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# Physicochemical Characterization and Acute Toxicity Evaluation of a Positively-charged Submicron Emulsion Vehicle

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**Abstract**—Fine, homogeneous, positively-charged emulsions with a mean droplet size of 138 ± 71 nm and a zeta potential value of 41·06 mV were prepared using a combination of emulsifiers comprising phospholipids, poloxamer 188, and stearylamine. The pH of these emulsions decreased with time. However, the extent of decrease was dependent on the storage temperature. The mean droplet size of the emulsions that had been prepared with 1% poloxamer began to increase slightly after six months' storage, particularly when stored at 23 and 37°C. However, emulsions prepared with 2% poloxamer remained stable for at least 10 months at 4°C, suggesting that the poloxamer 188 concentration is critical for prolonged emulsion stability. The results of the ocular tolerance study in rabbit eye indicate that hourly administration of a positively-charged emulsion vehicle was well tolerated without any toxic or inflammatory response to the ocular surface during the five days of the study. Scanning electron microscopy revealed a normal corneal surface, which was not different from that of the animals treated with physiological saline. No marked acute toxicity was observed when 0·6 mL of positively-charged emulsion was injected intravenously to BALB/c mice. Furthermore, no difference was noted between this group of animals and the group injected with the marketed Intralipid emulsion. These results were further confirmed in a rat study where there were no deaths following intravenous injection of 3·3 mL per rat of the positively-charged emulsion or Intralipid. Neither emulsion elicited any hepatotoxic or nephrotoxic effects. The overall results suggest that the novel positively-charged emulsion is suitable for parenteral use, and for ocular application.

There has been renewed interest in the emulsion as a vehicle for delivering drugs to the body. Emulsions are well accepted as intravenous delivery systems for either lipophilic or hydrophobic drugs such as cytotoxic drugs, (Prankered et al 1988; Stella et al 1988), amphotericin B (Davis et al 1987; Kirsh et al 1988; Levy et al 1993), diazepam (Levy & Benita 1989), prostaglandin $E_1$ $PGE_1$ (Japan Pharmaceutical Reference 1993), and propofol (Dundee & Clarke 1989). Other authors (Rubinstein et al 1991; Myers & Stella 1992; Kleinstern et al 1993) have used submicron emulsion delivery systems to prolong the pharmacological effect of drugs with short biological half-lives or poor bioavailability following oral administration. Muchtar et al (1992) have demonstrated that submicron emulsions can also be used as an ocular delivery system for the lipophilic antiglaucoma drug, tetrahydrocannabinol (THC). They showed that the emulsion was able to elicit a long-lasting antidepressant effect on the intraocular pressure of rabbits following a single instillation. These overall results underline the promising properties of emulsion drug carriers as therapeutic delivery systems for a variety of drugs.

The previously mentioned submicron emulsion formulations were stabilized by various combinations of surface-active agents which exhibited a high-negative zeta potential value able to avoid droplet coalescence upon random collisions.

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Recently, a positively-charged submicron emulsion, the biological fate of which is expected to be different from the previously described negatively-charged emulsion formulations, was briefly reported (Elbaz et al 1993). This formulation was based on three surface-active agents, Lipoid E-80, poloxamer 188 and stearylamine. Lipoid E-80, is a mixture of phospholipids from egg yolk sources. The major component of the mixture is phosphatidylcholine, which is zwitterionic in form and neutral over a wide pH range. In addition, the mixture contains negatively-charged phospholipids such as phosphatidylethanolamine, phosphatidylserine and phosphatidic acid and ionization that is markedly pH dependent (Bangham 1968; Rydhag & Wilton 1981; Davis 1982). Poloxamer 188 is a non-ionic block copolymer of polyoxyethylene-polyoxypropylene which stabilizes the emulsion through a steric repulsion mechanism, as previously described (Levy et al 1991). The third surface active agent is stearylamine, a cationic lipid with a $pK_a$ of 10·6 which contributes the overall positive charge to the oil droplet interfaces over a wide pH range owing to its quaternary ammonium group.

