizer with an interaction chamber. When a filter immersed in nitrogen is used to remove the big particles from the core emulsion, a homogeneous nanoemulsion is formed. A unique feature of the microfluidizer is that, its fixed-geometry interaction chamber, which ensures consistent processing conditions across batches and scales. Therefore, the resulting formulations typically exhibit narrow sized particle distribution and thereby it can enhance the stability, and making the technology particularly suitable for applications that demand high reproducibility and product uniformity. [22]

6.2 Low-energy methods

Low-energy methods for preparing multiple nanoemulsion relays on physiochemical principles rather than mechanical forces. These methods are more energy efficient, also it is cost effective and they are commonly used for compounds like pharmaceuticals, cosmetics and nutraceuticals.

6.2.1 Phase inversion temperature method

Phase inversion temperature method formulates nanoemulsions by taking advantages of the changes in the solubility of surfactants in oil and water as the temperature fluctuates. This process entails an orderly transformation from a water-in-oil emulsion to oil-in-water emulsion or the other way around through an intermediary bicontinuous phase. Typically, a combination of water, oil and surfactants is heated beyond an appropriate temperature known as the PIT, which is unique to the formulation being used, and then it is rapidly cooled. A shift in temperature from low to high represents the opening and reversal of the interfacial structures, leading to phase inversion. Additionally, rapid cooling a cause the interfacial structure to close once more, encapsulating the oil or water. This method is a bottom-up technique, and the freshly formed droplets remains stable for an extended period of time because of ample surfactant coverage. Since heat input is required, PIT technique may not be suitable for thermosensitive drugs. Additionally, the good mutual solubility of oil, water, surfactant, and drug is essential to ensure a smooth phase transition. Any destabilization that occurs is governed by Ostwald ripening. [22,21,24]

6.2.2 Spontaneous emulsification.

The process of spontaneous emulsification is comparable to the nanoprecipitation technique used to generate polymeric nanoparticles. Yet, oil is utilized in the place of polymer. Two phases are prepared during the process: an aqueous phase containing a hydrophilic surfactant and an organic or oil phase, such as mygliol which comprises a medication, an oil-soluble surfactant like (span), and an organic solvent which is somewhat water miscible like (acetone or ethyl acetate). To formulate tiny nanoscale emulsions, organic phase is added dropwise to the aqueous stirring phase (but the opposite, that is adding water to oil, is equally possible in the case of W/O emulsions).

6.2.3 Solvent evaporation method

This process involves creating a pharmaceutical solution and then emulsifying it in a liquid that isn't the solvent for the drug. The solvents dissipate causing the drug to precipitate. Strong shearing forces are produced by a high-speed blender so that is can be used to regulate crystalline formation and to reduce the size of particles.

6.2.4 Hydrogel method

This is equivalent to the solvent evaporation technique. The main difference between the two approaches is that drug solvent and drug anti-solvent are miscible. Strong shear pressure prevents Ostwald crystallization and ripening. [21,22]

Table 1.3 method of preparation of multiple nanoemulsion

Method	Droplet size tunning	Advantages	Limitations	Reference
High-pressure homogenization	Control via applied pressure, number of passes and temperature	Industrial-scale feasibility, Rapid process, tunable droplet size in nanometer range.	High-energy cost, heat-sensitive payload degradation	25
Microfluidization	Number of passes allows size tunning	Extreme narrow size distribution, uniform droplets	Expensive, risk of clogging, lower throughput	26
Ultrasonication	Adjust input energy, ultrasonic frequency, sonication time, amplitude	Simple lab-scale setup, scalable for small batches	Limited to small volumes, potential for thermal degradation, oxidation, limited scalability	27
Spontaneous emulsification	Droplet size depends on oil-to-surfactant ratio, injection rate	No external energy, simple, low thermal input. Energy-efficient, suitable for some lab applications	Require precise formulation design, stability issues such as Ostwald ripening	28
Phase inversion method	Tuned by temperature (PIT), droplet size controlled by inversion point design	Low energy – smaller droplet sizes achieved, minimal mechanical equipment	High surfactant requirement, narrow process window, scale-up challenge.	29

7. CHARACTERISATION TECHNIQUES USED FOR MULTIPLE NANOEMULSIONS

Characterization techniques provide a comprehensive understanding of stability, performance and efficacy of multiple nanoemulsions in pharmaceutical formulations [30-39].

