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An overview of Preparation, Evaluation and Applications of Multiple Emulsions

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ABSTRACT

Multiple emulsions are also known as emulsions of emulsions, liquid membrane system or double emulsion. Multiple emulsions are polydispersed systems where both oil in water & water in oil emulsions exist simultaneously. This review focuses on preparation, characterization and potential applications of multiple emulsions. Multiple emulsions can be classified as water-in oil-in water (W/O/W) or oil-in-water-in-oil (O/W/O) emulsions. This review described five methods to prepare multiple emulsions viz. two-step emulsification method, modified two-step emulsification method, phase inversion method, membrane emulsification & micro channel emulsification method. The Multiple emulsion is characterized by average globule size & size distribution, area of interfaces, number of globules, rheological evaluation, zeta potential, percentage drug entrapment, *In-vitro* drug release. Multiple emulsions have been proposed to have numerous uses including their use as prolonged drug delivery system.

KEYWORDS

Multiple Emulsions, Emulsifying agent, Membrane emulsification.

INTRODUCTION

Multiple emulsions are complex systems, termed "emulsions of emulsions", i.e. the droplets of the dispersed phase contain even smaller dispersed droplets themselves. Each dispersed globule in the double emulsion forms a vesicular structure with single or multiple aqueous compartments separated from the aqueous phase by a layer of oil phase compartments.[1,2,3] Multiple emulsions are also known as emulsions of emulsions, liquid membrane system or double emulsion.

The basic rationale for the use of W/O/W & O/W/O type multiple emulsions as a means of prolonged delivery of drugs is that the drug contained in the innermost phase is forced to partition itself through several phases prior to release at the absorption site. Thus the partition & diffusion coefficient of the drug & the strength of the middle membrane phase, which is a multimolecular layer of oil, water & emulsifier molecules at both the interfaces of multiple emulsion system, controls the drug release from these system. [4]

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Types of Multiple Emulsions[5]

The two major types of multiple emulsions are the water-oil-water (w/o/w) and oil-water-oil (o/w/o) double emulsions.

Water-in-Oil-in-Water (W/O/W) Emulsion System

In W/O/W system, an organic phase (hydrophobic) separates internal and external aqueous phase. In other words, W/O/W is a system in which oil droplets may be surrounded by an aqueous phase, which in turn encloses one or several water droplets.

Oil-in-Water-in-Oil (O/W/O) Emulsion System

In O/W/O systems, an aqueous phase (hydrophilic) separates internal and external oil phase. In other words, O/W/O is a system in which water droplets may be surrounded in oil phase, which in turn encloses one or more oil droplets.

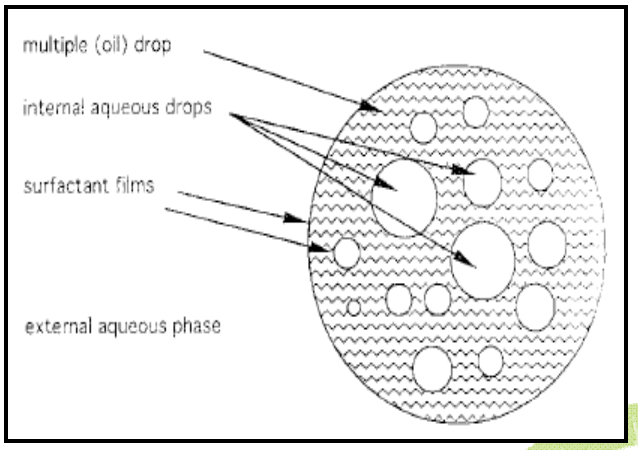

Figure 1: Schematic Representation Of A W/O/W Double Emulsion Droplet.

The objectives will be to produce a multiple emulsion system that has a high yield of multiple droplets containing the drug entrapped in the innermost phase, and for such a system to have good stability in vitro and the desired release characteristics in vivo.[6]

Formulation of Multiple Emulsions

Florence and Whitehil[3] described three different types of multiple emulsions, which they termed A, B, and C. Type A multiple emulsions were those in which only one large internal drop was contained in the secondary emulsion droplet. In type B emulsions, there were several small internal droplets contained in the secondary emulsion droplet, and type C emulsions were those with a large number of internal droplets present. Only the type C systems have applications in drug delivery and drug targeting.[6]

Oil Phase

The oil phase to be employed in a pharmaceutical emulsion must be nontoxic. The various oils of vegetable origin (soybean, sesame, peanut, safflower, etc.) are acceptable if purified correctly. Refined hydrocarbons such as light liquid paraffin, squalane, as well as esters of fatty acids (ethyl oleate and isopropyl myristate) have also been used in double emulsions [1]. Oils derived from vegetable sources are biodegradable, whereas those based on mineral oils are only removed from the body very slowly.

