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MICRO-EMULSION: AN OVERVIEW

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ABSTRACT

Micro-emulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a co-surfactant with a droplet size usually in the range of 20-200 nm. They can be classified as oil-in-water (o/w), water-in-oil (w/o) or bi-continuous systems depending on their structure and are characterized by ultra low interfacial tension between oil and water phases. These adaptable delivery systems provide protection against oxidation, enzymatic hydrolysis and improve the solubilization of lipophilic drugs and hence enhance their bioavailability. Micro-emulsion formulated with suitable excipients may also prove to be suitable vehicles for delivery of labile (peptide) and poorly soluble drugs. Micro-emulsions have higher solubilising capacities than simple micellar solution and their thermodynamic stability offers advantages over unstable dispersions, such as emulsions, suspensions because they can be manufactured with little energy input and have a long shelf life, the enormous interfacial areas associated with micro-emulsions would influence the transport properties of drug, an important factor in targeted and sustained drug delivery through ophthalmic, dental, pulmonary, vaginal and topical routes.

Keywords: Micro-emulsion, Thermodynamic stability, co-surfactant, phase behavior study, Targeted delivery.

INTRODUCTION

Our exclusive reliance on traditional dosage form is being currently overcomed by the novel carrier systems. Recent day's micro-emulsion is a novel drug carrier system. The micro-emulsion was first introduced by Hoar and Schulman in 1943[1]. Micro-emulsions are clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant [2]. The aqueous phase may contain salt(s) and/or other ingredients, and the "oil" may actually be a complex mixture of different hydrocarbons and olefins. In contrast to ordinary emulsions, micro-emulsions form upon simple mixing of the components and do not require the high shear conditions generally used in the formation of ordinary emulsions. Micro-emulsion has low viscosity with Newtonian behavior and is highly flexible. The three basic types of micro-emulsions are direct (oil dispersed in water, o/w), reversed (water dispersed in oil, w/o) and bi-continuous.

Successful delivery of drugs has always remained a challenge to the drug delivery field, since approximately 40% of the new drug candidates have poor water solubility, and thus topical delivery is frequently associated with implications of low bioavailability.

Micro-emulsions have attracted considerable amount of interest as potential drug delivery vehicles, largely due to their simplicity of preparation, clarity and ability to be filtered and incorporate a wide range of drugs of varying solubility. Oil-in-water (o/w) micro-emulsion is the most suitable formulation, which is expected to increase the solubility by dissolving compounds with low water solubility into an oil phase. They can also enhance bioavailability by reducing the droplet size (20-200 nm), and hence increase the rate of absorption due to surfactant-induced permeability changes [3]. There is growing recognition of the potential benefits of micro-emulsions in the field of cosmetics and other topical dosage forms in addition to drug delivery. They can be used to optimize drug targeting without a concomitant increase in systemic absorption.

The chemistry of micro-emulsions is at an incredibly exciting stage of development. The advent of systems that are easy to handle allows those without specialist knowledge of the field to use them for the first time. Because of its versatility and thermodynamic stability, the micro-emulsion systems find potential applications in pharmaceutical, oil recovery, as food additives and as reaction media, etc [4].

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Table 1: Comparison of Micro-emulsion with Conventional Emulsion [3]

S. No.	Property	Micro-emulsion	Emulsion
1	Appearance	Transparent (or translucent)	Cloudy
2	Optical Isotropy	Isotropic	Anisotropic
3	Interfacial tension	Ultra low	High
4	Microstructure	Dynamic (interface is continuously and spontaneously fluctuating)	Static
5	Droplet size	20-200nm	> 500 nm
6	Stability	Thermodynamically stable, long shelf-life	Thermodynamically unstable (kinetically stable),will eventually phase separate
7	Phases	Monophasic	Biphasic
8	Preparation	Facile preparation, relatively lower cost for commercial production	Require a large input of energy, higher cost
9	Viscosity	Low viscosity with Newtonian behavior	Higher viscosity

ADVANTAGES OF MICRO-EMULSION

Micro-emulsions exhibit several advantages over conventional dosage form,

- Micro-emulsions are thermodynamically stable systems exactly opposite to emulsions and stability allows for self-emulsification of the system whose properties are not dependent on the process followed.
- Micro-emulsions act as 'super solvents' of drug. Both hydrophilic & lipophilic drugs can be delivered by micro-emulsion.
- As compared to emulsion or suspension, the mean diameter of micro-emulsion droplets is below 0.22 m and hence such systems can be sterilized by filtration.
- Because of thermodynamic stability, micro-emulsions are easy to prepare and hence no significant energy contribution is required for the preparation.
- Micro-emulsions have low viscosity as compared to other emulsions.
- The use of micro-emulsions as delivery systems can improve the efficacy of drug as the total dose is reduced thereby minimizing the side-effects.
- The formation of micro-emulsions is reversible. These may become unstable at low temperature & as temperature is brought to stability range, micro-emulsions are reformed.
- Provides protection from hydrolysis and oxidation as drug in oil phase in O/W micro-emulsion is not exposed to attack by water and air [5, 6].
- Helpful in taste masking.
- Micro-emulsions have advantages over both colloidal systems under investigation and conventional emulsions, suspensions and micellar solutions and may provide alternative drug carriers.
- They are promising delivery systems which allow sustained or controlled drug release for percutaneous, peroral, topical, transdermal, ocular and parenteral administration of medicaments [7].

DISADVANTAGES OF MICRO-EMULSION

- Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
- Limited solubilizing capacity for high-melting substances.
- The surfactant must be nontoxic for using pharmaceutical applications.
- Micro-emulsion stability is influenced by environmental parameters such as temperature and pH. These parameters change upon micro-emulsion delivery to patients.
- For unique dosage preparation in gelatin capsules, it may produce softening or hardening effect on capsule shell, so for long term storage it is undesirable [8-10].

