ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 5, 2015

ISSN - 0974-2441

Review Article

MICROEMULSIONS: PLATFORM FOR IMPROVEMENT OF SOLUBILITY AND DISSOLUTION OF POORLY SOLUBLE DRUGS

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Received: 07 April 2015, Revised and Accepted: 12 May 2015

ABSTRACT

This study reviews that solubilization of lipophilic drugs with low aqueous solubility has been a major trust area in recent years. It can be seen that there is a real and continuing need for the development of effective drug delivery systems for poorly water-soluble drugs to enhance their absorption and bioavailability. One such approach might be pharmaceutical microemulsions as they have emerged as potential solubility enhancing technologies. Microemulsion system has considerable potential to act as a drug delivery vehicle by incorporating a wide range of drug molecules. Microemulsion has got advantage like excellent thermodynamic stability, high drug solubilization capacity, improved oral bioavailability and protection against enzymatic hydrolysis. This review focuses on the basic concept, formulation, characterization, and recent advances in microemulsions as novel drug delivery system.

Keywords: Microemulsion, Lipophilicity, Solubilization, Bioavailability, Phase behavior.

INTRODUCTION

Successful oral 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 oral delivery is frequently associated with implications of low bioavailability. Today, a large percent of the new chemical entities in addition to many existing drugs often show poor solubilization behavior, which lead to poor oral bioavailability with wide intra- and inter- subject variation and present formulators with considerable technical challenges. The selection of an appropriate dosage form is critical because a dosage form with poor drug delivery can make a useful drug worthless.

In the past decades, ongoing efforts have been made to develop systems or drug carrier capable of delivering the active molecules specifically to the intended target organ while increasing the therapeutic efficacy and the therapeutic efficacy relies on the bioavailability of the active molecule. Bioavailability is defined as the rate and extent to which the active drug is absorbed from a dosage form and becomes available at the site of drug action. Bioavailability has important clinical implications as both pharmacologic and toxic effects are proportional to both dose and bioavailability. Bioavailability depends on several factors, the important ones being, drug solubility in an aqueous environment and drug permeability through lipophilic membranes [1].

Many approaches have been meticulously explored to improve the oral bioavailability of poorly soluble drugs including particle size reduction (micronization or nanosizing), complexation with cyclodextrins, salt formation, solubilization based on cosolvents, surfactants, etc. Modification of the physicochemical properties, such as by salt formation and particle size reduction of the drug may improve the dissolution rate of the drug but these methods are not always practical, for example, salt formation of neutral compounds is not feasible. Moreover, the salts of weak acid and weak base may convert back to their original acid or base forms and lead to aggregation in the gastrointestinal (GI) tract. Particle size reduction may lead to build up of static charges, present handling difficulties and is not desirable where poor wettability are experienced for very fine powders. To overcome these limitations, various other formulation strategies have been attempted such as use of cyclodextrins, nanoparticles, solid dispersions and permeation enhancers. Indeed, in some selected cases, these approaches have been successful [2]. Some of the approaches have been highlighted in Fig. 1.

Microemulsions have been widely studied to enhance the bioavailability of the poorly soluble drugs. These systems are known to enhance drug solubility, increase the stability, and modify drug release [3,4]. Microemulsion allows the incorporation of hydrophilic as well as lipophilic compound depending on their internal structure [5]. These aggregates have been described as reservoir systems, which allow slow release of drugs, thus providing prolonged effects and avoiding high concentration in the blood [6-8]. They offer a cost effective approach in such cases. Microemulsions have very low surface tension and small droplet size which results in high absorption and permeation. Interest in these versatile carriers is increasing and their applications have been diversified to various administration routes in addition to the conventional oral route. This can be attributed to their unique solubilization properties and thermodynamic stability which has drawn attention for their use as novel vehicles for drug delivery. The results obtained have been indeed very promising.

Microemulsion formulation made the bioavailability and plasma concentration profiles of the drug more reproducible which is clinically important in the case of drugs showing serious adverse effects. This is a significant step forward in the delivery of poorly soluble drugs. Microemulsion systems are also now being increasingly investigated for transdermal, ocular, nasal, pulmonary, vaginal, rectal and intravenous drug delivery [9].

The microemulsion concept was introduced as early as the 1940s by Hoar and Schulman who generated a clear single-phase solution by titrating a milky emulsion with hexanol [10]. Schulman *et al.* subsequently coined the term microemulsion [11], and it has since been defined and indeed redefined on many occasions. Microemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a cosurfactant with a droplet size usually in the range of 20-200 nm. These homogeneous systems, which can be prepared over a wide range of surfactant concentration and oil to water ratio, are all fluids of low viscosity. Microemulsions as drug delivery tool show favorable properties like thermodynamic stability (long shelf-life), easy

formation (zero interfacial tension and almost spontaneous formation), optical isotropy, ability to be sterilized by filtration, high surface area (high solubilization capacity) and very small droplet size. The small droplets also provide better adherence to membranes and transport drug molecules in a controlled fashion.

In practice, the key difference between emulsions and microemulsions are that the former, whilst they may exhibit excellent kinetic stability, are fundamentally thermodynamically unstable and will eventually phase separate. Another important difference concerns their appearance; emulsions are cloudy while microemulsions are clear or translucent. In addition, there are distinct differences in their method of preparation, since emulsions require a large input of energy while microemulsions do not. The latter point has obvious implications when considering the relative cost of commercial production of the two types of system (Table 1) [12].

POTENTIAL ADVANTAGES OF MICROEMULSIONS [13,14]

Microemulsions are potential drug carrier systems for various routes of administration. These are having advantages when compare to the other dosage forms.

