Expert Opinion

Microemulsions as novel drug carriers: the formation, stability, applications and toxicity

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A microemulsion, made from water, oil, surfactants and cosurfactant is a thermodynamically stable system. The presence of the cosurfactant is often required in order to lower the interfacial tension of this interface, because a low interfacial tension is essential for the formation of microemulsions. The transparency of microemulsions arises from their small droplet diameter. The droplet diameter in stable microemulsions is usually within the range of 10 – 140 nm. Microemulsions are graphically represented as stability areas in triangular phase diagrams where each triangular corner designates a certain component. Microemulsions are actually quaternary (pseudoternary) systems. In pharmaceutical fields, the interest in microemulsions is increasing and, thus, they are applied to various administration routes.

Keywords: characterization, cytotoxicity effect, drug delivery, microemulsion, phase behaviour

Expert Opin. Drug Deliv. (2008) 5(1):119-135

1. Introduction

A microemulsion is a thermodynamically stable system composed of at least water, oil and surfactants. The microemulsion concept, generated as a clear single-phase system by titrating a milky emulsion with hexanol, was introduced as early as the 1940s by Hoar and Schulman [1-4].

The simplest representation of the structure of microemulsions is the droplet model in which microemulsion droplets are surrounded by an interfacial film consisting of both surfactant and cosurfactant molecules. There are three different basic structural types of microemulsions: water-in-oil (w/o), oil-in-water (o/w) and finally bicontinuous structures (**Figure 1**). They might be stabilized either by single surfactant, mixture of surfactants, or cosurfactant/surfactant combination [2,5-7].

Microemulsions are actually quaternary (pseudoternary) systems composed of an oil phase, a water phase, surfactant/s and a cosurfactant. These spontaneously formed systems possess specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability. The transparency of microemulsions arises from their small droplet diameter. Droplet diameter in stable microemulsions is usually within the range of 10 – 140 nm [2,8-10].

Microemulsions are graphically represented as stability areas in triangular phase diagrams where each triangular corner designates a certain component [11,12]. An example phase diagram is illustrated in **Figure 2**.

Microemulsions have favorable solvent properties due to the potential incorporation of a large fraction of lipophilic and/or hydrophilic phases. Moreover, investigations have indicated that the unique structural organization of the phases in microemulsions may contribute to additional solubility regions, increasing the load capacity of microemulsions, compared with non-structured systems containing the same fraction of constituents [13-16].

Due to their unique characteristics, microemulsions have been increasing in popularity and garnering more attention in recent years. Such interest has come

10.1517/17425247.5.1.119 © 2008 Informa UK Ltd ISSN 1742-5247

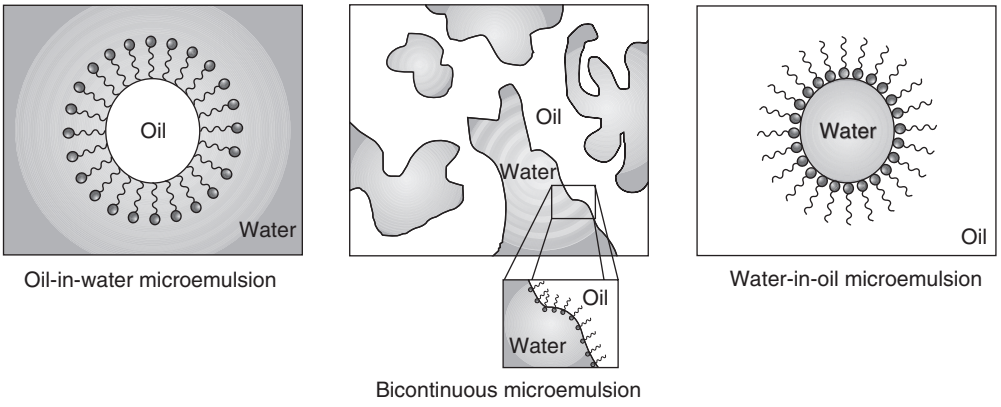

Figure 1. A diagrammatic representation of microemulsion structures. A. Water-in-oil microemulsion droplet; **B.** Oil-in-water microemulsion droplet; **C.** Irregular bicontinuous structure.
Figure adapted from [2].

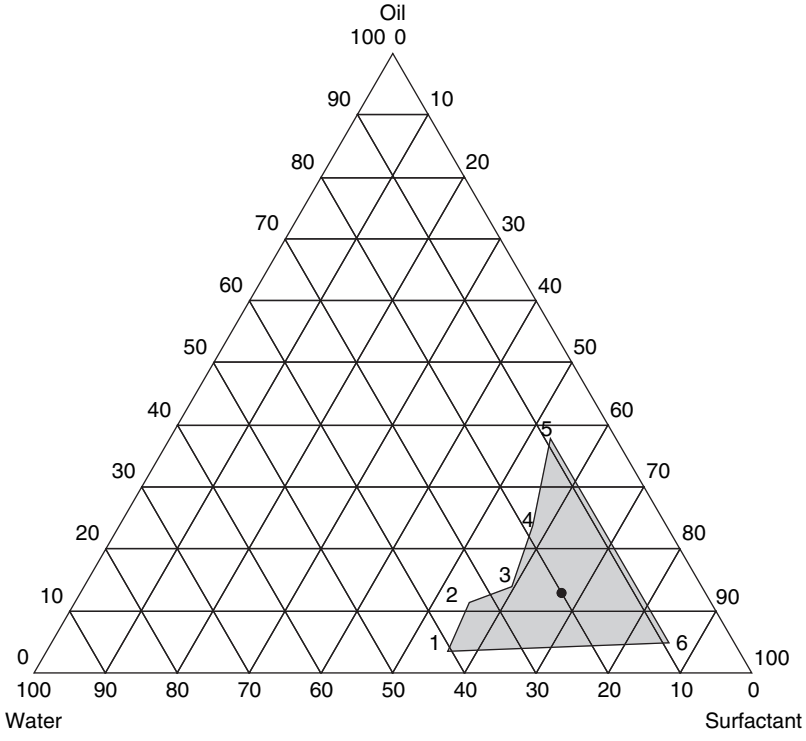

Figure 2. Pseudoternary phase diagrams indicating the microemulsion regions.
Figure adapted from [33].

from industrial laboratories, as well as academic researchers, and in pharmaceutical fields this has lead to their application in various administration routes.

This paper reviews the recent literature with respect to use of microemulsions for drug delivery, and discusses the influence of microemulsion composition.

2. The formation of a microemulsion

According to Danielsson and Lindman [17], microemulsions can be defined as systems of water with or without electrolyte, oil and nonionic surfactant or surfactants, which are single isotrophic and thermodynamically stable liquid systems.

There are three kinds of microemulsions: o/w (normal microemulsion), bicontinuous microemulsion, and w/o (reverse microemulsion) [2,6].

Few theories have tried to explain the formation of microemulsions; however, three approaches have been used to explain formation and stability [7,18,19]. These are:

i) interfacial or mixed film theories;
ii) solubilisation theories;
iii) thermodynamic treatments.

The formation of microemulsions usually involves a combination of three to five components: namely, oil, water, surfactant, cosurfactant and electrolyte. Whether the systems form w/o or o/w microemulsion is dependent on the properties of the oil and the surfactant, the water-to-oil ratio and the hydrophilic–lipophilic balance (HLB) temperature or phase inversion temperature. Nonionic surfactants are conveniently classified on an empirical scale known (HLB) ranging from 1 to 20. In general, w/o microemulsions are formed using surfactants that have an HLB in the range of ~ 3 – 6, and o/w microemulsions are formed using surfactants that have an HLB value in the range of ~ 8 – 18 [7,20,21].

Temperature usually exerts an effect on the formation and region of existence of microemulsions, as the HLB of surfactants can change with temperature and destabilize the surfactant interface. The stability of the final microemulsion formulation should always be examined within the temperature ranges of storage and application [22,23].

The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. An isothermal phase diagram of three or four components can represent the region of a transparent, isotropic and low-viscosity microemulsion phase along with those of a liquid crystalline phase or a lamellar phase [11,24-26]. 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 [27]. In general, an o/w microemulsion is prepared in the water-rich region, and a w/o microemulsion in the organic-rich region. An essential requirement for their formation and stability is the attainment of a very low interfacial tension. As microemulsions have a very large interface between oil and water, because of the small droplet size, they can only be thermodynamically stable if the interfacial tension is low. The role of the surfactants in the system is, thus, to reduce the interfacial tension between oil and water. The purpose of using the cosurfactant – usually a short chain alcohol – is to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film, due to the void space among surfactant molecules [28,29]. Careful consideration should also be given to the choice of cosurfactant. The inclusion of medium chain length alcohols as cosurfactants limits the potential use of the microemulsion, due to their toxic and irritant properties [5]. However, the use of cosurfactant in a microemulsion is not required, and alcohol-free self-emulsifying drug delivery systems (SEDDSs) have been described in the literature [30-32].

A highly schematic pseudoternary phase diagram is presented in **Figure 2** [33]. In this study of the controlled release of methotrexate from a w/o microemulsion, soybean oil was used as the oil phase, and Cremophore EL® (BASF) and Span 80 were the surfactants. Isopropyl alcohol was used as a cosurfactant. To investigate the microemulsion formulation areas, phase diagrams were constructed by titration of a series of surfactant/cosurfactant mixtures with 0.2 M NaOH solution at 25ºC. The boundaries of the microemulsion domains were determined and the ideal surfactant/cosurfactant weight ratios and microemulsion areas were detected by the aid of phase diagrams using a computer programme [34]. After the identification of the microemulsion region in the phase diagrams, the ideal microemulsion formulation was developed using the gravity center of the microemulsion formation area (**Figure 2**). In this formulation, the surfactant/cosurfactant weight ratios were 2:1 (w/w) and the Span 80-Cremophore EL weight ratio was 7:1 (w/w). For the preparation of this microemulsion, surfactants were mixed and melted at 60ºC, then added into the appropriate amount of soybean oil. The cosurfactant was added into this mixture and the formulation was carried out by slowly titrating with 0.2 M NaOH solution, while stirring the mixture with a stirring bar using a magnetic stirrer (9 rpm) until turbidity was observed.

