ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 43 (1997) 179–183

European Journal of Pharmaceutics and Biopharmaceutics

Development and characterization of microemulsions for ocular application

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Received 9 May 1996; accepted 20 November 1996

Abstract

Water-continuous microemulsions for ocular application were developed and the physico-chemical parameters characterized. These microemulsions have favourable features for ocular use. They show an acceptable physico-chemical behaviour, especially pH value, refractive index and viscosity, and a good physiological compatibility. A prolonged pilocarpine release from the microemulsions with lecithin was shown in in vitro experiments. The miotic activity was measured on albino rabbits. For ophthalmological use the miotic retard effect of pilocarpine in microemulsions turns out to be advantageous, but the effect on the intraocular pressure of glaucoma patients still has to be tested. © 1997 Elsevier Science B.V.

Keywords: Microemulsions; Pilocarpine; Physico-chemical parameters; In vitro release; Physiological compatibility; Miotic activity

1. Introduction

Microemulsions are surfactant-containing multicomponent systems which were brought before the public by Hoar and Schulman for the first time in 1943 [1]. During the last two decades they have also become the object of pharmaceutical research in order to improve drug formulations.

Microemulsions could become especially favourable for water-continuous ophthalmological carrier systems because of their aqueous consistence, their transparency and thermodynamical stability. Further advantages result from a possible improvement of solubility and stability of drugs with a potential increase in bioavailability, especially for poorly soluble drugs. In addition, no impairment of visibility can be expected in comparison with eye oils. Because of these circumstances the compliance to the patient could be improved.

Surfactant-containing multicomponent systems for possible ocular application have already been developed and characterized [2–4]. The problem generally is to find suitable components with physiological compatibility, especially with regard to a relative high percentage of surfactants. The aim of the present work was the development of a microemulsion system for ocular use containing pilocarpine hydrochloride as a drug. Such a preparation could be an alternative to pilocarpine eye oil, which affects visibility, and also an alternative to aqueous drops with no retarding effect. Several of the systems which have been developed show a suitability for eye application and therefore were chosen for further measurements. No phase studies were, however, made. Their physico-chemical characteristics, in vitro liberation behaviour, physiological compatibility and miotic activity in rabbits' eyes have been studied.

2. Materials and methods

2.1. Substances

The following components for the microemulsions were used in the supplied quality: lecithin from eggs (E.

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PII S0939-6411(96)00036-7

Merck, D-Darmstadt) and Macrogol-1500-glyceroltriricinoleate (BASF AG, D-Ludwigshafen) as surfactants, polyethylene glycol 200 (Caeser and Loretz GmbH, D-Hilden) and propylene glycol (Carl Roth GmbH + Co, D-Karlsruhe) as cosurfactants. The lipophilic component was isopropylmyristate (Caeser and Loretz GmbH, D-Hilden). Aqua bidestillata formed the hydrophilic component.

2.2. Preparation of microemulsions

For the preparation of the microemulsions a basemixture (BM) without water was made by weighing the components (w/w %). Lecithin was dissolved in isopropylmyristate and mixed with the other components under agitation. The water was added to the BM and rotated until a transparent multicomponent system was formed.

2.3. Physico-chemical parameters

The pH values were measured by a pH-meter CG 825 (Schott-Geräte GmbH, D-Hofheim a. Ts). With an ABBE-Refraktometer (Carl Zeiss, D-Jena) the refractive indices were determined at 25°C. Measurements of osmolality were made with Knauer Osmometer Automatic (Knauer, D-Berlin). The average diameters of particles were measured by Photon Correlation Spectroscopy (PCS) (Coulter N4 MD, 4 mW He-Ne-Laser, D-Krefeld) at an angle of 90° and a temperature of 25°C. The microemulsions were filtered before every measurement through a 0.22-μm membrane filter. The surface tension was determined by a Lauda-Tensiometer (TE 1/2 with SAE + KM3, Meßgeräte-Werk Lauda, Dr. R. Wobser GmbH and Co. KG, D-Lauda-Königshofen) at room temperature. A Rheolab MC 100 (Physica Meßtechnik GmbH, D-Stuttgart) was used for rheological measurements.

2.4. In vitro release

Drug release was determined by flow dialysis equipment with a donor volume of 2 ml at 32°C. Nephrophan® (Filmfabrik D-Wolfen), a hydrophilic pore membrane (pore diameter 2.4 nm) consisting of regenerated cellulose was used. The acceptor medium was always water. Sink conditions were guaranteed by an acceptor medium changed every 30 min.