As a general rule, mineral oils produced more stable multiple emulsions (w/o/w) than those produced from vegetable oils [2]. The order of decreasing stability and percentage entrapment has been found to be light liquid paraffin > squalane > sesame oil > maize or peanut oil. [7]

Nature and Quantity of Emulsifying Agents

Two different emulsifiers (lipohilic and hydrophilic) are required to form a stable emulsion. In general, for a w/o/w emulsion the optimal HLB value will be in the range 2-7 for the primary surfactant and in the range 6-16 for the secondary surfactant. The concentration of the emulsifiers can also be varied. Too little emulsifier may result in unstable systems, whereas too much emulsifier may lead to toxic effects and can even cause destabilization. [8] An excess of lipophilic surfactant can cause the inversion of w/o/w emulsion to simple o/w emulsion.

Phase Volume

It is very important to have proper order of phase addition while formulation and dispersed phase should be added slowly into the continuous phase for the formulation of a stable multiple emulsion. An optimal (22-50%) internal phase volume can be utilized for the emulsion formulation. Very high phase volume ratio (70-90%) had also been reported to produce a stable multiple emulsion.

Factor Affecting Stability of Multiple Emulsion

Nature of Entrapped Material

When formulating a w/o/w system the presence of the drug and other components (especially electrolytes) needs to be considered. The nature of drug (hydrophilic or hydrophobic) also is

considered.[6] Due to the nature of the multiple emulsions, the middle phase acts as a membrane, and osmotic effects may become significant. The entrapped solutions may interact with the surfactant or the surface active drugs may be adsorbed at the inter phase, resulting in decreased stability. [3]

Shear/Agitation

High shear disrupts the large percentage of multiple oil drops and hence results in the instability of system due to tremendous increase in effective surface area. Therefore, with increased homogenization time, the yield of the system falls rapidly. Generally high agitation speed is used for primary and low speed is used for secondary emulsification for the preparation of multiple emulsions.

Temperature

Temperature has only an indirect effect on emulsification that is attributed to its effect on viscosity, surfactant adsorption and interfacial tension. Generally, for the primary emulsion formulation temperature is kept at 70˚C, whereas for multiple emulsion preparation it is kept at 10˚C. Large temperature variations during manufacturing, storage, transport and use leads to drastic modifications within emulsions.

Rheology

The rheological properties of emulsions are influenced by a number of factors, including the nature of the continuous phase, the phase volume ratio, and to lesser extent by particle size distribution. For low internal phase volume emulsions, the consistency of the emulsion similar to the continuous phase; thus, o/w/o emulsions are generally thicker than w/o/w emulsions, and the consistency of a w/o/w system can be increased by the addition of gums, clays.

Effect of Lipophilic Emulsifier

As the concentration of lipophilic surfactant is increases, the swelling capacity of oil globule is increases, and the more the release is delayed.

The influence of the lipophilic surfactant concentration on the swelling of the oil globule can be explained by two different mechanisms. The first one consists in an increase of the rigidity of the second interface by the progressive migration of the lipophilic surfactant. During the second step of multiple emulsion preparation, lipophilic surfactant molecules can diffuse from the first to second interface, were they produce a synergistic effect resulting in membrane strengthening. The second one involves a delay in the in the aqueous droplet coalescence. In course of swelling of the oil globule, the lipophilic surfactant molecules, which are in excess in oily phase, can diffuse to the first interface to fill up free spaces caused by swelling, when required.[9]

Added Stabilizing Components

The stabilizers are added for improve the stability of multiple emulsions. These include gelling or viscosity-increasing agents added to internal and/or external aqueous phases (e.g., 20% gelatin, methylcellulose, and similar thickening agents, as well as complexing agents that will lead to liquid crystalline phases at the o/w interface (e.g., 1-3% cetyl alcohol) and gelling agents for the oil phase (e.g., 1-5% aluminum monostearate).[2]

Methods of Preparation

Two-Step Emulsification Method (Double Emulsification) [2]

It is the most common method because it is very easy and gives high yield with reproducibility. Multiple emulsions prepared by re-emulsification of a primary emulsion. In this method two stages involved.