TYPES OF MICRO-EMULSION

Micro-emulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating [11].

Wisnor defined the four general types of micro-emulsion

Wisnor Type I (O/W): With two phases, the lower (o/w) micro-emulsion phases in equilibrium with the upper excess oil.

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Wisnor Type II (W/O): With two phases, the upper micro-emulsion phase (w/o) micro-emulsion phases in equilibrium with lower excess water.
Wisnor Type III (B.C.): With three phases, middle micro-emulsion phase (o/w plus w/o, called bicontinous) in equilibrium with upper excess oil and lower excess water.
Wisnor Type IV (Isotropic micellar solution): In single phase, with oil, water and surfactant homogenously mixed.
Type I and II are two-phase system while Type III and IV are three-phase system [12].

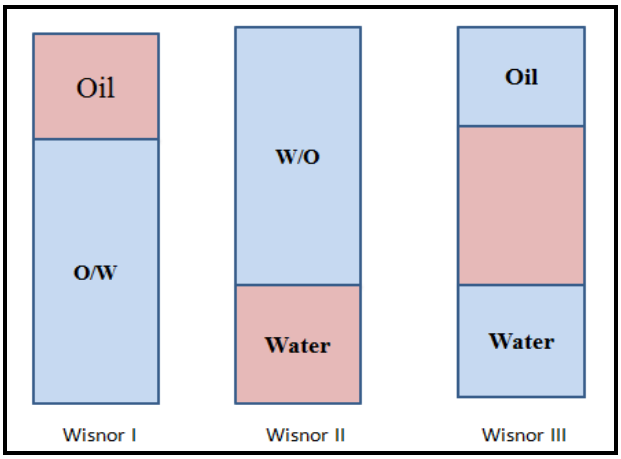

Figure 1: Types of emulsion

Interconversions among the above mentioned phases can be achieved by varying the proportions of constituents. Phase transitions are brought about by increasing either electrolyte concentration (in the case of ionic surfactants) or temperature (for non-ionics). Non-ionic surfactants form water-oil micro-emulsions (and emulsions) with a high temperature sensitivity. In particular, there is a specific phase inversion temperature (PIT) and the film curvature change from positive to negative. This critical point was defined by Shinoda et al. [13].

COMPONENTS OF MICRO-EMULSION FORMULATIONS

The micro-emulsion formulation is specific to the nature of the oil/surfactant pair, the surfactant concentration and oil/surfactant ratio, the concentration and nature of co-surfactant and surfactant/co-surfactant ratio and the temperature. These important discoveries were further supported by the fact that only very specific combinations of pharmaceutical excipients led to efficient systems [14, 15].
The main components of micro-emulsion system are:
1) Oil phase
2) Surfactant
3) Co-surfactant

1. Oil Phase

The selection of oil depends on mainly the solubility of drug. The drug should be highly soluble in oil-surfactant system, since the most of drugs are soluble in the O/W micro-emulsion. The solubility criteria may enhance the release of drug from system, increases the concentration gradient and enhances the penetration of drug through various biological membranes. A large number of oils and surfactants are available which can be used as components of micro-emulsion systems but their toxicity, irritation potential and unclear mechanism of action limit their use. One must choose materials that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range that will result in mild and non-aggressive micro-emulsions. The emphasis is, therefore, on the use of generally regarded as safe (GRAS) excipients.

Table 2: Various Oils and required HLB value

CLASS	Required HLB
Vegetable oils	6
Silicon oil	8-12
Petroleum oil	10
Ester emollients	12
Fatty acids and alcohols	14-15

The oil chain length affects the curvature of monolayer. Increasing the length of oil chain causes more positive curvature which causes the less penetration of surfactant tail region [16].

2. Surfactant

Surfactants are molecules that typically contain a polar head group and an apolar tail. They are surface-active and microstructure-forming molecules with a strong chemical dipole. They are mainly of two types,

- Ionic (cationic or anionic)
- Non ionic (zwitterionic)

The surfactant molecules can arrange themselves in a variety of shapes. They can form spherical micelles, rod-shaped micelles, a hexagonal phase (consisting of rod-shaped micelles), lamellar (sheet) phases, reverse micelles, or hexagonal reverse micelles.

The hydrophilic-lipophilic balance (HLB) of the surfactant can be taken into account to try to rationalize the surfactant's behaviour. It is generally accepted that a surfactant with HLB from 3-6 will favour the formation of water-inoil (w/o) micro-emulsions, where as surfactants with HLB from 8-18 are preferred for oil-in water (o/w) micro-emulsions. It must be noted, though, that micro-emulsions are only obtained under certain carefully defined conditions, and the HLB of the surfactant can only be used as a starting point in the selection of components that will form a micro-emulsion.