2.5. Determination of pilocarpine

Pilocarpine was measured by an UV-Vis scanning spectrophotometer (UV-2101PC, Shimadzu Corporation, J-Kyoto) at 214 nm.

2.6. Physiological compatibility

2.6.1. HET-CAM test

The testing of physiological compatibility was made by the HET-CAM test [5]. The tests were made by Confarma GmbH (D-Geretsried-Gelting). The HET-CAM test is made with incubated hen's eggs. First the shell of an inner egg skin was removed to obtain the free chorionallantoic membrane. A dosage of 0.2 ml of the microemulsion was given to each of the six test membranes and monitored at 30, 120 and 300 s after application in view of vascular injection, bleeding and vessel lysis and coagulation.

2.6.2. Draize test

The physiological compatibility on rabbits' eyes was tested by three male and three female New Zealand rabbits weighing 2.1–2.5 kg. Fifty μl was instilled into the conjunctival sac of one eye. The other eye was used as a control.

Draize [6] described a scale of weighed scorces for grading the severity of ocular lesions. The cornea (opacity and area of cornea involved), the iris (values) and the conjunctiva (redness, chemosis and discharge) were determined, and the maximum total score is the sum of all scorces.

2.7. Miotic activity

Miosis was measured on white New Zealand rabbits weighing 2.1–2.5 kg. Six rabbits, three male and three female, were used.

3. Results and discussion

3.1. Preparations

The following microemulsions were selected from a multitude of systems developed for the further experiments:

Microemulsions (ME) with lecithin:

ME 1: 1.7% lecithin from eggs; 13.4% macrogol-1500-glyceroltriricinoleate (MG-1500); 10.0% polyethylene glycol 200 (PEG 200); 6.7% isopropylmyristate (IPM); 68.2% water

ME 2: 1.7% lecithin; 13.4% MG-1500; 10.0% propylene glycol (PG); 6.7% IPM; 68.2% water.

ME without lecithin:

ME 3: 20.0% MG-1500; 5.0% PEG 200; 5.0% IPM; 70.0% water

ME 4: 20.0% MG-1500; 5.0% PG; 5.0% IPM; 70.0% water

Table 1
Physico-chemical parameters of the lecithin-containing ME 1 and ME 2

Parameter	ME 1	ME 2
pH	5.5–6.0	5.5–6.0
Refractive index	1.37–1.38	1.37–1.38
Osmolality [mOsm/kg]	1200–1300	2300–2400
Average diameter [nm]	45–55	28–29
Dynamic viscosity [mPa·s]	7.0–9.0	7.0–9.0
Surface tension [mN/m]	31–32	31–32

3.2. Microemulsions without drug

3.2.1. Physico-chemical parameters

The parameters pH value, refractive index and osmolality were determined. The characterization of particles took place by PCS [7]. Furthermore, the surface tension and the rheological behaviour of the systems were determined. Tables 1 and 2 show the results.

The microemulsions, both with and without lecithin, are clear and show acceptable parameters. All systems can be diluted with water infinitely without becoming unstable. The pH values lie within the physiological range and the refractive index of all microemulsions is not expected to give impairment of visibility. All systems show ideal viscous behaviour. The dynamic viscosity is in the favourable range of 7 to 13 mPa·s. This low viscosity allows sterile filtration and dispensing as eye drops, while the Newtonian behaviour ensures that blinking should have no effect on viscosity [8]. To maintain this viscous behaviour the amount of lecithin cannot be increased, since with increasing amounts of lecithin the systems become highly viscous.

The osmolality depends on the cosurfactant. Microemulsions with propylene glycol (ME 2 and ME 4) have double the osmolality of those with polyethylene glycol 200 (ME 1 and ME 3). This is in accord with the molecular mass of the two cosurfactants and well known for aqueous solutions. The resulting osmolality is generally higher than in pure aqueous systems.

The average diameter of the particles (publication in progress) of the four undiluted microemulsions is between 27 nm to 55 nm. The measurements of the particle size were made on single concentrations of the microemulsions (measurements on diluted systems, see [7]). The stated droplet sizes are apparent values and there may arise errors from interparticle interactions in these concentrated systems. The surface tension is not different in the four systems and is ≈ 30 mN/m. A low surface tension guarantees a good spreading effect on the cornea and mixing with the film constituents, thus possibly improving the contact between the drug and the corneal epithelium [9].

