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Review article

The potential of lipid emulsion for ocular delivery of lipophilic drugs

Shunmugaperumal Tamilvanan[a], Simon Benita[b,*,1]

[a]Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Punjab, India
[b]Department of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel

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Abstract

For nearly a decade, oil-in-water lipid emulsions containing either anionic or cationic droplets have been recognized as an interesting and promising ocular topical delivery vehicle for lipophilic drugs. The aim of this review is to present the potential of lipid emulsions for ocular delivery of lipophilic drugs. The review covers an update on the state of the art of incorporating the lipophilic drugs, a brief description concerning the components and the classification of lipid emulsions. The ocular fate following topical instillation, safety evaluation experiments and the applications of lipid emulsions are thoroughly discussed.

Keywords: Emulsion; Anionic; Cationic; Ocular bioavailability; Safety; Review

1. Introduction

For the treatment of different extra- and intra-ocular aetiological conditions such as glaucoma, uveitis, keratitis, dry eye syndromes, cytomegalovirus retinitis, acute retinal necrosis, proliferative vitreoretinopathy, macular degenerative disease, etc. a lot of lipophilic and poorly water soluble drugs have become available in recent years. These drugs represent a formulation challenge for scientists who design simple eye drop aqueous solutions with adequate drug concentrations because of aqueous solubility limitations. Dosage forms for ocular topical application of lipophilic drug are comprised of oil solutions, lotions, ointments, gels and ocular delivery systems [1]. However, most of the traditional ophthalmic dosage forms are clearly not only uncomfortable for the patient, but also not efficient in combatting some of the current virulent ocular diseases. Furthermore, in ophthalmology, the low viscosity topical formulations either in aqueous-based eye drops or in liquid retentive suboptimal forms are generally preferred to provide local drug concentrations in the precorneal or aqueous humor part of the eye.

In the last decade, oil-in-water (o/w) type lipid emulsions, primarily intended for parenteral applications, have been investigated and are now exploited commercially as a vehicle to improve the ocular bioavailability of lipophilic drugs [2–4]. The natural biodegradability, nanometer droplet size range, sterilizability and substantial drug solubilization either at the innermost oil phase or at the o/w interface, and improved ocular bioavailability are thus making the lipid emulsion a promising ocular delivery vehicle. For the first time, an anionic lipid emulsion containing cyclosporine A 0.05% (Restasis™, Allergan, Irvine, USA) was approved for clinical use by the FDA in December 2002, and is now available in the US for the treatment of chronic dry eye disease (available at www.restasis.com and www.dryeye.com, accessed on 15/12/2003). Furthermore, an over-the-counter product that features a non-medicated (blank) anionic emulsion formulation, Refresh Endura®, has been launched in the US market for eye lubricating purposes in patients suffering from moderate to severe dry-eye syndrome [5,6].

A number of reviews and entire issues of journals have been devoted solely to the subject of ophthalmic drug delivery systems [7–13]. Furthermore, as one of the non-invasive, topical drug delivery vehicles to treat ocular pathologies, the efficacy of o/w lipid emulsions has also

* Corresponding author. Department of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem, P.O. Box 12065, 91120 Jerusalem, Israel. Tel.: +972-2-675-8668; fax: +972-2-534-3167.
E-mail address: benita@cc.huji.ac.il (S. Benita).
[1] Affiliated with the David R. Bloom Center for Pharmacy at The Hebrew University of Jerusalem.

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been the focus of recent reviews [2–4,14]. Thus, keeping in mind the potential of lipid emulsions, the purpose of this review is to report on the most recent findings on the mode of ocular active lipophilic drug incorporation into lipid emulsions, to classify the lipid emulsions, to describe briefly the major components needed to formulate ocular compatible lipid emulsions, to relate the ocular biofate or concomitant protective mechanism factors faced by lipid emulsions following ocular topical application, to offer a short overview on safety assessment made so far with regard to the ocular topical route and finally to describe the ocular topical delivery of lipophilic drugs through o/w type lipid emulsions. The focus of this review is only on preformed submicron emulsions. They should not be confused with self-microemulsifying drug delivery systems or preformed microemulsions that are transparent thermodynamically stable dosage forms. The size and the size distribution of the submicron emulsions obviously depend on the specific formulation. Irrespective of the formulation, most of the lipid emulsions exhibit a narrow size distribution range which may vary from 50 to 700 nm with a mean droplet size of about 200 nm.

2. Ocular lipophilic drug incorporation pattern into lipid emulsion

There are three different approaches to incorporate lipophilic drugs into the lipid emulsions.

2.1. Extemporaneous drug addition

Cohen et al. [15], when looking for a new galenic presentation form for amphotericin B with better ocular tolerance over the commercial Fungizone® eye drops, incorporated the drug directly to the preformed 20% emulsion, Intralipid®. However, after addition of the solid drug particles or drug solution, several physical changes such as phase separation, nanoprecipitation or creaming may occur within lipid emulsions thus limiting such practices in ocular lipid emulsion preparations. Therefore, ocular active lipophilic agents are not normally incorporated into the lipid emulsions by this extemporaneous addition method.

2.2. De novo emulsion preparation

In principle, the drug molecules should be incorporated by a de novo process. Thus, the drug is initially solubilized or dispersed together with an emulsifier in suitable single oil or oil mixtures by means of slight heating. The water phase containing the osmotic agent with or without an additional emulsifier is also heated and mixed with the oil phase by means of high speed mixers. Further homogenization takes place to obtain the needed small droplet size range of the emulsion. A terminal sterilization by filtration or steam then follows. The lipid emulsion thus formed contains most of the drug molecules within its oil phase. This is a generally accepted and standard method to prepare lipophilic drug-loaded lipid emulsions for ocular use as shown in Fig. 1. This process is normally carried out under aseptic conditions and nitrogen atmosphere to prevent both contamination and potential oxidation of sensitive excipients.