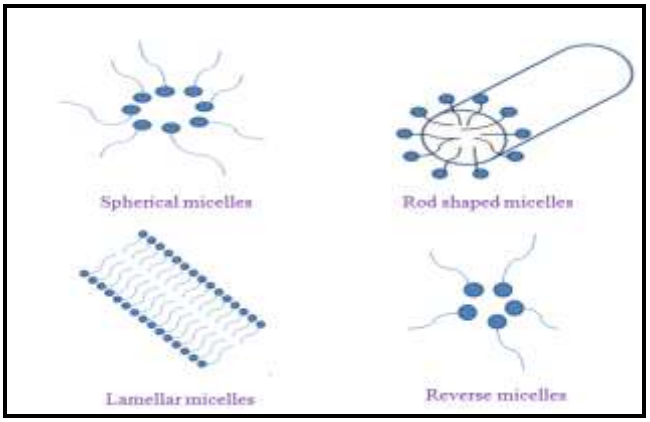

Figure 2: General Structures of micelles formed by ionic surfactants

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Ionic surfactants can be cationic, anionic, or zwitterionic. Cationic surfactants generally fall into the class of quaternary ammonium alkyl salts. Alkyl ammonium halides and tetra alkyl ammonium halides are the most numerous in this class. Alkyl ammonium halides are excellent hydrogen bond donors and interact strongly with water. The most well known examples from the cationic surfactant class are hexadecyl trimethyl-ammonium bromide (CTAB) and didodcecyl ammonium bromide (DDAB). Although less numerous, phosphorous can be quaternarized with alkyl groups to create alkyl phosphonium cationic surfactants as well [17]. Dioctyl sodium sulfo-succinate (DOSS) is the most widely studied anionic surfactant. It has twin tails and is a particularly good stabilizer of w/o micro-emulsion. Zwitterionic surfactant contains positive and negative charged groups. Phospholipids, such as lecithin, are common zwitterionic surfactants. They have certain advantages over other surfactants such as biocompatibility, nontoxic [18].

Non ionic

Non ionic surfactant differs from ionic surfactant as these does not contains any charge and no electrostatic force on the head group [17]**.** Ethoxylated alcohols are the most common nonionic surfactants. These alcohols contain a wide ranging degree of ethoxylation, where ethylene oxide is added to fatty acids to make them more water-soluble. They are considered "amphiphiles", with oil loving hydrocarbon tail group and water loving ethoxylated alcohol group [19]. Examples of non-ionic surfactants include polyoxy ethylene surfactants, such as Brij 35, or sugar esters, such as sorbitan mono oleate (Span 80). Polyoxy ethylene sorbitan mono oleate (Tween 80) and polyoxy ethylene-sorbitan mono laurate (Tween 20) appear safe and acceptable for oral and parenteral use [1, 20].

Table 3: A series of non ionic surfactant

HLB	Non ionic surfactant
HLB2	8% SPAN®80/ 92% SPAN 85
HLB4	88% SPAN80/ 12% SPAN 85
HLB6	83% SPAN 80/ 17% TWEEN® 80
HLB8	65% SPAN 80/ 35% TWEEN 80
HLB10	46% SPAN 80/ 54% TWEEN 80
HLB12	28% SPAN 80/ 72% TWEEN 80
HLB14	9% SPAN 80/ 91% TWEEN 80
HLB16	60% TWEEN 20 / 40% TWEEN 80

3. Co-surfactant

Sometimes the use of surfactant alone may not lead to the effective formation of micro-emulsion and micro-emulsion forming regions. To prepare an optimum micro-emulsion, sometimes there is need of addition of second surfactant with low molecular weight amphiphile such as alcohol derivatives. Co-surfactants increase the fluidity of hydrocarbon chain of primary surfactants.

Co-surfactants help to further reduce the surface tension and fluidize the surfactant film, which increases the entropy of the system leading to its thermodynamic stability. Co-surfactants increase the flexibility of the surfactant film around the micro-emulsion droplet. Short and medium chain alcohols, such as butanol, pentanol, ethanol, iso-propanol, or propylene glycol, are commonly added as co-surfactants. But the use of co-surfactant in excess quantity may cause the irritation to the biological system. And uses of some of them are reported as toxic.

The effect co-surfactant on the formation of micro-emulsion of amino-silicon oil is reviewed. Fig.3A, the pseudo-ternary phase diagram of amino-silicon oil, water, and complex surfactant, shows that to form micro-emulsion of amino-silicon a high concentration of surfactant is requires. While Fig.3B, shows that the use of cosurfactant1-pentanol is helpful to form a stable and transparent amino-silicon oil micro-emulsion. Acting a co-surfactant, 1- pentanol can influence the formation of micro-emulsion by both interfacial and bulk effects [21].

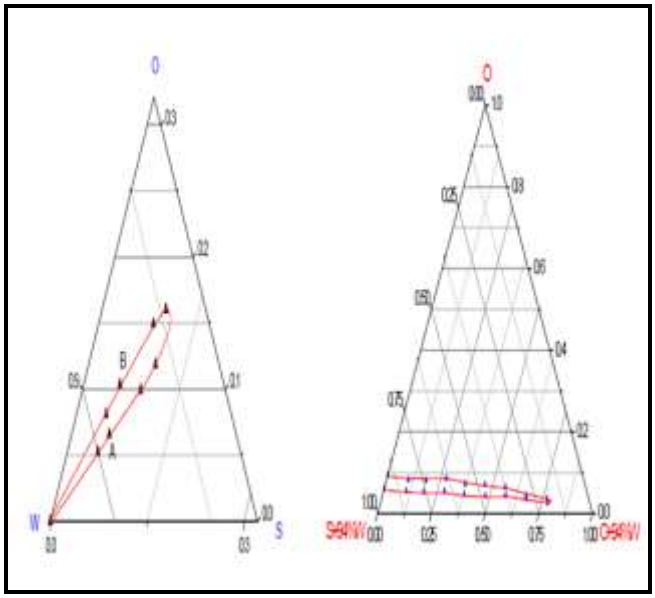

Figure 3: (A) The part pseudo-ternary phase diagram micro emulsion amino silicone oil (O), water (W) and complex surfactants (S) (B) The part pseudo-ternary phase diagram micro emulsion amino silicone oil (O), water (W), complex surfactants (S), and 1-pentanol (C)

THEORIES

The formation of micro-emulsion can be illustrate by three theories,

- Interfacial or mixed film theories
- Solubilisation theory
- Thermodynamic treatment.

The surfactant lowers the interfacial tension between droplets of oil which gives the amount of free energy of micro-emulsion formulation and change in the entropy can be given by equation [22].

