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The stability of piroxicam incorporated in a positively-charged submicron emulsion for ocular administration

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Abstract

A comprehensive physicochemical characterization of a positively-charged submicron emulsion containing piroxicam was carried out in an attempt to identify optimal experimental conditions. An appropriate oil phase was selected from the results of the piroxicam solubility study of various oil and phase compositions containing piroxicam. Stable monodispersed positively-charged emulsions with a mean droplet size of 100 nm were obtained setting an adequate homogenization processing time. It was observed that after autoclave sterilization, a progressive reduction in pH occurred during the storage period, especially in the emulsions which were adjusted to an initial pH of 7.5 and stored at 37°C. The fall in pH was most probably due to the formation of free fatty acids (FFA), the origin of which should be attributed to chemical changes in the phospholipids present in the emulsion formulation. It was shown that the emulsion containing piroxicam was very sensitive to the adjusted initial pH and to the formation of FFA which reduced its pH over prolonged storage times. However, the decrease in pH did not appear to affect the zeta potential, which remained positive, and led to the precipitation of piroxicam, easily detected by visual and microscopic observations. As revealed by the monolayer studies, the drug was capable of interacting with the film-forming components which could explain the observed stability of the emulsions.

Keywords: Emulsion; Submicron; Piroxicam; Positive charge; Surface pressure

1. Introduction

Piroxicam, an oxicam derivative, is a nonsteroidal anti-inflammatory drug (NSAID) with a pKa of 5.1. The drug exhibits anti-inflammatory, analgesic and antipyretic activities. Although the exact mechanism of its actions has not yet been clearly established, many of them appear to be principally associated with the inhibition of prostaglandin synthesis, as has already been established for other NSAIDs (McEvoy, 1990). Twelve NSAIDs (including members of each of the main chemical groups) were studied in vitro and examined for their effect on the oxidative burst induced by the receptor stimulus, platelet-activating factor (PAF), and by two post-receptor stimuli, fluoride and dioctanoylglycerol. The re-

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sults of these studies indicate that piroxicam causes a significant decrease in the O_2^- response induced by both PAF and fluoride, while it has no significant effect on dioctanoylglycerol-stimulated O_2^- production (Twomey and Dale, 1992). These data may have some clinical implications in the therapy of inflammatory disorders such as rheumatoid arthritis or ocular inflammations (uveitis). Those NSAIDs that cause an increased O_2^- response, while providing temporary relief of symptoms, could be exacerbating the underlying inflammatory condition and associated tissue damage. Although it is an appropriate NSAID candidate, no intravenous or ocular preparation of piroxicam is available in the market due to its poor solubility in aqueous solutions. Consequently, the incorporation of piroxicam into positively-charged submicron emulsions intended for either intravenous administration or ocular application has promising therapeutical applications. The positive charge of the emulsified oil droplets is conferred to them by the incorporation of a cationic lipid, stearylamine (SA) (Elbaz et al., 1993).

The rationale to design a novel submicron emulsion vehicle bearing a positive, instead of either a negative or a neutral charge, comes from the results published in the liposome research literature (Gregoriadis and Neerunjun, 1974; Steger and Desnick, 1977; Meisner et al., 1989). Gregoriadis and Neerunjun showed that the rate of removal of liposomes from circulation is dependent, not only on the vesicle size but also on the vesicle surface charge. Since positive liposomes are cleared less rapidly than negative ones, it is believed that positively-charged submicron emulsions may alter the pharmacokinetic profile of the selected incorporated drugs and enhance localization of higher drug concentrations in targeted organs. This hypothesis has also been advanced by other authors who investigated charge-reversed submicron emulsions (Davis et al., 1992). Furthermore, Meisner et al. (1989) reported on the significance of surface charge for ophthalmic drug delivery systems and showed that, compared to neutral or negative liposomes or solution, atropine-base loaded positively-charged liposomes generate longer pharmacological effects. Thus, a positively-charged submicron emulsion appears to be a suitable topical ocular drug delivery device provided that the emulsion used is capable of binding to the negatively-charged corneal surface without causing a toxic response. Such a hypothesis seems to be plausible (Rojanasakul and Robinson, 1989) and is currently being investigated.

Positively-charged liposomes prepared with SA were reported to be toxic both in cell culture systems (Magee and Miller, 1972; Campbell, 1983; Mayhew et al., 1987) and in vivo (Adams et al., 1977). Their ocular toxicity was also investigated (Lee and Carson, 1986; Tanaguchi et al., 1988) and despite the lack of clear conclusions regarding their toxic effects, numerous authors are convinced that further work should be done to evaluate the suitability of positive liposomes as ocular drug vehicles.