Depending on the physico-chemical properties of the constituents and composition, the stability of a microemulsion may be affected by the addition of buffers, electrolytes, preservatives, polymers and drugs, which may alter the microemulsion structure, and region of existence. Microemulsion systems based on nonionic surfactants are generally less affected by additives and changes in pH than ionic surfactants [35,36].

3. The characterization of microemulsions

As the size of microemulsion aggregates is smaller than the wavelength of visible light, and the structures can be altered by changes in composition and temperature, the direct examination of microemulsion structures is very difficult. Therefore, many techniques have been employed with varying success in the size analysis of microemulsions [37-42].

It is known that particle size distribution is one of the most important elements of a microemulsion to characterize, for the evaluation of its stability [43] and *in vivo* fate [44]. Ozguney *et al.* [45] have shown that the mean droplet diameters of microemulsions prepared with isopropyl alcohol and with propanol without diclofenac sodium were 11.7 and 14.45 nm, whereas with diclofenac sodium these were 9.19 and 12 nm, respectively. The mean droplet size of microemulsions incorporating diclofenac sodium is smaller

compared with the mean droplet size of microemulsions without the drug. Presently, it was not clear by which mechanism the droplet size is decreased. However, two possibilities have been considered: i) a portion of the undissolved drug could act as an emulsifying agent by the deposition of drug particles at the interface of the microemulsion; ii) the deposition of drug at the interface of the microemulsion, and the reduced motility of the surfactant, has been thought to decrease the particle size of drug-loaded microemulsions, as has previously been demonstrated [46].

Microemulsions have been evaluated using a wide range of different techniques over the years, but a combination of these methods is generally required in order to fully characterize these systems. At the macroscopic level viscosity, conductivity and dielectric methods provide useful information [47-55]. Viscosity measurement for example can indicate the presence of rod-like or worm-like reverse micelles [48-50], and conductivity measurements provide a means of determining whether a microemulsion is oil-continuous or water-continuous [51-53]. Dielectric measurements are a powerful means of probing both the structural and dynamic features of microemulsion systems [54-55].

As has been previously reported, in order to study the electrical conduction of nonionic microemulsions, a small amount of aqueous electrolyte must be added to provide the charges necessary for charge transport [56,57]. However, the addition of salt, especially sodium chloride, can significantly affect the phase behaviors and structural properties of microemulsions [58], and may even result in phase separation. Because of this, in a study by Kantarci *et al.* [53], the conductivity measurements were performed without deliberate incorporation of an electrolyte. Low conductivity values were obtained with microemulsion formulations without a water fraction. It is known that autoprotolysis constants (K) for aliphatic alcohols are -logK = ~ 20, and for water, -logK = 14. Therefore, solutions of alcohols are better conductors than water, and the conductivities of alcohols are also greatly increased by the presence of water. The appropriate electrical conductivity values for this particular conductivity study were obtained with formulations without water and diclofenac sodium, primarily enabled by the presence of alcohols; then the electrical conductivity values of the microemulsions with sufficient electrical conductivity values with and without diclofenac sodium were compared. As shown in **Table 1**, the conductivity values of unloaded microemulsions were 16.9 – 17.9 μScm^{-1}, and drug-loaded microemulsions 18.8 – 20.2 μScm^{-1}. From viscosity measurements, it was observed that the viscosity values of drug-loaded microemulsions were higher than the values for unloaded formulations (**Table 1**).

At relatively low dispersed phase volume fractions, the microemulsion generally contains nanometer-sized droplets of oil or water. If the droplets are non-interacting the resulting microemulsion will be of low viscosity and may, therefore, be suitable for oral, parenteral, pulmonary or even ocular delivery. When water is present in a microemulsion system, it can be either free or bound water depending on the state of the system. Bulk (free) water is assumed to have physicochemical properties similar to those of pure water. Bound water on the other hand is strongly influenced by the surfactants present in the samples and its properties will differ from those of pure water (i.e., the presence of a nearby surfactant alters its thermodynamic properties, such as freezing point, melting point, enthalpy and heat capacity) [10,59,60].

In one study, the viscosity measurements of methotrexate-loaded microemulsion (M-MTX) were examined as a function of shear rate [33]. **Figure 3** shows that M-MTX was a pseudo-plastics non-Newtonian fluid – this type of fluid has a decreasing viscosity with an increasing shear rate. This flow behavior is sometimes called 'shear thinning', and the viscosity of such fluids changes as the shear rate is varied. Thus, the experimental parameters of the viscometer model, spindle and speed all have an effect on the measured viscosity of a non-Newtonian fluid. Previous studies on microemulsion rheology have indicated that bicontinuous or middle-phase microemulsions may be weakly shear thinning. However w/o and o/w microemulsions remain Newtonian at high shear rates. It is well understood that the rheological behavior of a liquid depends upon how the shear rate compares with the rate of various relaxation processes in the system. As presented in **Figure 3**, the study by Karasulu *et al.* demonstrated that, at high shear rates, the microemulsion viscosity remained nearly constant as the shear rate was varied, as with a Newtonian fluid. The viscosity of M-MTX was 143 ± 3 cP.

The isotropic nature of microemulsions and their optical clarity requires the use of spectroscopic techniques for their study, particularly when making comparisons with conventional macroemulsions [2,59,60]. The measurement of self-diffusion coefficients can be performed by a number of nuclear magnetic resonance (NMR) spin-echo methods, the neutron spin-echo method, tracer techniques involving radioactive labeling of the compound, and transient optical grating methods [2,5,37,50,61-63]. Scattering methods have also been invaluable in elucidating the structure of microemulsions, and these include small angle X-ray scattering (SAXS), small angle neutron scattering (SANS), and static and dynamic light scattering. These techniques have a lower size limit of ~ 2 nm, and upper limit of ~ 100 nm for SANS and SAXS, and a few microns for light scattering [64-67].

Microemulsions offer several potential advantages as drug delivery systems due to their solubilization capacity, transparencies, high stability and simplicity of manufacture. However, the most critical problem regarding to the microemulsion-based drug carriers is the toxicity of the components. Recent efforts have been focused on how to decrease or eliminate the toxicity or irritation of the microemulsion formulations [68,69]. A microemulsion system free of alcohols has been investigated as a potential drug

Table 1. The conductivity, viscosity and observed pH values of w/o microemulsions with/without diclofenac sodium.

Formulations	Unloaded microemulsions			DS – loaded microemulsions		
	Conductivity σ (μS/cm)	Viscosity ή (cps)	pH	Conductivity σ (μS/cm)	Viscosity ή (cps)	pH
M (ethanol)	16.9	87	6.70	18.8	176	6.70
M (isopropyl alcohol)	16.9	80	6.75	20.2	174	6.85
M (propanol)	17.9	90	6.75	18.8	194	6.70

Table adapted from [53].
DS: Diclofenac sodium; M: Microemulsion.

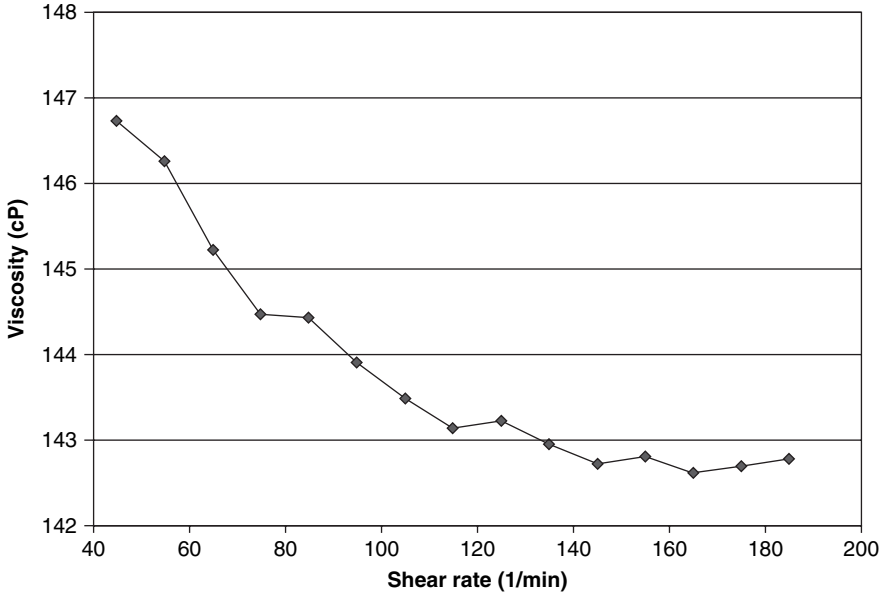

Figure 3. Variation of viscosity as function of the shear rate for the microemulsion system.
Figure adapted from [33].

delivery system [30-32], and in these studies, the components were all food-grade agents and were almost completely free from causing irritation or toxicity, which will be important for the improvement of microemulsion formulations in future. Other important questions surrounding microemulsion-based drug delivery systems are where the drug is solubized and how the stability of the drug is increased [68]. The use of ^{1}H NMR spectroscopy, high performance liquid chromatography assay and dynamic light scattering experiments have proven to be useful in these fields [35,70-72].

4. Microemulsions in drug delivery

Microemulsions have received recent interest as potential drug delivery systems [13-16,21,22,28-33,35,36,42-46,49,53,70,72]. Drugs incorporated in microemulsions will partition between the aqueous and hydrophobic phases, depending on their lipophilicity, and the influence of the partition coefficient of a drug on its release characteristics has been reported by Trotta *et al.* [73]. The attraction of o/w microemulsion systems lies in their ability to incorporate drugs into the apolar oil phase, thereby enhancing their solubility [13,74-76]. Hydrophilic drugs can be incorporated into the dispersed aqueous phase of w/o microemulsion droplets, to afford some protection from enzymatic degradation when orally administered [77-86]; Celebi *et al.* [84] prepared a stable microemulsion formulation effective in preventing the enzymatic degradation of epidermal growth factor in the gastrointestinal tract, and examined the effects of different epidermal growth factor formulations administered by different routes on the healing of acute gastric ulcers in rats. Several workers have reported studies in which the lipophilicity of drugs has been increased to enhance their solubility in the dispersed oil droplets. In this way, a reservoir of the drug is produced and

Table 2. Permeation paremeters of diclofenac sodium from different bases through rat skin*.

