Ocular drug deliver and the importance of microemulsion as a potential delivery system

Habib Fawzia¹, El-Mahdy Mona² and Maher Shaheer³

¹Professor of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt.
²Lecturer of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt.
³Lecturer Assistant, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

ABSTRACT
Conventional dosage forms such as eye drops are the most used dosage form by ocular route, in spite of their low bioavailability and the pulsed release of the drug where it is expected that direct application of the drug to the eye will give maximum response; however after instillation of an eye drop, about 70% of the administered volume can be seen to be lost by different factors. For this reason new ocular drug delivery vehicles have been developed in order to minimize the amount of the drug lost from the eye and at the same time provide maximum response with reduced frequency of administration. Among such delivery systems are microemulsions. Microemulsions are a promising dosage form for ophthalmic application because their industrial production and sterilization are relatively simple and inexpensive; they have good thermodynamic stability and inherently provide the capacity to make soluble lipophilic drugs. At the same time, the in vivo results and preliminary studies on healthy volunteers have shown a delayed effect and an increase in the bioavailability of the drug. The proposed mechanism is based on the adsorption of the nanodroplets, representing the internal phase of the microemulsion and acting as drug reservoir, on the cornea and thus increasing the time in which the drug is available for absorption. This review will discuss the important characteristics of ocular delivery systems, factors that reduce drug availability to the eye and methods to improve ocular bioavailability. It will also focus on microemulsions, their preparation methods, their components and their applications as drug carrier for ocular use.

Keywords: Ocular delivery system, Microemulsions, Phase diagram and Sustained release.

1. Introduction
“If a physician performed a major operation on a seignior (a nobleman) with a bronze lancet and has saved the seignior’s life, or he opened the eye socket of a seignior with a bronze lancet and has saved the seignior’s eye, he shall receive ten shekels of silver. But, if the physician in so doing has caused the seignior’s death or has he destroyed the seignior’s eye, they shall cut off his hand” the foregoing excerpts are from 282 laws of King Hammurabi’s Code. They show how delicate and important eye treatment was¹. The eye is unique in its therapeutic challenges. An efficient system, that of tears and tear drainage, which quickly eliminates drug solutions which makes topical delivery to the eye somewhat different from most other areas of the body². Preparations for the eye comprise a variety of different types of products; they may be solutions (eye drops or eyewashes), suspensions, or semisolids like ointments and gels. Any modern text on drug product design and evaluation must place into perspective, the unique nature of the ophthalmic dosage form more specifically. It must consider that, the bodily organ which probably better than any other, serves as a model structure for the evaluation of drug activity, the eye. In no other organ can the practitioner, without surgical or mechanical interaction, so well observe the activity of the drug being administered. Most ocular structures can be readily viewed from cornea to retina and in doing so; any signs of ocular or
systemic disease can be detected long before sight-threatening or certain health threatening disease states become intractable. Behind the relative straightforward composition nature of ophthalmic solutions and ointments, however, lie many physicochemical parameters which affect drug stability, safety and efficacy as do most other products. Additionally, specialized dosage forms such as parenteral type ophthalmic solutions for intraocular, subtenons, and retrobulbar use; suspensions for insoluble substances such as hydrocortisone; and solids for reconstitution such as ecothiophate iodide and tetracycline, all present the drug product designer with composition and manufacturing procedure challenges in the development of pharmaceuticals.

Ophthalmic products, like most others in the medical armamentarium, are undergoing a process termed optimization. New modes of delivering a drug to the eye are being actively explored ranging from a solid hydrophobic device which is inserted into the ophthalmic cul-de-sac, to conventionally applied dosage forms which, due to their formulation characteristics markedly increase the drug residence time in the orbit of the eye, thus providing drug absorption for prolonged period of time and reducing the frequency with which a given drug product must be administered.

Ocular diseases are mainly treated topically by the application of drug solutions as eye drops. These conventional dosage forms account for 90% of the available ophthalmic formulations due to the simplicity and convenience of such forms.