The results of the ocular tolerance study in rabbit eyes (Klang et al., 1994) indicated that hourly administration of a positively-charged emulsion containing SA was well tolerated and did not induce a toxic or inflammatory response during the 5 days of the study (40 instillations). When 0.6 ml of a positively-charged emulsion was injected i.v. to BALB/c mice, it did not cause any visual acute toxicity. Also, no difference was noted between this emulsion and the marketed negatively-charged Intralipid® emulsion injected to the same animal species. These results were further confirmed in a rat study where no death was noted following i.v. infusion of 3.3 ml per rat of the current positively-charged emulsion and Intralipid®. As confirmed by biochemical analysis, the emulsion did not elicit any hepatotoxic or nephrotoxic effects. The overall results of the early stage of research suggest that this novel positively-charged emulsion may be considered suitable for parenteral use and for ocular application (Klang et al., 1994).

Thus, the objective of the present communication is to characterize these positively-charged submicron piroxicam emulsions by using a physico-chemical evaluation of its surface and dielectric properties.

2. Materials and methods

2.1. Evaluation of the film-forming components

Surface tension measurements were performed using the Wilhelmy plate method. A thin platinum plate was attached to a force transducer and the signal was recorded after amplification on a chart recorder (Santos Magalhaes et al., 1991; Korner et al., 1994). The phospholipid, stearylamine and their mixtures in the molar ratio of 4:1 were first dissolved in a 9:1 (v/v) chloroform-methanol mixture and then spread on 20 ml of the aqueous subphase with an area of 11.7 cm^2 by means of a Gilson Microman pipette. The addition of the spreading solvent alone to a clean surface produced no measurable change in the surface tension of the water. The surface pressure π was calculated as the difference between the surface tension in the absence of the monolayer film and that of the film-covered surface: $\pi = \gamma_{H2O} - \gamma_F$. To study the change in surface pressure of a monolayer in the presence of piroxicam, measurements were made by injecting piroxicam solution through a side arm at 45° to the vertical in a glass vessel, positioned below the water-air interface. The vessel was mounted on a magnetic stirrer and a Teflon-coated stirrer bar was slowly rotated (so as not to disturb the monolayer) for 2 min. The surface tension was then measured until the equilibrium was reached.

To study the change in the surface pressure of a monolayer due to the presence of poloxamer and piroxicam molecules in the aqueous phase, the surface tension of the system was recorded, first by injection of poloxamer solution and then, after equilibrium was reached, by addition of piroxicam.

The change in the surface pressure $\Delta\pi$ was then calculated from the difference between the surface tension of the solution in the presence of the mixed monolayer with piroxicam, and of that in the absence of piroxicam:

$$\Delta\pi = \pi_{F+\text{piroxicam}} - \pi_F = \gamma_F - \gamma_{F+\text{piroxicam}}.$$

2.2. Materials

Medium-chain triglyceride (MCT) was purchased from Societe des Oleagineux (St. Laurent de Blangy, France). MCT consisted of no less than 95% esterified fatty acids, composed of between 8 and 10 carbon atoms according to manufacturer specifications. Lipoid E-80 was purchased from Lipoid AG (Ludwigshafen, Germany) and contained, according to manufacturer specifications, about 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% non-polar lipids and about 2% sphingomyelin. Polyoxyethylene-polyoxypropylene emulsifier was poloxamer 188 (Pluronic F68®) furnished by BASF (Parsippany, NJ), and stearylamine was purchased from Sigma (MO, USA). Other emulsion ingredients were all pharmaceutical grade products. Piroxicam, which conformed to BP specifications, was kindly provided by Agis Pharmaceutical Industries Ltd. (Yerucham, Israel).

2.3. Solubility determination

The solubility of piroxicam in both aqueous and oily phases was determined by the addition of an excess of the drug to a sample of each medium tested. The mixtures were shaken at room temperature (25°C) for 48 h until equilibrium was reached. Then, the samples were centrifuged at 2000 rpm for 5 min, and the supernatant liquid was assayed spectrophotometrically at 326 nm and 355 nm for organic and aqueous solutions vs. their corresponding blank solutions using appropriate calibration curves for five known concentrations.

