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MICROEMULSION DRUG CARRIER SYSTEM : A REVIEW

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ABSTRACT

Microemulsions are class of microheterogenous systems having unique features of stability, solubilisation capacity, Structural morphology, physical properties and applicability. Microemulsions are clear, transparent, thermodynamically stable dispersions of oil and water, stabilized by interfacial film of surfactant frequently in combination with a co-surfactant with a droplet size usually in the range of 10-100 nm. They can be classified as oil-in-water (o/w), water-in-oil (w/o) or bicontinuous systems depending on their structure and are characterized by ultra low interfacial tension between oil and water phases. Recently, there has been a considerable interest for the microemulsion formulation for the delivery of hydrophilic as well as lipophilic drug as drug carriers because of its improved drug solubilisation, long shelf life and ease of preparation and administration and improvement of bioavailability. Microemulsion is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. Microemulsion received much attention not only for prolonged release, but also for targeting of drugs to a particular site. The formulation of microemulsions for pharmaceutical use requires a thorough understanding of the properties, uses and limitations of microemulsions. The important applications of microemulsions in enhanced petroleum recovery, biotechnology, pharmaceutics, nanoparticles preparation, corrosion inhibition etc.

KEYWORDS : *Microemulsions, Thermodynamically stable, micelle, corrosion inhibition.*

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INTRODUCTION

Microemulsions were first introduced by Hoar and Schulman in 1943[1]: they are isotropically clear, and thermodynamically stable dispersions of two immiscible liquids such as oil and water, stabilized by relatively large amount of surfactant and usually in conjugation with a co-surfactant, typically a short to medium chain alcohols[2]. Microemulsions are isotropic, thermodynamically stable solutions in which substantial amounts of two immiscible liquids (i.e., water and oil) are brought into a single phase by means of an appropriate surfactant or surfactant mixture. Although microemulsions are macroscopically homogeneous on a microscopic level they are heterogenous, because a surfactant monolayer separates water- and oil-rich domains. It is now generally recognized that the spontaneous curvature of the surfactant monolayer at the oil/water interface dictates phase behavior and microstructure[3,4]. Microemulsions have been widely studied for drug targeting to the brain and to enhance the bioavailability of the poorly soluble drugs. 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 carrier for drug targeting to the brain.

Intranasal drug delivery is one of the focused delivery options for brain targeting, as the brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation[5]. The key differences between ordinary emulsions (macro emulsions) and microemulsions are shown in Table **1**.

Table 1: Emulsion Vs Microemulsion

Property	Emulsion	Microemulsion
Composition	Water, oil and emulsifier	Water, oil, emulsifier and cosurfactant
Appearance	Semi transparent to cloudy	Transparent homogenous liquid
Viscosity	Viscous liquid	Less viscous
Particle size	1-20 um	10-100 nm
Interfacial tension	5-50 dynes/cm	10^{-2} - 10^{-3} dyne/cm
Interfacial film	Tough	Highly flexible
Manufacturing	Tedious, high sheer needed	Easy and spontaneous
Free energy	More	Zero or negative
Stability	Thermodynamically unstable	Thermodynamically stable

The use of a microemulsion gel as vehicle may enhance transdermal penetration by various mechanism, many molecules or solubilised in microemulsion in addition microemulsion induce a change in the thermodynamic activity of the drug they contain, modifying their partition coefficient and thus favour penetration of the stratum corneum. Furthermore, their component surfactant reduces the functional barrier of stratum corneum. This latter function may be more or less important depending on the nature of the surfactant used [6].

FACTORS AFFECTING OF MICROEMULSIONS

The formation of microemulsion will depend on the following factors

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 association's leadings to the formation of microemulsion[7].

Critical packing ratio is given by:

c.p.p= V **/** (a × l)

Where, V = volume of surfactant molecule

a = head group surface area

l = length

• If c.p.p is between 0-1, interface curves towards water (positive)

• If c.p.p is greater than 1, interface curves towards oil (negative)

• If c.p.p is equal to 1, then either bicontinuous or lamellar structure.

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 this group, 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[7].

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 favoured, while longer chain co surfactant favors w/o type w/o type by alcohol swelling more in chain region than head region[7].