- These are thermodynamically stable and require minimum energy for formation
- Ease of manufacturing and scale-up
- This system is reckoned advantageous because of its wide applications in colloidal drug delivery systems for the purpose of drug targeting and controlled release
- Improvement in oral bioavailability: Microemulsion is a new approach to improve the water solubility and ultimately, bioavailability of lipophilic drugs by solubilized and micro emulsified form in GI tract and increase in specific surface area enables more efficient drug transport through intestinal aqueous boundary layer and through the absorptive brush border membrane leading to improved bioavailability
- Improvement of bioavailability of antifungal and anti-inflammatory drug by topical micro emulsion
- Reduction in inter-subject and intra-subject variability: There are several drugs which show large inter- and intra-subject variation in absorption, leading to decrease performance of drug and patient non-compliance. Microemulsion drug delivery system is a proven approach to overcome inter and intra-subject variation
- As solid dosage form for oral administration: Microemulsion can be converted into the various solid dosage forms by adsorbing onto the solid surface
- Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT
- Reduction of food effects: Microemulsion is independent of food and that microemulsion offer reproducibility of plasma profile
- No influence of lipid digestion process: Microemulsions are not necessarily digested before the drug is absorbed, as they present the drug in microemulsified form, which can easily penetrate the mucin and water unstirred layer.

ADVANTAGES OF MICROEMULSION BASED SYSTEMS

- Microemulsions exhibit several advantages as a drug delivery system
- Microemulsions are thermodynamically stable system, and the stability allows self-emulsification of the system whose properties are not dependent on the process followed
- Microemulsions act as super solvents of the drug. They can solubilize hydrophilic and lipophilic drugs including drugs that are relatively insoluble in both aqueous and hydrophobic solvents. This is due to the existence of microdomains of different polarity within the same single-phase solution
- The dispersed phase, lipophilic or hydrophilic (oil-in-water, O/W, or water-in-oil, W/O microemulsions) can behave as a potential reservoir of lipophilic or hydrophilic drugs, respectively. The drug partitions between dispersed and continuous phase, and when the system comes into contact with a semi-permeable membrane, the

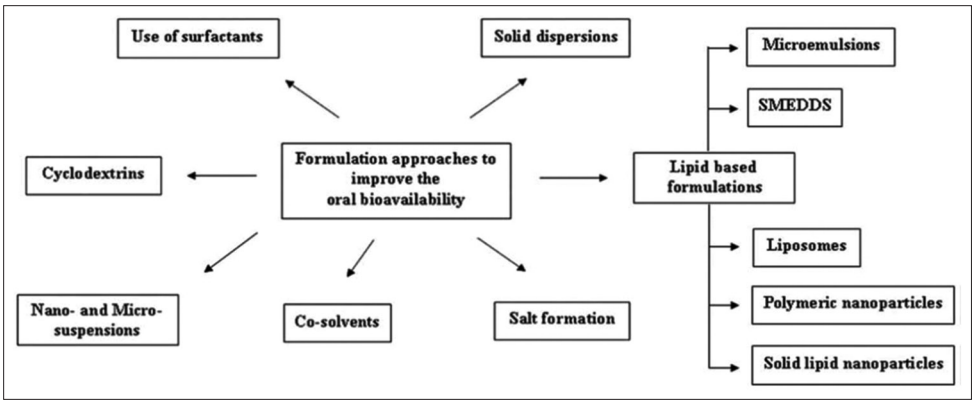

Fig. 1: Some of the formulation approaches to improve the oral bioavailability of poorly water soluble drugs

Table 1: Differences between microemulsion and macroemulsion

Serial number	Property	Microemulsion	Macroemulsion
1	Appearance	Transparent	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	20-200 nm	>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	Higher viscosity

drug can be transported through the barrier. Drug release with pseudo-zero-order kinetics can be obtained, depending on the volume of the dispersed phase, the partition of the drug and the transport rate of the drug

- The mean diameter of droplets in microemulsions is below 0.22 mm; they can be sterilized by filtration. The small size of droplet in microemulsions e.g. below 100 nm, yields very large interfacial area, from which the drug can quickly be released into external phase when absorption (*in vitro* or *in vivo*) takes place, maintaining the concentration in the external phase close to initial levels.
- Same microemulsions can carry both lipophilic and hydrophilic drugs
- Because of thermodynamic stability, microemulsions are easy to prepare and require no significant energy contribution during preparation. Microemulsions have low viscosity compared to other emulsions
- The use of microemulsion as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects
- The formation of microemulsion is reversible. They may become unstable at low or high temperature but when the temperature returns to the stability range, the microemulsion reforms.

STRUCTURE OF MICROEMULSION

Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating [15]. Structurally, they are divided into oil-in-water (o/w), water in-oil (w/o) and bicontinuous microemulsions. In w/o microemulsion, water droplets are dispersed in the continuous oil phase while o/w microemulsion is formed when oil droplets are dispersed in the continuous aqueous phase. In systems where the amounts of water and oil are similar, a bicontinuous microemulsion may result. In all three types of microemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants. The mixture of oil, water and surfactants is able to form a wide variety of structures and phases depending upon the proportions of the components. The flexibility of the surfactant film is an important factor in this regard. A flexible surfactant film will enable the existence of several different structures like droplet like shapes, aggregates and bicontinuous structures, and therefore broaden the range of microemulsion existence. The microemulsion structure is shown in Fig. 2.

The schematic representation given in Fig. 3 gives an indication of a few of the wide variety of possible self-association structures that surfactants can form in the presence of water, oil or combinations of all three.

The possible structural representations of the three different types of microemulsions which are most probably likely to be formed depending on their individual composition are represented in Fig. 4. It can be seen that while the oil-in-water (o/w) and water-in-oil composition (w/o) droplets are represented as spheres, they may be also asymmetric in shape, frequently looking like the shape of a prolate ellipsoid. The likely presence of o/w microemulsion droplets is a feature in microemulsions where the volume fraction of oil is low. Conversely, w/o droplets are likely when the volume fraction of water is low and in systems where the amount of water and oil are similar, a bicontinuous microemulsion may result.

THEORIES OF MICROEMULSION FORMULATIONS [16,17]

Historically, three different approaches have been proposed to explain microemulsion formation and the stability aspects:
(i) Interfacial or mixed film theories,
(ii) Solubilization theories and
(iii) Thermodynamic treatments.

