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Development of pharmaceutical multiple emulsion: An overview

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Abstract
Multiple emulsions are novel carrier system which are multiphase, complex and polydispersed in nature where both w/o and o/w emulsion exists simultaneously in a single system. Multiple emulsions are also called as emulsions of emulsions, liquid membrane system or double emulsion. It is important to prevent the problem of oral drug delivery system and they are stabilized by using of combination of both hydrophilic and lipophilic surfactant. It finds wide range of applications in targeted drug delivery, vaccine adjutants, delivery of proteins and peptides, taste masking, treatment of drug overdose, controlled or sustained drug delivery, bioavailability enhancement, enzyme immobilization and as intermediate step in microencapsulation process. Also in cosmetics, these systems can prevent degradation of an active ingredient and release it at a controlled rate. Progression in techniques for preparation, stabilization and rheological characterization of multiple emulsions, it will be able to provide a new carrier system for drugs, cosmetics and pharmaceutical agents. The aim of this article was to provide a detailed review of types, formulation approaches, preparation methods, evaluation parameters and applications of multiple emulsions.

Keywords: multiple emulsion, carrier, hydrophilic and lipophilic surfactant, stability, drug delivery

Introduction

An Emulsion is a two-phase system consisting of two incompletely miscible liquid, stabilized by the presence of third component called as emulsifying agent. These two liquids are also chemically non-reactive. Simple emulsion, Multiple emulsion, Microemulsion and Nanoemulsion are types of emulsion. Multiple Emulsions are heterogeneous systems in which dispersed phase contain smaller droplets that have the same composition as the external phase. In double emulsion each dispersed globule forms a vesicular structure with single or multiple aqueous compartments separated from the aqueous phase by a layer of oil phase compartments. [1-4]. They can consider as liquid membrane system because the two miscible phases are separated by an immiscible phase which acts as a thin semi-permeable film through which solute must diffuse in order to traverse from one phase to another. They encapsulate both hydrophilic and lipophilic compounds with high encapsulation efficiency also have been described as potential matrices for the encapsulation of bioactive compounds and for a controlled release of such compounds. Multiple emulsions are formulated in a two step manner. Firstly we have to prepare a simple emulsion then the inner emulsion is dispersed into an outer continuous phase and for two step method it require at least two emulsifiers one that has a low hydrophilic-lipophilic balance value to stabilize the primary w/o emulsion and one that has a high hydrophilic- lipophilic balance value to stabilize the secondary o/w emulsion. In addition to the emulsion composition, the stability of such systems also depends heavily on the emulsion matrix, influenced by the dispersing methods used. Multiple emulsion stability is correlated with the interfacial film strength (measured by interfacial elasticity) of the hydrophobic surfactant at the mineral oil/external continuous aqueous phase interface [5-8].

Types of Multiple Emulsions: The two major types of multiple emulsions are water-oil-water (w/o/w) and oil-water-oil (o/w/o)

1. 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.

2. 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 droplet [9-11].

Formulation of Multiple Emulsions

Copolymers, polymers and surfactants etc. are used to provide kinetic stability to maintain their structure for relatively long period of time because emulsions are thermodynamically unstable systems and hence emulsifiers, such as The main objective of multiple emulsion is that to produce high yield of multiple droplets containing drug engaging in the innermost phase, and for such a system to have good stability in-vitro and the desired release characteristics in-vivo. The following factors are considered important in multiple emulsion formulation [12-13].

1. Emulsifiers [14-21]
In the formulation of multiple emulsion, optimized concentration of surfactant is used for emulsification on the basis of the hydrophilic-lipophilic value (HLB) of oil phase.

Hydrophobic emulsifier with a low HLB value of 2-7 is used to stabilize the primary emulsion, while a hydrophilic emulsifier with a high HLB value of 6-16 is used to stabilize the secondary emulsion. Moreover, it has been found out majority of the cases that the most stable emulsions are formed when both emulsifying agents have the same hydrocarbon chain length, for instances pan 80 as hydrophobic emulsifier and Tween 80 as hydrophilic emulsifier. At higher concentrations the primary surfactant gets incorporated in secondary surfactant micelles and leads to instability of multiple emulsion. Non-ionic emulsifier alone or in combination are used because of their low toxicity and they are less likely to interact with other compound. Also, non-ionic surfactant gives better yield in comparison to ionic surfactants**.**

2. Quantity of emulsifiers

Quantity of emulsifiers plays prime role in stability of emulsion. Insufficient amount of emulsifier may result in unstable systems, whereas excessive amount of emulsifier may lead to toxic effects and even cause destabilization. In these emulsion, primary emulsion W/O is first prepared using water and low-HLB surfactant solution in oil.