The rationale to design a novel submicron emulsion vehicle bearing a positive charge instead of either a negative or a neutral charge should be sought in the extensive and interesting results published in the literature on liposome research (Gregoriadis & Neerunjum 1974; Steger & Desnick 1977; Meisner et al 1989). As an example, Gregoriadis & Neerunjum (1974) found that the rate of removal of liposomes from the circulation was dependent, not only on the vesicle size, but also on the vesicle surface charge. Positive

liposomes cleared less rapidly than negative ones. Therefore, it is believed that, in contrast to negatively-charged submicron emulsions, positively-charged submicron emulsions may alter the pharmacokinetic profile of the incorporated selected drugs resulting in enhanced localization of higher drug concentrations in targeted organs. This hypothesis has already been proposed by other authors who investigated charge-reversed submicron emulsions (Davis et al 1992).

However, positively charged stearylamine liposomes were reported to be toxic either in cell culture systems (Magee & Miller 1972; Campbell 1983; Mayhew et al 1987)) or in-vivo. Most of the liposome publications which outlined the animal toxicity of positively charged stearylamine liposomes referred to the work of Adams et al (1977). Those authors have shown that following intracerebral injection of stearylamine liposomes to mice, most of the animals died almost immediately apparently due to respiratory failure. The ocular toxicity of positively-charged stearylamine liposomes was also investigated. Lee & Carson (1986) have suggested that positive liposomes, particularly when administered on a multiple-dose basis to the eye are by no means inert insofar as tear flow and ocular surface characteristics are concerned. The nature of the effects which cause these changes was not clearly defined and the authors emphasized that further work should be done before a decision could be made on the suitability of positive liposomes as an ocular drug vehicle. Furthermore, in another study, no histological alterations in ocular tissues were observed following topical administration of either neutral or positively-charged stearylamine liposome to the eyes (Tanaguchi et al 1988). No clear conclusion could be drawn from all cited publications on the toxic effect of stearylamine liposomes. Since the present positively-charged submicron emulsion also contains stearylamine, and was designed as a vehicle for either intravenous or ocular drug administration, the potential induced toxicity should be assessed. It is, therefore, the objective of the present paper to describe the acute toxicity of the positively-charged submicron emulsion following intravenous (mice and rats) or ocular (rabbits) administration. In addition, a physicochemical evaluation of the emulsion was also carried out.

## Materials and Methods

*Emulsion ingredients*

Medium-chain triglyceride (MCT) was purchased from Societe des Oleagineux (St Laurent Blangy, France). MCT consisted of not less than 95% esterified fatty acids, comprising 8 and 10 carbon atoms according to the manufacturers specifications. Lipoid E-80 was purchased from Lipoid Ag (Ludwigshafen, Germany). The Lipoid E-80, according to manufacturer specifications, comprised about 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3·6% nonpolar lipids, and about 2% sphingomyelin. Polyoxyethylene-polyoxypropylene emulsifier, poloxamer 188 (Pluronic F68) was furnished by BASF (Ludwigshafen, Germany). Stearylamine was purchased from Sigma (MO, USA). All other ingredients were pharmaceutical grade.

*Emulsion preparation*

The nonionic emulsifier (poloxamer), and the osmotic agent (glycerol) were dissolved in the aqueous phase. The pH of the aqueous phase was adjusted to 4·0. The Lipoid E-80, anti-oxidant ($\alpha$-tocopherol) and the cationic surfactant stearylamine were dissolved in the MCT oil phase. Both phases were heated separately to 70°C, then the two phases were mixed and stirred with a magnetic stirrer. The resulting mixture was further heated to a temperature of 85°C. At this temperature, the coarse emulsion which was obtained was further mixed by a high-shear mixer Polytron (Kinematica, Luzern, Switzerland) for 5 min and rapidly cooled to below 20°C. After cooling, the emulsion was homogenized using a two-stage homogenizer valve assembly (Gaulin Homogenizer, APV Gaulin, Hilversum, The Netherlands) at 9000 pounds $in^{-2}$ for 5 min. After further rapid cooling below 20°C, the pH of the emulsion was adjusted to the desired value with 0·1 M HCl. The emulsion was filtered through a TE membrane filter (Schuell & Schleicher, Dassel, Germany) with a pore size of 0·45 $\mu$m, packed under nitrogen atmosphere in siliconized glass bottles and then sterilized by steam autoclave at 121°C for 15 min. The various emulsion batches were stored at three different temperatures, over prolonged periods of time.