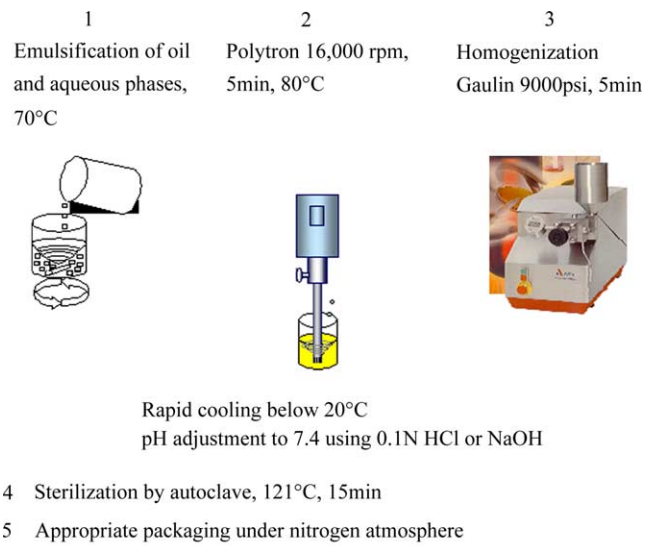

Fig. 1. Description of the de novo formulation process and selected equipment needed to prepare an homogeneous submicron emulsion.

2.3. Interfacial incorporation approach

Since many drugs of commercial interest including ocular therapeutic agents generally have a solubility that is too low in FDA approved oils, Lance et al. [16] proposed a method to incorporate such drugs into the interfacial o/w layer of the emulsion droplets. This can be achieved by initially dissolving the drug along with the phospholipid in an organic solvent, instead of oil. Following the solvent evaporation, the obtained phospholipids/drug co-mixture is used in the de novo production of the lipid emulsions [17]. However, this approach suffers from possible drug nanocrystal formation inside the lipid emulsions and from the use of organic solvent during the emulsion preparation process. To overcome such drawbacks, a novel SolEmul® technology has been developed in which an additional high speed homogenization step is included to mix the drug with lipid emulsions. The drug particles are in fact micronized to the nanosize range prior to incorporation into the lipid emulsions. By this technique, adequate amounts of lipophilic drugs can be substantially incorporated into the lipophilic core or intercalated between the selected emulsifier molecular films at the o/w interface of the lipid emulsions. The drugs reported to

have been incorporated by this novel approach are amphotericin B, carbamazepine and itraconazole [18–21]. However, it should be emphasized that all the lipophilic drug molecules that have been incorporated into the lipid emulsions by SolEmul® technology are meant only for parenteral use and so far no ocular active agents have been incorporated by this approach although there is no regulatory reason to exclude this technical improvement when designing ophthalmic emulsion formulations.

3. Classification of lipid emulsions

Based on the emulsifier combinations used in the formation of submicron emulsion droplets, the o/w lipid emulsions can easily be classified into two types: one having emusifiers with the capacity to produce a negative charge at the o/w interface termed anionic and another possessing emulsifiers able to provide a positive charge at the o/w interface called cationic. It is clear from lipid emulsion literature that neither triglycerides nor phospholipidic emulsifier's components of the conventional or anionic lipid emulsions are able to significantly sustain the incorporated lipophilic drug release in simulated or real physiological environments where full sink conditions prevail. Therefore, in an attempt to prolong and/or optimize the drug release, cationic lipid or polysaccharide emulsifiers should be added to the lipid emulsions to elicit mucoadhesion with anionic ocular tissues by an electrostatic adhesion. Indeed, cationic lipid emulsions prepared on the basis of stearylamine, oleyamine and chitosan can serve this purpose. It was initially believed and now has become clearer from many reports in the literature that an occurrence of electrostatic attraction between the cationic emulsified droplets and anionic cellular moieties of the ocular surface tissues such as the cornea will enhance the bioavailability of lipid emulsions containing lipophilic drugs [2,3,12].

4. Excipients for the manufacturing of ocular compatible lipid emulsion

Despite the similarity with parenteral lipid emulsions, topical ophthalmic lipid emulsions should be formulated with compatible vehicles and additives. The components of the internal and external phases of lipid emulsions should be chosen to confer enhanced solubility and/or stability to the incorporated ocular active lipophilic drug. In addition, it should also be designed to influence ocular biodistribution or therapeutic index. This section is a comprehensive presentation of the general considerations concerning excipient selection and optimum concentrations mainly in relation to the oil phase, the aqueous phase and the emulsifiers.

Prior to the formulation design of the lipid emulsions data are needed concerning the drug solubility in the oil vehicle. Additionally, prerequisite information is needed on compatibility of the oil vehicle with other formulation additives and the established ocular tissues-oil vehicle matching before the dosage form can be prepared. Table 1 lists the common emulsion excipients and the oils suitable for dissolving or dispersing lipophilic drugs of ocular interests. Since fatty oils are triglycerides, care must be taken to minimize or eliminate oxidation. α-Tocopherol is a good example of an antioxidant used to obtain a desired stabilized lipid emulsion under prolonged storage conditions. Therefore, α-tocopherol (0.001–0.002%, w/w) should be included in a typical lipid emulsion formulation for ocular use. The final oil phase concentration in ocular lipid emulsions is now widely accepted at or even below 5% (w/w) taking into account that the lipid emulsion must be kept in a low viscosity range, of between 2 and 3 cP, which is considered an adequate viscosity for ocular preparations [22]. Sometimes, a mixture of oils rather than a single oil is employed since drug solubilization in the oil phase is a prerequisite to exploit the lipid emulsion advantages. Jumaa and Müller [23,24] reported the effect of mixing castor oil with medium chain triglycerides on the viscosity of