$\delta G_f = \gamma \delta A - T\, \delta S$

Where, G_f = free energy of formation,

γ = surface tension of the oil-water interface,

A = change in interfacial area on micro emulsification,

δS = change in entropy of the system which is effectively the dispersion entropy,

T = temperature.

It should be noted that when a micro-emulsion is formed the change in A is very large due to the large number of very small droplets formed. In order for a micro-emulsion to be formed (transient) negative value of was required, it is recognized that while value of is positive at all times, it is very small and it is offset by the entropic component [23].

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The formulation of micro-emulsion can be described by following representation-

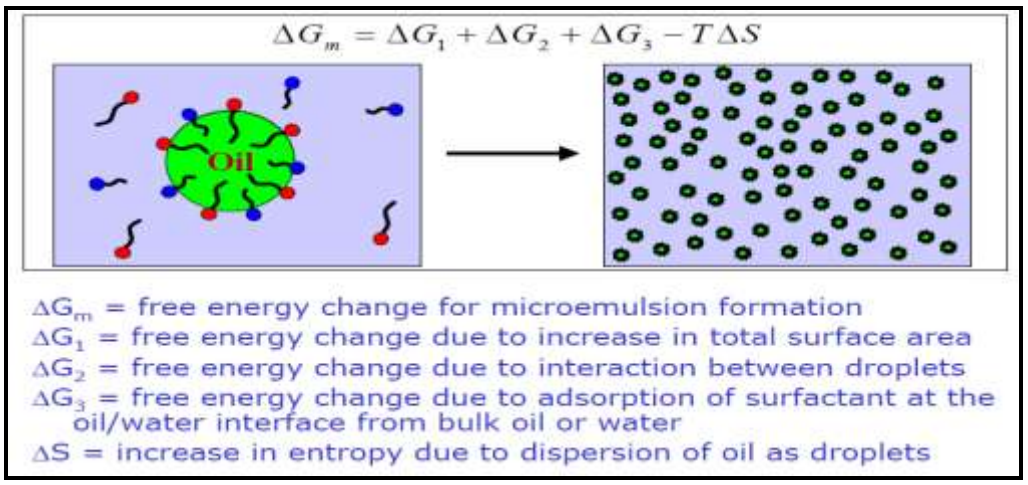

Figure 4: Formation of micro-emulsion

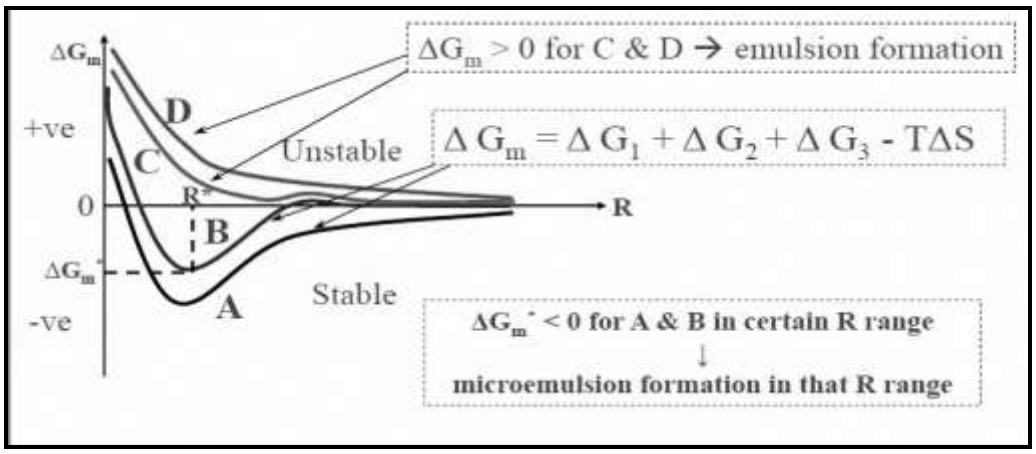

Figure 5: Schematic representations showing the stability of micro-emulsion

PHASE BEHAVIOUR STUDY

The effect of pressure, temperature, concentration on the formation of micro-emulsion can be illustrated with the help of phase diagram or pseudoternary diagram. This diagram contains triangle of which three corners are represented by water, oil, and surfactant: cosurfactant ratio. Construction of phase diagram is time consuming and not every combination of concentration produce micro-emulsion. A series of binary composition is formed and is titrated with the third phase. The end point is determined by careful observation of turbidity and phase separation. A hypothetical phase diagram is shown in figure 6 consist of existing fields with inverse micelle formation in W/O type of micro-emulsion. From the end point composition of titrated samples, the mass percent composition of the components like oil, surfactant, and water is calculated and is plotted on triangular coordinates to construct pseudoternary phase diagrams.

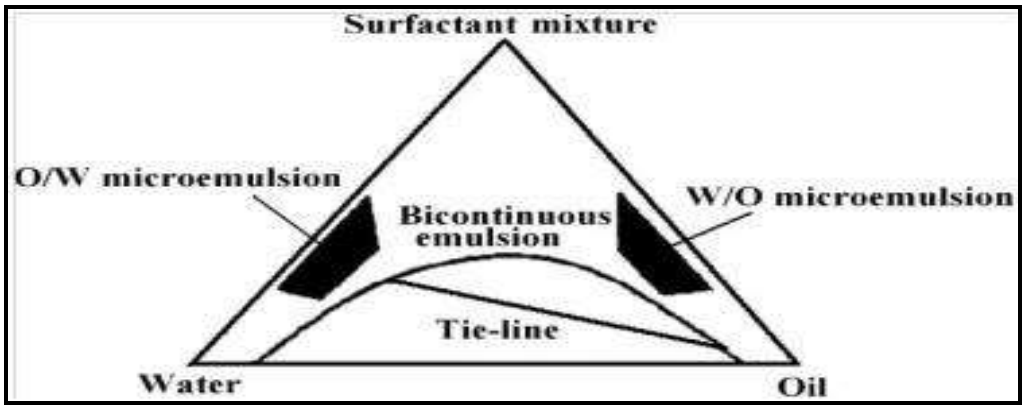

Figure 6: Pseudoternary phase diagram

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Each corner of triangle represents the 100% concentration of particular component. The co-surfactant is also strains impose a physical limit on the length of time. Amphiphilic with an affinity for both the oil and systems can be left to equilibrate and consequently aqueous phases. Co-surfactant mainly contains non ionic surfactant, alcohols, alkyl amines, and alkanediols [24].