A typical formulation (% w/w) consisted of MCT 8·5, Lipoid E-80 1·2, stearylamine 0·3, $\alpha$-tocopherol 0·02, poloxamer 188 1 or 2, glycerol 2·25 and double-distilled water to 100.

The emulsions containing 2% poloxamer were prepared using identical experimental conditions as described above.

*Particle size analysis*

The droplet size and size distribution were determined by means of a photon correlation instrument (Coulter Counter Supernanosizer N4SD, Luton, UK). Each sample was diluted to the appropriate concentration with a filtered isotonic solution (2·25% w/v glycerol in water). The measurement was carried out at 25°C and each emulsion was analysed twice.

*Zeta potential*

The zeta potential was measured with Malvern Zetasizer (Malvern, UK). The nature of the charge was assessed using a complementary method of moving-boundary electrophoresis (Shaw 1969). Appropriate experimental conditions able to yield accurate electrophoretic mobility data have already been established and described elsewhere (Benita et al 1986).

*pH*

The pH of the emulsion samples was measured and recorded at given time intervals using a pH meter (Corning pH meter 245 USA).

*Stability assessment*

The various emulsion properties described above were followed over 250 to 400 days upon storage at 4, 23, and 37°C. The degrees of creaming and phase separation were assessed visually at given time intervals. All other visible changes were recorded.

*Ocular irritation study*

Ocular irritation was evaluated in four albino male rabbits

(2–2·5 kg). Either saline or positively-charged emulsion was instilled randomly into four eyes, such that no rabbit received two of the same type of drops and each formulation was given to two right and two left eyes.

New bottles of non-preserved saline or positive emulsion were used daily. Each eye randomly received a drop of either saline or positively charged emulsion, eight times a day, between 0800 and 1600 h for five consecutive days. Eyes were evaluated under topical anaesthesia (one drop of 1% oxybuprocaine HCl) on the third and fifth days. Each examination included biomicroscopic eye examination with slit lamp and operating microscope. Eye-lids, conjunctiva, cornea and anterior chamber were inspected for inflammatory or toxic response. Topical fluorescein drops were used to detect epithelia defects.

After the final examination, on the fifth day, the rabbits were killed using an overdose of pentothal. The eyes were enucleated, placed individually in 2% glutaraldehyde, and studied using scanning electron microscopy (SEM) following cornea drying with Freon 113.

*Acute toxicity study in mice*

Male albino BALB/c mice (AnNHsd type, supplied by Harlan Sprague Dawley, ID, USA) 20 g, were injected with increasing doses of one of the following types of formulations through the tail vein: positively-charged emulsion, Intralipid (a commercial negatively-charged emulsion) or physiological saline. Each dosage was administered by single bolus injections (0·2 mL) to 10 mice. When the value of one single injection exceeded 0·2 mL, appropriate consecutive injections at 10-min intervals were performed. Survival was followed up to 30 days.

*Acute neurotoxicity evaluation in rats*

Keeping in mind the neurotoxicity results reported by Adams et al (1977) on the stearylamine liposomes, acute preliminary neurotoxicity evaluation of the positively-charged submicron emulsion was necessary.

*Animals and operation.* Male Lewis rats, 200–240 g, were acquired from the Animal Breeding Unit of the Hebrew University-Hadassah Medical School, Jerusalem, Israel. They had an indwelling cannula implanted in their right jugular vein under light ether anaesthesia one day before the intravenous administration. The cannulae were filled with saline without heparin (Hoffman & Alfon 1992). During the experimental period, all animals were housed in individual cages and had free access to food and water. Rectal temperature was monitored before the experiment.

*Emulsion infusion technique.* Positively-charged submicron emulsion, Intralipid 10% or saline (control) was administered as a continuous intravenous infusion for 2 h by a syringe infusion pump (Pump 22, Harvard Apparatus, South Natick, MA) at a rate of 0·0274 mL $min^{-1}$ (total infusion volume 3·3 mL).