- Obtaining an ordinary W/O or O/W primary emulsion wherein appropriate emulsifier system is utilised.
- The freshly prepared W/O or O/W primary emulsion is re-emulsified with an excess of aqueous phase or oil phase. The final prepared emulsion could be W/O/W or O/W/O respectively.

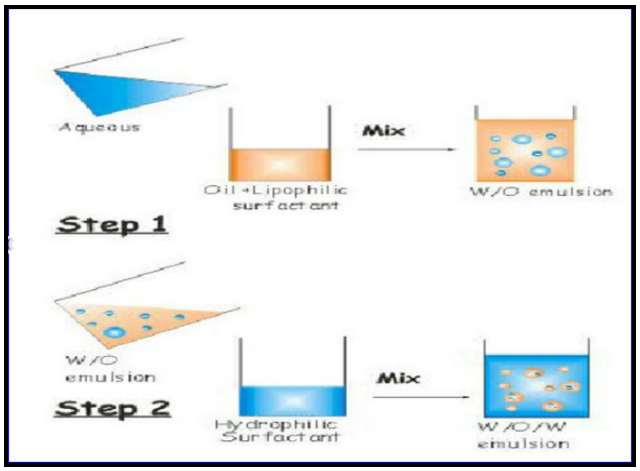

Figure 2: Two-step emulsification method

Modified Two-Step Emulsification Technique [5]

This method is different from the conventional two-step technique in two points.

- Sonication & stirring are used to obtain fine, homogenous & stable W/O emulsion.
- A continuous phase is poured into a dispersed phase for preparing W/O/W emulsion.

Moreover, the composition of internal aqueous phase-oily phase-external phase is fixed at 1:4:5, which produce most stable formulation as reported for most of W/O/W emulsions.

Figure 3: Modified two-step emulsification technique

Phase Inversion Technique (One Step Method) [5,22,23]

Preparation of W/O/W double emulsions which included strong mechanical agitation of the water phase containing a hydrophilic emulsion and an oil phase containing large amount of hydrophobic surfactant. An increase in volume concentration of dispersed phase which subsequently leads to the formation of multiple emulsions.

- A well-defined volume of oil phase is placed in a vessel of pin mixer.
- An aqueous solution of emulsifier is then introduced successively to the oil phase in the vessel at a rate of 5ml/min, while the pin mixer rotates steadily at 88 rpm at room temperature.

When volume fraction of the aqueous solution of hydrophilic emulsifier exceeds 0.7, the continuous oil phase is substituted by the aqueous phase containing a number of the vesicular globules among the simple oil droplets, leading to phase inversion and formation of W/O/W multiple emulsion.

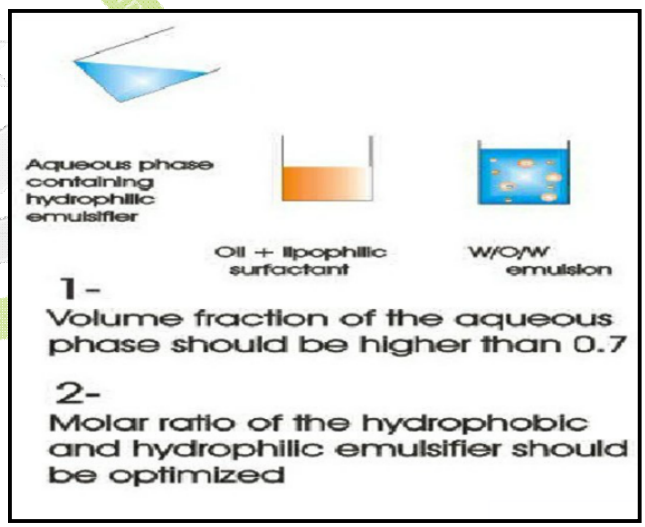

Figure 4: Phase inversion technique (one step method)

Membrane Emulsification Technique [11,12,35]

In this method, a W/O emulsion (dispersed phase) is extruded into an external aqueous phase (a continuous phase) with a constant pressure through a Porous Glass Membrane, which should have controlled & homogenous pores. The particle size of the W/O/W emulsion can be controlled with the proper selection of porous glass membrane. The relation between membrane

pore size and particle size of emulsion exhibits good correlation as described by the formula:

$$Y = 5.03X + 0.19$$

Where, X= the pore size

Y= the mean particle size

A micro porous glass membrane with narrow pore size range was used successfully for preparing stable simple (o/w) and water-oil-water (w/o/w) type emulsions.