2.4. Emulsion preparation

The non-ionic emulsifier (poloxamer), and the osmotic agent (glycerol) were dissolved in the aqueous phase. The pH of the aqueous phase was adjusted to 4.0 with 1 N HCl. Lipoid E-80, anti-oxidant (α-tocopherol), cationic surfactant stearylamine and the piroxicam were dissolved in the MCT oil phase. Both phases were heated separately to 70°C and then mixed and stirred with a magnetic stirrer. The resulting mix-

ture was further heated to 85°C. At this temperature, the resulting coarse emulsion was further mixed for 5 min using a high shear mixer Polytron™ (Kinematica, Luzern, Switzerland) and then rapidly cooled to below 20°C. After cooling, the emulsion was homogenized at 9000 pounds·inch^{-2} for 5 min using a two-stage homogenizer valve assembly (Gaulin Homogenizer, APV Gaulin, Hilversum, The Netherlands) and then again rapidly cooled to below 20°C. The pH of the emulsion was adjusted to a desired value using 0.1 N hydrochloric acid. Then, the emulsion was filtered through a TE membrane filter (Schuell and Schleicher, Dassel, Germany) with a pore size of 1 μm. Two different concentrations of poloxamer 188 (1 and 2%) were used. The emulsion was packed under nitrogen atmosphere in glass vials and then sterilized at 121°C for 15 min in a steam autoclave. The emulsion batches (500 ml) were stored at a desired temperature over prolonged periods of time.

A typical formulation (% w/w) consisted of piroxicam q.s., MCT 8.5, Lipoid E-80 1.2, stearylamine 0.3, α-tocopherol 0.02, poloxamer 188 1 or 2, glycerol 2.25 and bidistilled water to 100.

2.5. Emulsion characterization

The emulsion zeta potential was measured with the Malvern Zetasizer™ (Malvern, UK) while its pH was recorded at given time intervals using a pH meter (Corning pH meter 245, USA).

2.6. Particle size analysis

The emulsion droplet size and their size distribution were determined by means of photon correlation spectroscopy (Coulter Counter Supernanosizer N4SD™, Luton, UK). Each emulsion sample was diluted to the appropriate concentration with a filtered isotonic solution (2.25% w/v glycerol in water) and measurements were carried out at 25°C. Three analyses were performed for each emulsion sample.

2.7. Drug content

Piroxicam content was analyzed using a HPLC system consisting of a Milton Roy HPLC (Model Constametric 3000, Milton Roy, Riviera Beach, FL, USA) equipped with a variable wavelength ultraviolet detector (Spectro Monitor, 3100, Milton Roy) and a Milton Roy Integrator, coupled with a 100 mm reversed-phase column Spherisorb ODS-2, 5 μm (Altech, IL, USA). The column was eluted with a freshly prepared solution containing a mixture (60:40 v/v) of methanol and phosphate buffer at pH 7.0. The eluent was run at a rate of 1 ml/min and monitored at 254 nm following the injections of 20 μl of piroxicam standard solutions in isopropyl alcohol and of emulsion samples. The calibration curve, comprised of data points from five known concentrations, was linear within the 1–12 μg/ml concentration range. Prior to injection, piroxicam emulsion was diluted with isopropyl alcohol (1:100) to dissolve the oil phase. Each sample was analyzed twice.

2.8. Piroxicam distribution phase partitioning study

The low pressure ultrafiltration method, previously used for determination of phase partition profiles of other drugs in submicron emulsions, was used to evaluate the relative amount of piroxicam in the oil and aqueous phases of the emulsion. Amicon ultrafiltration membrane YM-100, 62 mm was soaked in the deionized water for at least 1 h to remove all water-soluble extractables and then placed into a stirred filtration cell Model 8200, (Amicon, Danvers, MA). As shown by other authors (Shimamoto et al., 1973; Teagarden et al., 1988; Benita and Levy, 1993) this ultrafiltration technique has to be validated prior to its use. The amount of bound drug and its depletion from a membrane was determined by the ultrafiltration of an aqueous solution containing the drug at the concentration of 1 mg/ml at pH 7.4. For these tests, 50 ml of emulsion to be filtered were placed into a stirred cell and pressurized under 20–40 pounds/inch^2 of nitrogen to generate the ultrafiltration process. The samples of clear filtrate (approx. 0.5 ml) were collected until 15–

20% of the emulsion had been ultrafiltered. Each of these samples was assayed spectrophotometrically for piroxicam and no interference of other soluble emulsion excipients was observed. Prior to filtration, the stock emulsion was assayed for piroxicam concentration using HPLC.

2.9. Stability assessment

The drug content, pH and droplet size distribution were monitored over long periods of time in the emulsions stored at 4, 23 and 37°C. The degree of creaming and the phase separation were assessed visually at given time intervals. All other visible changes were recorded.

To evaluate its mechanical and physical resistance, the emulsion was subjected to an accelerated mechanical stress and its particle size distribution was measured before and after shaking at 100 strokes/min over 48 h at room temperature.