The animals' behaviour was closely observed throughout the infusion period. After 24 h, light anaesthesia of the rats with ether was followed by withdrawal of maximal volume of blood (for serum) from the abdominal aorta to ensure death. The animals were dissected, and their internal organs were examined visually. Livers were separated, compressed slightly between paper towels, and weighed. The biological samples were frozen (−20°C) pending biochemical analysis. Data values of tests which were performed with commercially available kits (Hoffman & Alfon 1992) are expressed as the mean ± standard deviation. The data were analysed for statistical significance using either Student's unpaired *t*-test, or the Mann-Whitney test (two-sided $P$, $\alpha = 0{\cdot}1$). A statistically significant difference was taken as $P < 0{\cdot}05$ for Student's *t*-test.

## Results

*Physical emulsion evaluation*

The well-established combined emulsification technique for sizing to the submicron range, yielded fine homogeneous emulsions with a mean droplet size of 138 ± 71 nm, and a zeta potential value of + 41·06 mV at a pH of 7·15. The positive surface charge was confirmed by using the electrophoretic mobility technique which showed that Intralipid (a negatively-charged emulsion) and the emulsion containing stearylamine moved in opposite directions. The values of zeta potential and mean droplet size did not differ markedly from batch to batch (± 5%), indicating that the experimental conditions were well controlled.

*Evaluation of long-term stability*

*Effect of sterilization on pH.* The influence of steam-autoclave sterilization on pH was tested on different emulsion vehicles, the pH of which was adjusted to different values. Irrespective of the initial adjusted pH, the sterilization process decreased the pH by 1·6–1·7 units. Initial adjusted pH 8·80 and 8·41 were reduced to 7·15 and 6·70, respectively. This pH reduction is explained by hydrolysis of the phospholipids during the heat-sterilization process and formation of free fatty acids (Washington & Davis 1987; Herman & Groves 1993). This reduction in pH can significantly affect the physical stability of the emulsion, as will be further explained.

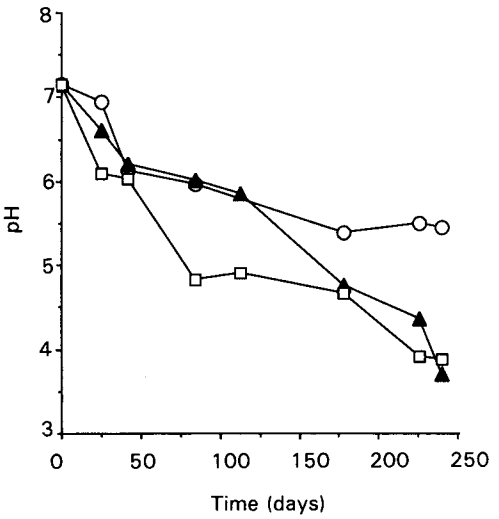


FIG. 1. pH variation in a positively-charged emulsion, prepared using the typical formulation and 1% poloxamer 188 stored at different temperatures, as a function of time. ○ 4°C; ▲ 23°C; □ 37°C.

*Effect of storage temperature on pH changes as a function of time.* The effect of storage temperature on pH reduction is shown in Fig. 1. It can be noted that at 4°C, the pH gradually declined reaching a minimum value of 5·4–5·5. After 175 days, the pH remained stable, and no further reduction was noted. On the other hand, at room temperature (23°C) and 37°C, the pH reduction continued, regardless of length of storage time.

*Effect of storage temperature on mean droplet size as a function of time.* The effect of storage temperature on mean droplet size is shown in Table 1. The emulsions were found to be stable for up to six months, at which time a slight increase in droplet size and distribution were noted. To determine whether the elevation is dependent on the poloxamer 188 concentration, a new batch of positive emulsion, containing 2% poloxamer 188, was manufactured. The droplet size and distribution profile were monitored at 4°C. The results, shown in Table 2, indicate that 2% poloxamer emulsion is stable for at least 10 months, suggesting that the poloxamer 188 concentration is critical for prolonged stability of the emulsion.

The pH variation of this emulsion at 4°C was followed. The initial decrease in pH was slower than that for the 1% poloxamer emulsion. After 180 days, the pH of 2% poloxamer emulsion remained stable and no further reduction was observed, as was noted for the 1% poloxamer emulsion (Fig. 2).