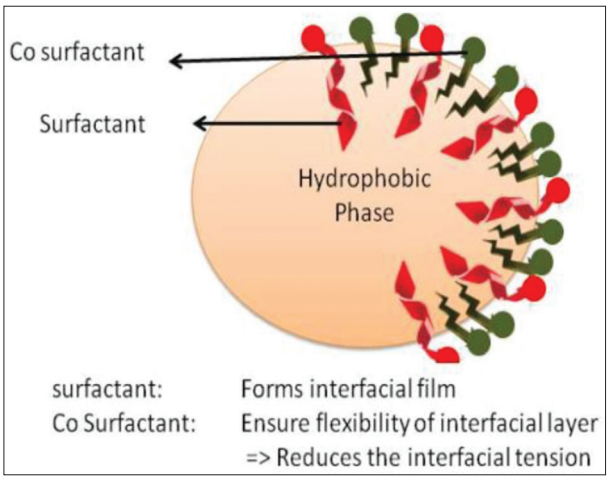

Fig. 2: Microemulsion structure

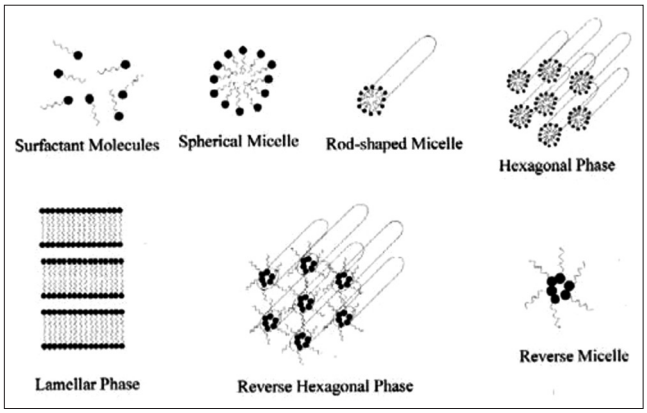

Fig. 3: Schematic representation of the most commonly encountered self-association structures in water, oil or a combination

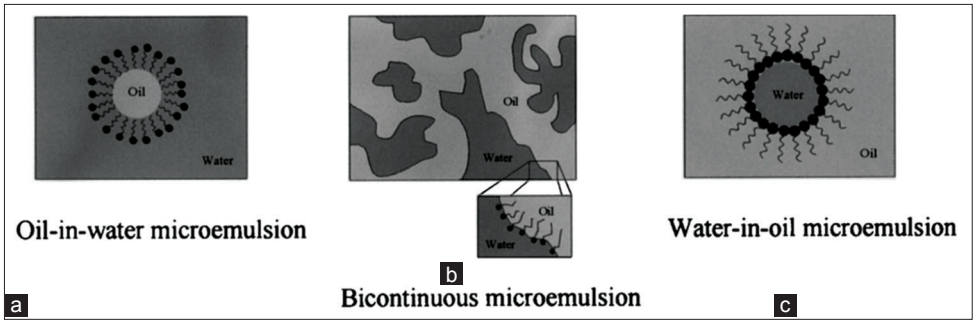

Fig. 4: Schematic representation of the three most commonly encountered microemulsion microstructures: (a) oil-in-water, (b) bicontinuous, and (c) water-in-oil microemulsion

The important features of the microemulsion are thermodynamic stability, optical transparency, large overall interfacial area (about 100 m^2/ml), variety of structures, low interfacial tension and increased solubilization of oil/water dispersed phase. Microemulsion requires more surfactant than emulsion to stabilize a large overall interfacial area.

The interfacial tension between the oil and water can be lowered by the addition and adsorption of surfactant. When the surfactant concentration is increased further, if lowers the interfacial tension till Critical Micelle Concentration (CMC). The micellar formation commences beyond this concentration of surfactant. This negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. The large interfacial area formed may divide itself into a large number of closed shells around small droplets of either oil in water or water in oil and further decrease the free energy of the system. In many cases, the interfacial tension is not yet ultra-low when the CMC is reached.

It has been studied and observed by Schulman *et al.* that the addition of a cosurfactant (medium sized alcohol or amine) to the system results in virtually zero interfacial tension. The further addition of a surfactant (where, interfacial tension (γ) is zero) leads to negative interfacial tension.

Interfacial/mixed film theories

The relatively large entropy of mixing of droplets and continuous medium explains the spontaneous formation of microemulsion. Schulman emphasized the importance of the interfacial film. They considered that the spontaneous formation of microemulsion droplets was due to the formation of a complex film at the oil-water interface by the surfactant and co-surfactant. This caused a reduction in oil-water interfacial tension to very low values (from close to zero to negative), which is represented by following equation.

$$\gamma i = \gamma o/w - \pi i \quad (1)$$

Where,

γo/w = Oil-water interfacial tension without the film present
πi = Spreading pressure
γi = Interfacial tension

Mechanism of curvature of a duplex film

The interfacial film should be curved to form small droplets to explain both the stability of the system and bending of the interface. A flat duplex film would be under stress because of the difference in tension and spreading of pressure on either side of it. Reduction of this tension gradient by equalizing the two surface tensions is the driving force for the film curvature. Both sides of the interface expand spontaneously with penetration of oil and co surfactant until the pressures become equal. The side with higher tension would be concave and would envelop the liquid on that side, making it an internal phase. It is generally easier to expand the oil side of an interface than the waterside and hence W/O microemulsion can be formed easily than O/W microemulsion.

Solubilization theories

Microemulsions are considered to be thermodynamically stable monophasic solution of water-swollen (W/O) or oil swollen (O/W) spherical micelles. The relationship between reverse micelles and W/O microemulsion can be studied with the help of phase diagrams. The inverse micelle region of ternary system i.e. water, pentanol and sodium dodecyl sulfate (SDS) is composed of water solubilized reverse micelles of SDS in pentanol. Addition of O-xylene up to 50% gives rise to transparent W/O region containing a maximum of 28% water with 5% pentanol and 6% surfactant (i.e. microemulsions). The quaternary phase diagram constructed on adding p-xylene shows relationship of these areas to the isotropic inverse micellar phase. These four component systems could be prepared by adding hydrocarbon directly to the inverse micellar phase by titration. Thus, the system mainly consists of swollen inverse micelle rather than small emulsion droplets [18].