3. Emulsifying equipment

By using laboratory mixer or homogenizer we can prepare primary emulsion in order to provide a good dispersion of droplets within appropriate continuous phase. In the secondary emulsification stage, primary emulsion must disperse into droplets of suitable size for use in delivery vehicles. Too much mixing, especially at high shear, can cause the primary emulsion droplets to rupture, so to avoid these problem low shear mixers which having low speed should be used, or the system can be shaken by hand. Ultrasonic homogenizers must be used with care for the secondary emulsification step.

4. Oil Phase

In a pharmaceutical emulsion oil phase is important due to its stability to solubilize the desired quantity of the lipophilic active compounds for transport through the intestinal lymphatic system. The various oils of vegetable origin (soybean, sesame, peanut, safflower, etc.) are acceptable if purified properly. Refined hydrocarbons such as light liquid paraffin, squalene, as well as esters of fatty acids (ethyl oleate and isopropyl myristate) have also been used in double emulsions. As a general rule, mineral oils produced more stable multiple emulsions (w/o/w) than those produced from vegetable oils. The order of decreasing stability and percentage entrapment has been found to be light liquid paraffin > squalene > sesame oil > maize or peanut oil.

5. Phase Volume

It is very important to have proper order of phase addition. For the formulation of stable multiple emulsion, dispersed phase should be added slowly into the continuous phase. Therefore the optimal internal phase volume ratio (22-50%) that can be utilized for the emulsion formulation to ensure good stability upon storage. Also very high phase volume ratio is 70-90% had also been reported to formulate a stable multiple emulsion**.**

6. Nature of entrapped material

In nature of entrapped material other components, such as electrolytes, proteins, or sugars needs to be considered, along with the nature of drug, i.e. hydrophilic or lipophilic, in formulating W/O/W emulsion. If oil phase of W/O/W emulsion is under the influence of osmotic gradient, then it acts semipermeable membrane between two aqueous phases, which results into passage of water across oil phase. If osmotic pressure is higher in internal aqueous phase, water may pass to this phase resulting in swelling of internal droplets, which may burst to release contents.

7. Added stabilizing components

In order to improve stability of multiple emulsion stabilizers are added which include gelling or viscosity increasing agents added to internal 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 O/W interface (e.g., 1-3% cetyl alcohol) and gelling agents for oil phase (e.g., 1-5% aluminium monostearate).

8. Shear/Agitation

Very high and low shear rate drastically affect stability of emulsion system hence shearing/agitation time should be optimized. High shear causes disruption of multiple oil droplets which results into instability of system due to tremendous increase in surface area. Generally, high agitation speed is used for primary emulsification and low speed is used for secondary emulsification. Due to higher shear stress there is incorporation of air and excessive frothing which results into loss of surfactant at water-air interface while low shear does not reduce size of globules. Thus high shear combined with air lead to instability of multiple emulsions.

9. Temperature

Temperature is a critical parameter during emulsion formation and needs to be precisely controlled. Rise in temperature augments the lipophilic character of the hydrophilic emulsifier, as it tends to precipitate. At 700^0C primary emulsion formulation take place whereas for multiple emulsion preparation it is kept at 100 ^{0}C.

10. Rheology

Rheological properties of emulsions are influenced by number of factors, including nature of continuous phase, phase volume ratio, and to lesser extent by particle size distribution.

11. Effect of Lipophilic Emulsifier

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 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.

Methods of Preparation

Multiple emulsions can be prepared by the re-emulsification of a primary emulsion or they can be produced when an emulsion inverts from one type to another, for example W/O

to O/W. The O/W emulsions have small size of internal dispersed phase therefore; it is not used in therapeutics.