*Animal studies*

*Ocular evaluation.* Biomicroscopic examination of the rabbit eyes under a slit lamp at days 3 and 5 revealed no signs of toxicity or inflammation. The ocular surface had remained intact without erosions, or hyperaemia of conjunctiva or eye-lids. The corneas were crystal clear, and the anterior chamber was also free of inflammatory signs. Scanning electron microscopy of the corneal surface revealed normal epithelium and no signs of toxicity (Fig. 3).

*Acute toxicity study.* The acute toxicity results are shown in Table 3. The positively-charged emulsion was tolerated at the same high doses of Intralipid and no animal deaths were observed over a period of 30 days.

*Acute neurotoxicity evaluation.* No signs of neurotoxicity were noticed during the infusion period. Serum biochemical indices are shown in Table 4. Total serum protein values were somewhat lower (although statistically significant according to the *t*-test) in the positively-charged emulsion and the Intralipid groups, than the control group. However, this reduction is a well known phenomenon which has already been observed (Stein 1982) during the investigation of fat parenteral nutrition and is confirmed in this study. No significant difference was noted in any biochemical parameter evaluated using the Mann-Whitney test.

Table 1. Effect of storage temperature on the mean droplet size of a positively-charged emulsion prepared using the typical formulation and 1% poloxamer 188 with time.

| Storage time (days) | Mean droplet size (nm) Temperature (°C) 4 | 23 | 37 |
|---|---|---|---|
| 0 | 138 ± 71 | 138 ± 71 | 138 ± 71 |
| 86 | 158 ± 47 | 154 ± 44 | 141 ± 64 |
| 167 | 135 ± 62 | 131 ± 42 | 151 ± 44 |
| 213 | 191 ± 140 | 255 ± 93 | 262 ± 110 |

Values are mean ± s.d.

Table 2. Mean droplet size variation with time of a positively-charged emulsion prepared using the typical formulation and 2% poloxamer stored at 4°C.

| Storage time (days) | Mean droplet size (nm) |
|---|---|
| 0 | 127 ± 46 |
| 28 | 124 ± 23 |
| 109 | 137 ± 55 |
| 284 | 140 ± 56 |
| 320 | 136 ± 18 |

## Discussion

*Physicochemical evaluation of the emulsion*

The inherent instability of a fluid emulsion is a result of the system's tendency to reduce its free energy by progressively increasing the particle size and broadening the distribution until the dispersed particles separate out as free liquid. Thus, if this tendency could be drastically reduced, it could lead to acceptable kinetic stability in a pharmaceutical dosage form which does not require thermodynamic stability (Rieger 1986). During the ageing process, the physical instability of the emulsion is evidenced by creaming, flocculation and coalescence. An emulsion is stabilized by the addition of emulsifying agents which lower the interfacial tension and form a film at the oil-water interface which acts as a mechanical barrier to droplet coalescence. Furthermore, flocculation can be prevented by producing repulsive electrical forces between approaching droplets. The electrical surface charge of droplets is produced by the composition of

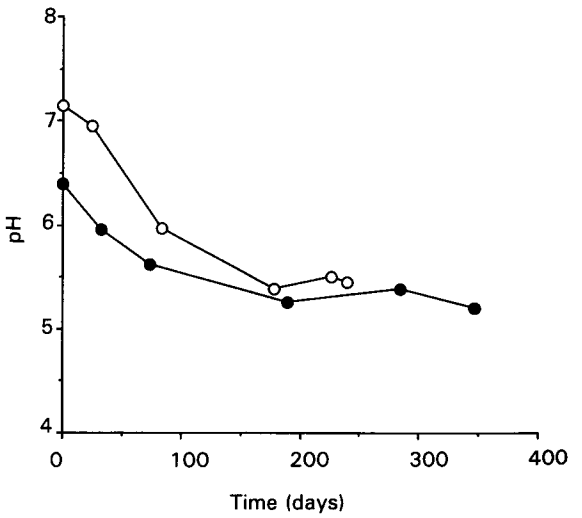


FIG. 2. pH variation in positively-charged emulsions prepared using typical formulation, and 1 or 2% poloxamer 188 stored at 4°C as a function of time. ● 2% poloxamer; ○ 1% poloxamer.