Thermodynamic theories

This theory explains the formation of microemulsion even in the absence of co surfactant. The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers the surface tension of the oil-water interface and the change in entropy of the system such that,

$$\Delta Gf = \gamma \Delta A - T\Delta S \quad (2)$$

Where,

ΔGf = Free energy of formulation
γ = Surface tension of the oil-water interface
ΔA = Change in surface area on microemulsification
ΔS = Change in entropy of the system T = Temperature

Thermodynamic theory takes into account entropy of droplets and thermal fluctuations at the interface as important parameters leading to interfacial bending instability. Originally workers proposed that in order for a microemulsion to be formed a negative value of γ was required, it is now recognized that while value of γ is positive at all times, it is very small, and is offset by the entropic component. The dominant favorable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, there are also expected to be favorable entropic contributions arising from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achiever when large reductions in surface tension are accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable. Later it was shown that accumulation of the surfactant and co-surfactant at the interface results in a decrease in chemical potential generating an additional negative free energy change called as dilution effect. This theory explained the role of co-surfactant and salt in a microemulsion formed with ionic surfactants. The co-surfactant produces an additional dilution effect and decreases interfacial tension further. The addition of salts to system containing ionic surfactants causes similar effects by shielding the electric field produced by the adsorbed ionic surfactant the adsorption of large amount of surfactant.

COMPONENTS OF MICROEMULSION FORMULATIONS

A large number of oils and surfactants are available which can be used as components of microemulsion 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 microemulsions. The emphasis is, therefore, on the use of generally regarded as safe excipients.

The main components of microemulsion system are:
1) Oil phase
2) Surfactant
3) Co-surfactant.

Oil phase

The oil represents one of the most important excipients in the formulation not only because it can solubilized the required dose of the lipophilic drug, it can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride [19].

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short

chain oils penetrate the tail group region to a greater extent than long chain alkanes, and hence swell this region to a greater extent, resulting in increased negative curvature (and reduced effective hydrophile - lipophile balance [HLB]). Saturated (for example, lauric, myristic and capric acid) and unsaturated fatty acids (for example, oleic acid, linoleic acid and linolenic acid) have penetration enhancing property of their own and they have been studied since a long time. Fatty acid esters such as ethyl or methyl esters of lauric, myristic and oleic acid have also been employed as the oil phase. Lipophilic drugs are preferably solubilized in o/w microemulsions. The main criterion for selecting the oil phase is that the drug should have high solubility in it. This will minimize the volume of the formulation to deliver the therapeutic dose of the drug in an encapsulated form.

Surfactants

The surfactant chosen must be able to lower the interfacial tension to a very small value which facilitates dispersion process during the preparation of the microemulsion and provide a flexible film that can readily deform around the droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region [20]. Surfactants used to stabilize microemulsion system may be:

i. Non-ionic,
ii. Zwitterionic,
iii. Cationic, or
iv. Anionic surfactants.

The surfactant used in microemulsion formation could be ionic or nonionic, which determines the stabilizing interactions of the hydrophilic end of the surfactant with the aqueous phase. Thus, while a non-ionic surfactant is stabilized by dipole and hydrogen bond interactions with the hydration layer of water on its hydrophilic surface, an ionic surfactant is additionally stabilized by the electrical double layer. Thus, the effect of salt concentration on the stability of an emulsion or a microemulsion is more profound in the case of ionic surfactant than non-ionic surfactants. However for pharmaceutical applications, ionic surfactants are not preferred due to toxicological concerns. Non-ionic surfactants are generally considered to be acceptable for oral ingestion, and the emergence of several successful marketed products has given the industry confidence in lipid-based products. The oral and intravenous LD_{50} values for most non-ionic surfactants are in excess of 50 g/kg and 5 g/kg respectively, so 1 g surfactant in a formulation is well-tolerated for uses in acute Oral Drug Administration.

Non-ionic surfactants in commercially available solubilized oral formulations include polyoxyl 35 castor oil (cremophor EL), polyoxyl 40 hydrogenated castor oil (cremophor RH 40), polysorbate 20 (Tween 20), polysorbate 80 (Tween 80), d-α-tocopherol polyethylene glycol 1000 succinate, Solutol HS-15, sorbitan monooleate (Span 80), polyoxyl 40 stearate, and various polyglycolyzed glycerides including Labrafil M-1944CS, Labrafil M-2125CS, Labrasol, Gellucire 44/14, etc. [21].

It is generally accepted that low HLB (3-6) surfactants are favored for the formulation of w/o microemulsion, whereas surfactants with high HLB (8-18) are preferred for the formation of o/w microemulsion. Surfactants having HLB >20 often require the presence of co-surfactants to reduce their effective HLB to a value within the range required for microemulsion formation.

Co-surfactants

In most cases, single-chain surfactants alone are unable to reduce the o/w interfacial tension sufficiently to enable a microemulsion to form. The presence of co-surfactants allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition. If a single surfactant film is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidizing groups (e.g. unsaturated bonds). Short to medium chain length alcohols (C3-C8) are commonly added as co surfactants which further reduce the interfacial tension and increase the fluidity of the interface. Typical co-surfactants are short chain alcohols (ethanol to butanol), glycols such as propylene glycol (PG), medium chain alcohols, amines or acids. The role of co-surfactant is to destroy liquid crystalline or gel structures that form in place of a microemulsion phase and co-surfactant free microemulsion in most system cannot be made except at high temperature. The role of a co-surfactant is as following [22]:

1) Increase the fluidity of the interface
2) Destroy liquid crystalline or gel structure which would prevent the formation of microemulsion
3) Adjust HLB value and spontaneous curvature of the interface by changing surfactant partitioning characteristic.

The production of an optimum microemulsion requires relatively high concentrations (generally more than 30% w/w) of surfactants. Organic solvents such as, ethanol, PG, and polyethylene glycol (PEG) are suitable for oral delivery, and they enable the dissolution of large quantities of either the hydrophilic surfactant or the drug in the lipid base. These solvents can even act as co-surfactants in microemulsion systems.

FACTORS AFFECTING FORMATION AND PHASE BEHAVIOUR OF MICROEMULSIONS

Factor affecting formation of microemulsion system [22,23]

The formation of oil or water swollen microemulsion depends on the packing ratio, property of surfactant, oil phase, temperature, the chain length, type and nature of co-surfactant.

Packing ratio

The HLB of surfactant determines the type of microemulsion through its influence on molecular packing and film curvature. The analysis of film curvature for surfactant associations leading to microemulsion formation has been called as critical packing parameter (CPP).

$$CPP = v/a * l \quad (3)$$

Where, v is the partial molar volume of the hydrophobic portion of the surfactant, a is the optimal head group area and l is the length of the surfactant tail.