3. Results and discussion

3.1. Surface activity of piroxicam

To gain an overall comprehension of the complex molecular interactions occurring between various components that constitute the interfacial film around the emulsified oil droplets we applied a monolayer approach. It should be noted that such studies have already been shown to explain certain factors influencing emulsion stability, especially for the systems constituted of phospholipids, fatty acids and triglycerides (Levy et al., 1991; Weingarten et al., 1991). Recently, the interactions between mixed monolayers of Lipoid E-80, stearylamine and poloxamer were studied and reported (Korner et al., 1994). The results of this study clearly demonstrated the existence of strong molecular interactions between stearylamine and phospholipid molecules. Mixed phospholipid-stearylamine monolayers exhibited a non-ideal behavior, which resulted in a significant increase in both the surface pressure and surface potential above the values corresponding to the pure compounds. The deviation from the ideal behavior appeared to be due to the strong interactions between the positively-charged stearylamine head groups and phospholipid polar groups occurring in the electrostatic double layer of the monolayers. The injection of poloxamer into the aqueous subphase, beneath the mixed monolayer, yielded an important increment of the surface potential of Lipoid E-80-SA monolayers which was 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 o/w emulsions was explained by the molecular arrangements at the interfacial regions of emulsion droplets.

Although the drug did not display any surface activity in aqueous solutions (data not shown here), in the presence of a stearylamine monolayer with the surface density of 1.01×10^{14} molecules/cm^2, the surface tension of the system was reduced to 52.3 mN/m which corresponds to the surface pressure value of: $\pi = 72.1 - 52.3 = 19.80$ mN/m.

The change in the surface pressure of the SA monolayer as a result of an interaction with increasing solution concentrations of piroxicam is shown in Fig. 1A.

The surface pressure of the mixed monolayer of stearylamine and Lipoid E-80 (molar ratio 1:4) corresponding to the surface density of 1.7×10^{14} molecules/cm^2 was found to be equal to 12.7 mN/m. On the injection of piroxicam into the subsolution the increments of the surface pressure

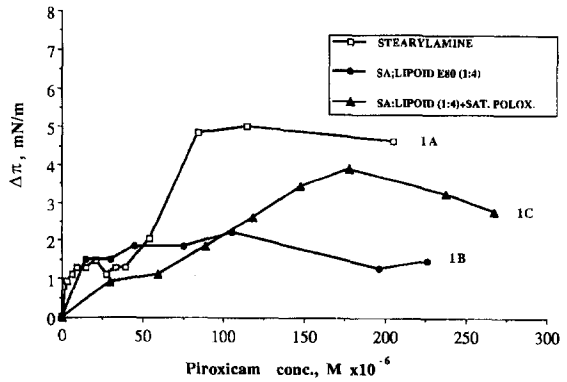

Fig. 1. Effect of piroxicam concentration on the surface pressure of monolayers.

calculated from the equation $\Delta\pi = 60.52 - \gamma_{F+\text{piroxicam}}$ increased with the increase in piroxicam concentration as shown in Fig. 1B.

Finally, the surface pressure of the mixed monolayer of stearylamine and Lipoid E-80 in the molar ratio 1:4 in the presence of poloxamer was measured and was obviously higher than that of the same monolayer in the absence of poloxamer. This gave rise to the surface pressure of 22.97 mN/m.

The change in surface pressure $\Delta\pi$ for this three component monolayer on the injection of piroxicam is shown in Fig. 1C as a function of drug concentration in solution.

Careful examination of the $\Delta\pi$ data in Fig. 1 shows that piroxicam interacts with the three studied monolayers. This interaction seems however to be enhanced when stearylamine is the only film forming component of the system. The effect is all the more pronounced within the drug concentration ranging from 5×10^{-5} to 10^{-4} M. A marked increase in $\Delta\pi$ values observed for stearylamine monolayers may be explained by their high compressibility in the conditions in which they are formed, e.g. at constant area and in the absence of any compression (Korner et al., 1994). When the phospholipid is added to form a mixed film with stearylamine, the penetration of piroxicam dramatically decreases as a result of reduced compressibility of the monolayer. It is worthwhile to note that at low piroxicam concentrations ($< 5 \times 10^{-5}$ M) the effect is not significant enough to discern the difference in behavior between the mixed stearylamine-phospholipid monolayer and that composed of only pure stearylamine. This, in fact, may be essentially due to the interaction of the drug at the level of polar head groups. Evidently, a steep rise in $\Delta\pi$ exhibited by the monolayer of stearylamine in the range of piroxicam concentrations 5×10^{-5}–10^{-4} M would strongly suggest that, in addition to interaction at the level of polar head groups of the monolayer, the interaction of the drug with its hydrophobic parts also takes place. The levelling-up of $\Delta\pi$ observed for the mixed stearylamine-phospholipid monolayers may thus indicate that such intercalation cannot occur. The highly incompressible state of the monolayer would explain this situation.