MECHANISM OF FORMING MICROEMULSIONS

All types of emulsions should be prepared with a certain amount of surfactant. Surfactants can promote the formation of emulsion as they reduce the interfacial tension between oil and water by attaching on to the liquid-liquid interface[8]. Surfactants can be thought of as “pollywogs” with hydrophilic head and hydrophobic tail. There are three types of surfactants: anionic, cationic and nonionic surfactants. Anionic surfactants have a negative charge on the hydrophilic part and cationic surfactants have a positive charge on the hydrophilic part. Nonionic surfactants have no charge on the molecules. In the pharmaceutical field, nonionic surfactants are widely used as they are less irritative than ionic surfactants. Ionic surfactants are used rarely in special cases[9]. When the surfactant concentration exceeds a certain value, aggregates of surfactant called “micelle” are formed. The critical concentration of surfactant where micelles are formed is called critical micelle concentration (CMC). The surfactant distributes in an energetically favorable way. In water, the hydrophilic heads of the surfactant molecules are surrounded by water molecules and the hydrophobic tails of the surfactant molecules are gathered up in the inner portion of the micelles. In oil, the hydrophilic heads of the surfactant molecules are inside the micelles (reverse micelles) and the hydrophobic tails of the surfactant molecules extend away form the core of the micelles to the oil phase[10]. The main difference between surfactant micelles and emulsion is the liquid phase. Typically, micelles are formed by adding surfactant to a single liquid phase, either oil (reversed micelles) or water whereas emulsions are prepared by adding surfactant to a double liquid phase, such as soybean oil and water.

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 (GRAS) excepients.

Oil Phase

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 HLB)[11]. 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.

Aqueous Phase

Aqueous phase may contain hydrophilic active ingredients and preservatives. Some workers have utilized buffer solutions as aqueous phase.

Primary 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. It is generally accepted that low HLB surfactants are favoured for the formulation of w/o microemulsion, where as surfactants with high HLB (>12) are preferred for the formation of o/w microemulsion. Surfactants having HLB greater than 20 often require the presence of co surfactants to reduce their effective HLB to a value within the range required for microemulsion formation. The surfactants are generally ionic, non ionic or amphoteric. The surfactants chosen are generally for the non ionic group because of their good cutaneous tolerance. Only for specific cases, amphoteric surfactants are being investigated[12, 13].

Secondary surfactant (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[14-18]. The presence of cosurfactants allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition[11, 19, 20]. If a single surfactant film is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidising 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.

PREPARATION OF MICROEMULSION

Phase 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.(**1**)