Phase inversion technique or single step technique

This method involves the addition of an aqueous phase containing the hydrophilic emulsifier (Tween 80/Sodium Dodcedyl Sulphate) to an oil phase consisted of liquid paraffin and containing liophillic emulsifier (Span 80). 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 5 ml/min, while the pin mixer rotates steadily at 88 rpm at room temperature. When volume fraction of the aqueous solution 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 [22-27].

Two-step emulsification

Multiple emulsions are usually formed by a two-step emulsification process using conventional rotor-stator or high pressure valve homogenizers. The primary W/O or O/W emulsion is prepared under high-shear conditions to obtain small inner droplets, while the secondary emulsification step is carried out with less shear to avoid rupture of the liquid membrane between the innermost and outermost phase. However, the second step often results in highly polydisperse outer drops (if homogenizing conditions are too mild) or in small encapsulation efficiency (if homogenization is too intensive [28-32].

Membrane emulsification technique

In this method particle size of the resulting emulsion can be controlled with proper selection of porous glass membrane. The relation between membrane pore size and particle size of W/O/W emulsion exhibits good correlation as described by the following equation:

$Y = 5.03X + 0.19$

Where, X is the pore size, Y is particle size of the multiple emulsions [33-36].

Evaluation of multiple emulsions [37-44]

1. Average Globule Size and Size Distribution

Calibrated ocular and stage micrometer can be utilized for globule size determinations. Based on this technique, multiple emulsions can be classified as coarse (>3 μm diameter), fine (1-3 μm diameter) and micro-multiple emulsion (<1 μm diameter). Bright field micrographs have been used to characterize internal droplet of multiple emulsions. Various other techniques like Coulter counter, freeze fracture electron microscopy and SEM is used to determine average globule size and size distribution of multiple emulsions**.**

2. Area of interfaces

Average globule diameter can be used in calculation of total area of interface using the formula: $S = 6/D$ Where, S = Total area of interface (sq.cm) D = Diameter of globules (cm).

3. Number of globules

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

No. of globules/mm3 = $\frac{No.\ of globules \times Dilution \times 4000}{No of small squares counted.}$

4. Rheological evaluation

Viscosity of multiple emulsions can be measured by Brookfield rotational Viscometer. Samples are sheared for one min at 100 rpm, using an appropriate spindle and readings are taken after equilibrium of indicator dial. Interfacial film strength can be evaluated by interfacial rheology measurements, i.e. elasticity of W/O and O/W components of W/O/W multiple emulsions and these data may relate to emulsion stability.

5. Zeta potential

Zeta potential measurements are pivotal in designing of surface modified or ligand anchored multiple emulsions. It can be calculated by using Smoluchowski equation from mobility and electrophoretic velocity of dispersed globules using Zeta-potentiometer, calculated by the formula:

$\zeta = 4\pi\eta\mu\ \varepsilon E \times 103$

Where, ζ = Zeta potential (mV) η = Viscosity of the dispersion medium (poise) μ =Migration velocity (cm/s) International Journal of Advances in Pharmaceutics 4 (6)2015 100ε = Dielectric constant of the dispersion medium E = Potential gradient (Voltage applied**)**

6. Percentage drug entrapment

Percentage drug entrapment or active moiety in multiple emulsions is generally determined using dialysis, centrifugation, filtration and conductivity measurements. Recently an internal tracer/marker was used to evaluate entrapment of impermeable marker molecule contained in inner aqueous phase of W/O/W emulsion.

Applications of multiple emulsions [45-54]

Multiple Emulsion are finding immense use because of their vesicular structure with innermost phase closely similar to that of liposomal vesicles and the selective permeability characteristics of liquid membrane.

Controlled and sustained drug delivery

The basic potential of multiple emulsion in clinical therapeutics is in the prolonged and controlled release of drugs. In both systems drug contained in innermost phase partitions through several phases prior to release at the site of absorption and the rate of release is governed by its ability to diffuse through various phases and cross interfacial barriers.

Targeting bioactives

An important prerequisite for success in application of pharmacologically active agents is site specificity. This is especially applicable to cancer chemotherapy in which supply of cytotoxic drugs into non-diseased tissues led to serious side effects. The administration of lipid vehicle

(O/W, W/O or W/O/W) systems intramuscularly or intraperitoneally results in emulsion droplets reaching lymphatic system and regional lymph nodes.