Rather unexpected contour of the $\Delta\pi = f(c)$ relationship is observed when poloxamer is first adsorbed into a mixed stearylamine-phospholipid monolayer and then the drug is injected into the subphase (Fig. 1C). Here, the gradual and important increase in $\Delta\pi$ occurs within a large range of drug concentrations ranging from the lowest studied concentration up to about 2×10^{-4} M. One possible explanation of this increase is an additional interaction of the drug with the hydrophilic poly(oxyethylene) moiety of poloxamer which overlaps monolayer head groups (Weingarten et al., 1991). Although it seems unlikely that after intercalation of hydrophobic poly(oxypropylene) anchors into the monolayer there is still enough room left for the drug to penetrate, one would speculate that the increase in $\Delta\pi$ is a direct result of the drug interaction with the hydrophobic poloxamer moiety. This suggestion is reinforced by two observations (I) piroxicam interaction with the adsorbed poloxamer layer is concentration dependent and up to about 10^{-4} M this interaction appears to be less effective than that with the mixed monolayer in the absence of poloxamer (Fig. 1B); (II) the interactions appear to be stronger at higher drug concentrations ($> 10^{-4}$ M) than in the absence of poloxamer (Fig. 1B). Since piroxicam molecules cannot intercalate hydrophobic groups of the monolayer (Fig. 1B), the comparison of the intermediate portion of the curve in Fig. 1C relative to those in Fig. 1A and Fig. 1B would strongly suggest that piroxicam interacts with the hydrophobic moiety of the adsorbed poloxamer.

These results suggest the possible existence of molecular interactions between various film-forming components at the o/w interface of the emulsified oil droplets. For all these reasons, despite solubility results which indicate that the 1:1 mixture of MCT and oleic acid (1:1) is the most favorable oil phase composition for the emulsion to allow maximum solubilization of piroxicam (Fig. 2), it has been decided to use only MCT, the best individual triglyceride solvent for piroxicam. The use of oleic acid was avoided since it produces a negatively charged o/w interface at pH 7.4 (Levy et al., 1994). Such a negative charge is capable of neutralizing the positive one resulting

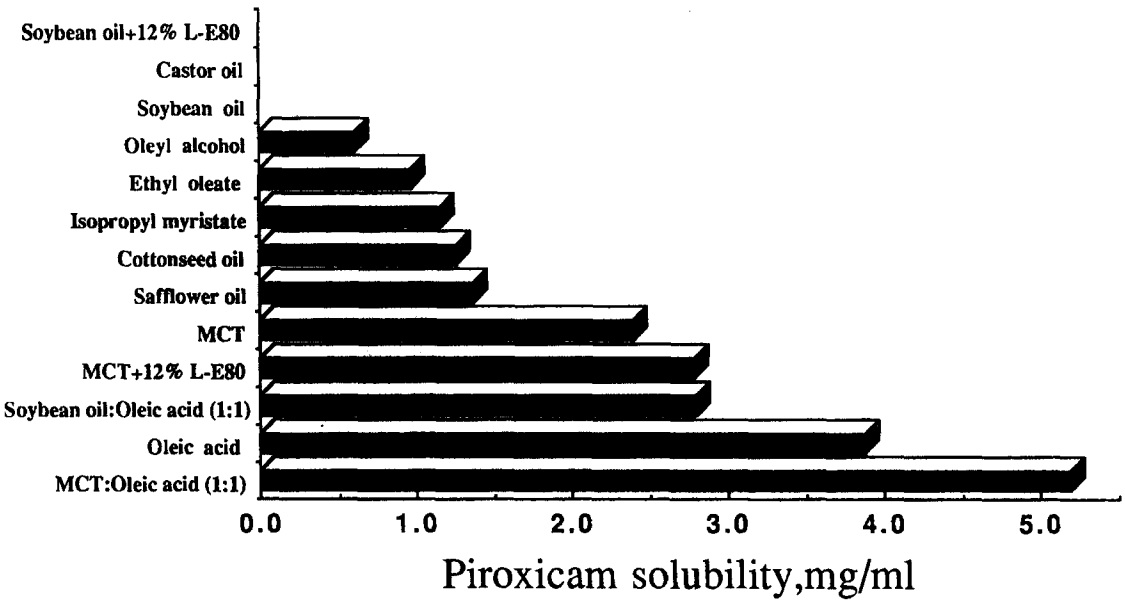

Fig. 2. Piroxicam solubility in different solvents and phase compositions.

in a marked diminution of the physical stability of the emulsion.