Micro Channel Emulsification [11,12]

The emulsion was prepared by first homogenizing a mixture of water & oil phase using conventional homogenizer. Then the W/O emulsion obtained was forced through the micro channel device into a second water phase containing a suitable emulsifier for oil phase stabilization.

Evaluation of Multiple Emulsions

Average Globule Size & Size Distribution

The optical microscopy method using calibrated ocular and stage micrometer can be utilized for globule size determinations of both multiple emulsion droplets as well as droplets of internal dispersed phase.

Bright field micrographs equipped with differential interference contrast optics have been used to characterize the internal droplet of multiple emulsions.

Various other techniques used to characterize colloidal carriers like Coulter counter, freeze-fracture electron microscopy and scanning electron microscopy are also used to determine average globule size and size distribution of multiple emulsions.

Recently, NMR self-diffusion methods are adapted to multiple emulsion characterization.

Area of Interfaces

The average globule diameter determined can be used in the calculation of the total area of interface using the formula

i. $S = 6/D$

Where,

S = Total area of interface (sq.cm)

D = Diameter of globules (cm)

Number of Globules

Number of globules per cubic millimetre can be measured using a haemocytometer cell after appropriate dilution of the multiple emulsions. The globules in five groups of 16 small squares (total 80 small squares) can be counted and the total number of globules in per cubic mm is calculated using the formula

$$\text{No. of globules/mm}^3 = \frac{\text{No. of globule} \times \text{dilution} \times 4000}{\text{No. of small squares counted}}$$

Rheological Evaluation

The rheology of multiple emulsions is an important parameter as it relates to emulsion stability and clinical performance. The viscosity and interfacial elasticity are two major parameters, which relate to product rheology.

The viscosity of the multiple emulsions can be measured by Brookfield rotational Viscometer. Samples are sheared for one min at 100 rpm, using an appropriate spindle.

Interfacial rheology (i.e., interfacial elasticity at the oil-aqueous interface) can be investigated at the mineral oil/water interface using an Oscillatory Surface Rheometer.

Zeta Potential

The zeta potential measurements are pivotal in the designing of surface modified or ligand anchored multiple emulsion systems. The zeta potential and surface charge can be calculated using Smoluchowski's equation from the mobility and electro phoretic velocity of dispersed globules using the Zeta-potentiometer. Zeta potential was calculated using following formula:

$$\zeta = \frac{4\pi\eta\mu \times 10^3}{\varepsilon E}$$

Where,

ζ = Zeta potential(mV)

η = Viscosity of the dispersion medium(poise)

μ = Migration velocity (cm/s)

ε = Di electric constant of the dispersion medium

E = Potential gradient (voltage applied/distance b/w electrodes)

Percentage Drug Entrapment

Percent entrapment of drug or active moiety in the multiple emulsions is generally determined using dialysis, centrifugation, filtration and conductivity measurements. Recently an internal tracer/marker was used to evaluate the entrapment of an impermeable marker molecule contained in the inner aqueous phase of W/O/W emulsion The unentrapped marker is calculated & the amount entrapped can be thus calculated by deducting unentrapped amount from the initially added amount.

In-Vitro Drug Release

The drug released from the aqueous inner phase of a W/O/W emulsion can be estimated using the conventional dialysis technique. The W/O/W emulsion was placed in the dialysis bag & dialyzed against 200 ml of phosphate saline buffer pH 7.4 at 37±1˚c & a sink condition was maintained while sink contents were stirred continuously using a magnetic stirrer. Aliquots were withdrawn at different time intervals and estimated using standard procedure and the data were used to calculate cumulative drug release profile.