Table 1
Excipients used for the formulation of ocular lipid emulsion

Oils	Emulsifiers	Cationic lipids and polysaccharide	Miscellaneous
Sesame oil	Cholesterol	Stearylamine	α-Tocopherol
Castor oil	Phospholipids (Lipoid)	Oleylamine	Glycerol
Soya oil	Polysorbate 80 (Tween 80)	Chitosan	Xylitol
Paraffin oil	Transcutol P		Sorbitol
Paraffin light	Cremophor RH		Thiomersal
Lanolin	Poloxamer 407		EDTA
Vaseline	Poloxamer 188		Methyl paraben
Corn oil	Miranol C_2M and MHT		Propyl paraben
Glycerin monostearate	Tyloxapol		
Medium chain monoglycerides			
Medium chain triglycerides			

castor oil. The oil combination, at the ratio of 1:1 (w/w) led to a decrease in the viscosity of castor oil and simultaneously to a decrease in the interfacial tension of the oil phase. This was related to the free fatty acids contained in castor oil, which can act as a co-emulsifier resulting in lower interfacial tension and, simultaneously in a more stable formulation in comparison with the other oil phases.

Traditionally, lecithins or phospholipids have been the emulsifiers of choice to produce ocular lipid emulsions. However, emulsifiers of this kind are not suitable to produce submicron sized emulsion droplets or to withstand the heat during steam sterilization. Therefore, additional emulsifiers preferably dissolved in the aqueous phase are usually included in the lipid emulsion composition. A typical example of the aqueous soluble emulsifiers is non-ionic surfactants (e.g. Tween 20®) after taking into consideration their non-irritant nature when compared to ionic surfactants. The non-ionic block copolymer of polyoxyethylene–polyoxypropylene, Pluronics F68 (poloxamer 188), is included to stabilize the lipid emulsion through strong steric repulsion. However, amphoteric surfactants, Miranol MHT (lauroamphodiacetate and sodium tridecethsulfate) and Miranol C_2M (cocoamphodiacetate) were also used in an earlier ophthalmic lipid emulsion [25]. It should be added that Restasis™ contains only polysorbate 80 and carbomer 1342 at alkali pH to stabilize the cyclosporin A-loaded anionic lipid emulsion. To prepare a cationic lipid emulsion, cationic lipids (stearylamine or oleylamine) or polysaccharides (chitosan) are added to the formulation. Strikingly, if chitosan is a choice of cation producing polysaccharide emulsifier molecules, there is no need to add amphoteric or non-ionic surfactants to the phospholipid or lecithin-stabilized lipid emulsions [26]. Conversely, a cationic emulsion based on an association of poloxamer 188 and chitosan without lecithin was prepared and also showed adequate physicochemical properties regarding stability and charge effects [24,27].

Additives other than antioxidants such as preservatives like benzalkonium chloride, chlorocresol, parabens, etc. are regularly included in ophthalmic lipid emulsions to prevent microbial spoilage of multi-dose ophthalmic lipid emulsions. The presence of components of natural origin like lecithin or oils with high calorific potential render the lipid emulsion a good medium to promote microbial growth when it is packed in multi-dose containers. Sznitowska et al. [28] studied the physicochemical compatibility between the lecithin-stabilized lipid emulsion and 12 antimicrobial agents over 2 years of storage at room temperature. Preliminary physicochemical screening results indicate that addition of chlorocresol, phenol, benzyl alcohol, thiomersal, chlorhexidine gluconate and bronopol should be avoided due to the occurrence of an unfavourable pH change followed by the coalescence of the lecithin-stabilized droplets of the lipid emulsion. Furthermore, the efficacy of antimicrobial preservation was assessed using the challenge test according to the method described in European Pharmacopoeia. Despite a good physicochemical compatibility, neither parabens nor benzalkonium chloride showed satisfying antibacterial efficacy in the lipid emulsion against the tested microorganisms and consequently did not pass the test. Therefore, higher concentrations of antimicrobial agents or their combination may be required for efficient preservation of the lecithin-stabilized lipid emulsions probably because of unfavorable phase partitioning of the added antimicrobials within the different internal structures of the lipid emulsions. It is interesting to note that benzalkonium chloride, a highly aqueous soluble drug, did not pass the standard challenge test even when incorporated in a cationic emulsion (unpublished data). This finding clearly indicates that the electrostatic attraction between the negatively charged lipid moieties of the mixed emulsifying film formed around the anionic emulsified oil droplets [28] and the quaternary cationic ammonium groups of the preservative, is not the plausible cause for the reduced activity of the benzalkonium chloride. Thus, the possible intercalation of this surfactant in either the cationic or the anionic interfacial mixed emulsifying film is likely to occur thereby preventing the benzalkonium chloride from eliciting its adequate preservative action. Overall, it is preferable to formulate lipid emulsions devoid of preservative agents and fill them in sterile single dose packaging units to prevent potential contamination due to repeated use of multi-dose packaging. It should be pointed out that the two available ocular emulsion products on the market are preservative free and packed in single use vials only.