FACTORS AFFECTING THE MICRO-EMULSION

The formation of micro-emulsion will depend on the following factors are:

a. Packing ratio

The HLB of surfactant determines the type of micro-emulsion through its influence on molecular packing and film curvature.

b. Property of surfactant, oil phase and temperature

The type of micro-emulsion depends on the nature of surfactant. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these groups, which are a measure of the differential tendency of water to swell head group and oil to swell the tail area, are important for specific formulation when estimating the surfactant HLB in a particular system. When a high concentration of the surfactant is used or when the surfactant is in presence of salt, degree of dissociation of polar groups becomes lesser and resulting system may be w/o type. Diluting with water may increase dissociation and leads to an o/w system. Ionic surfactants are strongly influenced by temperature. It mainly causes increased surfactant counter-ion dissociation. The oil component also influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chains oils penetrate the lipophilic group region to a great extent and results in increased negative curvature. Temperature is extremely important in determining the effective head group size of nonionic surfactants. At low temperature, they are hydrophilic and form normal o/w system. At higher temperature, they are lipophilic and form w/o systems. At an intermediate temperature, micro-emulsion coexists with excess water and oil phases and forms bicontinuous structure.

c. The chain length, type and nature of co-surfactant

Alcohols are widely used as a co-surfactant in micro-emulsions. Addition of shorter chain co-surfactant gives positive curvature effect as alcohol swells the head region more than tail region so, it becomes more hydrophilic and o/w type is favoured, while longer chain co-surfactant favours w/o type w/o type by alcohol swelling more in chain region than head region [25].

FORMULATION CONSIDERATIONS

The challenges in formulating topical micro-emulsions are:
1. Determining systems that are non-toxic, non-irritating, non-comedogenic and non-sensitizing.
2. Formulating cosmetically elegant micro-emulsions.

The micro-emulsion formulation must have low allergic potential, good physiological compatibility and high biocompatibility [26].

METHOD OF PREPARATION

1. Phase Titration Method

Micro-emulsions are prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components are mixed. Micro-emulsions are formed along with various association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) depending on the chemical composition and concentration of each component. The understanding of their phase equilibrium and demarcation of the phase boundaries are essential aspects of the study.

As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo ternary phase diagram is often constructed to find the different zones including micro-emulsion zone, in which each corner of the diagram represents 100% of the particular component Fig. (6).The region can be separated into w/o or o/w micro-emulsion by simply considering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the metastable systems are not included. The methodology has been comprehensively discussed by Shafiq-un-Nabi et al [17].

2. Phase Inversion Method

Phase inversion of micro-emulsions occurs upon addition of excess of the dispersed phase or in response to temperature. During phase inversion drastic physical changes occur including changes in particle size that can affect drug release both in vivo and in vitro. These methods make use of changing the spontaneous curvature of the surfactant. For non-ionic surfactants, this can be achieved by changing the temperature of the system, forcing a transition from an o/w micro-emulsion at low temperatures to a w/o micro-emulsion at higher temperatures (transitional phase inversion). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, promoting the formation of finely dispersed oil droplets. This method is referred to as phase inversion temperature (PIT) method.

Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone.

Additionally, a transition in the spontaneous radius of curvature can be obtained by changing the water volume fraction. By successively adding water into oil, initially water droplets are formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a w/o micro-emulsion to an o/w micro-emulsion at the inversion locus. Short-chain surfactants form flexible mono-layers at the o/w interface resulting in a bicontinuous micro-emulsion at the inversion point [26].

CHARACTERIZATION OF MICRO-EMULSION

Micro-emulsions have been characterized using a wide variety of techniques. The characterization of micro-emulsions is a difficult task due to their complexity, variety of structures and components involved in these systems, as well as the limitations associated with each technique but such knowledge is essential for their successful commercial exploitation. Therefore, complementary studies using a combination of techniques are usually required to obtain a comprehensive view of the physicochemical properties and structure of micro-

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emulsions. At the macroscopic level viscosity, conductivity and dielectric methods provides useful information [27].

(A) Phase Behavior Studies

Phase behavior studies are essential for the study of surfactant system determined by using phase diagram that provide information on the boundaries of the different phases as a function of composition variables and temperatures, and, more important, structural organization can be also inferred. Phase behaviour studies also allow comparison of the efficiency of different surfactants for a given application. In the phase behaviour studies, simple measurement and equipments are required. The boundaries of one-phase region can be assessed easily by visual observation of samples of known composition. The main drawback is long equilibrium time required for multiphase region, especially if liquid crystalline phase is involved.

Other useful means and ways of representing the phase behaviour are to keep the concentration of one component or the ratio of two components constant. As the number of components increases, the number of experiments needed to define the complete phase behaviour becomes extraordinary large and the representation of phase behaviour becomes extremely complex. One approach to characterize these multicomponent systems is by means of pseudo-ternary diagrams that combine more than one component in the vertices of the ternary diagram [28].