It is often assumed that, drugs administered topically to the eye are rapidly and totally absorbed and are available to the desirable site in the globe of the eye to exert their therapeutic effect. Indeed, this is generally not the case. When a quantity of topical ophthalmic dosage form is applied to the eye, generally, to the lower cul-de-sac, several factors immediately begin to affect the availability of the drug contained in that quantity of the dosage form. Upon application of 1 to 2 drops of a sterile ophthalmic solution, there are many factors, which will participate in the removal of the applied drops from the lower cul-de-sac. The first factor affecting drug availability is the loss of the drug from the palpebral fissure. This takes place by spillage of the drug from the eye and its removal via nasolacrimal apparatus. The normal volume of tears in human eye is estimated to be approximately 7 µl, and if blinking does not occur the human eye can accommodate a volume of 30 µl without spillage from palpebral fissure. With an estimated drop volume of 50 µl, 70% of the administered volume of 2 drops can be expelled from the eye by overflow. If blinking occurs, the residual volume of 10 µl indicates that 90% of the administered volume of two drops will be expelled.

The second factor is the drainage of the administered drop via the nasolacrimal system into the gastrointestinal tract which begins immediately upon instillation. This takes place when reflex tearing causes the volume of the fluid in the palpebral fissure to exceed the normal lacrimal volume of 7 – 10 µl. Fig 1 indicates the pathways of this drainage.

A third mechanism of drug loss from the lacrimal fluid is systemic absorption through the conjunctiva of the eye. The conjunctiva is a thin, vascularized membrane that lines the inner surface of the eyelids and covers the anterior part of the sclera. Due to the relative leakiness of the membrane, rich blood flow and large surface area, conjunctival uptake of a topically applied drug from the tear fluids is typically an order of magnitude greater than cornea uptake.

![Fig. 1: The pathways for drainage of drugs from the eye](image)
In competition with the three foregoing drug removal from the palpebral fissure is the transcorneal absorption of drug. The cornea is avascular body composed of lipophilic epithelium, Bowman’s membrane, hydrophilic stroma, Descemet’s membrane and lipophilic endothelium. Drugs penetrate across the corneal epithelium via the transcellular or paracellular pathway. Lipophilic drugs prefer the transcellular route. Hydrophilic drugs penetrate primarily through the paracellular pathway which involves passive or altered diffusion through intercellular spaces. For most topically applied drugs, passive diffusion along their concentration gradient, either transcellularly or paracellularly, is the main permeation mechanism across the cornea\(^2^,\,7\).

Physicochemical drug properties, such as lipophilicity, solubility, molecular size and shape and degree of ionization affect the route and rate of permeation in cornea\(^8\).

2. General requirements of ophthalmic dosage forms

Upon preparation of any ophthalmic dosage forms, there are general requirements that should be fulfilled, including:

1- Sterility

Every ophthalmic product must be manufactured sterile and proved sterile on a lot basis before release of the product to the marketplace. USP\(^9\) recognizes six methods of achieving sterilization (steam sterilization at 121 °C, dry heat sterilization, sterilization by filtration, gas sterilization, sterilization by ionizing radiation and aseptic processing). A combination of 2 or more of these methods is usually used. Sterility should be checked on a random sampling basis in the finished package\(^2^,\,3^,\,10\).

2- Ocular toxicity and irritation

Assessment of ocular irritation potential of ophthalmic products represents an extremely important step in their development. Since conventional ophthalmic products such as eyedrops are instilled in relatively, high concentrations directly on the highly innervated corneal surface it is not surprising that local irritation is sometimes encountered\(^8^,\,11\).

The evaluation of ophthalmic products can be traced by the biological response to the drug through examination of the conjunctiva, cornea, and iris. For this purpose, rabbits are currently used to test the ocular toxicity and irritation of ophthalmic formulations. Several articles relate to the use of rabbits as predictors for human response. The rabbit has obvious advantages associated with its use. It is readily available, docile, easily handled, relatively inexpensive, easy to maintain, has large eyes, both corneal surface and bulbar conjunctival areas are large and easily observed, and the iris is unpigmented allowing ready observation to the iridal vessels. The primary differences between rabbits and humans in ophthalmic studies relate to decreases tearing in rabbits and a slower re-epithelization of rabbit cornea.

3- Preservative and preservation

FDA requires that, all ophthalmic solutions should be manufactured sterile. Antimicrobial agents are included as a major component of multiple-dose eye solutions for the primary purpose of maintaining sterility of the product after opening and during use unless prepared in a unit-dose package\(^2\).

USP outlines a test for antimicrobial effectiveness and how to interpret the results. The testing of formulas is carried out as part of the formulation development sequence.