5. Ocular biofate of lipid emulsions following topical instillation

Considerations of ocular drug delivery are not detailed in this section. Pertinent information concerning factors affecting drug permeation or retention as well as eyes anatomy and physiology can be found in several reviews [29–33]. From a medical point of view, lipid emulsions for ophthalmic use aim to enhance drug bioavailability either by providing prolonged delivery to the eye or by facilitating transcorneal/transconjunctival penetration. Drugs incorporated in o/w type lipid emulsions are lipophilic in nature and, depending on the extent of lipophilicity, either the corneal or the conjunctival/sclera route of penetration may be favored [34]. For the more lipophilic drugs the corneal route was shown to be the predominant pathway for delivering drugs to the iris, whereas the less lipophilic drugs underwent the conjunctival/scleral penetration for delivery into the ciliary body [34]. Thus, transcorneal permeation has traditionally been the mechanism by which topically applied ophthalmic drugs are believed to gain access to the internal ocular structures. Relatively little attention has been given to alternate routes through which drugs may enter the eye. It was reported that drugs may be absorbed by the non-corneal route and appeared to enter certain intraocular tissues

through the conjunctiva/sclera [35–37]. Indeed when compared to the cornea, drug penetration through the conjunctiva has the advantage of a larger surface area and higher permeability, at least for drugs which are not highly lipophilic. Furthermore, the lasting presence of drug molecules in the lower conjunctival cul-de-sac of the eye could result in a reservoir effect. Nevertheless, the lipid emulsions more or less physically resemble a simple aqueous-based eye drops dosage form since more than 90% of the external phase is aqueous irrespective of the formulation composition. Hence, following topical administration, lipid emulsions would probably face almost similar ocular protective events as encountered with conventional eye drops into the eye. Lipid emulsions are likely to be destabilized by the tear fluid electrolytic and dynamic action. Because of constant eyelid movements, the basal tear flow rate (1.2 μl/min) and the reflex secretion induced by instillation (up to 400 μl/min depending on the irritating power of the topical ocular solutions [5]), topical eye drops dosage forms are known for being rapidly washed out from the eye. Therefore, the water phase of the emulsion is drained off while, most probably, the oil phase of the emulsion remains in the cul-de-sac for a long period of time and functions as a drug reservoir [5]. If the volume of instilled lipid emulsion in the eye exceeds the normal lachrymal volume of 7–10 μl, then the portion of the instilled lipid emulsion (one or two drops, corresponding to 50–100 μl) not eliminated by spillage from the palpebral fissure of conjunctiva, is drained quickly via the nasolacrimal system into the nasopharynx. In other words, the larger the instilled volume, the more rapidly the instilled lipid emulsion is drained from the precorneal area. Hence the contact time of the lipid emulsion with the absorbing surfaces (cornea and conjunctiva) is estimated to be a maximum of few minutes, well beyond the short residence time of conventional eye drops. In order to verify the extension of the residence time of the lipid emulsion in the conjunctival sac, Beilin et al. [38] added a fluorescent marker to the formulations. One minute after the topical instillation into the eye, 39.9 ± 10.2% of the fluorescence was measured for the lipid emulsion as compared to only 6.8 ± 1.8% for regular eye drops. In addition a study was carried out in male albino rabbits to compare the corneal penetration of indomethacin from Indocollyre® (a marketed hydro-PEG ocular solution) to that of negatively and positively charged submicron emulsions [39]. This comparison was made to gain insightful mechanistic information regarding the enhanced ocular penetration effect of the submicron emulsion as a function of dosage form and surface charge. The contact angle of one droplet of the different dosage forms on the cornea was measured and found to be 70 for saline, 38 for the anionic lipid emulsion and 21.2° for the cationic lipid emulsion. Respectively, the values of the calculated spreading coefficient were −47, −8.6 and −2.4 mN/m. It can be clearly deduced, due to the markedly low spreading coefficient values elicited by the emulsions, that both lipid emulsions had better wettability properties on the cornea compared to saline. The lipid emulsion may then prolong the residence time of the drop on the epithelial layer of the cornea, thereby enabling better drug penetration through the cornea to the internal tissues of the eye, as confirmed by animal studies [39]. It is therefore believed that drug is not released from the oil droplet and equilibrates with the tears but rather partitioned directly from the oil droplets to the cell membranes on the eye surface. Therefore, it is reasonable to assume that lipid emulsions have a definite advantage since they elicit an increased ocular residence time in comparison to conventional eye drops and thus significantly improve the ocular drug bioavailability [40]. This is also confirmed by the data shown in numerous cited papers reported in the present review.

In spite of a relatively rapid removal of conjunctivally absorbed lipid emulsion from the eye by local circulation, direct transscleral access to some intraocular tissues cannot be excluded especially if an electrostatic attraction does occur between the cationic oil droplets of lipid emulsions and anionic membrane moieties of the sclera as shown by some authors [3,12,39]. There is no doubt that the drug absorption from lipid emulsions through the non-corneal route needs to be investigated further as it may elicit insightful information on the potential of lipid emulsions to promote drug penetration to the posterior segment through a mechanism which bypasses the anterior chamber. In addition to all the above-described protective and elimination mechanisms of the eye, lipid emulsions remaining in the precorneal area may be subject to protein binding and to metabolic degradation in the tear film. Additional studies (at least in vitro) are necessary to clearly understand the lipid emulsion interaction with the ocular fluid components. Although it is unlikely to happen because of the low lipid emulsion volume remaining in the conjunctival sac, the fluid dynamics may be moderately altered by the physical and chemical properties of lipid emulsions, which include tonicity, pH, refractive index, interfacial charge, viscosity, osmolality and irritant ingredients. Thus, the formulations of ophthalmic drug products must take into account not only the stability and compatibility of a drug in the lipid emulsion, but also the influence of the lipid emulsion on precorneal fluid dynamics.