Formulation	Flux ± S.D (μg/cm^2/h)	Kp ± S.D (cm/h)
M	$4.9 \times 10^{-2} \pm 0.0040$	$4.9 \times 10^{-3} \pm 3.6 \times 10^{-4}$
M + DMSO	$5.3 \times 10^{-2} \pm 0.0120$	$5.3 \times 10^{-3} \pm 1.2 \times 10^{-3}$
Commercial	$2.7 \times 10^{-2} \pm 0.0070$	$2.7 \times 10^{-3} \pm 7.3 \times 10^{-4}$
Gel	$4.5 \times 10^{-2} \pm 0.0005$	$4.5 \times 10^{-3} \pm 4.5 \times 10^{-5}$

Table adapted from [45].
*Data are given as mean ± S.D. (n = 3).
DMSO: Dimthyl sulfoxide; Kp: Permeability coefficient; M: Microemulsion.

a sustained release effect is achieved, as the drug continuously transfers from the oil droplets to the continuous phase to replace drug released from the microemulsion [2,5,86,87]. Moreover, drug delivery forms based on w/o microemulsions can also be employed where dilution by the aqueous phase is less likely to occur, such as after intramuscular injection. Similarly, microemulsions and microemulsion gels for transdermal drug delivery are also under development [45,46,70,72,88]. These have been reported to significantly enhance the absorption of drugs compared with solution, gel or cream formulations. Oral, ocular, pulmonary, nasal, vaginal and intravenous routes are the main alternative administration routes to which the micoemulsion technique can be applied [29,46,59,69,89-94].

An interesting microemulsion formulation based on oleic acid/span 80/tween 80/isoropanol has been reported [95]. The microemulsion was prepared by the loading of an anticancer drug, mitomycin C (MC), into this oil/water system, and stability studies were perfomed in order to obtain the physical and physicochemical properties of the microemulsion. In this study, a new electrochemical detection method for the interaction of double-stranded DNA (dsDNA) with MC loaded into the microemulsion was employed, using differential pulse voltammetry with a disposable sensor and pencil graphite electrode. The magnitude of guanine oxidation was monitored before and after the interaction between MC and dsDNA. The effect of different experimental parameters such as MC concentration, MC interaction time with dsDNA, and dsDNA concentration were also studied to find the optimum analytical performance.

4.1 Microemulsions for dermal and transdermal delivery

Many studies have shown that microemulsion formulations possessed improved transdermal and dermal delivery properties, mostly *in vitro*, and several *in vivo*. The vast majority of drug delivery investigations with topical miroemulsions have been performed *in vitro*, using the classical Franz-type diffusion cells with various membranes. Although this method actually determines percutaneous, rather than cutaneous drug delivery, a good indication of the cutaneous drug delivery potential of microemulsions can be obtained from these studies. As demonstrated by recent publications, the intradermal permeation rates of a lipophililic drug are significantly increased from microemulsions, compared with commercial macroemulsions [35,45,53,70,72,88,96-98].

A study by Ozguney *et al.* [45] has been performed, which had the aim of improving the transdermal permeation of diclofenac sodium. Transdermal permeation studies were carried out using rat skin. Three topical formulations of diclofenac sodium (1%, w/w) were prepared: a gel, an emulsion and a microemulsion. Furthermore, the effect of dimethyl sulfoxide (DMSO), added as an enhancer into the microemulsion system, on the penetration rate of diclofenac sodium was examined. The commercial formulation of diclofenac sodium was also tested as a reference. It was found that the flux from the emulsion was 6.5×10^{-2} μg/cm^2/h, which was 2.4-times greater than that observed from the commercial dosage form (2.7×10^{-2} μg/cm^2/h). The flux values of the microemulsion and the microemulsion containing DMSO as an enhancer were 1.8- and 2.0-times greater than the commercial dosage form, respectively (**Table 2**). In a previous study, it was explained that the interaction of DMSO with the stratum corneum lipid alkyl chains resulted in decreased diffusion resistance of the barrier, with an observed drug partition increase into the skin [99]. In the same study, it was also found that the different formulations loaded with diclofenac sodium could significantly ($p < 0.05$) inhibit carrageenan-induced rat paw thickness. Furthermore the anti-inflammatory effects of the microemulsion, and microemulsion plus DMSO, formulations were statistically higher than the others, according to a Duncan test ($p < 0.05$). One reason for this effect is that the microemulsion had a very low interfacial tension, which allowed for excellent contact with the skin surface, allowing the vehicle to fill even microscopic gaps. This should enhance the vehicle skin drug transfer. The second possible mechanism is related to the high drug loading capacity of the microemulsion; and the third possibility is the penetration-enhancing effect of the microemulsion components. This mechanism can add to the explanation of the effect of cosurfactants, as they are known to act as skin penetration enhancers. Finally, the supersaturation process may be responsible, as it increases the thermodynamic activity and driving force for transdermal drug transfer [35,70,98,100].

Various lecithin (phosphatidylcholines)-based formulations have been proposed as dermal and transdermal drug delivery systems [101-104]. Paolino *et al.* [104] investigated the potential application of highly biocompatible o/w microemulsions as topical drug carrier systems for the percutaneous delivery of anti-inflammatory drugs. Microemulsions were made up of triglycerides as the oil phase, a mixture of lecithin and n-butanol as the surfactant/cosurfactant, and an aqueous solution as an external phase. To evaluate the percutaneous

Table 3. Human skin irritancy test of various topical formulations after 48 h of treatment.

Sample	Irritation evidence at 48 h								Score‡
	Number of cases*								
	Vesicles	Edema	Erythema	Flakiness	Dryness	Wrinkling	Glazing	No visible reaction	
OA 1%	–	–	6	7	–	1	1	15/30	16.27 ± 2.89
w/o	–	–	–	1	2	2	2	23/30	8.71 ± 3.15
o/w	–	–	–	1	1	2	2	24/30	6.17 ± 1.83
Gel	–	–	–	–	–	2	2	26/30	4.75 ± 2.06
SL ME	–	–	–	–	–	2	2	26/30	4.00 ± 1.83
OA-SL ME	–	–	–	1	1	2	1	25/30	5.60 ± 2.07

Table adapted from [104].

*The value reported in each column represents the number of subjects who showed skin reaction symptoms.

‡Non-parametric variable Kruskal-Wallis test provided: $p < 0.001$ for OA (1% w/w) aqueous dispersion and w/o cream versus all other samples; $p < 0.001$ for o/w cream versus gel and SL microemulsion; $p < 0.005$ for SL microemulsion versus OA-SL microemulsion.

ME: Microemulsion; OA: Oleic acid; o/w: Oil-in-water; SL: Soybean lecithin; w/o: Water-in-oil.

delivery-enhancing effect of oleic acid, this compound was used as a component of some of the o/w microemulsions. The topical carrier potential of lecithin-based o/w microemulsions were compared with respect to conventional formulations. The percutaneous adsorption of the various topical formulations was evaluated through healthy adult human skin. Ketoprofen-loaded microemulsions demonstrated enhanced permeation through human skin with respect to conventional formulations. The human skin tolerability of various microemulsion formulations was also evaluated in human volunteers, and displayed good tolerability (**Table 3**).

One of the problems associated with the use of microemulsions for topical drug delivery is the difficulty of using these vehicles on the skin, because of their fluidity. Gasco *et al.* [105] have recently addressed this problem with the development of a microemulsion for the topical administration of azelaic acid, which has showed therapeutic affects on some pageantry disorders and on acne vulgaris. The viscosity of the o/w microemulsions used in this study was increased with Carbopol® 934 (Lubrizol) to make them suitable for topical administration.

Several transdermal drug delivery studies, assessing drug levels in the systemic circulation, have been performed in rabbits [98,106,107]. In one such study [107], a microemulsion vehicle was investigated as a possible matrix for the transdermal delivery of theophylline. The existence of microemulsion regions were investigated in pseudoternary phase diagrams, and various microemulsion formulations were prepared using oleic acid, Cremophor RH40/Labrasol (Gattefosse; 1:2) and water. An *in vivo* study was performed using rabbits, and the results indicated that the $AUC_{0-\infty}$ of transdermal administration was 1.65-fold higher than that of oral solution administration. These studies showed that microemulsion systems might be promising vehicles for the transdermal delivery of theophylline.

In vitro and animal studies can provide very useful information and predictions about drug penetration and behavior in man. However, very few human investigations have been reported. Presumably, these studies have been investigated for cutaneous drug delivery and human skin tolerability [108-111].

4.2 Microemulsions for oral delivery

The assessment of microemulsions for oral delivery has centered on their potential use for the delivery of peptides/proteins, and in particular ciclosporin [2,5,44,78,80,83-85,93]. This formulation is generally administered as a soft capsule that contains an oil solution of a drug and surfactants. It converts into an o/w microemulsion in an aqueous environment (i.e., in the stomach and small intestine). It mimics bile salt micelles, which play an important role in the adsorption of poorly soluble drugs, and, thus, the absorption of such drugs can be significantly enhanced. It allows the plasma concentration profiles and bioavailability of drugs more reproducible. Tarr and Yalkowsky [44] have demonstrated enhanced intestinal absorption in rats through a reduction of emulsion droplet size by homogenization, which was explained in terms of the greater surface area of the dosage form. The results of this study are supported by a demonstration of the significantly higher bioavailability of ciclosporin when administered using w/o microemulsions.

An interesting microemulsion formulation was prepared by Formariz *et al.* [112]. The results of this work show that it is possible to obtain an o/w microemulsion stabilized by the mixed surfactant soya phosphatidylcholine/eumulgin/sodium oleate. The incorporation of doxorubicin in the microemulsion system increased the droplets size for all surfactant concentrations used. It was possible to conclude that the investigated microemulsion can be a very promising drug carrier for the administration of doxorubicin.