The choice of the antimicrobial agents in different ophthalmic products depends on safety, physical and chemical stability and compatibility with the drug and other excipients. Examples of the antimicrobial agents used: are benzalkonium chloride, chlorobutanol, organic mercurials, phenyl ethyl alcohols, mixture of methyl and propyl parabens and phenyl ethyl alcohol\(^12\).
4- Tonicity adjustment
It refers to the osmotic pressure exerted by a solution from solutes or dissolved solids present. Tear fluid and other body fluids exert osmotic pressure equal to that of normal saline or 0.9% sodium chloride solution. The eye can tolerate solutions having tonicity values ranging from equivalent of 0.5% to 1.6% sodium chloride without great discomfort. In preparing ophthalmic solutions, the tonicity of a solution can be adjusted to that of lacrimal fluid by addition of a suitable solute such as sodium chloride.

5- pH adjustment
pH of lacrimal fluid is close to 7.4. Adjustment of pH to a value close to that of tears is very important as it affects: the stability of the formulation, the comfort, safety and activity of the product, the aqueous solubility of the drug, the drug bioavailability and preservative efficacy. Ideally, pH of ophthalmic products should be adjusted to maintain the maximum shelf life. Sometimes drugs are not stable if prepared in solution with pH close to 7.4. In this case, they are buffered using low buffer capacity solutions thus enabling the tears to bring the pH of the eye back to the physiological range.

3. Approaches to improve ocular drug bioavailability
In ophthalmology, eye drops, has several disadvantages: the low bioavailability of the drugs that are absorbed systemically requires several daily administrations. The eye is not an easy-accessible organ, mainly because of the lachrymal drainage and corneal barrier.

In order to overcome such drawbacks of conventional dosage forms, various systems have been designed during the past two decades to maximize ocular absorption of ophthalmic drugs. There are two main strategies for improvements: increasing the corneal permeability and prolonging the contact time on the ocular surface. Most formulation efforts aim at maximizing the absorption through prolongation of the drug residence time in the conjunctival sac. The goal of ophthalmic drug delivery systems has traditionally been to maximize ocular drug absorption rather than to minimize systemic absorption.

Over the past 30 years, as the expenses and complications involved in marketing new drug entities have increased, with concomitant recognition of the therapeutic advantages of sustained drug delivery which provides medication over an extended period of time, greater attention has been focused on development of sustained or controlled release drug delivery systems. There are several reasons for the attractiveness of these dosage forms, among which are reduction of the frequency of dosing, increase effectiveness of the drug by localization at site of action, reducing total dose required or providing uniform dosage delivery. This can be achieve by maintaining the drug in the precorneal area and allow its diffusion across the cornea.

Different types of sustained delivery systems have been developed such as Ocusert-Pilo 20 or 40 or implantable polymer for endotoxin, however such systems have disadvantage of discomfort and difficulty to retain in the eye. The physiological requirement to preserve visual acuity poses significant problems in achieving sustained drug concentrations particularly associated with the need to provide a transparent formulation, reducing irritancy and avoiding rapid clearance. According to pharmaceutical formulators is to develop topical ocular delivery systems with improved ocular retention, increased corneal drug absorption and reduced systemic side effects whilst maintaining the simplicity and convenience of the dosage form as eye drops. Therefore, many strategies have been adopted to partially or fully achieve such target. These included the use of bioadhesive hydrogels formulation of temperature or pH-sensitive in situ gel forming systems, preparation of collagen shields, application of particulate and vesicular drug delivery systems such as
nanoparticles, liposomes and niosomes, or employing micellar solutions.\(^5\), \(^16\)-\(^18\).

4. Microemulsions
The term microemulsions were first described by Hoar and Schulmanin 1943\(^{[19]}\).

In spite of their similarity, the terms “microemulsion” and “emulsion”, characterize two very different systems both by their physical and thermodynamic properties and by their structure. In both cases, the systems consist of an aqueous phase, a lipophilic phase, and a surfactant agent. A co-surfactant is also required for some microemulsions. The addition of this compound results in a homogeneously dispersed system, which can diffuse the light, and, as opposed to emulsions, is more thermodynamically stable\(^{20}\).

It is also useful to mention that, self-microemulsifying drug delivery systems (SMEEDS) are not microemulsions, although they may be considered to be a closely related system. SMEED typically comprises a mixture of surfactant, oil and drug (known as the concentrate) which when introduced into the body is rapidly dispersed to form droplets of approximately the same size range as those observed in microemulsion systems. Once dispersed such systems would be expected to behave in vivo much the same way as oil-in-water (o/w) microemulsions\(^{21}\).