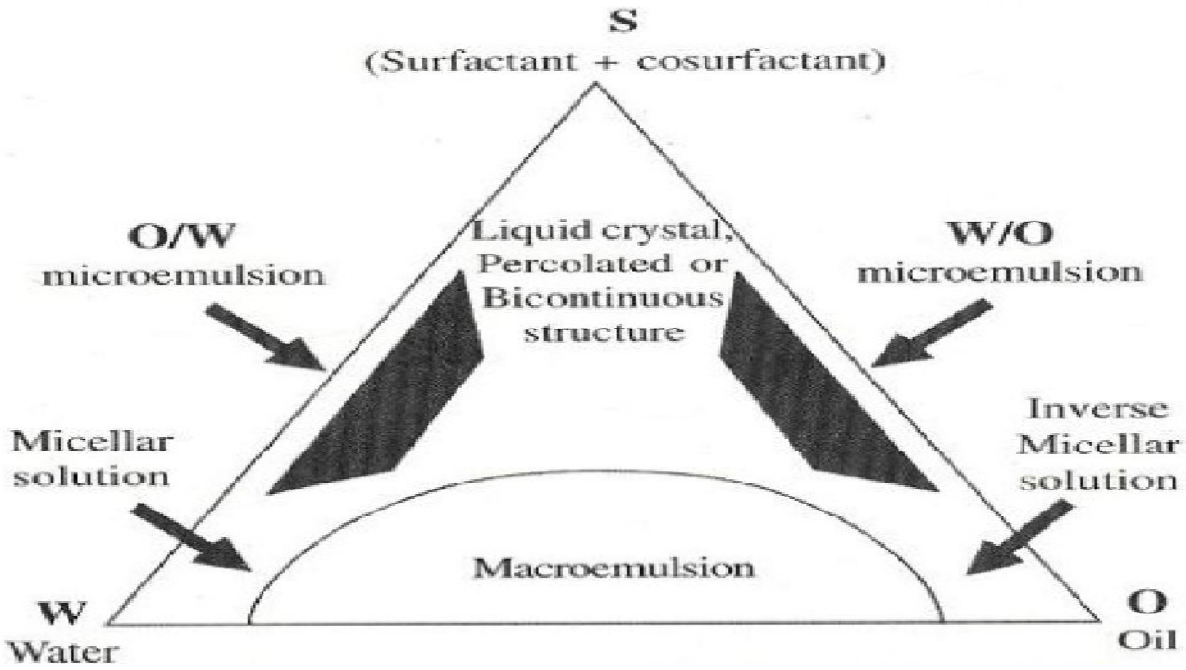

Fig.1 Pseudo ternary phase diagram of oil, water and surfactant showing microemulsion region.

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 metastable systems are not included. The methodology has been comprehensively discussed by Shafiq-un-Nabi et al[21].

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 (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[5].

Type of Microemulsions

a) o/w microemulsion

b) w/o microemulsion

c) O/W type, W/O type and bicontinuous microemulsion

Emulsions can be commonly classified as water-in-oil (W/O) emulsion or oil-in-water (O/W) emulsion. Generally speaking, hydrophilic surfactant forms O/W emulsion easily and hydrophobic surfactant is likely to form W/O emulsion[21].

Microemulsions have various textures such as oil droplets in water, water droplets in oil, bi continuous mixtures (Fig 2).