Self-microemulsifying drug delivery systems (SMEDDSs) and SEDDSs have been proposed to improve the bioavailability of poorly soluble drugs. SMEDDSs are mixtures of drugs (usually water insoluble), lipids, surfactants and cosurfactants. They form fine o/w microemulsions with a particle size of < 100 nm when exposed to aqueous media under conditions of gentle agitation or digestive motility that would be encountered in the gastrointestinal tract. The spontaneous formation of a microemulsion advantageously presents the drug in a dissolved form, and the resultant small droplet size provides a large interfacial surface area for drug release and absorption. In addition, the specific components of SMEDDSs promote the intestinal lymphatic transport of drugs. The main mechanisms include increasing the membrane fluidity to facilitate transcellular absorption, the opening of tight junctions to allow paracellular transport, inhibiting P-gp and/or CYP450 with surfactants to increase intracellular concentration and residence time, and stimulating lipoprotein/chylomicron production with lipids. The oral absorption of several drugs has been enhanced by SMEDDSs employing a single or combined mechanism [2,14,30,43,68]. SEDDSs are a mixture of oil and surfactant that contain drug, and they spontaneously form o/w emulsions in aqueous media under mild agitation. When these systems are orally administered, emulsion droplets should be smaller than those *in vitro*, because bile salts will be incorporated into the surfactant layers of the emulsion droplets [2,14,30,32,43,69,86]. SEDDSs have been reported to improve the reproducibility of the a drug's plasma profile and bioavailability [14,43]. In the study by Kawakami *et al.* [76], the absorption of the poorly soluble drug, nitrendipine, was enhanced significantly by employing a microemulsion formulation, compared with a suspension or oil solution (**Table 4**). The effect of a fed state on the oral absorption of nitrendipine became insignificant with microemulsion formulations, although it significantly affected the absorption from a suspension formulation. The absorption behavior also varied with the type of surfactant. The absorption from a Tween 80-based formulation was very rapid, but a HCO-60-based formulation displayed a prolonged plasma concentration profile. However, absorption from a BL-9EX (polyoxyethylene alkyl ether)-based formulation was hardly observed. In this study, damage to the gastrointestinal mucosa seemed to be an important problem associated with microemulsion formulations, because they contained a relatively large amount of surfactant. Tween 80-based and HCO-60-based formulations were mild to the organs in terms of toxicity, but BL-9EX-based formulations caused serious damage.

Another interesting study has been performed by Sha *et al.* [68]. The aim of this study was to investigate the effect of two novel SMEDDSs containing Labrosol on tight junctions, with different dilutions. The cytotoxicity of SMEDDSs and the effect of surfactants on the mitochondrial activity of Caco-2 cells were evaluated by using the MTT (3-[4,5 dimethylthiazol-2-yl]-2,3-diphenyl tetrazolium bromide) assay. This colorimetric method is based on the reduction of the tetrazolium ring of MTT by mitochondrial dehydrogenases, yielding a blue formazan product that can be measured spectrophotometrically; the amount of formazan produced is proportional to the number of viable cells. The results demonstrated that negatively charged SMEDDSs with different dilutions had no effect on transepithelial electrical resistance, but significantly increased the permeability of mannitol.

The most notable example of a SMEDDS relates to the oral delivery of ciclosporin A (CsA), in particular the commercial Neoral® (Novartis) formulation. CsA – a highly lipophilic undecapeptide – is commonly used as an immunosuppressant in transplantation surgery. The Neoral formulation uses an isotropic concentrated blend of surfactant based on medium chain length partial glyserides, a medium chain length triglyseride oil and drug [44,78,80,93]. The bioequivalence of generic formulations is established by measuring pharmacokinetic parameters in healthy volunteers [113]. CsA absorption and exposure is known to differ between healthy volunteers and transplant recipients. Therefore, bioequivalence testing may be inadequate to ensure therapeutic equivalence. In one study, the investigators sought to compare the efficacy of generic ciclosporin (ArpimuneME, RPG Life Sciences) versus Sandimmune® Neoral in *de novo* renal transplant recipients (**Table 5**). It was concluded that the use of a generic microemulsion form of CsA provided safe and effective immunosuppression compared with Sandimmune Neoral when drug monitoring was performed by C_2 levels.

The most significant problem with microemulsion systems is the lack of biological tolerance of for the excipients, such as the surfactant and cosurfactant. Karasulu *et al.* [33] have examined a microemulsion of methotrexate (M-MTX) and a solution of the drug (Sol-MTX) in a biological environmental model. For this purpose, a gastrointestinal cell culture model (Caco-2 cell line) was used to investigate the cytotoxic effects of the polymeric carrier and its effect on cell monolayer integrity. The results for the colorimetric assay revealed that for all empty microemulsion concentrations, the cell monolayers remained > 95% viable, when compared with the control, indicating that this system appears to possess very low cytotoxicity (**Figure 4**). Caco-2 viability experiments have been performed with M-MTX and Sol-MTX at the same concentrations. After the incubation of cells with Sol-MTX for 3 days, Caco-2 cell proliferation was significantly inhibited (determined by Tukey's test; $p < 0.05$) in a dose-dependent manner, to an extent of 38.11 ± 3.90 % at the highest concentration of 40 ng/75 µl. As is presented in **Figure 4**, the differences between the viability of cells for M-MTX and Sol-MTX were found to be significantly different when applied to ANOVA, according to a 2 × 8 factorial randomized design

Table 4. Pharmacokinetic parameters of an oral administration study of nitrendipine in rats.

Formulation	T_{max} (h)		C_{max} (µg/l)		AUC (µg/h/l)		AUC ratio
	Normal	Fasted	Normal	Fasted	Normal	Fasted	Normal/fasted
MC suspension	8.0 ± 0.0	1.5 ± 0.5	0.23 ± 0.03	0.04 ± 0.00	1.03	0.05	21.4
Oil solution	4.0 ± 0.0	3.5 ± 1.7	0.53 ± 0.09	0.30 ± 0.09	2.55	1.71	1.50
Tween 80 ME	1.3 ± 0.7	1.3 ± 0.7	0.36 ± 0.00	0.59 ± 0.12	2.09	2.50	0.84
C12E9 ME	4.3 ± 3.8	3.0 ± 1.0	0.22 ± 0.19	0.08 ± 0.00	0.58	0.47	1.25
HCO60 ME	≥ 8.0	≥ 7.0	≥ 1.45	≥ 1.44	7.70	6.43	1.20

Table adapted from [76].
AUC: Area under the concentration–time curve from 0 to 8, calculated by trapezoidal method; C_{max}: Maximum drug concentration (average ± S.E.); MC: Methylcellulose; ME: Microemulsion; T_{max}: Time to reach maximum drug concentration (average ± S.E.).

Table 5. The efficacy of two forms of ciclosporin A.

	Group A	Group B	p-Value
Mean CsA level (ng/ml)			
1 month	1419.1 ± 213.6	1460.5 ± 290.7	NS
3 month	1306.7 ± 254.4	1342.4 ± 303.4	NS
6 – 12 month	1061.3 ± 450.1	1066 ± 171.8	NS
Mean CsA dose (mg/kg)			
1 month	9.5 ± 1.5	8.7 ± 1.6	0.03
3 month	6.2 ± 1.4	5.9 ± 2.2	NS
6 – 12 month	4.2 ± 1.0	4.1 ± 1.6	NS
Mean creatinine (mg %)			
At discharge	1.6 ± 0.8	2.0 ± 1.4	NS
1 month			
12 month	1.4 ± 0.6	1.5 ± 1.6	NS
Mean cholestrol levels (mg %)	178.2 ± 24.4	179.1 ± 31.7	NS
Mean systolic BP (mmHg)	125.3 ± 13.5	129.4 ± 13.8	NS
Mean diastolic BP (mmHg)	81.7 ± 7.8	86.4 ± 7.1	NS
Antihypertensive drug requirement	1.95 ± 1.0	1.8 ± 0.8	NS

Table adapted from [113].
Group A patients doses 9.5 ± 1.5 mg/kg; Group B patients doses 8.7 ± 1.6 mg/kg.
BP: Blood pressure; CsA: Ciclosporin A.

($p = 0.016$, $\alpha = 0.05$, power = 0.695). When MTX was loaded into the microemulsion system, the toxicity to cells was significantly lower when compared with Sol-MTX ($p < 0.05$); however, this effect was not dose dependent. At the lowest M-MTX concentration of 0.5 ng/75 µl, MTX had clearly no antiproliferative effect. At dilution rates of M-MTX applied to Caco-2 cells of 2.5 – 40 ng/75 µl, Caco-2 cell proliferation was inhibited, but no significant difference could be determined by Dunnett test at these concentrations ($p > 0.05$). The results of the colorimetric assay revealed that, for M-MTX concentrations, cell monolayers remained > 72.11% viable when compared with control, indicating that this system appears to possess very low cytotoxicity compared with Sol-MTX (61.89 %). In conclusion, the microemulsion formulation of MTX had little cytotoxic effect on Caco-2 cells when compared with Sol-MTX. Therefore, it is possible that the therapeutic application of M-MTX will be associated with low cytotoxicity in normal cells, and low side effects may be expected.