7.1 Globule size and zeta potential

Small quantity of multiple nanoemulsions were placed in disposable sizing cuvettes. After this using a dynamic light scattering was used to evaluate the size of droplets as well as the zeta potential of multiple nanoemulsions

7.2 Surface morphology

Surface morphology is measured by SEM, TEM, AFM, light scattering microscope etc... Under the microscope a small quantity of prepared multiple nanoemulsions were placed on the slide and observed. After visual examinations data are taken.

7.3 Drug content determination

The drug content of multiple nanoemulsions is dispersed in appropriate solvents. Then the sample

was centrifuge. Then the extracted solvent was spectrophotometrically evaluated. Then absorbance was taken, and the observed absorbance was then converted to its corresponding concentration using a calibration curve, and then the exact amount of the drug in the formulation was then calculated.

7.4 Entrapment efficacy

The concentration of unentrapped drug was measured to calculate the percentage drug encapsulation efficiency. After separation of entrapped drug from the formulation the amount of drug encapsulated per unit weight of formulation was calculated using the equation:

%EE= (amount of drug added – free (unentrapped) drug) / (amount of drug added) ×100

7.5 pH of multiple nanoemulsion

pH of multiple nanoemulsion was calculated using digital pH meter

7.6 Refractive index measurement

Refractometry measures the extent to which light is refracted when it moves from air to a multiple nanoemulsion in order to assess their composition or purity. One drop of formulation was placed on the slide and refractive index was measured by digital refractometer.

7.7 Rheology measurement

Rheology is measured for understanding the flow behavior and mechanical properties of multiple nanoemulsions. Oscillatory rheology, thixotropy test, steady shear flow test is used to determine the rheology of multiple nanoemulsions.

7.8 *In vitro* release study

In vitro release study is to evaluate the drug release profile from the nanoemulsion system under controlled laboratory conditions. Mainly this is performed using dialysis bag, Franz diffusion cells, and synthetic membranes. Here, the formulation is placed in donor compartment; where the recipient compartment contains buffer medium. Samples were withdrawn a regular interval and analyzed using UV-visible spectroscopy or HPLC

7.9 *Ex vivo* studies

Ex vivo studies are performed to assess the permeation, retention, and barrier interaction of the formulation across the biological tissues, most probably animal or human/mucosa. Frans diffusion cells are mainly used with excised skin. Here, the formulation is placed in the donor compartment. The excised skin was placed between the donor and the recipient chamber of the apparatus. The skin should be placed in a way that its epidermis faces the donor chamber and the dermal side faces the recipient compartment. The recipient compartment was filled with buffer medium. Samples were withdrawn at regular intervals and fresh medium was replaced and analyzed using UV-visible spectroscopy.

7.10 *In vivo* studies

In vivo studies are performed to evaluate the pharmacokinetics, therapeutic efficacy, biodistribution, toxicity, and biocompatibility of multiple nanoemulsions in living organisms. The main models used are rodents (rats/mice) are employed for dermal, oral, parenteral, or ocular routes. *In vivo* studies demonstrate the bioavailability enhancement, site-specific delivery, and reduction in systemic side effects. For performing in vivo studies, ethical approval is required.

7.11 Stability test

Stability studies are performed to evaluate the physical, chemical, and functional stability of multiple nanoemulsions under various environmental conditions over time. Some stability studies are centrifuge, heating-cooling cycles, freeze-thaw cycles, etc.

7.12 ATR-FTIR analysis

ATR-FTIR is a spectroscopic technique used to analyze the surface chemistry, functional group interactions, and structural integrity of multiple nanoemulsions. The ATR-FTIR spectra of the sample were taken using an IR spectrophotometer. The samples were placed on a zinc selenide crystal and then scanned between 4000-400cm^{-1}

7.13 Statistical analysis

Statistical analysis is essential for validating experimental results, determining significance and ensuring reproducibility of multiple nanoemulsions. The student's t test and one-way ANNOVA were used for the statistical analysis. The software can be used is GraphPad prism 10 software.