Microemulsions are an interesting alternative for conventional ophthalmic dosage forms. The industrial production and sterilization are relatively simple and inexpensive; they have good thermodynamic stability and inherently provide the capacity to make soluble lipophilic drugs, which depends also on the lipophilic phase used\(^{20}\), \(^52\).

The simplest representation of a microemulsion is the droplet model. These systems actually exist when they have a low percentage of oil or water in the internal phase (about 10%). These dispersions of oil or water nanodroplets in an external phase are stabilized by an interfacial film of surfactant and co-surfactant. In the case of larger quantities of oil or water, the structure of nanodroplets that was described above does not apply. As a result, a bicontinuous system has been proposed in which water and oil should be separated by a regular or irregular interfacial layer\(^{23}\).

4.1. Components of Microemulsion System:
1- The surfactant (S)
The preferential adsorption of the surfactant enables the modification of the physico-chemical properties of the interface due to its amphiphilic nature. While the surfactant concentration is 0.1% w/w in emulsions, it accounts for at least 10% w/w in microemulsions due to the increase of the interface area between the aqueous and oily phases. This high concentration of surfactants can lead to ocular toxicity. That is why, it might be better to decrease its quantity as possible\(^{20}\).

The role of surfactant in the formulation of microemulsion is to lower the interfacial tension which will ultimately facilitates dispersion process during the preparation of microemulsion and provide a flexible film around the droplets. The surfactant should have appropriate lipophilic character to provide the correct curvature at the interfacial region. Generally, low HLB surfactants are suitable for w/o microemulsion, whereas high HLB (>12) are suitable for o/w microemulsion.

Nonionic surfactants are the major type of surface active agents used in ophthalmic delivery systems since their advantages with respect to compatibility, stability, and toxicity are quite significant compared to the cationic, anionic, or amphoteric counterparts. They are generally less toxic, less hemolytic, and less irritating to the ocular surface, and tend to maintain near physiological pH values when in solution\(^{24}\).

Polyoxyethylated nonionic surfactants have been widely used in the topical delivery of ophthalmic drugs for the treatment of various ocular disorders such as dry eye, inflammation, allergy, ocular hypertension, glaucoma, etc. The dosage
forms in which these surfactants are applicable, are micellar solution, emulsion, microemulsion, suspension, noisome, and liposome. The functional roles these surfactants play in the ophthalmic preparations range from wetting, emulsification, solubilization, and permeability enhancement. Fig. 2 gives a schematic representation 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. 

**Fig. 2**: Gives a schematic representation 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.

**2- The co-surfactant (C)**

The co-surfactants have three functions:

(i) They provide very low interfacial tensions required for the formation of microemulsions and their thermodynamic stability.

(ii) They can modify the curvature of the interface based on the relative importance of their apolar groups. (iii) They act on the fluidity of the interfacial film.

If the film is too rigid, it prevents the formation of microemulsion and results in a more viscous and birefringent phase. The co-surfactant provides additional fluidity and is equivalent to a branched surfactant, fig. 3. The co-surfactants that are used consist of small molecules, which generally are alcohols and glycols such as sorbitan monoleate, sorbitan monoester, propylene glycol, propylene glycol monocaprylate (Capryol 90), 2-(2-ethoxyethoxy)ethanol (Transcutol) and ethanol. Some alcohols, such as pentanol and hexanol, are not used in pharmaceutical applications due to their highly irritating nature. Amines with short chains can also be used as co-surfactants.

**3- The oil phase (O)**

The choice of the oily phase (which is dispersed for ophthalmic drug delivery applications) is important because it conditions both the existence of the microemulsion and the solubilization of the drug. The oil component influences curvature by its ability to penetrate and swell the tail group region of the surfactant monolayer. Therefore, it is necessary to find the appropriate oil type. Oils with excessively long hydrocarbon chains do not result in microemulsions. The shorter the chain, the deeper the penetration of the organic phase into the interfacial film and more important the existence range of the microemulsion. On the contrary, the capacity of solubilization by the organic phase increases with the length of the chain. Therefore, the choice is based on the solubility of the drug.

The most often used oil phases consist of vegetable oils like soybean oil, castor oil, isopropyl myristate, fatty acids, such as oleic acid, and esters of saccharose, such as mono-, di- or tri-palmitates of saccharose. As these excipients are well tolerated by the eye, their degree of purity must be high in order to prevent any contamination with potentially irritating substances.
4- The aqueous phase
The aqueous phase must contain several additives, such as buffers, antibacterial and isotonic agents. They can affect the area of existence of the microemulsions, and therefore they must be studied in the presence of other constituents of the microemulsions[20].