(B) Scattering Techniques for Micro-emulsions Characterization

Small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and static as well as dynamic light scattering are widely applied techniques in the study of micro-emulsions. These methods are very valuable for obtaining quantitative informations on the size, shape and dynamics of the components. The major drawback of this technique is the dilution of the sample required for the reduction of interparticular interaction. This dilution can modify the structure and the composition of the pseudophases. Nevertheless, successful determinations have been carried out using a dilution technique that maintains the identity of droplets. Small-angle X-ray scattering techniques have been used to obtain information on droplet size and shape[29] .

Static light scattering techniques have also been widely used to determine micro-emulsion droplet size and shape. In these experiments the intensity of scattered light is generally measured at various angles and for different concentrations of micro-emulsion droplets. Dynamic light scattering, which is also referred as photon correlation spectroscopy (PCS), is used to analyze the fluctuations in the intensity of scattering by the droplets due to Brownian motion. The self-correlation is measured that gives information on dynamics of the system [30].

(C) Nuclear Magnetic Resonance Studies

The structure and dynamics of micro-emulsions can be studied by using nuclear magnetic resonance techniques. Self-diffusion measurements using different tracer techniques, generally radio labeling, supply information on the mobility of the components. The Fourier transform pulsed-gradient spin-echo (FT-PGSE) technique uses the magnetic gradient on the samples and it allows simultaneous and rapid determination of the self-diffusion coefficients (in the range of 10^{-9} to 10^{-12} m^2s^{-1}) of many components [31].

(D) Interfacial Tension: The formation and the properties of micro-emulsion can be studied by measuring the interfacial tension. Ultra low values of interfacial tension are correlated with phase behaviour, particularly the existence of surfactant phase or middle-phase micro-emulsions in equilibrium with aqueous and oil phases. Spinning-drop apparatus can be used to measure the ultra low interfacial tension. Interfacial tensions are derived form the measurement of the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with high-density phase [32].

(E) Viscosity Measurements

Viscosity measurements can indicate the presence of rod-like or worm-like reverse micelle. Viscosity measurements as a function of volume fraction have been used to determine the hydrodynamic radius of droplets, as well as interaction between droplets and deviations from spherical shape by fitting the results to appropriate models (e.g. for micro-emulsions showing Newtonian behaviour, Einstein's equation for the relative viscosity can be used to calculate the hydrodynamic volume of the particles) [33] .

(F) Simple tests

Dye Solubilization: A water soluble dye is solubilized within the aqueous phase of the W/O globule but is dispersible in the O/W globule. A oil soluble dye is solubilized within the oil phase of the O/W globule but is dispersible in the W/O globule.

Dilutability Test: O/W micro-emulsions are dilutable with water whereas W/O are not and undergo phase inversion into O/W micro-emulsion.

Droplet Size Determination: Size of droplet is measured by photon correlation spectroscopy (PSC) with Zetasizer. All measurements are carried out at scattering angle of 90° and 25°C temperatures. Prior to measurement, micro-emulsion is diluted in two steps with pure water then it is filtered through a 0.22um filter just before it is added to cuvette. In first step it is diluted with equal amount of water. In second step the mixture is further diluted to appropriate concentration for the measurement. That depends on droplet size (Usually diluted 100-200 times).

Refractive Index and Percent Transmittance: Refractive index and percent transmittance proved the transparency of formulation. The refractive index of the system is measured by refractometer by placing drop of solution on slide and it compare with water (1.333). The percent transmittance of the system is measured at particular wavelength using UV spectrophotometer keeping distilled water as blank. If refractive index of system is similar to the refractive index of water (1.333) and formulation have percent transmittance > 99%, then formulation have transparent nature.

Permeation Study: Now a day's micro-emulsion is arising as a topical drug delivery system. Micro-emulsion enhances the penetration of drug through various biological membranes. The permeation of drug through skin by micro-emulsion is mainly studied by using various diffusion cells such Franz's diffusion cell, Keshary- chein diffusion cell. For this mainly animal skin is used.

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Turbidity Measurement: This is to identify efficient self-emulsification by establishing whether the dispersion reaches equilibrium rapidly and in a reproducible time [24].

(G) Conductance Measurement

O/W micro-emulsion where the external phase is water are highly conducting whereas W/O are not, since water is the internal or dispersal phase. To determine the nature of the continuous phase and to detect phase inversion phenomena, the electrical conductivity measurements are highly useful. A sharp increase in conductivity in certain W/O micro-emulsion systems was observed at low volume fractions and such behaviour was interpreted as an indication of a 'percolative behaviour' or exchange of ions between droplets before the formation of bi-continuous structures. Dielectric measurements are a powerful means of probing both structural and dynamic features of micro-emulsion systems [34].

(H) Electron Microscope Characterization

Transmission Electron Microscopy (TEM) is the most important technique for the study of microstructures of micro-emulsions because it directly produces images at high resolution and it can capture any co-existent structure and micro-structural transitions. There are two variations of the TEM technique for fluid samples [35].

1. The cryo-TEM analyses in which samples are directly visualized after fast freeze and freeze fructose in the cold microscope.
2. The Freeze Fracture TEM technique in which a replica of the specimen is images under RT conditions.

(I) Stability Studies

The stability of the micro-emulsion has been assessed by conducting long term stability study and accelerated stability studies. In long term stability study, the system is kept at room temperature, refrigeration temperature (4-8 ˚C) and elevated temperature (50±2 ˚C). Over the time period, micro-emulsion systems are evaluated for their size, zeta potential, assay, pH, viscosity and conductivity. On long term study, the activation energy for the system and shelf life of the system may be calculated as like other conventional delivery system. Accelerated stability studies are the essential tools to study the thermodynamic stability of micro-emulsions. It can be done by centrifugation, heating/cooling cycle and freeze/thaw cycles [36].