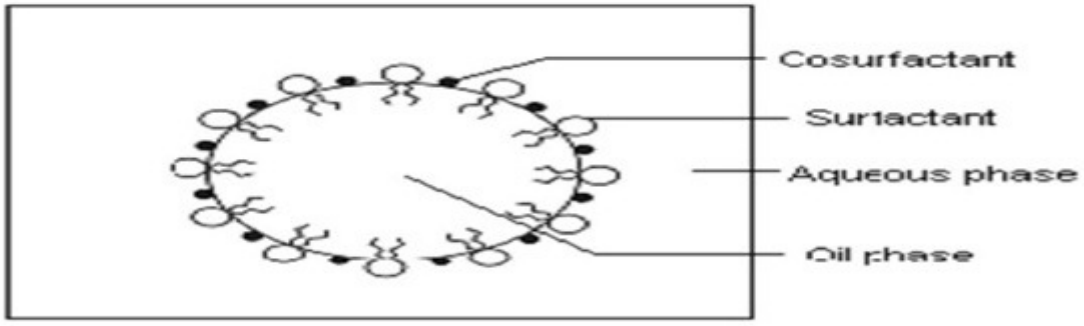

Fig.2 Oil in Water type microemulsions

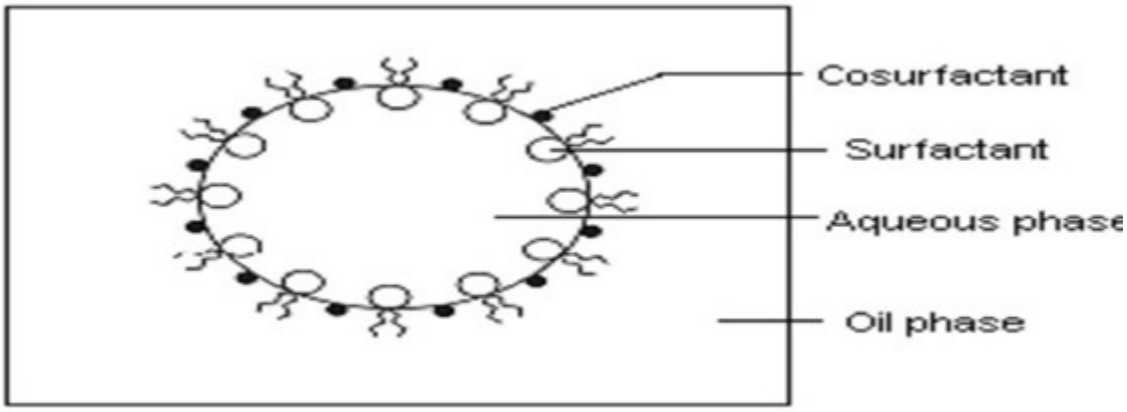

Fig. 3 Water in Oil type microemulsion

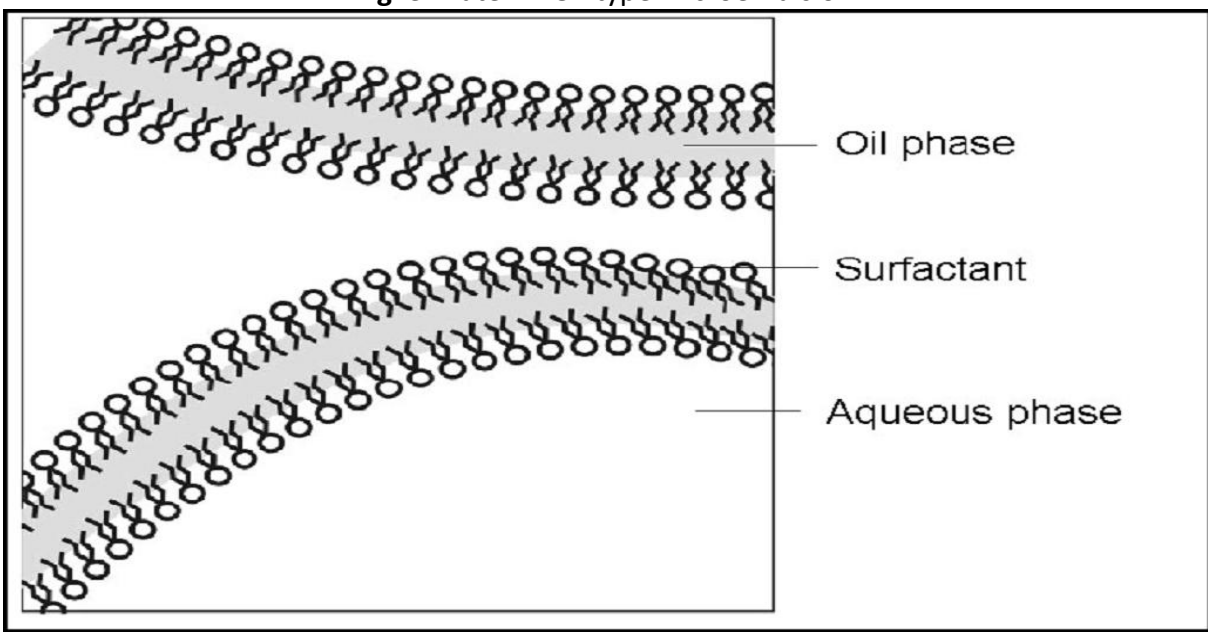

Fig. 4 O/W type, W/O type and Bicontinuous microemulsion

ADVANTAGES OF MICROEMULSIONS

In recent years microemulsions have attracted a great deal of attention because of their following advantages;

1. Ease of manufacturing and scale-up.
2. Wide applications in colloidal drug delivery systems for the purpose of drug targeting and controlled release.
3. Helps in solubilization of lipophillic drug hence Increase the rate of absorption and bioavailability of drugs.
4. Eliminates variability in absorption.

5. Provides an aqueous dosage form for water insoluble drugs.
6. Various routes like tropical, oral and intravenous can be used to deliver the drugs[22].
7. Rapid and efficient penetration of the drug moiety.
8. Helpful in taste masking.
9. Same microemulsions can carry both lipophilic and hydrophilic drugs[23].
10. Provides protection from hydrolysis and oxidation as drug in oil phase in O/W microemulsion is not exposed to attack by water and air.
11. Liquid dosage form increases patient compliance.
12. Less amount of energy requirement.
13. Microemulsion lower the skin irritation: alcohol-free microemulsions have been reported with much lower irritation potential.
14. Long shelf life as compared to other colloidal drug delivery system.
15. High drug loading.
16. Improve therapeutic efficacy of drugs and allow reduction in the volume of the drug delivery vehicle, thus minimizing toxic side effects[24].
17. Easy to administer in child and adults who have difficulty swallowing solid dosage forms.