3.2. Effect of droplet charge

The positive surface potential value of the droplets of the present emulsion depends mainly upon the extent of the ionization of stearylamine at the o/w interface. The dissolution of phospholipids and stearylamine in the oil phase and the adjustment of the aqueous phase to pH 4 yielded a blank emulsion with a positive zeta potential value of +38 mV. This would suggest that in these conditions, a better interfacial ionization of stearylamine was achieved. Furthermore, the increase of stearylamine concentration above 0.3% did not produce any additional increase in zeta potential, indicating that the generated interfacial area was saturated with stearylamine molecules and their excess most probably dissolved in the internal oil phase.

The zeta potentials at different adjusted initial pH values indicate that neither the incorporation of piroxicam nor the pH variation affected the surface charge of emulsions (Fig. 3). In the case of the present formulation, this could be explained by the fact that the decrease in pH concomitantly led to the decrease in the ionization of anionic phospholipidic components, and to the increase in the ionization degree of stearylamine.

3.3. Effect of pH

For a piroxicam emulsion prepared with 0.1% of piroxicam and 1% of poloxamer the pH varia-

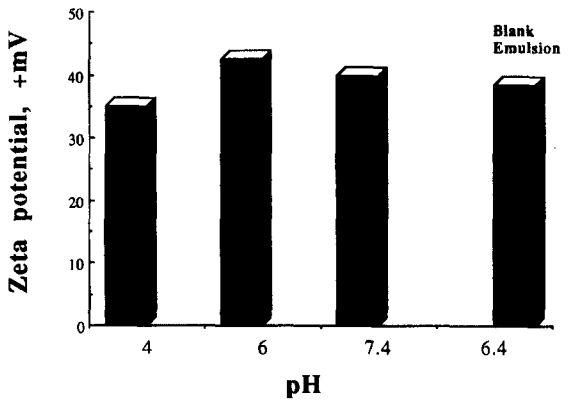

Fig. 3. Influence of initial adjusted pH on zeta potentials of piroxicam submicron emulsions.

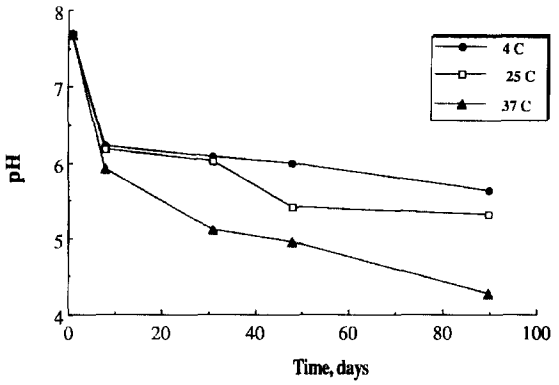

Fig. 4. pH variation with time for piroxicam emulsions containing 1% (w/w) poloxamer as a function of temperature.

tion with time at various storage temperatures is depicted in Fig. 4. From this figure it may be noted that the pH decreased rapidly within the first 10 days irrespective of the temperature and then more gradually with the slowest rate observed at 4°C (Fig. 4). For piroxicam emulsions prepared with 2% of poloxamer, the pH dependence on time at 4°C is shown in Fig. 5. Here, pH decreased progressively to the values of 5.5 and 6 in the blank and piroxicam emulsions in which the initial pH was adjusted to 7.2 and 7.4, respectively. The rapid initial decrease in pH was most probably due to the autoclaving sterilization and the formation of free fatty acids (FFA), the origin of which should be attributed to chemical changes

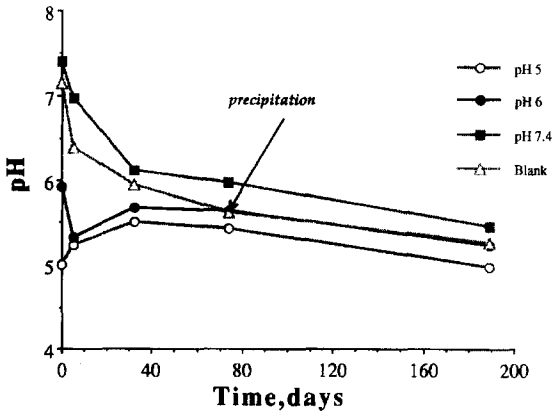

Fig. 5. pH variation of piroxicam submicron emulsions containing 2% (w/w) poloxamer as a funtion of length of storage at 4°C.