6. Safety assessments

One of the enduring features of any topical ophthalmic drug delivery system is the ocular tolerance following instillation into eye. In vitro, ex vivo and in vivo evaluations should be done on both drug free and drug-loaded ocular lipid emulsions. Table 2 lists the parameters investigated to determine lipid emulsion formulation safety. To be compatible and isotonic with ocular fluids, the lipid emulsion usually has a tonicity modifier like glycerol

Table 2
Investigated ocular parameters to assess the lipid emulsion formulation safety

Ocular parameters	Reference
Isotonicity of the preparation (hemolysis test)	[41]
Irritation effect on rabbit eyes, Draize test	[43]
Symptoms of ocular discomfort	[51]
Visual acuity	[51]
Search for adverse events by scanning electron microscopy of corneal surface	[51]
Ophthalmologic parameters like	
Rose Bengal staining	
Superfacial punctuate keratitis	
Schirmer tear test	
Ocular surface disease impact	
BUT	
Biomicroscopic evaluation of the lipid film	[6,59,60]

(2.25–2.5%, w/w) in the formulation (285–290 mosmol/kg for saline and 283.9 ± 1.73 mosmol/kg for glycerol 2.5%, determined by an osmometer [41]). Furthermore, the use of sorbitol or xylitol as suitable substances to isotonize lipid emulsions has also been recommended [41]. For ocular lipid emulsions, glycerol is the most widely used substance to adjust the tonicity of the vehicle with respect to tear fluid [39]. Irritation of ocular tissues may arise due to one of the components of lipid emulsions, usually from emulsifiers or a high concentration of oil. As previously noted, the oil concentration is limited to 5% (w/w) or less to prevent potential blurred vision. If the emulsifier molecules impart a potential irritation effect on the ocular surface tissues, a reflex lachrymation can be seen followed by inflammatory symptoms (edema) on the precorneal area of the eye, such as conjunctival congestion, swelling and corneal opacification, etc. Eye irritation assessments are important in establishing the safety profile and safe handling procedures for lipid emulsions. Despite interspecies differences in blink frequency, ocular surface permeability and aqueous humor dynamics between animals and humans [42], the rabbit is used as the standard animal model for determination of eye irritancy. Rabbits test procedures are based on methods originally developed by Dr John Draize and co-workers at the US Food and Drug Administration in the 1940s. The Draize rabbit test involves introduction of 0.1 ml of a test substance into the lower conjunctival cul-de-sac of albino rabbit eyes [43]. Responses of the cornea, conjunctiva and iris are graded at 1, 24 and 48 h (and longer if needed) after exposure to the test materials; a scoring system is used to assign numerical grades for ocular response. However, concerns have been expressed over Draize rabbit eye testing, from both the standpoint of animal use as well as the accuracy of the Draize procedure for prediction of human eye response. Objections and limitations to the Draize rabbit's eye test and the necessity to develop non-animal tests in assessing the ocular irritancy of a topically applied drug or chemical have been raised and discussed in book chapters [44,45].

Modified eye irritation tests have been proposed, including the low-volume eye test (LVET). In the LVET procedure [46], 0.01 ml or mg equivalent of test material is placed directly on the central cornea to obtain a better model of accidental human eye exposures [47]. In this study, groups of eight human volunteers and eight albino rabbits, under controlled laboratory conditions, were exposed in one eye without subsequent rinsing to the same concentrations and volumes of four prototype consumer products: fabric softener, shampoo, hand soap, and laundry detergent. Two irritation scales were employed with both human and rabbit eyes: the Draize scale by a technician and a medical scale used with slit lamp examination by an ophthalmologist. Eyes were examined by both graders before and after dosing at specified intervals until recovery. Mean and maximum irritation scores are presented for each grading time, method, and exposure, as are the mean hours to recovery (clearing) for each exposure. For surfactant-based consumer products, the LVET more accurately predicts human exposures and rates of recovery than does the Draize procedure. Additionally, the LVET meets criteria suggested by the Interagency Regulatory Alternatives Group for alternative tests to the Draize [48].

From 1992 to 1994 a world-wide international validation study on nine alternatives to the Draize eye test was conducted in 37 laboratories on 60 coded chemicals, sponsored by the European Union (EU) and the British Home Office [49]. The principal goal of the study was to establish whether or not one or more of the nine non-animal tests (Table 3) could be used to replace the Draize eye test for all severely irritating materials or for severely irritating materials belonging to specific classes and to replace the animal test completely for chemicals with or without regard to chemical class. However, with the possible exception of predicting the irritancy of surfactants, none of the nine tests met any of the four performance goals.

In this context, we have conducted a potential induced toxicity of a novel cationic emulsion vehicle prepared using a combination of emulsifiers consisting of phospholipids (Lipoid E 80), poloxamer 188 (Pluronics F68)

Table 3
In vitro methods evaluated in the EU/Home Office international validation study on alternatives to the Draize eye test for classification and labeling of chemicals [49]

Organotypical tests	Physicochemical and cellular tests
HET-CAM test[a]	EYTEX test (in vitro International, Irvine, USA)
Isolated rabbit eye test	Neutral red uptake cytotoxicity test
Chicken enucleated eye test	Red blood cell haemolysis test
Bovine corneal opacity	Fluorescein leakage test
Permeability test	Silicon microphysiometer test

[a] Hen's egg chorioallantoic membrane test for irritation potential.