4.2. Phase behavior
The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. Compositional variables can also be studied as a function of temperature and pressure; however, the large majority of systems are studied under conditions of ambient pressure and temperature. The phase behavior of simple microemulsion systems comprising oil, water and surfactant can be studied with the aid of ternary phase diagram in which each corner of the diagram represents 100% of that particular component. More commonly, however, and almost always in the case of microemulsions in pharmaceutical applications, the microemulsion will contain co-surfactant. In this case where four or more components are investigated, pseudo-ternary phase diagrams are used where a corner will typically represent a binary mixture of two components such as surfactant/co-surfactant or water/co-surfactant. The number of different phases present for a particular mixture can be visually assessed. A highly schematic pseudo-ternary phase diagram illustrating these features is presented in fig. 4. It should be noted that, not every combination of components produce microemulsions over the whole range of possible compositions; in some instances the extent of microemulsion formation may be very limited. The physico-chemical interaction between the components is too complex to provide a functional general mathematical guideline for prediction of microemulsion formation as a function of component properties27. It should be noted that, constructing phase diagrams is time consuming process; it will be described later in experimental part.

![Fig. 3: Pseudo-ternary phase diagram of microemulsion existence area](image)

4.3. Preparation of microemulsion
The preparation of microemulsions requires the determination of the existence range of microemulsions, which can be determined by visual observation of various mixtures of surfactant, co-surfactant, oily phase, and aqueous phase reported in a phase diagram. Two techniques are presented in the literature, each of them resulting in microemulsions: (1)"Exact" process by autoemulsification; (2) process based on supply of energy.

1- Autoemulsification
Due to the spontaneous formation of the microemulsions, they can be prepared in one step by mixing the constituents with magnetic stirrer. The order of the addition of the constituents is not considered a critical factor for the preparation of microemulsions, but it can influence the time required to obtain equilibrium. This time will increase if the co-surfactant is added to the organic phase, because its greater solubility in this phase will prevent the diffusion in the aqueous phase. This method is easier and much simpler then “supply of energy” method.

2- Process based on supply of energy
In this case, microemulsions are not obtained spontaneously. A decrease of the quantity of surfactants results in the use of high-pressure homogenizers in
order to obtain the desired size of droplets that constitute the internal phase as opposed to the former technique. Benita and Levy [28] have studied the efficacy of various equipment for obtaining particles of different sizes. Two steps are required: the first step produces a coarse emulsion (0.65 mm) by using a high-speed mixer. The second step consists of using a high pressure homogenizer. The dispersion of the oily phase in the aqueous phase is also facilitated by heating the phases before mixing them, the choice of the temperature depending on the sensitivity of the drug to heat. Cooling the preparation is required before its introduction in the high-pressure homogenizer, which can raise the temperature. A blue opalescent microemulsion is obtained.

4.4. Structure of microemulsion

The mixture of oil, water and surfactants is able to form a wide variety of structures and phases. Beside microemulsions, structural examinations can reveal the existence of regular emulsions, anisotropic crystalline hexagonal or cubic phases, and structures depending on the ratio of the components. Most of these different phases and structures are easily recognized by simple visual inspection of the compositions due to their physical appearance (e.g., emulsions are nontransparent and phases separate after a while; lamellar structures and cubic phases are highly viscous). However, even within the microemulsion regions, several different internal structures can form from the immiscible water and oil phase, and the interfacial surfactant film. The microemulsions structure is greatly influenced by the physico-chemical properties of the components used, and the ratios between the components.

In very dilute systems, with only a few percentages of oil or water, microemulsion structures may approach regular or reverse ‘swollen micelle’ droplet-like shapes [23]. However, the microemulsion components typically form non-spherical aggregates, which may be more or less continuous in the phase with highest volume fraction [29-30]. For the majority of microemulsion systems these aggregates fluently change into bicontinuous structures by titration with the phase of the lowest volume fraction, and through these structures, fluently invert to ‘reversed’ aggregates, fig. 5.

Thus, microemulsion systems do often not display emulsion-like behaviour with sudden inversion of the ‘swollen micelle’, and the emulsion terminology of characterizing the systems as oil-in-water (o/w), or water-in-oil (w/o), is therefore in many situations not applicable to microemulsions.