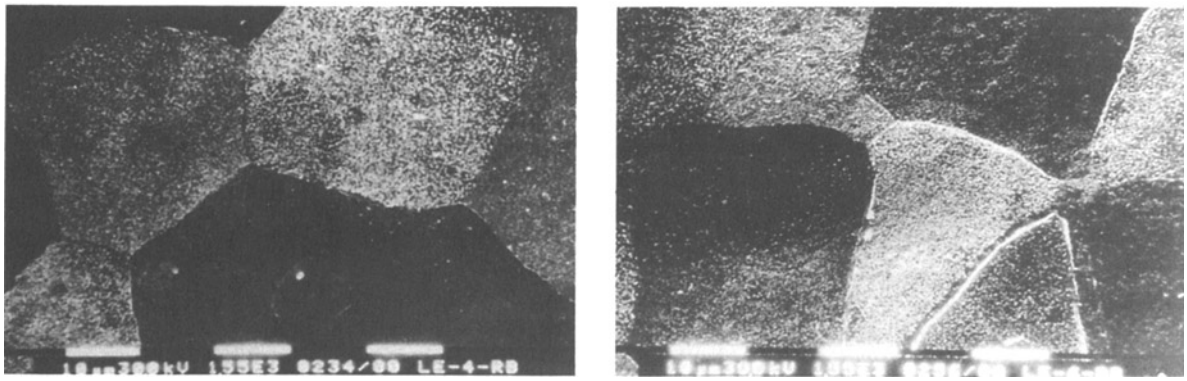


FIG. 3. Scanning electron photomicrograph of rabbit cornea surface following 40 repeated applications of saline and positively-charged blank emulsion (one drop was instilled every hour for 8 h over five consecutive days).

interfacial film-forming components. In the present study, the combination of Lipoid E-80, poloxamer 188 and stearylamine was shown to yield stable positively-charged homogeneous emulsions in the nanosize range. The actual emulsion was stabilized by a mixed surfactant monolayer formed around the emulsified droplets as was evidenced by the results of a TEM freeze-fracturing examination reported elsewhere (Zeevi et al 1994). No liquid crystal, or multilamellar liposomal structures were detected by the freeze-fracturing technique.

Attempts to gain an overall comprehension of all the complex molecular interactions occurring between various components forming an interfacial film around emulsified oil droplets through monolayer studies have been reported by some authors to be successful (Rubino 1990; Weingarten et al 1991; Levy et al 1991). Thus, a monolayer study under different experimental conditions was carried out between Lipoid E-80 and stearylamine (Korner et al 1994). The results clearly revealed strong molecular interactions between stearylamine and the phospholipid molecules. Mixed phospholipid-stearylamine monolayers exhibited non-ideal behaviour, which resulted in a significant increase in both surface pressure and surface potential above that corresponding to the pure compounds. The deviation from the ideal behaviour is mainly due to strong interactions between the positively-charged stearylamine head group and phospholipid polar group occurring in the electrostatic double layer. The injection of poloxamer into the aqueous subphase, beneath the mixed monolayer yielded an important increment of the surface potential of the Lipoid E-80-stearylamine monolayers which should be attributed to the interaction occurring at the level of hydrophilic groups in the electrostatic double layer. On the basis of these data, the prolonged stability of the oil/water emulsion could be attributed to the existence of molecular interactions among phospholipids, poloxamer and stearylamine. These interactions result in molecular arrangements in the interfacial regions leading to an effective combined steric/electrical energy barrier capable of preventing droplet coalescence upon random collisions. The overall positive charge exhibited by the emulsified droplets should be attributed to an excess in interfacial concentration of stearylamine, while the interfacial steric stabilization is conferred on the emulsified droplets by the hydrophilic polymeric moieties of poloxamer oriented towards the external aqueous solution. This hypothesis is further supported by the results presented in Tables 1 and 2, which show the necessity of the presence of at least 2% poloxamer in the typical formulation if there is to be prolonged stability of the emulsion. Such a minimum concentration is probably needed because it allows a better coverage of the generated interfacial surface by the emulsification process.