and stearylamine (SA) in rabbit and rat animal models [50]. Despite the presence of a cationic primary amine SA, which may be suspected of being an irritant in its pure form, in the emulsifier combination, the hourly instillation of SA-based cationic emulsion vehicle into rabbit eye was well tolerated without any evidence of toxic or inflammatory response to the ocular surface during the 5 days of the study (40 single drop instillations between 8 a.m. and 4 p.m. each day). Following 0.2, 0.4 and 0.6 ml single bolus injections of the same emulsion vehicle, representing a huge single administered dose of 30 ml/kg, no animal deaths were noted over a period of 30 days apparently indicating the absence of marked acute toxicity. Furthermore, the same SA-based cationic emulsion vehicle did not cause acute neurotoxicity in rats when a continuous intravenous infusion (3.3 ml) for 2 h at a rate of 27.4 μl/min was administered through jugular vein. A very recent study of the long-term subchronic toxicity in rabbit eye (healthy) elicited an almost similar non-irritating effect to eye tissues following thrice-daily one single drop topical instillation of SA-based emulsion as compared to the thrice-daily one single drop topical instillation of the normal saline treated control rabbit eyes (unpublished data). Similar experimental protocols were also followed in evaluating the ophthalmic tolerance of an amphotericin B ocular lipid emulsion which has been found to be better tolerated than the instillation of commercial Fungizone® eye drops in rabbits [15]. With a view to finding an irritation free ocular colloidal drug carriers, Calvo et al. [51] prepared an anionic lipid emulsion based on Miglyol 840 oil and lecithin. This formulation was tested on rabbit for acute ocular tolerance following topical instillation (30 μl) every 30 min for 6 h. A microscopic evaluation using a slit lamp, at 3, 6.5, and 24 h after the first instillation, was carried out to study possible damage in the conjunctiva, cornea, and iris and to determine the irritant indices according to a scale of predetermined scores [52]. Calvo et al. also showed the ocular lesion index (OLI) values of 1.2 ± 1.1, 5.6 ± 1.7, and 0, respectively, at 3, 6.5, and 24 h after the first instillation of the anionic lipid emulsion into rabbit eye. Nevertheless, the OLI values observed at all time points are less than 5% of the limit value of acceptability ($OLI_{max} = 110$). This means that the lipid emulsion, prepared with Miglyol 840 oil and lecithin, is well tolerated and can be useful for topical ophthalmic therapy. In addition, extensive preclinical long-term safety studies including histological evaluation of all tissues and organs (3 and 6 months ocular study on new Zealand white rabbits and 1 year ocular study on beagle dogs) have been carried out on either different cyclosporin A concentrations in the novel anionic emulsion formulation or the blank emulsion vehicle [53]. The preclinical safety data in rabbits and dogs have shown that cyclosporin A ophthalmic formulations at concentrations up to 0.4% dosed up to six times daily did not show any topical or systemic toxicity. The results of all of these safety evaluation studies support the statement of Rieger [6] that lipid emulsions most closely resemble natural tears.

An immortalized human corneal epithelial cell line has also been demonstrated to be an interesting tool for predicting topical irritation or toxicity of applied formulations [54]. This cell culture technique obviously reduces the time and expense that the formulator requires for animal experiments to assess the formulation irritation effect. Moreover, a mitochondrial assay based on the MTT formation is also a very useful tool to address the formulation toxicity or irritation. Furthermore, a human cell-based in vitro method, termed the tissue equivalent assay, is a valuable tool to screen for the ocular irritancy potential of various formulations including lipid emulsions to mucosal tissues such as cornea and conjunctiva [55]. Osborne et al. [55] developed methods for topical application (an exposure that mimics in vivo testing) and wash-off of test substances on the epithelial surface of human skin derived epithelial-fibroblast cocultures. These cultures contain non-cornified stratified squamous epithelium, providing a three-dimensional in vitro model that resembles non-cornified mucosal epithelium, such as cornea and conjunctiva. The hypothesis tested and confirmed in this work was that the rate of cytotoxicity induced by topical application of test substances to the stratified epithelial cell cultures would correlate with ocular irritancy.

Apart from the above-described general parameters concerning the ocular safety assessment of blank lipid emulsions, the concentration of drug obtained in vivo in aqueous humor, cornea, conjunctiva, vitreous, sclera, or retina can also be utilized to partially evaluate the safety assessment of the drug-loaded lipid emulsions. The intraocular pressure (IOP) as a function of time is also often considered for antiglaucoma agent. Muchtar et al. [56] conducted an ex-vivo permeation study with a new corneal diffusion model and recently, parameters such as miotic effects [57], alkali burn model and re-epithelization healing process [58] have been proposed depending on the intended pharmacological action of incorporated drugs. The most recent works have focused on ophthalmologic parameters such as Rose Bengal staining, break-up-time (BUT), biomicroscopic evaluation of the lipid film, superficial punctate keratis, Schirmer tear test and ocular surface disease impact [6,59,60].

7. Ocular delivery of lipophilic drugs by lipid emulsions

For convenience, we have divided this section into two parts based on the charge of the emulsified oil droplets: anionic and cationic lipid emulsions (Table 4).

7.1. Anionic lipid emulsions application

The in vivo data obtained from studies of early formulations confirmed that anionic lipid emulsions can

Table 4
Non-exhaustive list of oil-in-water (o/w) submicron lipid emulsion for ocular drug delivery

Emulsion type	Drug used	Reference
Anionic emulsion	Δ^8-THC	[61]
Anionic emulsion	Pilocarpine base and indomethacin	[70]
Anionic emulsion	Adaprolol maleate	[68,69]
Anionic emulsion	Indomethacin	[51,55,67]
Anionic emulsion	Synthetic HU-211 and pilocarpine base	[25,62,63]
Anionic emulsion	Pilocarpine base	[57,64–66,71]
Anionic emulsion	Cyclosporin A	[60,72–80]
Cationic emulsion	Piroxicam	[58]
Cationic emulsion	Indomethacin	[39]
Cationic emulsion	Miconazole	[85]
Cationic emulsion	Cyclosporin A	[81,82]

Δ^8-THC and synthetic HU-211 are derivatives of *cannabis sativa*.