The flexibility of the surfactant film is an important factor, which determines the possible structures and ways of structural transitions by changes in component ratios, for a given microemulsion system. A very rigid surfactant film, will likely result in droplet-like shapes and will not enable existence of bicontinuous structures. This will impede the range of existence; microemulsions will only form in very narrow composition ranges. A more flexible surfactant film, will most likely enable the existence of several different structures like aggregates and bicontinuous structures, and therefore broaden the range of existence, enabling formation of microemulsion with a wide variety of compositions.
4.5. Microemulsion in vitro experiments

In a study of diffusion kinetics through an artificial membrane, Siebenbrodt and Keipert [29] compared the kinetic release of chloramphenicol, indomethacin and sodium diclofenac which were obtained from aqueous solutions, microemulsions, aqueous solutions containing 1% hydroxyethylcellulose and with the same viscosity as the microemulsions at 25 °C. The studies of diffusion kinetics are conducted by means of an artificial membrane. The single-layer hydrophilic artificial membrane is placed between the donor and the receiver compartments. The diffusion and kinetic release decreased for each drug when they were formulated as microemulsions as opposed to the formulations of aqueous solutions. The results obtained with aqueous solutions, which have a similar viscosity with microemulsions, were reported only for sodium diclofenac (4.5 times less drug released from the microemulsion after 6 h).

Nevertheless, the authors specified that, although the kinetic releases of the three drugs was significantly slower when they were incorporated in aqueous solutions containing hydroxypropylcellulose compared to aqueous solutions, the decrease in the kinetic release was insignificant compared to the decrease in the microemulsions.

Hasse and Keipert [31] compared the release kinetics of the pilocarpine hydrochloride from an aqueous solution and from microemulsions. The experimental conditions were similar to those developed by Siebenbrodt and Keipert namely the use of a hydrophilic artificial membrane. Two microemulsions were prepared. Egg yolk lecithin was incorporated in the first, and PEG 1500 and 200 were used in the second. The diffusion kinetics of pilocarpine hydrochloride from the aqueous solution and microemulsion no. 2 did not show any difference between the two formulations. However, the diffusion kinetics of the pilocarpine hydrochloride from the microemulsion containing lecithin was significantly different. For microemulsion no. 1, 25% less pilocarpine hydrochloride
was diffused through the artificial hydrophilic membrane after 6 h than for the same concentration of drug from the microemulsion no. 2 and from aqueous solution. Nevertheless, although the microemulsion containing lecithin seemed to prolong the diffusion of the drug in vitro, the results were less pronounced than those observed with the sodium diclofenac. Feng, Xiong et al.\(^\text{[32]}\) prepared gelled microemulsion containing water-soluble sodium salicylate in the presence of the low molecular weight gelator N-stearyl-N-stearine-N-steryl-L-phenylalanine. The microemulsion consists of i-propyl myristate, Tween 80, propylene glycol and water. The release rates decreased with an increase of the gelator and isopropyl myristate contents. The release profiles exhibited a controlled release and followed the first-order release kinetics. Muchtar et al.\(^\text{[33]}\) studied the diffusion of drugs through a rabbit cornea used as a membrane between the two compartments. The endothelium was placed at the receiver. The experiments were conducted using a microemulsion and an aqueous solution containing indomethacin. The coefficients of permeability of the indomethacin incorporated in a microemulsion were greater than that of the conventional aqueous eye drops containing the same drug. Nevertheless, as the two compositions did not have the same pH (3.8 for the microemulsion and 6.8 for the aqueous eye drops); it is difficult to reach conclusions. The pH of the aqueous solution is high due to the large proportion of ionized indomethacin at this pH (pKa=4.5) and its hydrosolubility. Besides, the microemulsion was not formulated at pH 6.8 for reasons of instability. Fialho and Armando\(^\text{[34]}\) developed an oil-in-water microemulsion containing dexamethasone. They used isopropyl myristate, Cremophor EL as surfactant, propylene glycol as co-surfactant and water as aqueous phase. The developed system showed an acceptable physico-chemical behavior and presented good stability for 3 months. The ocular irritation test used suggested that, the microemulsion did not provide significant alteration to eyelids, conjunctiva, cornea and iris. This formulation showed greater penetration of dexamethasone in the anterior segment of the eye and also release of the drug for a longer time when compared with a conventional preparation. Djordjevic et al.\(^\text{[35]}\) studied the influence of microemulsion vehicles containing PEG-8 caprylic/capric glycerides (surfactant), polyglyceryl-6 dioleate (co-surfactant), isopropyl myristate and water on the in vitro release rate of amphiphilic drug diclofenac diethylamine (DDA). The study showed that, the release rate of DDA from the investigated systems depends significantly on both the drug/vehicle interactions and the water volume fraction. Monzer\(^\text{[36]}\) studied diclofenac sodium solubilization capacity in microemulsions for oral use. Microemulsions were composed from biocompatible components that are water/sucrose laurate/ethoxylated mono-di-glyceride/oil and ethanol. The oil was R(+-)limonene or isopropyl myristate. The solubilization capacity of the drug in microemulsions was very much higher than its solubility in R(+-)limonene or isopropyl myristate.