DISADVANTAGES OF MICROEMULSIONS

1. In many cases high concentration of surfactant and co-surfactants is required to formulate a stable microemulsion.
2. A relatively small number of pharmaceutically acceptable excepients are available to be used in microemulsion formulation

APPLICATIONS

Microemulsions have found numerous applications in different field, and in this respect they are among the most useful microheterogenous systems. A concise account of number of important applications and uses of microemulsions is presented.

Liquid membrane

Microemulsion can function as liquid membrane. Both the WI (O/W) and WII (W/O) types have been considered as dispersed liquid membrane which facilitates the transfer the solute by convenient uptake and release. Tondre et al. have studied various separation processes with the help of microemulsion[25, 26]. Acetic acid has been separated from water by using microemulsion [27].

Microemulsion have also been found to be efficient in the extraction of the heavy metals (e.g. Hg) involving oleic acid from contaminated water[28]. They have been used in chromatography, e.g., microemulsion electrokinetic chromatography. By this method solute hydrophobicity can be obtained. Al^{3+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+} ions have been estimated by using microemulsion which are useful as spectral shift reagents, intensity amplification agents, as a media etc.[29]. Selective transport of amino acids e.g., tryptophan and p-iodophenylalanine through AOT containing microemulsion (WII) liquid membrane, has been investigated. The microemulsion was used as AOT/isooctane/aqueous phosphate buffer (0.1M). The two amino acids have different hydrophobicities but their partition coefficients between water and microemulsion have no direct relation to the transport properties. The selectivity seems to depend upon AOT concentration of the microemulsion[30].

Biotechnology

Microemulsion has been used for selective protein extraction from fermenter liquids. The protein and enzyme have been found to retain their normal activities [31-33]. The pH. Ionic strength, salt type, temperature, solvent concentration etc. affect the partition of the protein. Moreover, it has been shown that the chiral epoxide can be produced by using mycobacterium in W/O microemulsion. This is important because many biotransformation processes are difficult to carry out because of the poor water solubility of the substrate or product. If microemulsions are used, such problem can potentially be overcome[34].

Enhanced petroleum recovery

Microemulsions have been suggested to be used in the enhanced tertiary oil recovery. Approximately 20% difficult to recover underground oil can be recovered by this method. Because of high

interfacial tension (IFT) between crude oil and brine, the oil remains trapped in the fine pores of the geological oil reservoirs. By decreasing IFT to very low value (□10$^{□3}$ mN m$^{□1}$), a large amount of crude oil can be recovered[35]. This is known as surfactant-polymer flooding process.

Fuel, Lubricant and Corrosion inhibitors

The microemulsions are used as effective lubricants, cutting oils, and corrosion inhibitors. The systems have thermodynamic stability, and the surfactants help in the corrosion inhibition and higher water content (i.e. higher heat capacity which provides advantage in their uses as cutting oils). Corrosive agents get solubilzed in the microemulsions, surfactant gets adsorbed on the metal surface and hence the corrosion is reduced[36].

Detergency

Microemulsions are good for detergency. It does not contain both polar and nonpolar liquids and hence can dissolve both polar (e.g., salt, pigment, protein) and nonpolar (e.g., grease, oil etc.) substances. Microemulsions, particularly the middle phase microemulsion, are good for detergency for effective soil removal from textiles, wool and also for skin degreasing. Microemulsions are efficient color remover from textile waste-water. The effect of co-surfactant in the microemulsion on efficiency of color removal process was studied. It was observed that in the presence of isoamyl alcohol as co-surfactant, the ionic surfactant were somewhat more efficient in color removal than the n-butyl alcohol or n- octyl alcohol. Various different color were used e.g., procion yellow, H-E4R (CI Reactive Yellow 84); procion blue H-ERD (CI reactive blue 160), etc.[37].