be effective topical ophthalmic drug delivery systems [61] with a potential for sustained drug release [62]. Naveh and co-workers [63] have also noted that the IOP-reducing effect of a single, topically administered dose of pilocarpine-loaded anionic lipid emulsions lasted for more than 29 h in albino rabbits whereas that of the generic pilocarpine lasted only 5 h. Zurowska-Pryczkowska et al. [64] studied how lipid emulsions as a vehicle influence chemical stability of pilocarpine, as well as how the drug may affect the physical stability of lipid emulsions. In a subsequent paper [65] from the same group on in vivo evaluation using normotensive rabbits, it was shown that anionic lipid emulsions formulated with pilocarpine hydrochloride at pH 5.0 could be indicated as a preparation offering prolonged pharmacological action (miotic effect) together with satisfactory chemical stability. However, the ocular bioavailability arising from such a formulation did not improve significantly when compared to an aqueous solution of the same drug. The same authors concluded from their further studies that an anionic lipid emulsion with an improved oily phase distribution of pilocarpine resulting either from pilocarpine prodrugs [57] or from pilocarpine ion pair [66] did not achieve a better ocular bioavailability in comparison to the generic solution form of the drug. Inadequate precorneal residence time of their egg lecithin-stabilized lipid emulsion formulations is the most probable reason for such observations. On the other hand, Calvo et al. [51,67] observed an improvement in indomethacin ocular bioavailability when the drug was incorporated in a lipid emulsion compared to the commercial aqueous drops following topical application into rabbit eye.

Beilin et al. [38] who showed, as previously mentioned that a lipid emulsion increased ocular residence time in comparison to eye drops, correlated the delayed pharmacological action to the delayed residence time. Anselem et al. [68] and Melamed et al. [69] prepared an anionic lipid emulsion containing adaprolol maleate, a novel soft β-blocking agent and observed a delayed IOP depressant effect in human volunteers. A similar pharmacological effect was also observed in ailing human volunteers by Aviv et al. [70] using pilocarpine base-loaded lipid emulsion. Another randomized human trial, conducted by Garty et al. [71] compared the activity of the pilocarpine base incorporated lipid emulsion instilled twice daily with a generic dosage form instilled four times a day in 40 hypertensive patients for seven days. No local side effects were observed. The IOP decreased 25% in both of the formulations during this time period. No significant difference was noticed between the two treatments. These results proved that the lipid emulsion extended the action of the drug and two daily administrations have the same result as four instillations of regular eye drops.

A novel anionic lipid emulsion incorporating the immunomodulatory agent cyclosporin A was developed and its clinical efficacy was investigated for the treatment of moderate to severe dry eye disease in animals and humans [72–74]. The novel cyclosporin A ophthalmic dosage form represents a breakthrough in the formulation of a complex, highly lipophilic molecule such as cyclosporin A within an anionic lipid emulsion.

Ding and Coll [72] have developed a castor oil in water emulsion stabilized by polysorbate 80. The cyclosporin penetrated into rabbit extraocular tissues (cornea, lachrymal glands, conjunctiva) at concentrations adequate for local immunosuppression activity while penetration into intraocular tissues was much lower and absorption into blood was minimal [74]. Following thorough validations of this formulation through several clinical studies in various countries [60,75–80], an anionic o/w type lipid emulsion containing cyclosporine A 0.05% (Restasis™, Allergan, Irvine, USA) was approved by the FDA and is now on the market. Sasaki et al. [5] have assumed that when these blank castor oil-based emulsions interact with the tears in the eye, the electrolytes in the tears elicit a physical emulsion instability resulting in some release of the oil. The oil can then migrate towards the lower lid where it may reside longer than aqueous fluids and supplement the lipid layer in the tears. This indicates that lipids containing eye drops such as lipid emulsions have moved a step closer to natural tears even in terms of ocular tolerability and therefore should not be expected to produce any ocular discomfort [6]. Indeed, the lipid substances normally present in tears, such as phospholipids, saturated and unsaturated fatty acids and triglycerides, are currently used in the preparation of most lipid emulsions. Therefore, the lipid emulsions closely correspond to the natural tear fluid and seem to participate in forming a physiological tear film.

7.2. Cationic lipid emulsions application

The potential of a cationic submicron emulsion suitable for ocular application of piroxicam was reported [50]. It was shown that the piroxicam positively charged emulsion was the most effective formulation in lowering the ulcerative

cornea score following alkali burn of rabbit corneas. An increased uptake of the positive oil droplets by the negatively charged cornea is a plausible explanation for the resulting enhancement of the lipophilic drug ocular disposition. Furthermore, the blank emulsion showed a very rapid healing rate over the first three days, with a breakdown on day 14 and then complete re-epithelialization on day 28. The same behavior but less pronounced, was noted in piroxicam emulsion [50]. In addition as previously mentioned, a study was carried out in male albino rabbits to compare the corneal penetration of indomethacin from Indocollyre® (a marketed hydro-PEG ocular solution) to that of anionic and cationic submicron emulsions [39]. Regardless of the preparation instilled, the highest concentration of indomethacin was achieved in the cornea, followed by conjunctiva, sclera retina and aqueous humor. However, the cationic emulsion provided significantly higher drug levels than the control solution and anionic emulsion only in the aqueous humor and sclera-retina. Furthermore, when compared to the anionic lipid emulsion, the submicron cationic lipid emulsion enhanced the ocular bioavailability of cyclosporin A [81,82] following one single drop dose instillation into the rabbit eye (Fig. 2).