The *in vitro* release behavior of MTX from a microemulsion and a solution has also been examined using a dialysis tube method [33]. The release behavior of MTX from a microemulsion exhibited a slow and continuous release for 36 h, and the release of MTX from solution

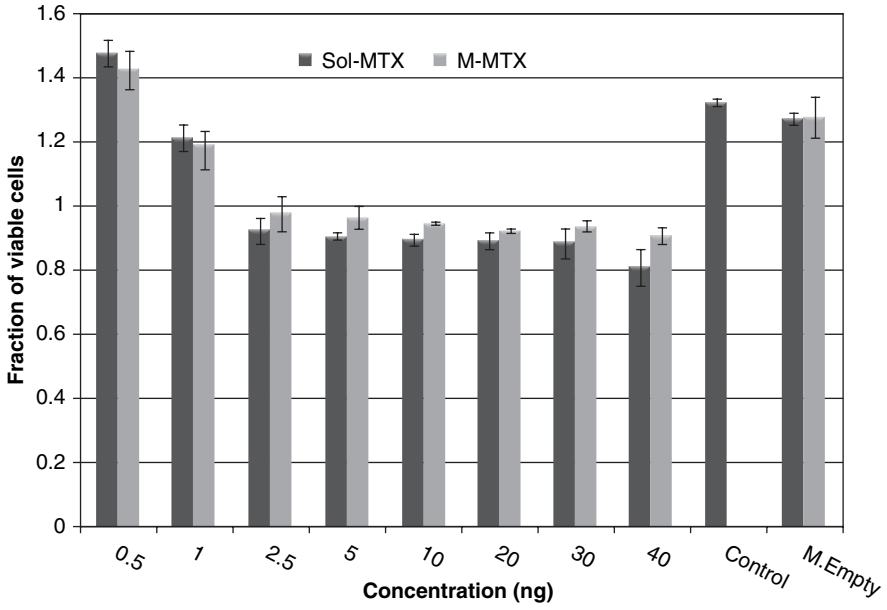

Figure 4. Cytotoxicity assays of Caco-2 cells treated with Sol-MTX, M-MTX and empty microemulsion (no drug loaded) at different concentration. The viability was measured by MTT test. The values represent the mean of three independent experiments (mean ± S.D., n = 3).

Figure adapted from [33].

M-MTX: Methotrexate microemulsion; Sol-MTX: Methotrexate solution.

lasted for 6 h (**Figure 5**). This might suggest that the release rate of MTX from a microemulsion could be controlled by this formulation.

4.3 Microemulsions for parenteral, ocular and pulmonary delivery

The parenteral administration of poorly soluble substances, especially by the intravenous route, is a major problem in the pharmaceutical industry, and several solubilizing techniques have been used in the past. Microemulsions also have great potential as intravenous vehicles for poorly soluble drugs, because of their high solublization capacity. However, the pharmaceutically acceptable microemulsions designed for intravenous administration have recently not only been formulated and characterized, but also tested *in vivo* for homodynamic response. The main drawback with microemulsions has been the high concentrations of surfactant that are required and the types of oil phase used. Some recent studies have shown that microemulsions can be formulated using a medium-chain triglyceride as a nonpolar component, with phosphatidylcholine and a short-chain alcohol (C_3 or C_4) as the surfactant and cosurfactant, respectively. The C_3 or C_4 chain alcohols are not acceptable for intravenous uses, in contrast to the medium-chain triglycerides and phosphatidylcholine that are used in intravenous nutrition emulsions [20,114].

A pharmaceutically acceptable microemulsion system composed of a medium-chain triglyceride has been presented and characterized in terms of phase behavior, microstructure, solubilization capacity and *in vivo* effects after intravenous administration to conscious rats [114]. This microemulsion can be administered by intravenous infusion to conscious rats in a dose of up to 0.5 ml/kg without producing any significant effect on the acid–base balance, blood gases and plasma electrolytes. The main conclusion that can be drawn from this work is that it is possible to formulate a microemulsion of pharmaceutically acceptable compounds.

Kang *et al.* [115] have developed an optimal paclitaxel microemulsion prepared by SMEDDSs, which is a mixture of paclitaxel, tetraglycol, Cremophor ELP and Labrafil 1944 (Gattefosse), and a paclitaxel microemulsion containing poly(D,L-lactide-co-glycolide) (PLGA) in order to achieve the controlled release of paclitaxel. Paclitaxel has shown significant anti-tumor activity against various tumors. Paclitaxel injection is presently the only dosage form available for clinical use (Taxol®; Bristol-Myers Squibb), and is a solution of paclitaxel in 50% Cremophor EL and 50% alcohol. A variety of approaches to avoid using Cremophor EL have been investigated to deliver paclitaxel with high therapeutic efficacy, such as liposomes, nanospheres and parenteral emulsions [116-118]. These drug delivery systems have disadvantages. Liposomes have a poor shelf life and insufficient loading for lipophilic drugs. Nanospheres have poor loading efficiency and are associated with the problem of eliminating residual solvents. Emulsions are not appropriate for the parenteral delivery of anti-cancer drugs,

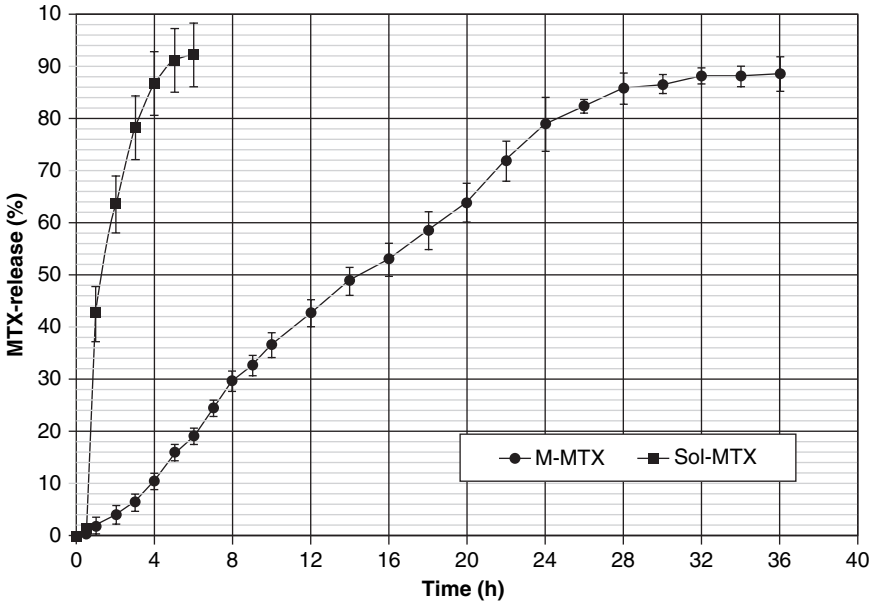

Figure 5. Release profiles of methotrexate from a methotrexate microemulsion and a methotrexate solution.
n = 3.
Each value represents the mean ± S.D.
Figure adapted from [33].
M-MTX: Methotrexate microemulsion; Sol-MTX: Methotrexate solution.

because of rapid release. Among the emulsifying methods, SMEDDSs are worthy of note. The results obtained from one investigation [115] have established the potential use of microemulsions incorporating PLGA for the sustained release of lipophilic drugs such as paclitaxel. In this study, the release behavior of paclitaxel from a microemulsion containing PLGA exhibited a biphasic pattern characterized by a fast initial release during the first 48 h, followed by a slower and continuous release for 144 h, in contrast to the release of paclitaxel from a microemulsion without PLGA (24 h duration). This result was identical to that of the anti-tumor activity *in vitro* of paclitaxel from a microemulsion containing PLGA against the human breast cancer cell line MCF7, and this formulation enhanced anti-tumor activity *in vivo* compared with a microemulsion without PLGA against SKOV-3 human ovarian cancer cell bearing nude mice model.

w/o microemulsions have been described in the literature as drug carriers of water-soluble molecules for oral or intramuscular delivery [16,46]. In the case of w/o microemulsions, phase inversion is an interesting property, and the resulting o/w microemulsions can be used for parenteral drug delivery [20,46]. It has been found that w/o microemulsions can easily be inverted into o/w microemulsions and/or multiple water-oil-water (w/o/w) emulsions upon dilution with excess aqueous phase [114]. An injectable microemulsion of arsenic trioxide (As_2O_3-M) has been prepared for intratumoral injection, and its suppressive effect on human breast cancer cells (MCF-7) was compared with those of a solution of the drug [49]. The microemulsion was made up of soybean oil as the oil phase, a mixture of Brij 58 and Span 80 as surfactants, absolute ethanol as a cosurfactant, and bi-distilled water containing As_2O_3 solution as the aqueous phase. The microemulsion formulation contained 5×10^{-6} molar As_2O_3, and the pH of As_2O_3-M was adjusted to 7.35 ± 0.1, and the physicochemical stability of the formulation was observed. The formulation was physically stable for 12 months at room temperature when kept in ampule forms, as well as after autoclaving at 110°C for 30 min. The antitumor effects of As_2O_3-M were examined on MCF-7 cells. It was clearly demonstrated that As_2O_3-M had a significant cytotoxic effect on breast cancer cell lines, and this effect was significantly more than that of regular As_2O_3 solutions. Even ~ 3000-times diluted microemulsion formulations loaded with 5×10^{-6} molar As_2O_3 showed cytotoxic effect. As a result, this diluted concentration (~ 1.6×10^{-9} M) was found to be 1000-times more effective than regular As_2O_3 solutions (5×10^{-6} M). **Figure 6** shows that the ~ 1.6×10^{-9} M microemulsion form of As_2O_3 exerted a highly cytotoxic effect and killed 80% of tumor cells. A significant difference was determined by using Scheffe's F-test ($p < 0.05$). According to the *in vitro* cytotoxicity studies, it can be concluded that when As_2O_3 was incorporated into the microemulsion, which is a new drug carrier system, it suppresses tumour cell growth in multiple tumor lines.

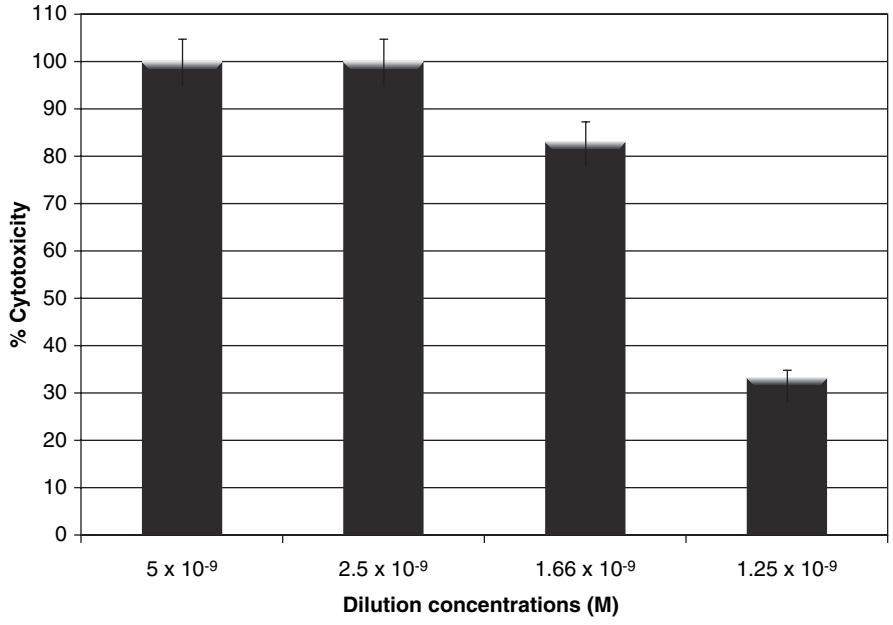

Figure 6. The cytotoxicity of tumor cells treated with As_2O_3-M. Cytotoxicity was assessed by a tryptan blue dye exclusion test following 72 h culture.
Each point represented the mean ± S.D.
Figure adapted from [49].