If CPP has value between 0 and 1 interface curves towards water (positive curvature) and o/w systems are favored but when CPP is >1, interface curves spontaneously towards oil (negative curvature) so w/o microemulsions are favored. At zero curvature, when the HLB is balanced (p is equivalent to 1), then either bicontinuous or lamellar structures may form according to the rigidity of the film (zero curvature).

Property of surfactant, oil phase and temperature

The type of microemulsion 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, microemulsion coexists with excess water and oil phases and forms bicontinuous structure.

The chain length, type and nature of co-surfactant

Alcohols are widely used as a co-surfactant in microemulsions. 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 favored, while longer chain co-surfactant favors w/o type w/o type by alcohol swelling more in chain region than head region.

Factor affecting phase behavior

Salinity

At low salinity, the droplet size of o/w microemulsion increases. This corresponds to increase in the solubilization of oil. As salinity further increases, the system becomes bi- continuous over an intermediate salinity range. Increase in salinity leads to formation of continuous microemulsion with reduction in globule size. Further increase in salinity ultimately results in complete phase transition.

Alcohol concentration

Increasing the concentration of low molecular weight alcohol as a co surfactant leads to the phase transition from w/o to bicontinuous and ultimately to o/w type microemulsion. Exactly opposite phase transition is noticed in case of high molecular weight alcohol.

Surfactant hydrophobic chain length

The increase in length of hydrophobic chain length of the surfactant shows the change of o/w microemulsion to w/o via bi continuous phase.

pH

Change in pH influences the microemulsions containing pH sensitive surfactants. This effect is more pronounced in case of acidic or alkaline surfactants. Carboxylic acids and amines change the phase behavior from w/o to o/w by increasing the pH.

Nature of oil

Increase in the aromaticity of oil leads to phase transition from o/w to w/o and is opposite to that of increase in the oil alkane carbon number.

Ionic strength

As the ionic strength increases the system passes from o/w microemulsion in equilibrium with excess oil to the middle phase and finally to w/o microemulsion in equilibrium with excess water.

METHOD OF MICROEMULSION FORMULATION

Phase titration method (water titration method)

Microemulsions 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. Microemulsions 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 equilibria 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 microemulsion zone, in which each corner of the diagram represents 100% of the particular component Fig. 5. The region can be separated into w/o or o/w microemulsion by simply considering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the meta stable systems are not included [24].

Phase inversion method

Phase inversion of microemulsions 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 microemulsion at low temperatures to a w/o microemulsion 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 method. Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone. In addition, 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 microemulsion to an o/w microemulsion at the inversion locus. Short-chain surfactants form flexible monolayers at the o/w interface resulting in a bicontinuous microemulsion at the inversion point.

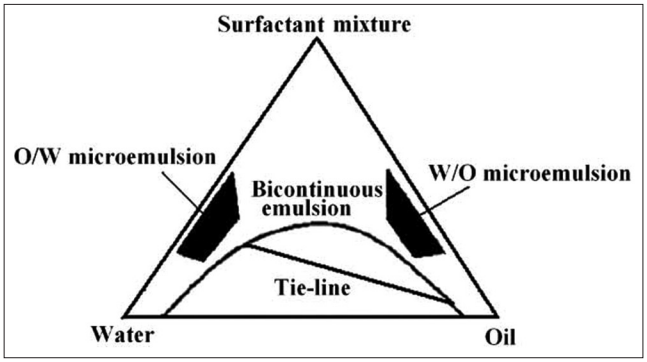

Fig. 5: Pseudoternary phase diagram of oil, water and surfactant showing microemulsion region

CONSTRUCTION OF PHASE DIAGRAM [25]

When water, oil and surfactants are mixed, microemulsion is only one of the association structures. Preparation of a stable, isotropic homogeneous, transparent, non-toxic microemulsion requires consideration of a number of variables. Construction of phase diagrams reduces a number of trials and labor. Phase diagrams help to find the microemulsion region in ternary or quaternary system and also help to determine the minimum amount of surfactant for microemulsion formation.

Pseudo-ternary phase diagrams of oil, water, and co-surfactant/ surfactants mixtures are constructed at fixed cosurfactant/surfactant weight ratios. Phase diagrams are obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of monophasic/biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the microemulsion region of existence. Fig. 6 shows hypothetical pseudo-ternary diagram at constant surfactant to co-surfactant ratio. It also shows that single phase or multiphase regions of microemulsion domains are near the center of diagram in areas containing large amounts of surfactant that is toxic. The phase behavior of surfactants, which form microemulsion in absence of co-surfactant, can be completely represented by ternary diagram.

TYPES OF MICROEMULSION SYSTEMS

Within phase regions of the ternary phase diagram, microemulsions can exist in equilibrium with excess water or oil phases. These

multiphase systems can be conveniently described using the Winsor classification [26,27] Fig. 7.

According to Winsor, there are four types of microemulsion phases exists in equilibria, these phases are referred as Winsor phases. They are;

Winsor I: The microemulsion composition corresponding to Winsor I is characterized by two phase, the lower oil/water (O/W) microemulsion phase in equilibrium with excess oil.

Winsor II: The microemulsion composition corresponding to Winsor II is characterized by very low interfacial tension and maximal solubilization of oil and water for a given quantity of surfactant. Since, in this phase, microemulsion coexists with both excess phases, no one can distinguish the dispersed phase from the continuous phase.

Winsor III: This phase comprises of three phases, middle microemulsion phase (O/W plus W/O, called bicontinuous) in equilibrium with upper excess oil and lower water.