APPLICATIONS OF MICRO-EMULSION

Micro-emulsions have shown great potential in the area of pharmaceuticals. They can be applied to a wide variety of dosage forms including oral, topical, ocular, parenteral, periodontal, buccal, and nasal formulations. Oral delivery offers the opportunity to deliver peptide and protein drugs. Usually when peptides and proteins are delivered orally, they are degraded in the GI and are not therapeutically active. Delivery of these molecules using micro-emulsions, though, increases their bioavailability.

Oral Delivery

The development of the effective oral delivery systems has always been the main goal because drug efficacy can be severely limited by instability or poor solubility in the gastrointestinal fluid. Biopharmaceutical Classification System (BCS) is a useful guidance by US FDA and it takes into account contributions of three major factors, dissolution, solubility, and intestinal permeability, which affect oral drug absorption. According to the BCS, drug substances are classified as follows:

Class I - High Permeability, High Solubility
Class II - High Permeability, Low Solubility
Class III - Low Permeability, High Solubility
Class IV - Low Permeability, Low Solubility

Knowledge of BCS help the formulation scientists to develop a dosage form based on mechanistic, rather than empirical approaches. Drug substances are considered highly soluble when the largest dose of a compound is soluble in <250 mL of water over a range of pH from 1.0 to 7.5 and highly permeable when they show >90 percent absorption of the administered dose [37-38].

In contrast, compounds with solubility below 0.1mg/mL provide significant dissolution related problems, and often, even compounds with solubility below 10mg/mL present difficulties related to solubilization during formulation. A major technological hurdle for routine clinical use of many drugs is their very poor solubility in water. Micro Emulsions have the potential to enhance the solubilization of the poorly soluble drugs and overcome the dissolution related bioavailability problems. This is particularly important for the BCS class II or class IV drugs. The successful formulation of such drugs is highly dependent on the performance of the formulated product. Micro-emulsions act as super solvent of these drugs and can be optimized to ensure consistent bioavailability. In addition, they can be used for the delivery of hydrophilic drugs including macromolecules such as proteins and peptides. This is due to the existence of polar, nonpolar and interfacial domains which allow encapsulation of drugs with varying solubility. Moreover, these systems have been reported to protect the incorporated drugs against oxidation, enzymatic degradation and enhance the membrane permeability [39,40].

a) Bioavailability Enhancement of Poorly Water Soluble Drugs.
b) Controlled and Sustained Release of Drugs

Parenteral Delivery

The formulation of lipophilic and hydrophobic drugs into parenteral dosage forms has proven to be difficult. O/w micro-emulsions are beneficial in the parenteral delivery of sparingly soluble drugs where the administration of suspension is not desirable. They provide a means of obtaining relatively high concentration of these drugs which usually requires frequent administration. Other advantages are that they exhibit a higher physical stability in plasma than liposomes or other vesicles and the internal oil phase is more resistant against drug leaching [41].

Several sparingly soluble drugs have been formulated into o/w micro-emulsion for parenteral delivery. Micro-emulsions can also be used as intravenous delivery systems for the fat soluble vitamins and lipids in parenteral nutrition [42, 43].

Micro-emulsions are generally not dilutable with aqueous fluids, such as certain bodily fluids and buffer solutions, and form emulsions upon contacting such fluids. Various micro-emulsions are also sensitive to temperature and are not stable outside of room temperature conditions. US Patent provided drug delivery compositions in both concentrated and diluted forms for use as vehicles in the

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administration of various active agents. The concentrated drug delivery compositions were formulated with a phospholipid component, a component selected from propylene glycol or certain polyethylene glycol compounds, a high HLB surfactant, and the drug component, with water and/or an optional oil component. The concentrated drug delivery compositions could be diluted with an aqueous fluid to form an o/w micro-emulsion. These o/w micro-emulsions were characterized by their small particle size and their wide range of temperature stability, typically from about -20°- 50°C. They could be administered by intravenous, intraarterial, intrathecal, intraperitoneal, intraocular, intraarticular, intramuscular or subcutaneous injection [44].

Ophthalmic Delivery

In conventional ophthalmic dosage forms, water soluble drugs are delivered in aqueous solution while water insoluble drugs are formulated as suspensions or ointments. Low corneal bioavailability and lack of efficiency in the posterior segment of ocular tissue are some of the serious drawbacks of these systems. Recent research efforts have therefore focused on the development of new and more effective delivery systems. Micro-emulsions have emerged as a promising dosage form for ocular use [45].

Nasal Delivery

Micro-emulsions are now being studied as a delivery system to enhance uptake across nasal mucosa. Addition of a mucoadhesive polymer helps in prolonging the residence time on the mucosa. Nasal route for administration of diazepam might be a useful approach for the rapid onset of action during the emergency treatment of status epilepticus [46].

Periodontal Delivery

Periodontal disease is a collective term for a number of progressive oral pathological afflictions like inflammation and degeneration of the gums, periodontal ligaments, cementum and its supporting bone. It is a major cause of tooth loss. The invention of Brodin et al., included a novel pharmaceutical composition comprising local anaesthetizing oil form, surfactant, water and optionally a taste masking agent [47]. The composition was in the form of an emulsion or micro-emulsion and had thermo-reversible gelling properties i.e. it was less viscous at room temperature than after introduction onto a mucous membrane of a patient. The surfactant in the formulation imparted the thermo-reversible gelling properties.

Drug Targeting

Drug targeting has evolved as the most desirable but elusive goal in drug delivery. By altering the pharmacokinetics and bio-distribution of drugs and restricting their action to the targeted tissue increased drug efficacy with concomitant reduction of their toxic effects can be achieved. Drug targeting to diseased cells can be achieved by exploiting the presence of various receptors, antigens/proteins on the cell membrane which may be uniquely expressed or over expressed in these cells as compared to the normal cells. Specific antibodies to the surface proteins and ligands for the receptors can be used to target specific cells. Submicron size range of these systems confers excellent opportunities to overcome the physiological barriers and enables efficient cellular uptake followed by intracellular internalization.

