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# Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion pair

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**Abstract**

Aqueous dispersions of solid lipospheres containing up to 7.5% pilocarpine as lipophilic ion pairs were submitted to a preliminary evaluation. The lipospheres (diameter 75–85 nm) consisted mainly of stearic acid and egg lecithin; pilocarpine base was incorporated as ion pair with mono-octylphosphate, monodecylphosphate and monohexadecylphosphate. The following parameters were investigated: stability constants ($\beta$) and lipophilicity of the ion pairs, size, polydispersity and drug content of the lipospheres, pilocarpine release in vitro. The preparations might constitute a promising vehicle for sustained ocular delivery of pilocarpine.

*Keywords:* Liposphere; Pilocarpine; Ion pair; Mono-octyl phosphate; Monodecyl phosphate; Monohexadecyl phosphate; Stearic acid; Lecithin

The poor topical bioavailability of pilocarpine (Pi) instilled from conventional preparations is well documented (Schoenwald, 1993). Various approaches aimed at increasing the ocular retention of Pi, and hence its absorption, and/or at improving the transcorneal penetration properties of the drug, have been reported. Substantial improvements have been achieved, to mention only a few examples, with lipophilic prodrugs (Mosher et al., 1987), mucoadhesive complexes (Saettone et al., 1994), nanoparticles (Harmia et al., 1986), pH-sensitive latexes (Ibrahim et al., 1990), polymeric inserts, including the Ocusert® (Urquhart, 1980; Urtti et al., 1985), etc. None of the approaches reported hitherto, however, is exempt from disadvantages, and further investigation in this direction is still deemed useful.

Previous investigations have dealt with the formulation of selected $\beta$-blocking agents (timolol, levobunolol) as lipophilic ion pairs (Gallarate et al., 1988, 1993; Gasco et al., 1989; Cavalli et al., 1994). In rabbits, topical administration of timolol as ion pair resulted in a 3–4-fold bioavailability increase with respect to timolol alone (Gasco et al., 1989). Parallel studies were concerned with the preparation of solid colloidal lipospheres as carriers for different drugs (Cavalli et al., 1992, 1993).

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It was postulated, in analogy with a previous study on timolol (Cavalli et al., 1992), that formation of ion pairs would increase the lipophilicity of Pi, whose log $P$ is $-3.2$ (Leo et al., 1971), and that lipospheres would constitute an optimal carrier for the lipophilic complexes. This preliminary note is concerned with a physico-chemical evaluation of lipophilic ion pairs of Pi, which were incorporated into lipospheres. The in vitro drug release properties of the preparations were investigated, as an essential prerequisite to further studies in vivo.

The preparation of aqueous dispersions containing Pi ion pairs entrapped in lipospheres consisted of two steps: formulation of microemulsion containing stable, lipophilic ion pairs, and preparation of the lipospheres by dispersing the warm microemulsion in cold water. Pilocarpine base (PiB) was prepared from Pi hydrochloride (Sigma, St. Louis, USA). The following sodium salts of monoalkyl esters of phosphoric acid, used as as counterions, were prepared as indicated by Brown et al. (1955): mono-octyl phosphate (C-8), monodecyl phosphate (C-10) and monohexadecyl phosphate (C-16).

The stability of the PiB complexes with alkylphosphoric acids was determined by calculating their $\beta$ stability constants for the overall reaction ($\beta = K_1 K_2$) by potentiometry, as indicated by Irving and Rossotti (1953) and Rossotti and Rossotti (1961). The measurements were carried out in ethanol/water mixtures, and the $\beta$ values in water were obtained by extrapolation.

For the C-8/Pi complex, the titrations were performed both in water and in a series of ethanol/water mixtures (10, 20, 30, 40, 50, 60 and 70% v/v). The titrations of the C-10 and C-16 Pi complexes were carried out only in the ethanol/water mixtures in which they were soluble. As shown in Table 1, the $\beta$ values were quite high with C-8, and increased with increasing chain length, thus demonstrating the influence of this parameter on the stability of the ion pairs.

Subsequent efforts were directed at achieving the incorporation of a sufficient amount of PiB in the lipospheres, and at verifying the release characteristics in vitro. For this purpose, three microemulsions, stable at 70°C, and containing 2.1% w/w PiB (A, B, and C), were prepared using C-8, C-10 and C-16, respectively, as counterions. Microemulsions A and B consisted (all percentages w/w) of 7.3% stearic acid (Merck, Darmstadt, Germany) as internal phase, 4.9% purified egg lecithin as surfactant, 5.4% sodium taurodeoxycholate, TDC (Sigma, St. Louis, USA) and 4.4% butanol (Merck, Darmstadt, Germany) as cosurfactants, and 72.5% distilled water as continuous phase. PiB and the alkyl phosphates C-8 or C-10 were added to the microemulsions in the ratio 1:1.5. Microemulsion C consisted of 7.1% stearic acid, 4.8% purified egg lecithin, 6.4% TDC and 5.1% butanol and 71.3% distilled water. The PiB:C-16 molar ratio was 1:1.