Vaccine adjuvants
The vaccines contribute to both humoral as well as cell-mediated immune responses in protection against the infection. It was concluded that multiple emulsion-based vaccine could be successfully used in effective control of hemorrhagic septicemia.

Local Immuno suppression
A potential approach to avoid complication of systemic immuno suppression and simultaneously enhance immuno suppressive efficacy is to deliver immuno suppressive agents locally to the site of target organs. W/O/W multiple emulsion has been developed for the delivery of immuno suppressant**.**

Absorption enhancement via GIT
The various drugs have been incorporated in Multiple Emulsions for the enhancement of the increase of oral bioavailability from the stomach

Delivery of proteins and peptides
Multiple emulsions are unique in that a true liquid phase is maintained separate from an external aqueous phase. This may be especially important for bioactive molecules that cannot be appropriately stabilized in solid state. The separation of aqueous phases enables highly specialized environments, conducive to protein activity.

Oxygen delivery system
A multiple emulsion of aqueous oxygen carrying material in oil in outer aqueous phase is suitable for provision of oxygen for oxygen transfer processes. Haemoglobin 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.

Enzyme Immobilization
Enzymatic conversion of water insoluble, highly lipophilic substrates, such as steroids, can be carried out in multiple emulsions. Immobilized enzyme retains catalytic activity and recovered by simple mechanical destruction of liquid membrane. It is used mainly in kidney diseases.

Drug overdose treatment
Multiple emulsion can be utilized for the over-dosage treatment by utilizing the difference in pH.

Cosmetics and healthcare
The basis of most cosmetics and toiletries is an emulsion of either type O/W or W/O. These are also used for moisturizing, nutritive and protective action, when applied in forms of sunscreens, hand creams, makeup cleansers, shaving creams, antiperspirants etc. Use of stable multiple emulsion of O1/W/O2 has been reported as sun protection or makeup formulation**.**

Herbal Drugs
The formulation of multiple emulsion of herbal drugs strengthens the stability of the hydrolyzed materials, improve the penetrability of drugs to the skin and mucous and reduce the drugs stimulus to tissues.

Taste masking of drugs
Multiple emulsions has been employed for taste masking of drugs like chlorpromazine HCl and Chloroquine. By dissolving drug in inner aqueous phase of W/O/W emulsion under conditions of good shelf stability the formulation could be designed to release drug via oil phase in the presence of gastric fluid.

Food Industry
Sensitive food materials and flavors can be encapsulated in W/O/W emulsions.

Agrochemical
The multiple emulsion has been successfully applied to the agriculture products and the multiple emulsion are relatively stable even on storage at room temperature and for 30 days.

Diabetes
The formulation of s/o/w emulsion for oral administration of insulin was studied for their hypoglycemic properties.

Discussion
Multiple Emulsions have emerged as a delivery system which is simple, relatively cheaper, stable, amenable to controlled delivery, targeting and commercially a viable proposition. Natural constituents such as the protein, polisaccharides, starch, xanthan gum, agar gum are becoming widely acceptable for the formulation owing to the increasing health concerns. They also enhance the stability of emulsion formulations. Multiple emulsion have generally poor stability characteristics but if is well controlled would enhance its marketability and more stable systems can be created through the use of added complexing, gelling and polymeric agents. This study revealed that Multiple Emulsion can be optimized for good stability and higher entrapment efficiency by optimizing different formulations variables like type & proportion of primary & secondary emulsifier.

Conclusion
The Multiple Emulsions are largely advantageous to be formulated for human consumption. Various beneficial active compounds especially for use in pharmaceutical, nutraceutical and food industry were successfully formulated by multiple emulsion. It is one of the superior drug delivery systems for the enhancement of the various characteristics of the drugs like bioavailability, taste, release rate etc. As well as multiple emulsion is one of the advanced drug delivery systems in which advances include various novel formulations for betterment of the drug administration and improvement in the palatability of drug by incorporating them into various formulations. It also has a remarkable degree of biocompatibility, completely biodegradable, hydrophilic and lipophilic drugs can be entrapped, protection from inactivation by the endogenous factors etc. so it can be used in many pharmaceutical applications.

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