Pharmaceuticals

A wide range of water-soluble and insoluble acidic drugs have been resolved by a high-pH MEEKC method using a single set of operating conditions[38]. These include a range of related cephalosporins acetylsalicylic acid and insoluble drugs such as ibuprofen, indomethacin and

troglitazone. The method was used to quantify levels of troglitazone in a tablet formulation. A result of 199.4 mg troglitazole was obtained compared with the label claim of 200 mg[38]. MEEKC separations of a range of watersoluble and insoluble basic drugs (including terbutaline, bupivacaine and amitryptyline) were also resolved by the same standard operating conditions with no evidence of peak tailing. The separation of basic analytes achieved in MEEKC is based on solute partitioning into the droplet, ion pair interaction with the surface of the droplet and electrophoretic migration of the positively charged compound. To eliminate the ion-pair and migration aspects it is possible to employ high-pH (pH 13)[39] microemulsions where the basic drug will be neutral and will separate solely by partitioning with the droplet. Fast separation can be achieved using a low conductivity microemulsion buffer prepared with ethyl acetate[40]. Ethyl acetate has a lower surface tension than oils such as heptane and octane. Therefore, a lower concentration of surfactant can be used allowing high voltages to be applied across the capillary without generating excessive current levels. Analysis of neutral components such as guaiphenesin, 4-hydroxyacetophenone and benzophenone was possible in a very short time[40]. When using a high-speed separation buffer the injection repeatability was excellent over a range of 70 runs using the same set of vials.

Analgesic cardiac and glycosides steroid drugs are highly insoluble, neutral and possess limited chromophores[41]. MEEKC has been successfully used to separate a range of related glycosides with detection at low UV wavelength[41].

CONCLUSION

Microemulsions offer an interesting and potentially quite powerful alternative carrier system for drug delivery because of their high solubilisation capacity, transparency, thermodynamic stability, ease of preparation and high diffusion and absorption rates through skin when compared to solvent without the surfactant system. Parameters such as temperature and pH will influence the solubility of drug inside the microemulsion system. Microemulsions are optically isotropic and thermodynamically stable liquid solutions of oil, water and amphiphile. Drug delivery through

microemulsions is a promising area for continued research with the aim of achieving controlled release with enhanced bioavailability and for drug targeting to various sites in the body. The numerous applications of microemulsions mean that these microheterogenous system will be continue to be a rich field for exploration for scientists will continue to create interest among industrial technologists.

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REFERENCES

1. Hoar T.P, Schulman J.H, Transparent water in oil dispersions: the oleopathic hydro-micelle, Nature 152 ;1943: 102–103.
2. Ho H.O, Hsiao C.C, Sheu M.T, Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs, J. Pharm. Sci. 85;1996: 138– 143.
3. Safran S.A, Tlusty T, Curvature elasticity models of microemulsions. Ber. Bunsenges. Phys. Chem. 100; 1996:252-263.
4. Gradzielski M, Langevin D, Parago B, Experimental investigation of the structure of nonionic microemulsions and their relation to the bending elasticity of the amphiphilic film. Prog. Colloid. Polym. Sci. 100;1996:162-169.
5. Dhakar R. C, Maurya S. D, Gupta A. K, Jain A, Kiroriwal S, Gupta M, Microemulsions a Carrier for Nose to Brain Targeting: a review and update, Int. Journal of Pharm Sci.2(1);2011:49-70.
6. Nielloud F, Marti-Mestres G. Pharmaceutical Emulsion and Suspensions. Drugs and the pharmaceutical sciences, 2-9,20.
7. Shinoda K, Solvent Properties of Surfactant Solutions,1967:234 – 237.
8. Becher P, Emulsions: Theory and Practice. American Chemical Society, 1966 42-46.
9. James WM. Colloid Science, 16-17.
10. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Me´ndez J: Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur. J. Pharm. Biopharm, 43;1997: 37-42.
11. Rao Y. S, Deepathi K. S, Chowdary K. P. R, Microemulsion a Novel Drug Carrier System, Int. Journal of Drug Delivery Technology, 1(2);2009:39-41.
12. P.K, Murthy, R.S, Microemulsions: A potential drug delivery system. Curr. Drug Deliv. 3(2);2006:167-180.
13. Corswant C, Thoren P, Engstrom S. Triglyceride-based microemulsion for intravenous administration of sparingly soluble substances, J. Pharm. Sci. 1998;87, 200.
14. Aboofazeli R, Patel N, Thomas M, Lawrence MJ. Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol and oil; the influence of oil. Int. J. Pharm. 125;1995: 107-116.
15. Bhargava HN, Narurkar A, Lieb LM. Using microemulsions for drug delivery. Pharm Tech. 11;1987: 46- 52.
16. Kreuter J. Microemulsions; In: Colloidal drug delivery systems. New York: Marcel Dekker 1994; 31-71.
17. Lawrence M.J. Surfactant systems: microemulsions and vesicles as vehicles for drug delivery. Eur J Drug Metab Pharmacokinet, 3;1994: 257-269.
18. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. Crit Rev Therapeut Drug Carrier Syst, 16;1999: 461-521.
19. Lawrence MJ, Rees GD. Microemulsion based media as novel drug delivery systems. Adv Drug Del Rev. 47;2000: 89-121.
20. Aboofazeli R, Lawrence CB, Wicks SR, Lawrence MJ. Investigations into the formation and characterization of phospholipid microemulsions III. Pseudo-ternary phase diagrams of systems containing water-lecithin-isopropyl myristate and either an alkanoic acid, amine, alkanediol, polyethylene glycol alkyl