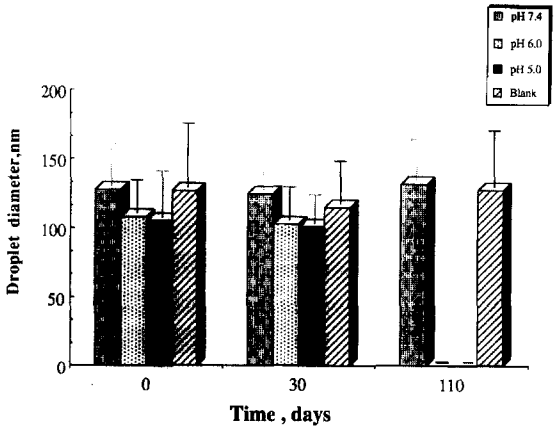

Fig. 6. Effect of initial adjusted pH on the mean droplet size of a piroxicam emulsion prepared with 2% (w/w) of poloxamer at 4°C as a function of storage time.

in phospholipids, caused by their oxidation and hydrolysis (Hansrani et al., 1983; Washington and Davis, 1987; Herman and Groves, 1993). It appears that the final pH of these emulsions, irrespective of the initially adjusted pH value, reached an ultimate value ranging from 5.1 to 5.6 (Fig. 5).

The effect of the storage time at 4°C on the mean droplet diameter of emulsions prepared with 2% of poloxamer and adjusted to different initial pH values is shown in Fig. 6. Although no marked changes were noted over a 1-month period of observation, a yellow sediment at the bottom of the bottles filled with emulsions adjusted to pH 5 and 6 was detected after 75 days of storage. The presence of small crystals in the sediment was confirmed by microscopic examination and was attributed to the precipitation of piroxicam. The piroxicam was easily identified by UV spectrophotometry after the crystals were isolated and subsequently dissolved in alcohol. The emulsions with the initial pH adjusted to 7.4 did not contain any sediment. It should be emphasized that piroxicam emulsions prepared with 1% of poloxamer presented a yellow sediment after 90 days of storage even at 4°C.

3.4. Piroxicam partitioning in the emulsion

The knowledge of partitioning of a drug among the various emulsion phases is necessary. Gener-

ally, ultrafiltration techniques are used to achieve such objectives. According to the literature data, piroxicam is sparingly soluble in water and slightly soluble in alkaline aqueous solutions. In the presence of moisture, the drug acquires a hydrate form which is less soluble than piroxicam itself, and this hydrate crystallizes out gradually (Nozawa and Ohaya, 1987).

The piroxicam binding to and depletion from an ultrafiltration membrane was studied with the aqueous solution of the drug at 1 mg/ml. The recovery curve for piroxicam in an aqueous solution is shown in Fig. 7. As indicated by the levelling off of the curve, the membrane appeared to be almost entirely saturated after 10% of the total volume had been filtered. The recovery which presented 100% of the theoretical value indicated that the depletion was non-existent. On the basis of these data it was concluded that piroxicam emulsion formulations do not require any correction, provided that at least 10% of the total volume was filtered to allow saturation of the membrane. A freshly prepared emulsion with 0.1% of piroxicam, 1% of poloxamer and adjusted to a pH value of 7.4 revealed that only 2.6% of the initial piroxicam concentration was localized in its external aqueous phase. The remainder of the piroxicam was in the oil phase and at the interface of the emulsion. However, as previously pointed out the pH of the emulsion decreased with time and reduced the solubility of piroxicam, leading to the formation of the yellow sediment.

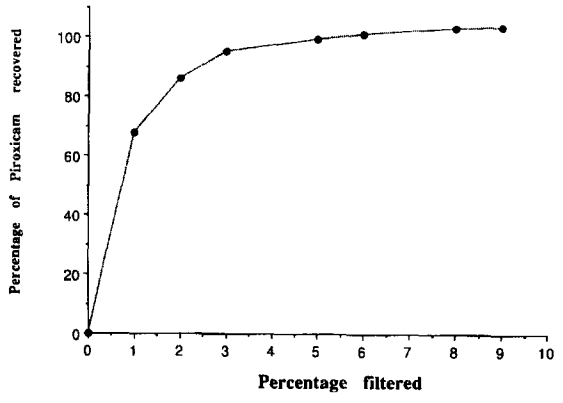

Fig. 7. Membrane recovery curve of piroxicam from aqueous solution (1 mg/ml) after ultrafiltration.