4.6. In vivo experiments on experimental animals

Naveh et al.\(^\text{[37]}\) conducted a randomized double blind trial on three groups, each consisting of twelve healthy rabbits, and determined the effects after a single administration of a microemulsion dosage form containing 1.7% pilocarpine base as opposed to an aqueous solution containing 2% pilocarpine hydrochloride (equivalent to 1.7% pilocarpine base). The eye drops modified the intraocular pressure (IOP) earlier (2 h after instillation), but the duration of its pharmacological action was shorter (5 h). Eleven hours after the instillation, the control values were the same as the values observed on the treated rabbits. The instillation of the microemulsion demonstrated a pharmacological action only 5 h later. However, IOP kept going down until the 29th hour. Furthermore, the
maximum reduction recorded for the intraocular pressure was only 18% for regular eye drops and 28.5% for microemulsion eye drops. The microemulsion dosage form provided a delayed pharmacological action compared to that of regular eye drops. This observation led to the conclusion that, the microemulsion eye drops have a real advantage compared to regular eye drops which must be administered four times a day due to the short duration of the pharmacological action. According to Naveh et al, it appeared that, the retention of pilocarpine content in the internal oil phase, and the oil-water interface of the emulsion are sufficient to concomitantly enhance the ocular absorption of the drug through the cornea, and increase the corneal concentration of pilocarpine. After comparing the diffusion profiles of two microemulsions preparations and an aqueous solution of pilocarpine, Hasse and Keipert studied their pharmacological effect in vivo by using six rabbits for each group. The obtained results were different from those observed in vitro. The two microemulsions provided a delayed release compared to the release of the drug incorporated in the aqueous solution.

Although the bioavailability of the microemulsion does not reach the maximum, its pharmacokinetic profile is different from the aqueous solutions. The absorption of the microemulsion takes longer time after instillation. Halbert et al. studies the incorporation of both etoposide and a methotrexate diester derivative in water-in-oil (w/o) microemulsion as potential carriers for cancer chemotherapy. Etoposide was rapidly lost from the microemulsion particles, whereas 60% of the methotrexate diester remained incorporated in the internal phase of the microemulsion. The methotrexate diester microemulsion showed an in vitro cytotoxic effect against mouse leukemia cells.

Kantarci et al. compared appropriate microemulsion formulations containing diclofenac sodium as drug carriers for topical application. Soybean oil was used as organic phase, Brij 58 and Span 80 as surfactants, ethanol, isopropyl alcohol and propanol were used as co-surfactants and water as aqueous phase. All the prepared microemulsions showed no irritation. Microemulsion prepared using propanol as co-surfactant showed significantly highest flux value. Also, it was observed that, there is a strong correlation between the water phase concentration and the flux value of the drug.

4.7. In vivo studies of microemulsions on man

No experimental study has been conducted with microemulsions prepared by autoemulsification. However, several trials were conducted with microemulsions prepared by supply of energy. Melamed et al prepared microemulsions containing adaprolol maleate. According to those authors, no ocular irritation was noticed in the group of forty healthy volunteers as opposed to regular eye droplets. A single instillation of microemulsion or corresponding placebo, namely microemulsion without any drug, was administered to twenty healthy volunteers. The determined parameters were the pupillary diameter and variation of IOP. The effect of the microemulsion which contains pilocarpine is obvious as compared to the placebo and was noticed within 1 h from instillation. The return to the initial values was noticed within 12 h. Another experimental trial conducted by Garty and Lusky, who used pilocarpine in ocular drug deliveries, was conducted in order to compare the activity of the microemulsion instilled twice a day with a generic dosage form instilled four times a day to forty hypertensive patients. All treatments were stopped two to three weeks before the trial. For seven days, the patients randomly received treatment with microemulsions or regular eye drops. No local side effect has been reported. The intraocular pressure decreased with 25% in both groups during all this period. No significant difference has been noticed
between the two treatments. These results proved that, the microemulsion extended the action of the drug and two daily administrations have the same result as four instillations of regular eye drops. Wang et al.\textsuperscript{42} prepared microemulsion in order to improve peroral bioavailability of silymarin. Ethyl linoleate was used as oil phase, Tween 80 as surfactant, ethyl alcohol as co-surfactant and water as aqueous phase. Pharmacokinetic parameters of silymarin loaded microemulsion; solution and suspension were compared in rabbits. Bioavailability of silymarin loaded microemulsion was about two times that of silymarin solution which in turn greater than suspension.