ether or alcohol as co-surfactant. Int J Pharm. 111;1994: 63-72.
21. Stilbs P, Lindman B, Rapacki K. Effect of alcohol cosurfactant length on microemulsion structure. J Colloid Interface Sci. 95;1983: 583-585.
22. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. Crit RevTherapeut Drug Carrier Syst. 16;1999: 461- 521.
23. Aboofazeli R, Lawrence MJ. Investigations into the formation and characterization of phospholipid microemulsions: I Pseudo-ternary phase diagrams of systems containing water–lecithin–alcohol–isopropyl myristate, Int. J. Pharm. 1993, 161–175.
24. Nastruzzi C Gambari R., Antitumar activity of transdermally delivered aromatic tetra-amidines. J Controll release; 1994; 29:53.
25. Tondre C, Xenakis A, faraday Dissess, Chem. Soc. 1984, 77,115.
26. Ismael M, Tondre C, J. Membrane Sci. 1992, 72, 181.
27. Wiencek J, Qutubudin S, Sep. Sci. technol, 1992, 27, 1211.
28. Larcen k, Wiencek J, Environm. Prog. 1994, 13, 253.
29. Ishihama Y, Oda N, Uchikawa K, Asakawa N, Anal. Chem. 1995, 67, 1588.
30. Hebrant M, Tondre C, Analyt. Sci. 1998, 14, 109.
31. Macozzi G, Correa N, Lussi P.L, Caselli M, Biotechnol. Bioeng. 1991, 38, 1239.
32. Kawakami L, Dungan S.R, Langmuir, 1996, 12, 4073.
33. Andrews B.A, Pyle D.L, Asenjo J.A, Biotechnol. Bioeng. 1994, 43, 1052.
34. Prichanont S, Leak D. J, Stucky D.C, Enzyme Microb. Tech. 2000, 27, 134.
35. Schramm L.L, Fisher D.B, Schurach S, Cameron A, Colloids Surf. A. 1995, 94, 145.
36. Paul B.K, Moulik S.P, Curr. Sci. 2001, 80, 990.
37. Beltrame L.T.C, de Castro dantas T.C, Dantas Neto A.A, de Barro Neto E.L, Lima F.F.S, J, Chem. Tech. Biotech. 2005, 80.92.
38. Gluck S.J et al., J. Chromatogr. A, 744;1996:141–146.
39. Altria K.D, J. Chromatogr. A, 892;2000:171–186.
40. Mahuzier P.E, Clark B.J, Altria K.D, Electrophoresis, 22;2001:3819–3823.
41. Debusschère L et al., J. Chromatogr. A, 779;1997: 223–227.

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