Table 1
Stability constants in water (25°C) and apparent partition coefficents ($P_{app}$, 70°C) of the PiB/alkyl phosphate complexes

| Alkyl phosphate | Log $\beta$ | $P_{app}$ |
|---|---|---|
| C-8 | 11.45 | 1.4 |
| C-10 | 12.08 | 2.5 |
| C-16 | 13.00 | 10.8 |

Solid lipospheres were obtained by dispersing the warm microemulsions in distilled cold water (2–3°C) under mechanical stirring. The aqueous liposhere suspensions were washed twice with distilled water by ultradiafiltration (Amicon TCF2A, Grace, Danvers USA), and then were freeze-dried (Modulyo freeze dryer, Edwards Crawley, UK).

To evaluate the increase in drug lipophilicity due to the presence of the counterion, the apparent partition coefficients ($P_{app}$) of PiB between stearic acid and water at pH 6.0 were determined at 70°C, at a pilocarpine/alkyl phosphate ratio of 1:2. After separation of the two phases, the drug concentration in the aqueous phase was determined by HPLC (Perkin Elmer Binary LC Pump 250 liquid chromatograph, Bio-Rad C-8 $\mu$-Bondapack column) as indicated by Durif et al. (1988). The eluent was 5% v/v methanol in 0.05 M phosphate buffer, adjusted to pH 2.5 with triethylamine. The analysis was run at a flow rate of 1.3 ml/min with the detector operating at 214

Table 2
Characteristics of the lipospheres containing different PiB/alkyl phosphate complexes

| Lipospheres | Average diameter (nm) | Polydispersity | PiB content (%) |
|---|---|---|---|
| A | 85 | 0.2 | 5.1 |
| B | 75 | 0.1 | 7.5 |
| C | 70 | 0.3 | 5.9 |

nm (Perkin Elmer LC UV-Visible spectrophotometer).

The $P_{app}$ values (cf. Table 1) were 0.9 for PiB alone, and 1.4, 2.5 and 10.8 for the PiB/C-8, C-10 and C-16 complexes, respectively. These results, which are correlated with the $\beta$ values of the complexes, are indicative of the increased lipophilicity of the ion pairs, when compared with PiB.

The average diameter of the three types of lipospheres, measured by photon correlation spectroscopy (Zetasizer 2C Malvern, Malvern, UK), was in the range 75 to 85 nm (Table 2). The amount of PiB incorporated into the lipospheres was determined by HPLC on samples (c.2 mg) of the freeze-dried products, after dissolution in octanol (5.0 ml) and extraction (2 × 5.0 ml) with pH 2.5 phosphate buffer. As reported in Table 2, the percent PiB incorporated appeared to increase with increasing alkyl chain length from C-8 (5.1%) to C-10 (7.5%), while it was reduced to 5.9% in the case of C-16. The observed decrease was presumably due to the different PiB/alkyl phosphate ratio, which was 1:1 for C-16 and 1:1.5 in the case of the other two counterions.

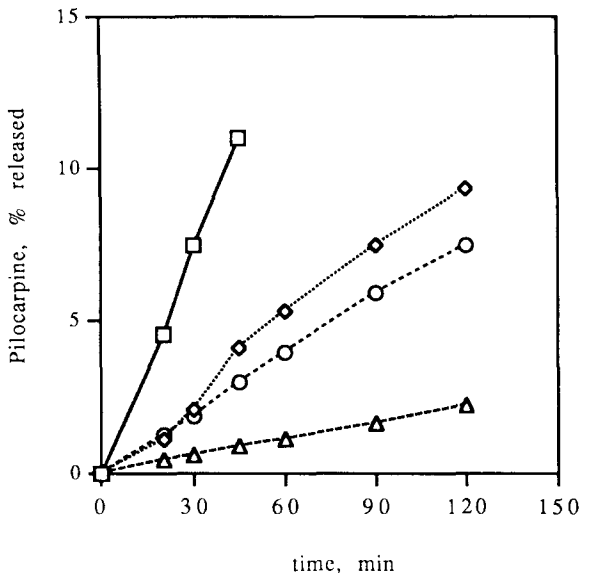


Fig. 1. Percent Pi released from an aqueous solution (□) and from lipospheres containing octyl phosphate (◇), decyl phosphate (○) and hexadecyl phosphate (△) ion pairs. The standard deviation of the data was smaller than the size of symbols ($n \geq 3$).

The release of Pi from the lipospheres was investigated using the method described in a previous paper (Cavalli et al., 1992). As shown in Fig. 1, the amount of PiB diffused through a cellophane membrane (dialysis tubing, Sigma, St. Louis, USA) after 120 min was 9.2 and 7.5% for lipospheres A and B, respectively, and 2.2% for the C lipospheres. Under the same conditions, the percent PiB released from a simple solution was 29.3.

On the basis of the reported preliminary data, the presently described liposphere suspensions containing PiB as ion pair might constitute a promising sustained-release ocular formulation. In vivo tests, now underway, will be the objective of a forthcoming paper.

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