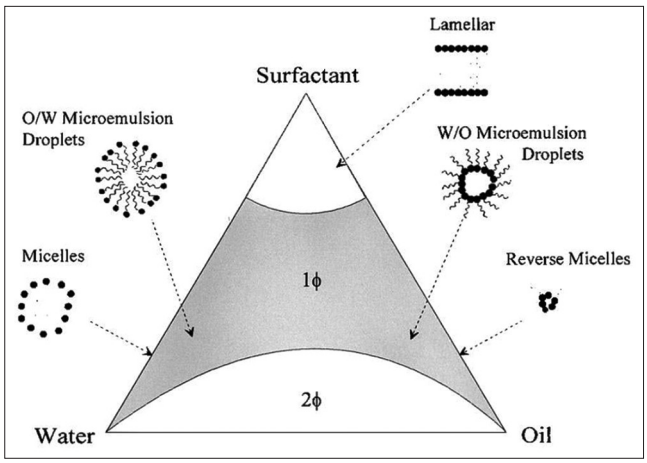

Fig. 6: A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion emulsion phases. Within the phase diagram, existence fields are shown where conventional micelles, reverse micelles or water-in-oil (w/o) microemulsions and oil-in-water microemulsions are formed along with the bicontinuous microemulsions. At very high surfactant concentrations two phase systems are observed

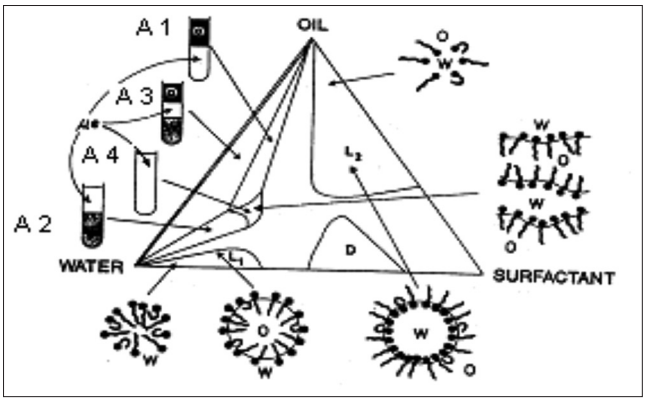

Fig. 7: Schematic ternary phase diagram of water-oil-surfactant mixtures representing Winsor classification and probable internal structures. L1, a single phase region of normal micelles or oil-in-water (O/W) microemulsion; L2, reverse micelles or water-in-oil (W/O) microemulsions; D, anisotropic lamellar liquid crystalline phase

Winsor IV: Microemulsions can be distinguished from the micelles by its inner core swollen with oil. The microemulsion structure depends on the chemical composition, temperature and concentration of the constituents.

Different surfactants stabilize different microstructures due to aggregation. This aggregation phenomenon leads to a system with minimum free energy and thermodynamic stability. Even though the spherical micelles are considered to have minimal water-hydrocarbon contact area for a given volume, the inter micellar free energy and the impossibility of the existence of voids in the hydrophobic region leads to other amphiphillic assemblies like cylinders and planes. They are organized in the form of liquid crystalline phases or liquid isotropic phases. A wide variety of surfactant molecules obeys the geometric rules embodied in the packing parameter.

CHARACTERIZATION OF MICROEMULSIONS

In contrast to their ease of production, microemulsions are very difficult to characterize principally because of their wide variety of structures. For this reason, the use of several techniques is often required in order to characterize microemulsion systems. An understanding of the properties of the vehicle is an important requirement for optimizing drug delivery. Additionally, factors affecting drug release, stability, and structure need to be understood in order to establish the potential, and also limitations of microemulsion formulations. A variety of techniques, such as nuclear magnetic resonance (NMR) spectroscopy, electrical conductivity, self-diffusion, small-angle neutron scattering, quasi-elastic light scattering, and fluorescence spectroscopy, have been employed to characterize these systems.

MICROSCOPY

Although polarizing microscopy confirms the optical isotropy of the microemulsion system, conventional optical microscopy cannot be used for studying microemulsion systems because of the small droplet size diameter which is typically <150 nm. However, transmission electron microscopy combined with freeze fracture techniques have been successfully applied for the study and characterization of microemulsions [28]. The sensitivity of microemulsion structure to temperature and the potential introduction of experimental artefacts during manipulation are of some concern with this approach. Other problems are: (1) high microemulsion vapor pressure, which is not compatible with low pressures used in microscopy, (2) electrons may induce chemical reactions, thus, altering microemulsion structure, and (3) lack of contrast between the microemulsion structure and its environment. The introduction of controlled environmental chambers as well as improvements in thermal fixation now permit very fast sample cooling rates to be achieved without crystal formation. The techniques of Cryo-transmission electron microscope (TEM) and freeze fracture-TEM, which have evolved from these advances, permit direct visualization of the microemulsion structure with fewer problems of artifactual results [29].

NMR

Self-diffusion is the random movement of a molecule in the absence of any concentration gradient, and this movement reflects the environment where the molecule is localized. If a molecule is confined in a close aggregate, such as micelles, its self-diffusion will be two or three orders of magnitude lower than the expected self-diffusion coefficient from a pure solvent. Therefore, in w/o microemulsions, the self-diffusion of water molecules is slow, whereas, the diffusion of the oil molecules is high. Conversely, for O/W microemulsions the reverse is found. In bicontinuous structures, both oil and water molecules exhibit high self-diffusion coefficients. Microemulsion structure has been characterized as using self-diffusion measurements of the components, obtained by proton Fourier transform pulse-gradient spin-echo NMR (PGSE NMR) [30].

CONDUCTIVITY AND VISCOSITY

The nature of the microemulsion and detection of phase inversion phenomena can be determined using classical rheological methods and by conductivity determination. Viscosity determination also provides useful information on how the colloidal systems may influence drug release. The likely systems present are, for example, vesicles with multilamellar layers, rod-like or worm-like reverse micelles. Water-continuous microemulsions display high conductivity values, whereas oil-continuous systems should have poor or no conductivity [31].

FLUORESCENCE SPECTROSCOPY

Fluorescence spectroscopy measures the ease of movement of the fluorescent probe molecules in the microemulsions. This is controlled by diffusion, which varies inversely with the viscosity of the medium and with the microemulsion type. In water-continuous microemulsions, the propagation of the excitation is inhibited because of the slow diffusion of the water-insoluble fluorescent (e.g. pyrene) molecules. On the other hand, oil continuous microemulsions should produce a similar excimer formation to that of the pure oil [32].