Applications of Multiple Emulsions

Controlled and Sustained Drug Delivery

The basic potential of multiple emulsions (both w/o/w and o/w/o) in clinical therapeutics is in the prolonged and controlled release of drugs. In both systems drug present in innermost phase has to cross several phases before it is available for absorption for the system. W/O/W emulsions for parenteral delivery are more convenient to handle, use, and inject due to lower viscosity of these systems.

Inverse Targeting

Regarding this approach Talegaonkar and Vyas were prepared poloxamer 403 containing spherein oil-in-water (s/o/w) multiple emulsion of diclofenac sodium by gelatinization of inner aqueous phase and they examined the effect of poloxmer 403 on surface modification for inverse targeting to reticuloendothelial system-rich organs. The results concluded that this multiple emulsion system containing poloxamer has capability to retards the RES uptake of drugs mainly to liver, brain and targeting to non-RES tissues such as lungs, inflammatory tissue. [12]

Vaccine Adjuvant

The use of w/o/w multiple emulsion as a new form of adjuvant for antigen was first reported by Herbert[13]. These emulsions elicited better immune response than antigen alone. Rishendra and Jaiswal[14] developed a multiple emulsion vaccine against Pasteurella multocida infection in cattle. This vaccine contributed both humoral as well as cell-mediated immune responses in protection against the infection. It was concluded that this multiple emulsion-based vaccine could be successfully used in the effective control of hemorrhagic septicemia. Recently, multiple water-in-oil in- water (w/o/w) emulsion formulations, containing influenza virus surface antigen hemagglutinin was prepared and was characterized in-vitro and in-vivo in wistar albino rats. SDS-PAGE technique was used for evaluating hemagglutinin and in vitro release of antigen respectively. Results suggested that multiple emulsion formulations carrying influenza antigen have advantage over conventional preparation and can be effectively used as one of the vaccine delivery system with adjuvant properties. In an another report by the same researchers they concluded that multiple emulsion and nanoparticle formulations containing influenza virus surface antigen Hemagglutinin were more effective in eliciting an immune response in rats than the conventional vaccine.[15,16,29,32]

Oxygen Substitute

A multiple emulsion of aqueous oxygen carrying material in oil in outer aqueous phase is suitable for provision of oxygen for oxygen transfer processes. A hemoglobin multiple emulsion in physiologically compatible oil in an outer aqueous saline solution is provided in sufficiently small droplet size to provide oxygen flow through blood vessels to desired body tissues or organs thereby providing a blood substitute. A process is provided wherein hemoglobin, a fragile material, is formulated into high hemoglobin content water-in-oil-in-water multiple emulsions while maintaining high yields and high oxygen exchange activity.

Multiple Emulsion for Local Immunosuppression

A potential approach to avoid the complication of systemic Immunosuppression & simultaneously enhance immunosuppressive agents locally to the site of the target organs. W/O/W multiple emulsion has been developed for the delivery of immunosuppressant.

Bioavailability Enhancer

Multiple emulsions have also been used to improve bioavailability of lipophilic drugs, which have high first pass metabolism. Multiple emulsion increases bioavailability of drugs either by protecting drugs in physiological, ionic/enzymatic environment in the GIT where otherwise these gets degraded like proteins, peptides or by passing the hepatic first pass metabolism.

Enzyme Immobilization

Enzymatic conversion of water insoluble, highly lipophilic substrates, such as steroids, can be carried out in a multiple emulsion. The enzyme is contained in a microdroplet 'water pool', whereas the organic phase contains the substrate solution. For example hydrocarbon based liquid surfactant membranes have been used to immobilize Urease.

Drug over Dosage Treatment

This system could be utilized for the over dosage treatment by utilizing the difference in the pH. For example:-barbiturates. In these emulsions, the inner aqueous phase of emulsion has the basic buffer and when emulsion is taken orally, acidic pH of the stomach acts as an external aqueous phase. In the acidic phase barbiturate remains mainly in unionized form, which transfers through oil membrane into inner aqueous, phase and gets ionized. Ionized drug has less affinity to cross the oil membrane thereby getting entrapped. Thus, entrapping excess drug in multiple emulsions cures over dosage.[17,24,26]

Taste Masking

Multiple emulsions of chloroquine, an antimalarial agent has been successfully prepared and had been found to mask the bitter taste efficiently[18,19,21]. Taste masking of chlorpromazine, an antipsychotic drug has also been reported by multiple emulsions.

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