The direct injection of various anticancer agents into tumors has several advantages over systemic administration. For example, lower doses can be injected into the tumor site, to reduce the side effects. However, most anticancer agents are composed of small molecules and their intratumoral clearance is relatively rapid [119]. To overcome this problem, lipid carrier systems have been used because of their favorable characteristics as a biodegradable drug reservoir. Thus, the local disposition characteristics of lipid carrier formulations after intratumoral injection have become an important issue in drug delivery.

In conclusion, it has been suggested that lower concentrations of As_2O_3-loaded microemulsion formulations could be effective in MCF-7 cell lines, and the As_2O_3-M system offers greater stability and a longer shelf-life when compared with the other emulsions.

The cornea and nasal mucosa both offer the possibility for simple and comfortable drug administration [69,89-91,120-122]. The development and characterization of o/w, and w/o microemusions designed for ocular use have recently been reported [69,120,122]. The instillation of the microemulsion 8-times a day for 5 days in 4 rabbits did not result in any irritation or inflammation. The cornea and its epithelium were not affected [122].

Eye drops are the most commonly used dosage form for the ocular route of administration, and chloramphenicol is one of the most effective drugs used in the eye drop. However, eye drops as drug delivery systems have several disadvantages, such as the very low bioavailability of the drugs, which must be absorbed at this site and must be applied several times a day. In addition, the effective component, chloramphenicol, has very low solubility in water, and easily hydrolyzes. In this area, an interesting formulation has been developed by Lv *et al.* [69]. In another study, a microemulsion composed of Span 80, Tween 20, isopropyl myristate was investigated as a potential drug delivery system for eye drops [120]. The system is important in that all its components are food grade so that the microemulsion is almost free of toxicity and irritancy. The phase transition was investigated using electrical conductivity measurements. The location of the chloramphenicol molecules in the microemulsion formulations was determined by dynamic light scattering and ^{1}H NMR spectroscopy. Its stability was investigated by the high performance liquid chromatography. The results implied that the stability of chloramphenicol in the microemulsion formulation was remarkably increased. A further study investigated a microemulsion containing pilocarpin, developed using Brij 35P and Span 80 as the surfactants, propanol as the cosurfactant, and soybean oil as the oil phase. The test microemulsion was non-irritant in rabbit eyes and a prolonged pharmacodynamic effect was observed *in vivo* compared with the drug administered as a simple solution or gel. Neat fluorocarbons are being investigated for the pulmonary delivery of drugs and genes, triggering interest in reverse water-in-fluorocarbon emulsions as delivery systems for hydrophilic bioactive materials to the lung. In one such study [89], the ability of a series of perfluoroalkylated amphiphiles with a dimorpholinophosphate polar head

group were examined. F8H11DMP was found to allow the obtaining of both stable water-in-fluorocarbon emulsions and of a microemulsion. This study allowed the selection of F8H11DMP as the choice emulsifier candidate for the preparation of water-in-perflourooctyl bromide emulsions, as it yielded finer, more narrowly dispersed and more stable emulsions than any of the other F*n*H*m*DMPs investigated. Using F8H11DMP, a whole range of reverse water-in-perflourooctyl bromide macro-, mini- and microemulsions have been obtained and characterized.

5. Conclusions

Microemulsions that are made of water, oil, surfactant and occasionally alcohol as a cosurfactant have unique properties as drug carriers. Microemulsions have been increasing in popularity and garnering more attention in recent years because of their solubilization capacity, transparencies, high stability and simplicity of manufacture. Microemulsions may be prepared by many different aqueous solutions, surfactant and oil constitutes, and according to the properties of the applied constituents, microemulsions can exist with a wide range of compositions. Due to the possible concurrent incorporation of oil and water in microemulsions, the vehicles have excellent solubility properties for both lipophilic and hydrophilic drugs.

Various theories concerning microemulsion formation, stability and phase behavior have been proposed over the years. An essential requirement for their formation and stability is the attainment of a low interfacial tension. Lowering of the interfacial tension and the fluidity of the interfacial surfactant film is usually achieved by introducing a short chain cosurfactant to the surfactant film. Careful consideration should also be given to the choice of cosurfactant. However, the use of cosurfactant is not required, and alcohol-free SEDDSs or SMEDDSs have been described in the literature.

Furthermore, it had been proven that it was possible to formulate preparations suitable for most routes of administration from microemulsions. A number of comparative investigations exist in the literature, some of which have evaluated the use of microemulsion formulations against alternative delivery systems. However, there is still a considerable amount of fundamental work to be done in order to establish the physicochemical behavior of microemulsions, before they can live up to their potential as multipurpose drug delivery vehicles.

6. Expert opinion

For several years, microemulsions have been investigated as novel drug delivery systems, and their potential uses have been studied by several research teams. Formulations based on microemulsions have several interesting advantages, such as enhanced drug solubilization and bioavailability, good thermodynamic stability and ease of preparation.

The preparation of microemulsions requires only the most basic mixing equipment. Particularly, their manufacture is not dependent on the careful control of the manufacturing process, as with emulsions.

The optimization of the solubility of poorly water-soluble drugs in pharmaceutical dosage forms presents a challenge, due to the severe restrictions on the choice of solvents suitable for oral, topical or parenteral use. The finely dispersed oil droplets of o/w microemulsions offer a potential solvent system for drugs, although the toxicity of the microemulsion components still imposes limitations on their use.

The transparency of microemulsions enables them to be visually assessed for microorganism growth, and also allows inspection for the presence of undissolved drug. Their transparency is also of benefit in topical applications. The thermodynamic stability of microemulsions is clearly an important characteristic when compared with kinetically stabilized macroemulsions.

The main problem of microemulsion systems is the lack of biological tolerance of excipients such as the surfactant and cosurfactant. Recent efforts have been focused on how to decrease or eliminate the toxicity or irritation of microemulsions, and, towards this end, a microemulsion system free of alcohols has investigated.

Microemulsion formulations are considered to be a promising formulation technique, because the enhanced absorption and bioavailability of poorly-soluble drug becomes an increasingly problem to overcome. In addition, microemulsions have recently been suggested as carriers for the oral administration of peptide/protein drugs.

The favorable drug delivery and solvent properties, together with the ease of preparation and the infinite physical stability of these unique oil-water-surfactant mixtures, makes microemulsions very promising vehicles for future formulations.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Hoar TP, Schulman JH. Transparent water-in-oil dispersions: the oleopathic hydro-micelle. Nature 1943;152:102-3
2. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Del Rev 2000;45:89-121

•• **An important review article that presents general information about microemulsions and gives recent developments and future directions.**

3. Moulik SP, Paul BK. Structure: dynamics and transport properties of microemulsions. Adv Colloid Interface Sci 1998;78:99-195
4. Schwuger MJ, Stickdorn K. Microemulsions in technical processes. Chem Rev 1995;95:849-64
5. Attwood D. Microemulsions. In: Colloidal Drug Delivery Systems. Kreuter J, editor. New York: Marcel Dekker; 1994. p. 31-71

•• **This review article discusses the potential of microemulsion systems in drug delivery.**

6. Tenjarla S. An overview and pharmaceutical applications. Crit Rev Ther Drug Carrier Syst 1999;16:461-521
7. Warisnoicharoen W, Lansley AB, Lawrence MJ. Nonionic oil in water microemulsions: the effect of oil type on phase behavior. Int J Pharm 2000;198:7-27
8. Ceigle A, Das KP, Lindman B. Microemulsion structure in four-component systems for different surfactants. Colloids Surf 1987;28:29-40
9. De Gennes PG, Taupin C. Microemulsions and flexibility of oil/water interfaces. J Phys Chem 1982;86:2294-304
10. Watari H. Microemulsions in separation sciences. J Chromatogr A 1997;780:93-102
11. Atwood D, Mallon C, Ktistis G, Taylor JC. A study on factors influencing the droplet size in non-ionic oil in water microemulsions. Int J Pharm 1992;88:417-22
12. Taha MO, Al-Ghazawi M, Abu-Amara H, Khalil E. Development of quantitative structure–property relationship models for pseudoternary microemulsions formulated with nonionic surfactants and cosurfactants: application of data mining and molecular modeling. Eur J Pharm Sci 2002;15:461-78
13. Kawakami K, Yoshikawa T, Moroto Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs. I. Prescription design. J Control Rel 2002;81:65-74

• **This paper describes a screening process to design microemulsion formulations for oral administration and what characteristics of the excipients affected the formation of the microemulsion.**