INTERFACIAL TENSION

The formation and the properties of microemulsion can be studied by measuring the interfacial tension. Ultralow values of interfacial tension are correlated with phase behavior, particularly, the existence of surfactant phase or middle-phase microemulsions in equilibrium with aqueous and oil phases. Spinning-drop apparatus can be used to measure the ultralow interfacial tension. Interfacial tensions are derived from the measurement of the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with high-density 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 microemulsion system was observed at low volume fractions and such behavior was interpreted as an indication of a "percolative behavior" or exchange of ions between droplets before the formulation of bi-continuous structures. Dielectric measurements are a powerful means of probing both structure and dynamic feature of microemulsion systems [33].

SCATTERING TECHNIQUES FOR MICROEMULSION CHARACTERIZATION

Small-angle X-ray scattering techniques have been used to obtain information on droplet size and shape. Using synchrotron radiation sources, in which sample-to-detector distances are bigger, significant improvements have been achieved. With synchrotron radiation more defined spectra are obtained and a wide range of systems can be studied, including those in which the surfactant molecules are poor X-ray scatters. Small-angle neutron scattering, however, allows selective enhancement of the scattering power of different microemulsion pseudophases by using protonated or deuterated molecules.

Static light scattering technique has also been widely used to determine microemulsion droplet size and shape. In this technique, the intensity of scattered light is generally measured at various angles and for different concentration of microemulsion droplets.

Dynamic light scattering, which is also referred as photon correlation spectroscopy, is used to analyze the fluctuations in the intensity of scattering by droplets due to Brownian motion. The self-correlation is measured that gives information on dynamics of the system. This technique allows the determination of z-average diffusion coefficients D. In the absence of inter-particle interactions, the hydrodynamic radius of the particles, can be determined from the diffusion coefficient using the Stokes-Einstein equation as follows:

$$D = kT/6\pi\eta RH \quad (4)$$

Where, k is Boltzmann constant, T is the absolute temperature and η is the viscosity of the medium, RH is the relative humidity.

MICROEMULSION AS DRUG DELIVERY CARRIER

Microemulsions have generated considerable interest over the year as potential drug delivery systems. During the last two decades, microemulsions have been promisingly used as drug delivery system for its advantages include their thermodynamic stability, optical clarity and ease of penetration. The role of microemulsion as drug delivery carriers for pharmaceutical development shall be discussed herein.

ORAL DELIVERY [34]

The development of effective oral delivery systems has always been challenging to researchers because drug efficacy can be restricted by instability or poor solubility in the GI fluid. Microemulsions have the potential to enhance the solubilization of poorly soluble drugs (particularly BCS Class II or Class IV) and overcome the dissolution related bioavailability problems. Due to the presence of polar, nonpolar and interfacial domains, hydrophilic drugs including macromolecules can be encapsulated with varying solubility. These systems have been protecting the incorporated drugs against oxidation, enzymatic degradation and enhance membrane permeability. Presently, sandimmune neoral (R) (cyclosporine A), fortovase (R) (saquinavir), norvir (R) (ritonavir) etc. are the commercially available microemulsion formulations. Microemulsion formulation can be potentially useful to improve the oral bioavailability of poorly water soluble drugs by enhancing their solubility in GI fluid.

PARENTERAL DELIVERY [35]

The formulation of parenteral dosage form of lipophilic and hydrophilic drugs has proven to be difficult. O/w microemulsions are beneficial in the parenteral delivery of sparingly soluble drugs where the administration of suspension is not required. 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 liposome's or other vehicles and the internal oil phase is more resistant against drug leaching. Several sparingly soluble drugs have been formulated into o/w microemulsion for parenteral delivery. An alternative approach was taken in which C3-C4 alcohols were replaced with parenterally acceptable cosurfactants, PEG (400)/PEG (660) 12-hydroxystearate/ethanol, while maintaining a flexible surfactant film and spontaneous curvature near zero to obtain and almost balanced middle phase microemulsion.

TOPICAL DELIVERY [36]

Topical administration of drugs can have advantages over other methods for several reasons, one of which is the avoidance of hepatic first-pass metabolism, salivary and degradation of the drug in stomach and related toxicity effects. Another is the direct delivery and targetability of the drug to affected areas of the skin or eyes. Now a day, there have been a number of studies in the area of drug penetration into the skin. They are able to incorporate both hydrophilic (5-flurouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate) and lipophilic drugs (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide) and enhance their permeation. Since formation of microemulsion formation requires high surfactant concentration, the skin irritation aspect must be considered especially when they are intended to be applied for a longer period.

OPHTHALMIC DELIVERY [37,38]

In conventional ophthalmic dosage forms, water soluble drugs are delivered in aqueous solution while water insoluble drugs are formulated as suspension or ointments. Low corneal bioavailability and lack of efficiency in the posterior segment of ocular tissue are

some of the serious problem of these systems. Recent research has been focused on the development of new and more effective delivery systems. Microemulsions have emerged as a promising dosage form for ocular use. Chloramphenicol, an antibiotic used in the treatment of trachoma and keratitis, in the common eye drops hydrolyzes easily. The microemulsion composed of Span 20, Tween 20, isopropylmyristate and water as potential drug delivery systems for eye drops was investigated. Chlroamphenicol was entrapped in the o/w microemulsion free of alcohol. The authors revealed that microemulsion formulation content much lower glycol (main hydrolysis product) than that in the commercial eye drops at the end of the accelerated experiments. Thus, a remarkable increase in the Chloramphenicol stability was observed in the microemulsion formulations. The microemulsion based dexamethasone eye drops were studied which showed better tolerability and higher bioavailability. The formulation showed greater penetration in the eye which allowed the possibility of decreasing dosing frequency and thereby improve patient compliance.

NASAL DELIVERY

Recently, microemulsions are being studied as a delivery system to enhance uptake of drug through nasal mucosa. In addition with mucoadhesive, polymer helps in prolonging residence time on the mucosa [39]. Nasal route for administration of diazepam microemulsion might be a useful approach for the rapid onset of action during the emergency treatment of status epilepticus due to better penetration and improved bioavailability [40].

DRUG TARGETING

Drug targeting to the different tissues has evolved as the most desirable goal of drug delivery. By altering pharmacokinetics and biodistribution of drugs and restricting their action to the targeted tissue increased drug efficacy with concomitant reduction of their toxic effects can be achieved. A novel microemulsion formulation for tumor targeting of lipophilic antitumor antibiotic aclainomycin A was an effective way of targeting emulsion to tumor cells [41].