Table 3. Acute toxicity of various emulsion vehicles in BALB/c mice.

| Group (n = 10) | Survival after 30 days (%) |
|---|---|
| Saline control | 100 |
| Intralipid, negatively-charged emulsion | |
| 0·2 mL | 100 |
| 0·4 mL | 100 |
| 0·6 mL | 100 |
| Positively-charged emulsion | |
| 0·2 mL | 100 |
| 0·4 mL | 100 |
| 0·6 mL | 100 |

The results presented in Fig. 1 indicate that after the sterilization process a progressive reduction in pH occurred during storage. The fall in pH is due to the formation of free fatty acids (FFA), the origin of which should be attributed to chemical changes in phospholipids, owing to oxidation and hydrolysis (Hansrani et al 1983; Washington & Davis 1987). The principal degradation process is due to the hydrolysis of the diacylphosphatidylcholine and diacylphosphatidylethanolamine to their corresponding monoacyl (lyso-) derivatives and FFA moieties. FFA can also be formed by the hydrolysis of emulsified triglycerides to the corresponding mono- and diglycerides, although this reac-

Table 4. Effect of a positively-charged submicron emulsion and Intralipid infusion (0·0274 mL $min^{-1}$, 2 h) in male Lewis rats (n = 4).

| Parameter | Control (saline) | Intralipid 10% | Positively charged submicron emulsion |
|---|---|---|---|
| Weight (g) | 213·5 ± 7·5 | 225·5 ± 12 | 217·5 ± 14 |
| Rectal temperature (°C) | 37·9 ± 0·1 | 37·5 ± 0·3 | 37·6 ± 0·3 |
| Liver weight (g) | 8·83 ± 0·44 | 8·86 ± 0·35 | 8·74 ± 0·64 |
| Creatinine (mg, $dL^{-1}$) | 0·32 ± 0·07 | 0·28 ± 0·05 | 0·23 ± 0·10 |
| Serum alanine aminotransferase activity (units $dL^{-1}$) | 20·83 ± 5·10 | 20·88 ± 3·92 | 20·88 ± 3·92 |
| Urea nitrogen, (mg $dL^{-1}$) | 12·04 ± 0·73 | 14·13 ± 1·60 | 10·58 ± 1·63 |
| Serum total protein (g $dL^{-1}$) | 5·94 ± 0·37 | 5·69 ± 0·15* | 5·47 ± 0·21** |

Total infusion volume was 3·3 mL. *$P < 0·02$, **$P < 0·01$ compared with control.

tion is believed to be relatively slow compared with the breakdown of the diacylphosphatidyl derivatives in phospholipids, as far as long-chain triglycerides, such as soybean oil, are concerned (Herman & Groves 1992). However, in the present study, MCT was used and this is 100 times more soluble in water than soybean oil. Therefore, MCT is probably more sensitive to hydrolysis than soybean oil, and subsequently, the FFA degradation products are more soluble in water, and should contribute to a further decrease in pH. The decrease in pH is known to affect significantly the physical stability of the emulsion since it reduces the ionization of some phospholipids such as phosphatidylethanolamine, phosphatidylserine, and phosphatidic acid, resulting in a diminution of the negative zeta-potential value (Bangham 1968; Rydhag & Wilton 1981; Davis 1982).

However, with the present formulation, a decrease in pH concomitantly led to a decrease in the ionization of the anionic phospholipidic components, and to an increase in the ionization of stearylamine. This resulted in no marked effect on the zeta potential as already confirmed in a previous study (Zeevi et al 1994). This was again demonstrated in the present study, where at time zero, the zeta potential of the positively-charged emulsion was measured at pH 5·0, 6·0, and 7·0, and found to be 36·67, 38·50, and 41·06 mV, respectively. Neither creaming nor coalescence was noted, even though pH decreased to a value of 5·4–5·5. This is reflected by the lack of change in mean droplet size with time, as presented in Table 2.