8. APPLICATION OF MULTIPLE NANOEMULSIONS

Multiple nanoemulsion is typically oil-in-water (O/W/O) and water-in-oil (W/O/W) systems which is a sophisticated class of emulsion-based carrier which enables the simultaneous transport and encapsulation of both lipophilic and hydrophilic agents. They provide a number of benefits, including better stability, protection of labile substances, controlled medication release, and improved bioavailability of active ingredients, because of their structural complexity and nanoscale droplet size.

8.1 ORAL ROUTE OF DRUG DELIVERY

Table 1.4 examples of oral delivery of multiple nanoemulsions

Purpose	Drug examples	Nanoemulsion type	References
Enhance solubility, protect from degradation, sustain release	Insulin, valsartan, 5-FU	W/O/W	40
Increases membrane permeability, increases bioavailability, and enhances pharmacokinetic effects.	OXA/DCK and 5-FU	W/O/W	31
Enhance the oral absorption of polar drugs	Pharmaceutical grade excipients	W/O/W	30
Enhanced the intestinal permeability and oral bioavailability.	Etoposide (ETP)	W/O/W	41
Enhanced the intestinal permeability, enhance oral bioavailability.	oxaliplatin	W/OW	42

8.2 TOPICAL ROUTE OF DRUG DELIVERY

Table 1.5 examples of topical delivery of multiple nanoemulsions

Purpose	Drug examples	Type	Reference
Improve skin penetration, localized delivery	Chrysin, corticosteroids	W/O/W	43
Skin penetration potency of acyclovir, optimization of this multiple nanoemulsion formulation	Aciclovir	W/O/W	33

Control and regulate the premature release of the drug at the skin's surface	Itraconazole	O/W/O	32

8.3 FOR CANCER THERAPY

Table 1.6 examples of cancer therapy of multiple nanoemulsions

Purpose	Drug examples	Type	Reference
Dual drug loading, targeted release	Doxorubicin + paclitaxel, curcumin	W/O/W	44
Improve the therapeutic efficacy towards cancer cells, as well as lowered the toxicity effect towards normal cells	Cisplatin. Tocotrienol, caffeic acid	W/O/W	45
Inhibit tumor growth following oral administration	Deoxycholic acid	W/O/W	46

8.4 VAGINAL DRUG DELIVERY

Vaginal delivery of therapeutics aims to provide localized treatment with minimal systemic side effects. Using multiple nanoemulsion, particularly W/O/W systems, enhances controlled release, mucoadhesion, and pH-sensitive drug release – making them ideal for treating infections and diseases affecting the vaginal mucosa.

8.5 VACCINE AND PROTEIN DRUG DELIVERY

Delivering proteins, peptides, or antigens via mucosal or oral routes is challenging due to enzymatic degradation, poor permeability, and low bioavailability. Multiple nanoemulsions offer a protective barrier and controlled release mechanism, making them potential carriers for vaccines and biotherapeutics.

FUTURE PERSPECTIVE

In the upcoming years, research on MNEs is likely to focus on refining formulation parameters to enhance stability and scalability, integrating natural and biodegradable surfactants to improve safety profiles, and utilizing environmentally friendly synthesis techniques. Progress in nanotechnology, microfluidics, and computational modelling will support the precise design of MNEs with consistent drug release kinetics. Additionally, merging MNEs with stimuli-responsive systems, such as those triggered by pH or temperature changes, has the potential to transform personalized medicine. The industrial uptake will rely on addressing regulatory hurdles, establishing strong quality control protocols, and minimizing production expenses through advanced continuous manufacturing methods.

CONCULSION

Multiple nanoemulsions serve as a versatile and effective platform for administering therapeutic agents through various routes. Their capacity to encapsulate both water-soluble and fat-soluble drugs, safeguard sensitive compounds, and facilitate controlled release provides them with an advantage in contemporary drug delivery research. Despite ongoing challenges related to formulation stability and large-scale production, recent advancements and interdisciplinary strategies are swiftly tackling these issues. With sustained advancements, MNEs are likely to emerge as a crucial technology in the pharmaceutical sector,

leading to better treatment outcomes and improving the quality of life for patients.

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HOW TO CITE: Hendrieta Mary Bivera, Ajitha K Cherian, Vyshnavi R Nath, A Detailed Comprehensive Review of Multiple Nanoemulsions on Pharmaceutical Applications, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 9, 541-561. https://doi.org/10.5281/zenodo.17054542