14. Wu W, Wang Y, Que L. Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. Eur J Pharm Biopharm 2006;63:288-94
15. Gershanika T, Benita S. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. Eur J Pharm Biopharm 2000;50:179-88
16. Kim C-H, Cho Y-J, Gao Z-G. Preparation and evaluation of biphenyl dimethyl dicarboxylate microemulsion for oral delivery. J Control Rel 2001;70:149-55
17. Danielsson I, Lindman B. The definition of microemulsion. Colloids Surf A 1981;3:391-3
18. Schulman JH, Stoeckenius W, Prince LM. Mechanism of formation and structure of microemulsions by electron microscopy. J Phys Chem 1959;63:1677-80
19. Prince LM. A theory of aqueous emulsion. I. Negative interfacial tension at the oil/water interface. J Colloid Interface Sci 1967;23:165-73
20. Constantinides PP, Scalart J-P. Formulation and physical characterization of water-in-oil microemulsions containing long-versus medium-chain glycerides. Int J Pharm 1997;158:57-68
21. Muller-Goymann CC, Hamann H-J. Sustained release from reverse micellar solutions by phase transformations into lamellar liquid crystals. J Control Rel 1993;23:165-74
22. Trotta M, Gasco MR, Pattarino F. Diffusion of steroid hormones from o/w microemulsions: influence of the cosurfactant. Acta Pharm Technol 1990;36:226-31
23. Acosta E, Kurlat DH, Bisceglia M, et al. Indused electric birefringence and viscosity studies in microemulsions. Collids Surf A Physicochem Eng Aspects 1996;106:11-21
24. 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 cosurfactant. Int J Pharm 1994;111:63-72
25. Aboofazeli R, Lawrence CB, Wicks SR, 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 1995;125:63-72
26. Kale NJ, Alen LV. Studies on microemulsion using Brij 96 as surfactant and glycerin, ethylene glycol and propylene glycol as co-surfactants. Int J Pharm 1989;57:87-93
27. Kantarci G. Karasulu HY, Ozgüney I, Güneri T. Phase behaviour of microemulsion systems containing short-chain alcohols as co-surfactant. Acta Pharm Turcica 2002;44:77-85
28. Sintov AC, Shapiro L. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. J Control Rel 2004;95:173-83
29. Alany RG, Rades T, Nicoll J, Tucker IG, Davies NM. w/o microemulsions for ocular delivery: evaluation of ocular irritation andprecorneal retention. J Control Rel 2006;111:145-52
30. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and self-microemulsfying drug delivery systems. Eur J Pharm Sci 2000;11:93-98
31. Porter CJH, Kaukonen AM, Boyd BJ, Edwards GA, Charman WN. Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium chain lipid-based microemulsion formulation. Pharm Res 2004;21:1405-12
32. Osborne DW, Middleton CA, Rogers RL. Alcohol-free microemulsions. J Disper Sci Tech 1988;9:415-23
33. Karasulu HY, Karabulut B, Goker E, Guneri T, Gabor F. Controlled release of methotrexate from w/o microemulsion and its in vitro anti-tumor activity. Drug Deliv 2007;14:225-33

34. Ege MA, Karasulu HY, Guneri T. Triangle phase diagram analysis software. Acta Pharmaceutica Turcica (The 4th International Postgraduate Research Symposium on Pharmaceutics. Istanbul, Turkey). 2004;46:36

35. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Del Rev 2002;54:S77-S98

•• **A critical review of the literature with respect to the use of microemulsions for cutaneous drug delivery, and a discussion of the influence of microemulsion composition on drug delivery.**

36. Calfors J, Blute I, Schmidt V. Lidocaine in microemulsion-dermal delivery system. J Disper Sci Tech 1991;12:467-82

37. Goddeeris C, Cuppo F, Reynaers H, et al. Light scattering measurements on microemulsions: estimation of droplet sizes. Int J Pharm 2006;31:187-95

• **This paper focuses on the droplet size analysis in microemulsions, prepared after dilution of a SMEDDSs.**

38. Uskoković V, Drofenik M, Ban I. The characterization of nanosized nickel-zinc ferrites synthesized within reverse micelles of CTAB/1–hexanol/water microemulsion. J Magnetism Magnetic Materials 2004;284:294-302

39. Aikawa K, Kaneko K, Tamura T, Fujitsu M, Ohbu K. Formation of fractal porous silica by hydrolysis of TEOS in a bicontinuous microemulsion. Coll Surf A Physicochem Eng Aspects 1999;150:95-104

40. Mohapatra P, Mishra T, Parida KM. Effect of microemulsion composition on textural and photocatalytic activity of titania nanomaterial. Appl Catalysis A General 2006;310:183-9

41. Tomšič M, Bešter-Rogač M, Jamnik A, et al. Ternary systems of nonionic surfactant Brij 35, water and various simple alcohols: Structural investigations by small-angle X-ray scattering and dynamic light scattering. J Coll Interface Sci 2006;294:194-211

42. Kantarci G, Ozgungey I, Karasulu HY, Guneri T, Basdemir G. In vitro permeation of diclofenac sodium from novel microemulsion formulations through rabbit skin. Drug Dev Res 2005;65:17-25

43. Charman SA, Charman WN, Rogge MC, et al. Self-emulsifying drug delivery systems: Formulation and biopharmaceutical evaluation of an investigational lipophilic compound. Pharm Res 1992;9:87-93

44. Tarr BD, Yalkowsky SH. Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. Pharm Res 1989;6:40-3

45. Ozguney (Sarigullu) I, Karasulu HY, Kantarci G, et al. Transdermal delivery of diclofenac sodium through rat skin from various formulations. AAPS Pharm Sci Tech 2006;7:88-94

46. Park KM, Kim CH. Preparation and evaluation of flurbiprofen-loaded microemulsion for parenteral delivery. Int J Pharm 1999;181:173-9

47. Lam AC, Schechter RS. The theory of diffusion in microemulsion. J Coll Interface Sci 1987;120:56-63

48. Zhao X-Y, Xu J, Zheng L-Q, Livisk X-W. Preparation of temperature-sensitive microemulsion-based gels formed from a triblock copolymer. Coll Surf A Physicochem Eng Aspects 2007;307:100-7

49. Karasulu HY, Karabulut B, Kantarci G, et al. Preparation of arsenic trioxide loaded microemulsion and its enhanced cytotoxicity on MCF-7 breast carcinoma cell line. Drug Deliv 2004;11:345-50

50. Fanun M. Conductivity, viscosity, NMR and diclofenac solubilization capacity studies of mixed nonionic surfactants microemulsions. J Mol Liquids 2007;135:5-13

51. Mo C, Li X. Microstructure and structural transition in coconut oil microemulsion using semidifferential electroanalysis. J Coll Interface Sci 2007;312:355-62

52. Shrestha S, Yeung CMY, Nunnerley C, Tsang SC. Comparison of morphology and electrical conductivity of various thin films containing nano-crystalline praseodymium oxide particles. Sensors Actuators A Physical 2007;136:191-8

53. Kantarci G, Ozguney (Sarigullu) I, Karasulu Hy, Arzil S, Guneri T. Comparison of different w/o microemulsions contain diclofenac sodium; preparation, characterization, release rate and skin irritation studies. AAPS Pharm Sci Tech In Press

54. Sorichetti PA, Matteo CL. Low-frequency dielectric measurements of complex fluids usinghigh-frequency coaxial sample cells. Measurement 2007;40:437-49

55. Letamendia L, Louisor E, Pru-Lestret E, et al. Relaxation phenomena in critical microemulsion systems. Coll Surf A Physicochem Eng Aspects 1998;140:289-93

56. Ezrahi S, Wachtel E, Aserin A, Garti N. Structural polymorphism in a four-component nonionic microemulsion. J Coll Interface Sci 1997;191:277-90

57. Weigert S, Eicke H-F, Meier W. Electric conductivity near the percolation transition of a nonionic water-in-oil microemulsion. Physica A 1997;242:95-103

58. Paul BK, Moulik SP. Microemulsions; an overview. J Dispers Sci Tech 1997;18:301-67

•• **An interesting paper with respect to understanding the formation, phase behavior and characterization of microemulsions.**

59. Chan J, El Maghraby GMM, Craig JP, Alany RG. Phase transition water-in-oil microemulsions as ocular drug delivery systems: in vitro and in vivo evaluation. Int J Pharm 2007;328:65-71

60. Alany RG, Tucker IG, Davies NM, Rades T. Characterizing colloidal structures of pseudoternary phase diagrams formed by oil/water/amphiphile systems. Drug Devel Ind Pharm 2001;27:31-8

61. David G, Ozer F, Simionescu BC, Zareie H, Piskin E. Microemulsion photopolymerization of methacrylates stabilized with sodium dodecyl sulfate and poly(N-acetylethylenimine) macromonomers. Eur Polym J 2002;38:73-8

62. Seto H, Nagao M, Kawabata Y. Pressure-dependence of the bending modulus of surfactant monolayers in ternary microemulsion systems observed by neutron spin echo. Coll Surf A Physicochem Eng Aspects 2006;284-285:430-3

63. Farago B. Neutron spin echo study of well organized soft matter systems Physica B Condensed Matter 2006;385-386:688-91

64. Libster D, Aserin A, Garti N. A novel dispersion method comprising a nucleating agent solubilized in a microemulsion, in polymeric matrix. II. Microemulsion characterization. J Coll Interface Sci 2006;302:322-9

65. Pal A, Shah S, Devi S. Preparation of silver, gold and silver–gold bimetallic nanoparticles in w/o microemulsion containing TritonX-100. Coll Surf A Physicochem Eng Aspects 2007;302:483-7