Micro-emulsion mainly used now a day in 1) Cellular targeting 2) Brain targeting 3) Tumour targeting [48].

Antifungal

Antifungal agents e.g. miconazole, ketoconazole, and itraconazole being lipophilic in nature have been formulated as micro-emulsions to impart to them the advantages like ease of preparation due to spontaneous formation, thermodynamic stability, transparent and elegant appearance, increased drug loading, enhanced penetration through the biological membranes, increased bioavailability compared to conventional dosage forms [49,50].

Antiviral

A study was done to investigate and evaluate micro-emulsion and micro-emulsion-based hydrogel as a topical delivery system for penciclovir in comparison with a commercial cream. The results of permeation test in vivo in mice showed that as compared with the commercial cream, micro-emulsion based hydrogel and micro-emulsion could significantly increase the permeation of penciclovir into both epidermis and dermis. Stability tests showed that micro-emulsion-based hydrogel stored at 4 °C for 3 months had no significant change in physicochemical properties. Skin irritation test in rabbits demonstrated that single application or multiple applications of micro-emulsion-based hydrogel did not cause any erythema or edema. Thus, it can be that micro-emulsion based hydrogel could be a promising vehicle for topical delivery of penciclovir [51].

Antiacne

Novel drug delivery strategies like micro-emulsions can play a pivotal role in improving the topical delivery of antiacne agents by enhancing their dermal localization with a concomitant reduction in their side effects [52].

Micro-emulsions of azelaic acid, a bioactive molecule used in many skin disorders, prepared using its monosodium salt (AZA-Na) has been evaluated as delivery vehicles. Dialysis membrane experiments showed decreasing permeability to AZA-Na, and this was related to its partition at the micro-emulsion interface. The results suggested that micro-emulsions containing AZA-Na could be used to optimize drug targeting in acne treatment [53].

Antioxidants

Antioxidants have been used in dermatological and cosmetic products because of their property of scavenging and destroying aggressive oxidizing agents and free radicals that are involved in various skin conditions.

In animals, topical application of alpha-tocopherol has shown to exert photo protective effects by reducing the number of sunburn cells, UV induced damage and inhibiting photo carcinogenesis. An o/w or w/o micro-emulsion of vitamin E delivered the vitamin predominantly to the epidermis avoiding accumulation in organs other than the skin. The cream or lotion prepared with the same amount of vitamin results in excessive accumulation in the organs [54].

Newer studies show that combined applications of various antioxidants can increase their potency as compared with a single antioxidant alone. Branka Roz manet al have developed a temperature-sensitive micro-emulsion gel as

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an effective and safe delivery system suitable for simultaneous topical application of a hydrophilic vitamin C and a lipophilic vitamin E. By changing water content of liquid o/w micro-emulsion, a gel like micro-emulsion with temperature sensitive rheological properties was formed. The temperature driven changes in its microstructure were confirmed by rotational rheometry, viscosity measurements and droplet size determination. The release studies have shown that the vitamin release at skin temperature from

Gels like micro-emulsion were comparable to those from o/w micro-emulsion and were much faster and more complete than from o/w micro-emulsion conventionally thickened with polymer (carbomer) [55].

Spermicidal

O.D'Cruz described a formulation of novel gel micro-emulsions (GM) as nontoxic, dual-function intra-vaginal spermicides, which could be used as delivery vehicles for lipophilic drug substances targeting sexually transmitted pathogens. These GMs comprising oil-in water micro-emulsion and polymeric hydrogels were designed to solubilize lipophilic antiviral/antimicrobial agents and exhibited rapid spermicidal activity in human semen and was compared against nonoxynol-9-based detergent spermicide (Gynol II). Spermicidal GM has shown unprecedented potential as dual function microbicidal contraceptives to improve vaginal bioavailability of poorly soluble antimicrobial agents without causing significant vaginal damage [56].

Cosmetics

There is growing recognition of the potential benefits of micro-emulsions in the field of cosmetics in addition to drug delivery. They are now being widely investigated for preparing personal care products with superior features such as having improved product efficiency, stability, appearance and minimal irritation. They are well suited for the preparation of various cosmetic products such as moisturizing and soothing agents, sunscreens, antiperspirants, body cleansing agents, hair conditioners and after shave formulations. Micro-emulsions are also suitable in perfumery so as to minimize the quantity of organic solvents [57].

Other Applications

- Micro-emulsion in enhanced oil recovery.
- Micro-emulsions as fuels.
- Micro-emulsions as lubricants, cutting oils and corrosion inhibitors.
- Micro-emulsions as coatings and textile finishing.
- Micro-emulsions in detergency.
- Micro-emulsions in cosmetics.
- Micro-emulsions in agrochemicals.
- Micro-emulsions in food.
- Micro-emulsions in environmental remediation and detoxification.
- Microporous media synthesis (micro-emulsion gel technique).
- Micro-emulsions in analytical applications.
- Micro-emulsions as liquid membranes.
- Novel crystalline colloidal arrays as chemical sensor materials [58].

CONCLUSION

Micro-emulsions have been shown to be able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability. Furthermore, it has proven possible to formulate preparations suitable for most routes of administration. There is still however a considerable amount of fundamental work characterizing the physico-chemical behaviour of micro-emulsions that needs to be performed before they can live up to their potential as multipurpose drug delivery vehicles.

The availability of efficient, non toxic surfactants and co-surfactant now makes them a very attractive and feasible option to overcome the bioavailability problems frequently encountered in the development of modern drugs.

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