Dreher et al.\textsuperscript{43} compared 2 lecithin-based microemulsion gel to isopropyl palmitate (IPP) as vehicle for transdermal delivery of indomethacin and diclofenac. The authors found a significant increase (3–6-fold) in flux through excised human skin for both drugs applied in the microemulsion vehicle compared to the neat oil vehicle.

Kapoor et al.\textsuperscript{44} prepared nanostructured poly (2-hydroxyethyl methacrylate) (p-HEMA) hydrogels containing microemulsions of Brij 97 for extended ocular delivery of cyclosporine A. Results showed that, the surfactant and microemulsion-laden gels can deliver cyclosporine A at therapeutic dosages for a period of about 20 days.

Lv et al.\textsuperscript{45} investigated microemulsion systems composed of Span20/80, Tween20/80, n-butanol, H2O, isopropyl palmitate (IPP)/isoproplmyristate (IPM) as model systems of drug carriers for eye drops. The results showed that, the stability of the chloramphenicol in the microemulsion formulations was increased remarkably.

Chan et al.\textsuperscript{5} studied microemulsion systems for ocular delivery of pilocarpine hydrochloride (model hydrophilic drug). They used two non-ionic surfactants, sorbitan mono laurate and polyoxyethylene sorbitan mono-oleate with ethyl oleate (oil component) and water. These systems undergo phase change from microemulsion to coarse emulsion with a change in viscosity depending on water content. Drug release depended on the viscosity with lower release rates obtained from formulations with high viscosity. The miotic response and duration of action were greater in case of microemulsion formulations indicating high ocular bioavailability.

Alany et al.\textsuperscript{46} formulated microemulsion systems using Crodamol EO, Crill 1 and Crillet 4, an alkanol or alkanediol as cosurfactant and water. The ocular irritation potential of components and formulations was assessed using a modified hen's egg chorioallantoic membrane test and the pre-ocular retention of selected formulations was investigated in rabbit eye. Results showed that, Crill 1, Crillet 4 and Crodamol EO were nonirritant.

Hae\textsuperscript{47} prepared microemulsions for ocular application and the physicochemical parameters were characterized. These microemulsions have favorable features for ocular use. They show acceptable physico-chemical behavior, especially pH value, refractive index and viscosity, and a good physiological compatibility.

It is of interest to note that, the first study investigating the use of microemulsions as potential drug delivery vehicles was reported in 1974 by Attwood et al. However the field lay virtually dormant until a review on the subject was published by Bhargava et al. in 1987. Since then there has been a very gradual increase in the number of research papers published on the topic until 1994 since when about 20 papers detailing the pharmaceutical use of microemulsions have been published each year. Also, paucity of information is only available in literature regarding its use as ocphalthmic delivery system. This small number of papers contrasts very sharply with other drug delivery systems such as liposomes where the number of publications dealing specifically with their use as drug delivery vehicles runs into the order of 300 per year. This lack of research in the field does not mean that, microemulsions offer any less potential as delivery systems than liposomes, indeed it is pertinent to note that, it took considerably less time for a
microemulsion product (i.e. Neoral®) to get onto the market than the first liposomal drug delivery system [21].

**Current and Future Developments**

During the last two decades, a lot of research work has been carried out on microemulsion system for providing novel solutions to overcome the problems of poor aqueous solubility of highly lipophilic drug compounds and provide reproducible bioavailability.

From industrial point of view, microemulsion can be easily scaled up with considering relative cost of commercial production. Now a day, researcher work is focused on the production of safe, efficient and more compatible microemulsion constituents which will further enhance the utility of this novel delivery system 20.

**REFERENCES**

18. Vyas SP, Mysore N, Jaitley V, Venkatesan N. Niosome based
37. Naveh N, Weissman C, Muchtar S, Benita S, Mechoulam R. A submicron...