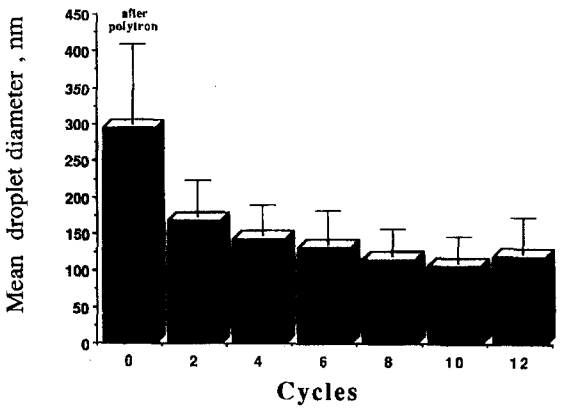

Fig. 8. Optimization of the homogenization processs of a positively-charged piroxicam submicron emulsion.

3.5. Stability evaluation

It should be noted that one of the main factors which can alter the final droplet size distribution of an emulsion during its manufacturing is the extent to which the generated droplets are covered by various emulsifier molecules. A partial interfacial coverage might lead therefore, to an increase in surface tension which in turn will be compensated by an increase in the droplet size. The smallest droplets are considered to be the most favorable for emulsion and the optimal conditions for manufacturing and processing are supposed to fulfill these requirements. The homogenization process is achieved by passing the emulsion repeatedly through a high pressure homogenizer. Ten cycles, equivalent to 5 min of homogenization time were necessary to reach the minimal mean droplet size (Fig. 8). Neither creaming nor coalescence was noted, even though pH decreased to a value of 5.0–5.5.

As shown in Table 1, the initial piroxicam content was affected by the sterilization process and depends to a high extent on the initial piroxicam concentration. Whereas piroxicam emulsions prepared with 2% poloxamer and adjusted to the initial pH values of 5 and 6 exhibited a drug loss of 46.4 and 9.6%, respectively, no marked changes in the drug content were observed for the emulsions prepared with 2 and 1% of poloxamer adjusted to the initial pH values of 7.4 and 7.6,

Table 1
Physicochemical characteristics of positively-charged submicron emulsions prepared at different piroxicam and poloxamer concentrations as a function of adjusted initial pH

Dosage form	Poloxamer concentration (% w/w)	pH value		Piroxicam concentration (% w/w)		Piroxicam concentration decrease (Δ%)	Mean droplet size (nm)	
		Initial adjusted value	After sterilization	Before sterilization	After sterilization		Before sterilization	After sterilization
Emulsion	1	7.6	6.6	0.103	0.101	2.0	123 ± 38	120 ± 27
Emulsion	2	5.0	5.2	0.097	0.052	46.4	111 ± 28	121 ± 27
Emulsion	2	6.0	5.3	0.073	0.066	9.6	118 ± 36	119 ± 52
Emulsion	2	7.4	7.0	0.142	0.133	6.3	104 ± 27	134 ± 16
Aqueous solution		7.3	7.0	0.105	0.098	6.7	-	-

respectively. The 6% drug loss observed in the emulsion prepared with 2% poloxamer was comparable to that observed in aqueous solution subjected to the same sterilization cycle as the piroxicam emulsion. Irrespective of the value of initial pH, there was no change in the mean droplet size of various emulsions before and after their sterilization (Table 1). These results clearly indicate that the changes observed in the drug content are essentially due to the intrinsic chemical sensitivity of piroxicam to withstand the autoclave cycling process. This was also confirmed by the lack of change in the mean droplet size during the excessive shaking (100 strokes/min over 48 h at 4°C) of the piroxicam emulsion prepared with 1% of poloxamer. It should also be pointed out that 1% of poloxamer appears to be insufficient in preventing piroxicam precipitation over the 3 months storage. For this emulsion, the pH decreased more rapidly than for the emulsion prepared with 2% of poloxamer (Table 1). It appears that only the emulsion prepared with 2% of poloxamer and 0.133% of piroxicam was stable enough and did not contain any yellow sediment over 4 months of storage at 4°C. This emulsion is currently being investigated and tested in animal experiments.

4. Conclusions

A physicochemical study of a positively-charged submicron emulsion containing piroxicam was carried out in an attempt to identify optimal experimental conditions of its fabrication. It has been shown that piroxicam was very sensitive to the initial pH of emulsion and to the formation of free fatty acids (FFA) which reduce its pH of the emulsion over prolonged storage times. The decrease in pH was found to be temperature dependent and led to the precipitation of the drug which was easily detectable by visual and microscopic observations. The results reveal that when the initial adjusted pH was at least 7.4, and the rate of hydrolysis of phospholipids and triglycerides was sufficiently reduced, satisfactory conditions for preparation of a positively-charged submicron piroxicam emulsion were established for its use as a parenteral and ocular dosage form.

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