A significant drug reservoir effect was noted in the cornea and conjunctiva even for more than 8 h following the instillation of the cyclosporin A-loaded cationic lipid emulsion [82]. This is probably due to the adhesion of the cationic oil droplets to the anionic corneal surface moieties as a result of electrostatic attraction. This hypothesis was supported by data from an ex-vivo study, which showed that the cationic lipid emulsion exhibited better wettability properties on albino rabbit eye cornea than either the saline or the anionic lipid emulsion [39]. Thus, a cationic lipid emulsion vehicle enables a regular or spontaneous spreading behavior over the precorneal area immediately upon its instillation into eye. Associated with poloxamer 188 and phospholipids, a cationic primary amine, stearylamine, has been used to obtain all the above-described cationic lipid emulsion effects. Moreover, the stability and ocular tolerance following topical instillation into eye of these cationic lipid emulsion vehicles were investigated [50,83,84] The promising results obtained with cyclosporin A-loaded cationic lipid emulsions paved the way for the formulation to recently obtain approval from regulatory authorities to undergo Phase-I clinical study for the free drug cationic lipid emulsion [85]. Furthermore, it is interesting to note that the cationic emulsion is promoting the penetration of indomethacin and of cyclosporin A (Fig. 3) to ocular tissues of the posterior segment following one single topical instillation [39,82]. It can be noted that the concentration of the respective drugs in the sclera/retina and in the optic nerve was higher with the cationic emulsion than with the anionic emulsion. Such relatively high concentrations in the posterior segment can be reached only by diffusion of the drugs through one of the following pathways: transcorneal, transconjunctival or through the blood circulation secondary to the systemic absorption. Since aqueous humor and blood levels of cyclosporine were found to be low, while with indomethacin the corneal concentrations of the anionic and cationic emulsions were not significantly different and the blood levels were also low, only the transconjunctival route can represent a plausible approach for the increased concentrations of the drugs in the posterior segment as previously suggested. However, a direct transscleral access to some ocular tissues in the back of the eye cannot be excluded. Further studies are needed to elucidate the mechanism by which drugs can reach the posterior segment of the eye.

A cationic lipid emulsion based on an association of poloxamer 188 and chitosan was also prepared and exhibited interesting physicochemical properties regarding stability and charge effects [24,27]. A remarkable fact from the data reported in the present review is that irrespective of

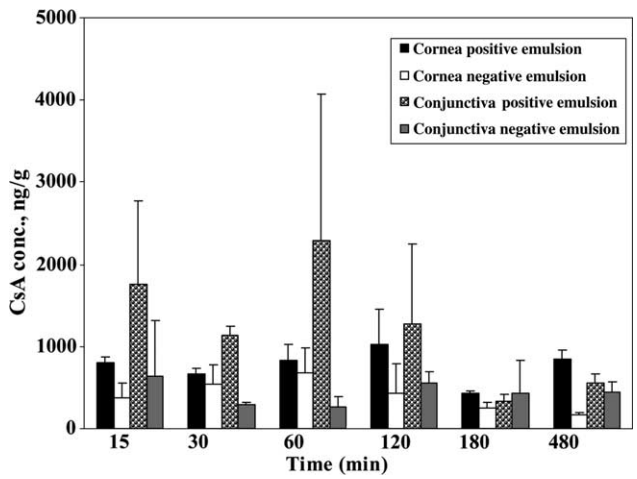

Fig. 2. Influence of emulsion's surface charges on cyclosporin A (CsA) concentrations in ocular surface tissues (cornea and conjunctiva) following one single dose (50 μl) instillation of CsA-loaded cationic (positive) and anionic (negative) submicron emulsions into albino rabbit eye [82].

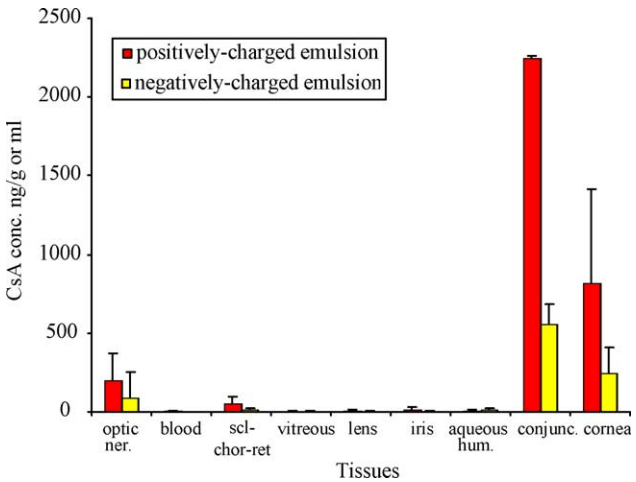

Fig. 3. CsA concentration in different ocular tissues after 60 min from instillation of the emulsions in the treated eye as a function of surface charge droplet [82].

the drug, the cationic emulsion provided higher drug levels than the anionic emulsion formulation. There are evidences that colloidal delivery systems can facilitate the penetration of drugs into ocular surface tissues through an endocytic mechanism [51,67]. The endocytic effect is probably more pronounced with the cationic emulsion as suggested [86]. All these studies stress the effectiveness of cationic lipid emulsions, which promote ocular drug absorption via internalization possibly through an endocytic process.

8. Conclusion

The o/w type lipid emulsion seems to offer a number of advantages in the treatment of various ocular pathologies by providing an altered ocular pharmacokinetics profile of the lipophilic drug incorporated in lipid emulsion following topical instillation into the eye. The potential role of lipid emulsions in the ocular topical delivery of lipophilic drugs to the posterior segment of the eye is currently under investigation. Attempts were being made to optimize the lipid emulsion formulations to elicit adequate therapeutic concentrations of effective drugs for the treatment of virulent eye pathologies especially in the posterior segment of the eye.

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