Table 2
Physical-chemical parameters of ME 3 und ME 4 (without lecithin)

Parameter	ME 3	ME 4
pH	6.5–7.0	6.5–7.0
Refractive index	1.37–1.38	1.37–1.38
Osmolality [mOsm/kg]	650–750	1250–1350
Average diameter [nm]	30–35	27–33
Dynamic viscosity [mPa·s]	11.0 13.0	11.0–13.0
Surface tension [mN/m]	32–33	32–33

3.2.2. Physiological compatibility

For developing water-continuous microemulsions we took components from monographs in international pharmacopeias. Egg lecithin (USP23 NF18) is an often used surfactant for parenteral nutrition. The purity of the egg lecithin was not given by the producer. MG-1500 (DAC 1986) is a surfactant which is without irritation to rabbits' eyes in a concentration below 30% according to the literature of the manufacturer [10]. We used PG (DAB 10) and PEG 200 (USP23 NF18) as cosurfactants and IPM (DAB 10) as the lipophilic component. Aqua ad iniectabilia (DAB 10) was used as the aqueous phase.

With favourable physico-chemical parameters for the microemulsions developed in view of ocular application, the physiological compatibility was tested next. As a prerequisite to testing with animals, we determined first the physiological compatibility of the microemulsions ME 1 and ME 2 by the HET-CAM test (hen's egg test on the chorionallantoic membrane). After applying the test solution observations were made to check for the irritation phenomenon of vascular injection, bleeding, vessel lysis or coagulation. The result of this testing was 'not irritant on mucous membrane'.

Because the HET-CAM test showed no irritation, the Draize test [6] on rabbits' eyes was carried out with the lecithin-containing systems as well as the systems without lecithin. This is necessary because the HET-CAM test is not a validated alternative method [5].

First experiments with the New Zealand rabbits were made with a physiological solution of sodium chloride. Furthermore, a fluorescein test followed to control the untreated cornea. It did not reveal corneal lesions. The application of the test microemulsions (each 50 μl) was made three times at intervals of 2 h always into the left eye of the rabbit. The right eye was used as a control. The rabbits' eyes were monitored during the first 8 h every h and than after 24 and 48 h. According to the observation of the rabbits' eyes, no irritations arose from all tested microemulsions. The microemulsions will be diluted in vivo after application to the eye. Comparable in vitro dilution with water shows that they still remain clear and even the in vivo application on rabbits' eyes shows no instability or clouding. Therefore, the developed systems qualified for application to the eye.

Table 3
Physico-chemical parameters of ME 1–4 each with 2% pilocarpine hydrochloride

Parameter	ME 1	ME 2	ME 3	ME 4
pH	4.5–5.0	4.5–5.0	5.0–5.5	5.0–5.5
Refractive index	1.37–1.38	1.37–1.38	1.37–1.38	1.37–1.38
Osmolality [mOsm/kg]	1400–1500	2400–2500	900 1000	1500–1600
Average diameter [nm]	30–45	25–28	30 - 33	42–44
Dynamic viscosity [mPa·s]	7.0–9.0	7.0–9.0	11.0–13.0	11.0–13.0

3.3. Microemulsions with pilocarpine

Because of the positive results of the physiological compatibility of the microemulsions, pilocarpine hydrochloride as a model drug was added to the systems and further studies on the physico-chemical properties, in vitro release of the drug, and testing of the miotic activity with rabbits followed.

3.3.1. Physical-chemical parameters

Two percent pilocarpine hydrochloride was added to the microemulsions by dissolving the drug into the whole system. Table 3 shows the physico-chemical parameters.

The added drug decreases the pH value by about one unit, but is still in the tolerable range and optimal for the stability of pilocarpine. The refractive index has not changed in comparison with the drug-free systems. The osmolality increased as expected by about 200 units and shows the same trends in relation to the surfactant/co-surfactant as systems without drug (Tables 1 and 2). The average diameters of 25–45 nm are comparable with the results of the drug-free systems. Also the dynamic viscosity is not influenced by pilocarpine hydrochloride. Because drug incorporation did not influence the pilocarpine parameters, the pilocarpine-MEs are likewise favourable for ocular use.