*Animal studies*

Muchtar et al (1992) have already demonstrated that submicron negatively-charged emulsions can be used as an ocular delivery system. The conventional ophthalmic dosage form, eye drops, has two major disadvantages: poor drug penetration into ocular tissues, and a short duration of action. One of the main reasons for poor penetration of the drugs into the eye is the decrease of medication concentration in the tear film as a result of the blinking reflex, tear production, and drainage through the nasolacrimal system. These are accompanied by unwanted entrance of the drug into the systemic circulation (Meisner et al 1989).

The need for safer, more efficacious and acceptable ocular therapeutic systems is obvious. The significance of surface charge had been reported by Meisner et al (1989) who showed that atropine-base encapsulated positively-charged liposomes provided the longest pharmacological effect, compared with neutral and negative liposomes or solution. The positive submicron emulsion can be suitable as a topical ocular drug delivery device, provided that the emulsion has affinity for, and is able to bind to, the corneal surfaces without causing any toxic response. Our study indicates that hourly administration of a positively-charged emulsion vehicle was very well tolerated without any evidence of any toxic or inflammatory response to the ocular surface during the five days of the study. Furthermore, scanning electron microscopy revealed a normal corneal surface, which was not different from that of the animals treated with saline (Fig. 3).

Adams et al (1977) reported generalized epileptic seizures, widespread tissue necrosis and some death in mice following intracerebral injections of liposomes containing stearylamine and diacetylphosphate. According to those authors, the amount of lipid injected ranged from 5 to 10 mg, corresponding to 0·22 to 0·44 mg stearylamine, respectively, per 20 g mouse. This dose was believed to be too large, since Kimelberg (1980) reported that one-thirtieth of the dose injected intracerebrally to a monkey did not produce any obvious toxic effect.

In contrast, no marked acute toxicity effect was observed in the present study when 0·6 mL positively-charged submicron emulsion, corresponding to a dose of 1·8 mg stearylamine was injected intravenously to mice (Table 3). Furthermore, no difference was noted between this group of animals and the group injected with the marketed Intralipid emulsion. These results were confirmed in the rat study, where no death was noted following administration of stearylamine doses equivalent to 10 mg per 250 g rat.

Thus, it appears that the stearylamine associated with the emulsion did not induce any of the effects reported by Adams et al (1977). This might be explained by the fact that the route of administration was different. Alternatively, it can be attributed to the strong molecular interactions occurring at the oil/water interface between the film-forming components. This prevented stearylamine from leaking and exerting any intrinsic toxicity which is usually anticipated in the case of injection of sensitive positive liposomes to animals. A final explanation could be the fact that the positively-charged emulsified droplets did not penetrate through the blood-brain barrier and, therefore, did not reach the brain, which is considered the organ where stearylamine side-effects were produced.

No hepatotoxicity or nephrotoxicity was detected according to the results of the blood tests presented in Table 4. No statistically significant difference in the creatinine values was noted between the control group and the group treated with Intralipid and positively-charged emulsion. The large stan-

dard deviation noted in the positive emulsion is not clinically indicative of any nephrotoxic sign, as the variation was in the normal range due to subject intervariation. A normal serum alanine aminotransferase value indicates that no rupture in the hepatocytes occurred. Serum total protein concentration in the group treated with the marketed Intralipid, and the group treated with positively-charged emulsion, was significantly lower than the concentration in the control group only according to the *t*-tests. This is probably due to the accumulation of fat in the liver following administration of emulsion. Subsequently, the whole body and plasma albumin synthesis rates are depressed because the tissues are semi-starved (Stein 1982). Nevertheless, the positively-charged submicron emulsion did not behave differently from the clinically well accepted marketed Intralipid emulsion.

Positively-charged liposomes of doxorubicin which were prepared with stearylamine, among other lipids, have been tested in a clinical phase I and II study (Treat et al 1989). The corresponding amount of stearylamine injected ranged from 52 to 156 mg per patient for one single cycle. These doses are well above the dose that would be expected to be administered with a positively-charged submicron emulsion (2–3 mg only). Furthermore, up to 19 cycles were administered in this study and the authors clearly reported that no hepatic renal, or pulmonary toxicity was seen. Thus, there is a reasonable chance that positively-charged submicron emulsions will not elicit any adverse effect following oral administration to patients.

These overall results suggest that our new positively-charged submicron emulsion is suitable for parenteral use of ocular application.

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