66. Frielinghaus H, Maccarrone S, Byelov D, et al. SANS studies of confined diblock

copolymers in microemulsions. Physica B Condensed Matter 2006;385-386:738-41

67. Hellweg T. Phase structures of microemulsions. Curr Opin Coll Interface Sci 2002;7:50-56
• **This paper focuses on the new developments in microstructures.**
68. Sha X, Yan G, Wu Y, Li J, Fang X. Effect of self-microemulsifying drug delivery systems containing labrosol on tight junctions in Caco-2 cells. Eur J Pharm Sci 2005;24:477-86
• **This article discusses the cytotoxicity of SMEDDSs and the effect of surfactants on Caco-2 cells.**
69. Lv F-F, Li N, Zheng L-Q, Tung C-H. Studies on the stability of the chloramphenicol in the microemulsion free of alcohols. Eur J Pharm Biopharm 2006;62:288-94
70. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterization and transdermal drug delivery potential of microemulsion systems. J Control Rel 2000;69:421-33
71. Soderman O, Nyden M. NMR in microemulsions. NMR translational diffusion studies of a model microemulsion. Coll Surf A Physicochem Eng Aspects 1999;158:273-89
72. Barolli B, Lopes-Quintela MA, Delgado-Charro MB, Fadda AM, Blanco-Mendes J. Microemulsiona for topical delivery of 8-methosalen. J Control Rel 2000;69:209-18
73. Trotta M, Gasco MR, Morel S. Release of drugs from oil-water microemulsions. J Control Rel 1989;10:237-43
74. Araya H, Tomita M, Hayashi M. The novel formulation design of o/w microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds. Int J Pharm 2005;305:61-74
75. Yuan Y, Li S-M, Mo F-K, Zhong DF. investigation of microemulsion system for transdermal delivery of meloxicam. Int J Pharm 2006;321:117-23
76. Kawakami K, Yoshikawa T, Hayashi T, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs. II. In vivo. J Control Rel 2002;81:75-82
77. Ke W-T, Lin S-Y, Ho H-O Sheu M-T. Physical characterizations of microemulsion systems using tocopheryl polyethylene glycol 1000 succinate (TPGS) as a surfactant for the oral delivery of protein drugs. J Control Rel 2005;102:489-50
78. Wang CH, Ko WJ, Chou NK, Wang SS. Efficacy and safety of tacrolimus versus cyclosporine microemulsion in primary cardiac transplant recipients: 6-month results in Taiwan. Transplant Proc 2004;36:2384-5
79. Lucangioli SE, Kenndler E, Carlucci A, et al. Relation between retention factors of immunosuppressive drugs in microemulsion electrokinetic chromatography with biosurfactants and octanol–water partition coefficients. J Pharm Biomed Anal 2003;33:871-87
80. Pally C, Tanner M, Rizvi H, Papageorgiou C, Schuurman H-J. Tolerability profile of sodium mycophenolate (ERL080) and mycophenolate mofetil with and without cyclosporine (Neoral) in the rat. Toxicology 2001;157:207-15
81. Cibulskyte D, Pedersen M, Hjelm-Poulsen J, et al. The pharmacokinetics and acute renal effects of oral microemulsion cyclosporin A in normal pigs. Int Immunopharmacol 2006;6:627-34
82. Margreiter R. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. Lancet 2002;359:741-6
83. Cilek A, Çelebi N, Tirnaksiz F, Tay A. A lecithin-based microemulsion of rh-insulin with aprotinin for oral administration: investigation of hypoglycemic effects in non-diabetic and STZ-induced diabetic rats. Int J Pharm 2005;298:176-85
84. Celebi N, Turkyilmaz A, Gonul B, Ozogul C. Effects of epidermal growth factor microemulsion formulation on the healing of stress-indused gastric ulcers in rats. J Control Rel 2002;83:197-210
85. Kim SK, Lee EH, Vaishali B, et al. Tricaprylin microemulsion for oral delivery of low molecular weight heparin conjugates. J Control Rel 2005;105:32-42
86. Spernath A, Aserin A. Microemulsions as carriers for drugs and nutraceuticals. Adv Coll Interface Sci 2006;128-130:47-64
•• **This review explores some of the new trends in microemulsion research through an analysis of some representative studies.**
87. Yamazaki K, Imai M, Suzuki I. Water solubilization capacity and mean emulsion size of phospholipid-based isooctane-alcohol w/o microemulsion. Coll Surf A Physicochem Eng Aspects 2007;293:241-6
88. Chen H, Chang X, Du D, et al. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm 2006;315:52-8
89. Courrier HM, Vandamme TF, Krafft MP. Reverse water-in fluorocarbon emulsions and microemulsions obtained with a fluorinated surfactant. Coll Surf A Physicochem Eng Aspects 2004;244:141-8
90. Zhang Q, Jiang X, Jiang W, et al. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. Int J Pharm 2004;275:85-96
91. Li L, Nandi I, Kim KH. Development of an ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam. Int J Pharm 2002;237:77-85
92. D'Cruz OJ, Uckun FM. Gel-microemulsions as vaginal spermicides and intravaginal drug delivery vehicles. Contraception 2001;64:113-23
93. Terek MC, Karabulut B, Selvi N, et al. Arsenic trioxide-loaded, microemulsion-enhanced cytotoxicity on MDAH 2774 ovarian carcinoma cell line. Int J Gynecol Cancer 2006;16:532-7
94. Hwang SR, Lim S-J, Park J-S, Kim C-K. Phospholipid-based microemulsion formulation of all-trans-retinoic acid for parenteral administration. Int J Pharm 2004;276:175-83
95. Karadeniz H, Alparslan L, Erdem A, Karasulu E. Electrochemical investigation of interaction between mitomycin C and DNA in a novel drug-delivery system. J Pharm Biomed Anal 2007;452:322-6
• **This paper focuses on the interaction between an anticancer drug and DNA in a drug carrier systems.**
96. Gupta RR, Jain SK, Varshney M. AOT water-in-oil microemulsions as a penetration enhancer in transdermal drug delivery of 5-fluorouracil. Coll Surf B Biointerf 2005;41:25-32
97. Djordjevic L, Primorac M, Stupar M, Krajisnik D. Characterization of caprylocaproyl macrogolglycerides based microemulsion drug delivery vehicles for an amphiphilic drug. Int J Pharm 2004;271:11-19
98. Kemken J, Ziegler A, Muller BW. influence of supersaturation on the pharmacodynamic effect of bupranolol

after dermal administration using microemulsions as vehicles. Pharm Res 1992;43:554-8

99. Anigbogu ANC, Williams AC, Barry BW, Edwards HGM. Fourier transform raman-spectroscopy of interactions between the penetration enhancer dimethyl sulfoxoide and human stratum corneum. Int J Pharm 1995;125:265-82

100. Dreher F, Walde P, Walther P, Wehrli E. interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J Control Rel 1997;45:131-40

101. Yajima I, Sakai H, Miyazawa K, et al. Preparation and properties of multiphase microemulsions with some phosphatidylcholines having different alkyl chains. Coll Surf B Biointerf 1997;9:177-86

102. Changez M, Chander J, Dinda AK. Transdermal permeation of tetracaine hydrochloride by lecithin microemulsion: in vivo. Coll Surf B Biointerf 2006;48:58-66

103. Mei Z, Chen H, Weng T, Yang Y, Yang X. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur J Pharm Biopharm 2003;56:189-96

104. Paolino D, Ventura CA, Nisticò S, Puglisi G, Fresta M. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int J Pharm 2002;244:21-31

• **This paper discusses the preparation, characterization and toxicity evaluation of lecithin-based microemulsions as percutaneous drug delivery systems.**

105. Gasco MR, Gallarate M, Pattarino F. In vitro permeation of azelaic acid from viscosized microemulsions. Int J Pharm 1991;69:193-6

106. Kemken J, Ziegler A, Muller BW. Investigation into the pharmacodynamic effects of dermally administered microemulsions containing beta-blokers. J Pharm Pharmacol 1991;43:679-84

107. Zhao X, Liu JP, Zhang X, Li Y. Enhancement of transdermal delivery of theophylline using microemulsion vehicle. Int J Pharm 2006;327:58-64

108. Escribano E, Calpena AC, Queralt J, Obach R, Doménech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharm Sci 2003;19:203-10

109. Boinpally RR, Zhou S-L, Devraj G, et al. iontophoresis of lecithin vesicles of cyclosporin A. Int J Pharm 2004;274:185-90

110. Schmalfuß U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Rel 1997;46:279-85

111. Peltola S, Saarinen-Savolainen P, Kiesvaara J, Suhonen TM, Urtti A. Microemulsions for topical delivery of estradiol. Int J Pharm 2003;254:99-107

112. Formariz TP, Sarmento VHV, Silva-Junior AA, et al. Doxorubicin biocompatible o/w microemulsion stabilized by mixed surfactant containing soya phosphatidylcholine. Coll Surf B Biointerf 2006;51:54-61

113. Sharma A, Shekhar C, Heer M, Minzcorswant M. Comparison of generic cyclosporine microemulsion versus neoral in de novo renal transplant recipients managed by 2-h postdose monitoring. Transplant Proc 2006;38:2051-3

114. Corswant CV, Thoren P, Engstrom S. Triglyceride-based microemulsion for intravenous administration of sparingly soluble substances. J Pharm Sci 1998;87:200-208

115. Kang BK, Chon SK, Kim SH, et al. Controlled release of paclitaxel from miroemulsion containing PLGA and evaluation and anti-tumor activity in vitro and in vivo. Int J Pharm 2004;286:147-56

• **This paper discusses the development of an optimal paclitaxel microemulsion by self-microemulsifying drug delivery systems and evaluation of anti-tumor activiy.**

116. Crosasso P, Cerutti M, Brusa M, et al. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. J Control Rel 2000;63:19-30

117. Feng SS, Huang G. Effects of emulsifiers on the controlled release of paclitaxel (Taxol) from nanospheres of biodegradable polymers. J Control Rel 2001;71:53-69

118. He L, Wang GL, Zhang Q. An alternative paclitaxel microemulsion formulation: hypersensitivity evaluation and pharmacokinetic profile. Int J Pharm 2003;250:45-50

119. Nomura T, Koreeda N, Yamashita F, Takakura Y, Hashida M. Effect of particle size and charge on the disposition of lipid carriers after intratumoral injection into tissue-isolated tumors. Pharm Res 1998;15:128-32

120. Ince i, Kirilmaz L, Ates H, Karasulu E. Development and validation of an acute glaucoma model to measure the ocular hypotensive effects and use of pilocarpine HCL microemulsion in rabbits. PSWC 2007 3rd World Congress of the Board of Pharmacutical Sciences of FIP; 2007 April 22 – 25; Amsterdam, Netherlands

121. Richter T, Keipert S. In vitro permeation studies comparing bovine nasal mucosa, porcine cornea and artificial membrane: androstenedione in microemulsions and their components. Eur J Pharm Biopharm 2004;58:137-43

122. Vandamme THF. Microemulsion as ocular drug delivery systems: recent developments and future challenges. Prog Retin Eye Res 2002;21:15-24

•• **A review that presents the recent developments and future challenges of microemulsion as ocular drug delivery systems.**

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