3.3.2. In vitro release

The in vitro release behaviour of pilocarpine hydrochloride out of ME 1 and ME 3 was measured in comparison with the aqueous pilocarpine standard solution by using a hydrophilic membrane (Fig. 1). The liberation rate amounts range from 63% (ME 1) to 86% (ME 3) after 6 h.

Only ME 1 is statistically significant to the standard solution and gives 21% less pilocarpine than the aqueous solution after 6 h. In contrast to this, about 2% more pilocarpine is released by the ME 3 without lecithin than by the aqueous solution after 6 h. The composition of ME 3 without lecithin causes a higher release rate of pilocarpine under in vitro conditions.

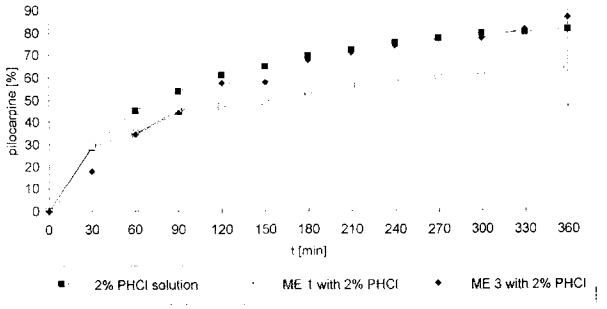

Fig. 1. In vitro release of pilocarpine from aqueous solution and ME 1 and ME 3.

3.3.3. Miotic activity

The pilocarpine bioavailability was measured by the miotic effect on rabbits' eyes. This is a commonly used method to determine the bioavailability of ophthalmic dosage forms. For example, Durrani et al. [11], have investigated the ocular bioavailability of pilocarpine nitrate entrapped in liposomes. Deshpande et al. [12] studied hydrogels. After obtaining results from in vitro release, tests on animals were made, because in vitro results are often different to in vivo measurements [13].

In contrast to the in vitro release which shows no relevant retard effect for pilocarpine from the systems ME 1 and ME 3 (Fig. 1) both systems have a longer miotic effect on rabbits compared with the aqueous pilocarpine solution (Fig. 2). The AUC of the lecithin-containing ME 1 is about 56%, and that of the lecithin-free system ME 3 68% higher than the AUC of the aqueous solution (Table 4). Even considering some 20% physiological fluctuation in in vivo experiments, the results of the microemulsions are significantly higher than those of the aqueous pilocarpine solution.

An increase of the AUC results from a longer effect in comparison with the standard solution, especially for ME 1 (Table 4). The miotic effect of pilocarpine is only about 3.5 h, whereas with ME 1 about 6.5 h and with ME 3 about 5 h were achieved. Furthermore, the

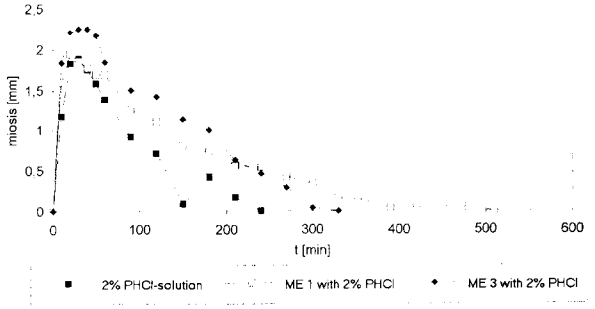

Fig. 2. Miotic activity of aqueous pilocarpine–HCl solution and of ME 1 and ME 3 with 2% pilocarpine–HCl.

Table 4
AUC of miotic activity effect of aqueous pilocarpine solution and pilocarpine in ME 1 and ME 3

System	I_{max} [S.D.]	TP	D	AUC	Relative AUC
Aqueous solution	1.88 [9.4]	30	210	200.7	1.00
ME 1	1.94 [13.7]	20	390	313.7	1.56
ME 3	2.25 [26.4]	30	300	338.1	1.68

I_{max}, peak height [mm].
S.D., standard deviation [%].
TP, time to peak [min].
D, duration of activity [min].
AUC, area under the curve [mm·min].

intensity of action is increased more for ME 3 than for ME 1. That means the bioavailability of the microemulsions is generally enhanced in comparison with the aqueous solution. Corresponding to previous experiments [13], a more intensive reduction of the intraocular pressure will be expected.

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