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. A novel pharmaceutical composition comprising local anesthetic in oil form, surfactant, water and optionally a taste masking agent was in the form of an emulsion or microemulsion 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 thermoreversible gelling properties. Preferred surfactants were Poloxamer 188®, Poloxamer 407® and Arlatone 289®. The composition could be used as a local anesthetic for pain relief within the oral cavity in conjunction with periodontal scaling and root planning and overcame the problem with the existing topical products (jelly, ointment or spray) such as lack of efficacy due to inadequate depth of penetration, too short duration and difficulties in administration due to spread, taste etc. [42].

CELLULAR TARGETING

Nucleic acids delivered to cells are promising therapeutics. The invention of insertion of nucleic acid into a reverse micelle for cell delivery proved very promising [43]. They referred w/o microemulsions to as reverse micelles. The reverse micelle had the property to compact the nucleic acid for easier delivery. To further enhance the delivery, other molecules such as a surfactant having a disulfide bond or a polyion might be added to the nucleic acid-micelle complex. Another advantage of the invention was the use of reverse micelles for gene delivery to the cells. The micelle containing the compacted polynucleotide could be utilized as a reaction vesicle in which additional compounds such as polycation could be added to the DNA. In addition, the polynucleotide/reverse micelle system was used as a vesicle for template polymerization of the DNA or caging of the DNA in which the polycation was cross linked. Another advantage was that the micelle might be cleaved under physiological conditions involved along the transfection (process of delivering a polynucleotide to a cell) pathway. Better recovery and purification of the biomolecules could be achieved by utilizing cleavable reverse micelles which was difficult earlier.

TUMOUR TARGETING

The utility of microemulsions as vehicles for the delivery of chemotherapeutic or diagnostic agents to neoplastic cells while avoiding normal cells was suggested [44]. A method was claimed for treating neoplasms, wherein neoplasms cells have an increased number of low density lipoprotein (LDL) receptors compared to normal cells. The microemulsion comprised of a nucleus of cholesterol esters and not more than 20% triglycerides surrounded by a core of phospholipids and free cholesterol and contained a chemotherapeutic drug. Microemulsions were similar in chemical composition to the lipid portion of LDL, but did not contain the protein portion. These artificial microemulsion particles incorporated plasma apolipoprotein E (apo E) on to their surface when they were injected in the bloodstream or incubated with plasma. The apo E served as a linking element between the particles of the microemulsion and the LDL receptors. The microemulsions could then be incorporated into cells via receptors for LDL and delivered the incorporated molecules. Thus, higher concentration of anticancer drugs could be achieved in the neoplastic cells that have an increased expression of the receptors. In this way toxic effects of these drugs on the normal tissues and organs could be avoided. In human subjects, they observed no change in the plasma kinetics of the radioactively labeled microemulsion containing carmustine or cytosine-arabinoside thereby confirming that the incorporation of these drugs did not diminish the capacity of the microemulsion to incorporate apo E in the plasma and bind to the receptors.

BRAIN TARGETING [45-47]

Intranasal administration confers a simple, practical, cost effective, convenient and non-invasive route of administration for rapid drug delivery to the brain. It allows a direct transport of drugs to the brain circumventing the brain barriers. A mucoadhesive microemulsion was prepared for an antiepileptic drug clonazepam. The aim was to provide rapid delivery to the rat brain. Brain/blood ratio at all sampling points up to 8h following intranasal administration of clonazepam mucoadhesive microemulsion compared to intra venous was found to be 2-fold higher indicating larger extent of distribution of the drug in the brain.

OCULAR AND PULMONARY DELIVERY [48]

For the treatment of eye diseases, drugs are essentially delivered topically. O/W microemulsions have been investigated for ocular administration, to dissolve poorly soluble drugs, to increase absorption and to attain prolong release profile. The microemulsions containing pilocarpine were formulated using lecithin, PG and PEG 200 as co-surfactant and isopropyl myristate as the oil phase. The formulations were of low viscosity with a refractive index lending to ophthalmologic applications. The formation of a water-in-HFA propellant microemulsion stabilized by fluorocarbon non-ionic surfactant and intended for pulmonary delivery has been described.

MICROEMULSIONS IN BIOTECHNOLOGY [49,50]

Many enzymatic and biocatalytic reactions are conducted in pure organic or aqua-organic media. Biphasic media are also used for these types of reactions. The use of pure apolar media causes the denaturation of biocatalysts. The use of water-proof media is relatively advantageous. Enzymes in low water content display and have:

1. Increased solubility in non-polar reactants
2. Possibility of shifting thermodynamic equilibria in favor of condensations
3. Improvement of thermal stability of the enzymes, enabling reactions to be carried out at higher temperatures.

Many enzymes, including lipases, esterases, dehydrogenases and oxidases often function in the cells in microenvironments that are hydrophobic in nature. In biological systems many enzymes operate at the interface between hydrophobic and hydrophilic domains and these usually interfaces are stabilized by polar lipids and other natural amphiphiles. Enzymatic catalysis in microemulsions has been used for a variety of reactions, such as synthesis of esters, peptides and sugar acetals transesterification; various hydrolysis reactions and steroid transformation.

CONCLUSION

The use of microemulsions as drug delivery vehicle has been an exciting and attractive area of research because of its any potential and extraordinary benefits. In microemulsions, one can design the interface of such nanometer sized droplets so that droplet stability and lifespan in humans can be made to last from a few milliseconds to minutes, or even to hours. The interfacial rigidity of the microemulsion droplets plays a key role in the flux of the drugs from such droplets to the cells and tissues. Tailoring of microemulsion systems to control the flux of the drugs can be done so as to customize drug delivery according to individual patient requirements or to specific pharmaceutical needs. Microemulsions offer an interesting and potentially quite powerful alternative carrier system for drug delivery because of their high solubilization capacity, transparency, thermodynamic stability, ease of preparation, and high diffusion and absorption rates when compared to solvent without the surfactant system. However the toxicological evaluation of the prepared microemulsion can be a research area in future.

ACKNOWLEDGMENT

The authors are thankful to Dr. K. P. Bhusari, Principal, Sharad Pawar College of Pharmacy for providing the necessary facilities for the work.

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