Tsuei et al 1980). Since its infusion through polyethylene catheters did result in significant sorption, errors could be avoided by the use of polyethylene infusion catheters and tubing. Correction of these losses is necessary to avoid the over-estimation of dose related pharmacokinetic parameters (Tsuei et al 1980).

Both drugs diffused across the septum separating the lumens of the thermodilution catheter, resulting in concentrations of the same order of magnitude as the blood concentrations.

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A gamma scintigraphic evaluation of the precorneal residence of liposomal formulations in the rabbit

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Multilamellar liposomes were prepared from dipalmitoyl phosphatidylcholine or egg lecithin in combination with cholesterol and either dicetyl phosphate or stearylamine. The size and charge of the colloidal preparations were characterized before labelling with [^{111}In]8-hydroxyquinoline. Freshly labelled liposomes were instilled into the eyes of unanaesthetized NZW rabbits and their disposition and drainage followed using gamma scintigraphy. A positive surface charge was found to affect significantly liposomal drainage rate, whereas an increase in size restricted drainage from the inner canthal region. Drainage of the suspending medium was directly compared with liposomes by labelling the medium with [^{99m}Tc] sodium pertechnetate and following the simultaneous change in removal of ^{99m}Tc and ^{111}In from the precorneal area. Slower drainage rates were obtained for the suspending medium compared with solutions of the isotopes suggesting that the liposomes restricted solution drainage.

The most commonly used and convenient route of drug administration to the eye is the topical application of ophthalmic formulations based on aqueous vehicles or petrolatum. Solutions have the disadvantage that most of the instilled drug is lost due to drainage via the nasolacrimal duct within the first 15 to 30 s after instillation (Shell 1982). Conversely, oily bases cause blurring of vision and gumming of the eyelids.

Biodegradable colloids such as liposomes have received attention recently as potential ophthalmic delivery systems. These can be easily prepared from non-toxic materials, which are non-irritant and do not obscure vision. Their surface properties may be altered to confer surface charges or ligands such as lectins to improve adhesion to the cell surfaces (Ketis & Grant 1982). However, there are two possible mechanisms since microparticles above a certain size may physically retard drainage by blocking the inner puncta (Sieg & Triplett 1980).

Schaeffer & Krohn (1982), using an *in-vitro* technique, have demonstrated that positively charged unilamellar liposomes enhance the corneal penetration of penicillin incorporated into the formulation to a greater extent than the drug encapsulated into either negatively charged or neutral liposomes. Positively charged small unilamellar vesicles (SUV) were found to be more effective than positively charged multilamellar vesicles

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(MLV), suggesting that a closer apposition of liposomes to cell membranes or increased surface charge density ensured a more efficient drug transfer.

In the present investigation, methods have been developed to produce vesicles with a variety of physical properties. After labelling the vesicles with a suitable radioisotope, the influence of charge and size on precorneal residence was investigated in the rabbit using the technique of gamma scintigraphy.

Materials and methods

DL-α-Dipalmitoylphosphatidylcholine (DPPC), dicetyl phosphate, Dowex 1-X8 resin, 8-hydroxyquinoline and nitrilotriacetic acid were all supplied by Sigma Chemical Company. Stearylamine, cholesterol, and chloroform were obtained from BDH Laboratories and Lucas-Meyer kindly donated the egg lecithin (87% phosphatidylcholine). [^{111}In]Indium chloride (specific activity greater than 300 MBq mL^{-1}) was obtained from Amersham International and [^{99}Tc]sodium pertechnetate was eluted from a generator as required (Elumatic 111, Amersham International). [^{99}Tc]-Labelled diethylenetriaminepentaacetic acid (DTPA) was prepared from a kit supplied by CIS (UK) Ltd, London UK. All reagents were used without further purification.

Formulations. Multilamellar vesicles (MLV) were prepared from natural lipids (egg lecithin) and synthetic lipids (DPPC) using the method described by Bangham et al (1965). The molar ratios of lipid and other constituents used to make liposomes from natural unpurified egg lecithin (87% phosphatidylcholine, 0·4% phosphatidyl ethanolamine, 1·34% lysophosphatidyl choline) were as follows: egg lecithin–cholesterol–stearylamine (5 : 2 : 1) for positively charged liposomes; egg lecithin–cholesterol–dicetyl phosphate (5 : 2 : 1) for negatively charged liposomes and egg lecithin–cholesterol (9 : 1) for neutral liposomes. Liposomes were also prepared from DPPC by substituting the synthetic lipid for egg lecithin in the above formulae.

Heterogeneous populations of MLVs were produced varying in size, encapsulation volume and number of lamellae. Samples of each preparation were characterized before radiolabelling for in-vivo use.

Particle sizing. A Coulter Counter (Model TA11) fitted with a 30 or 100 μm tube was used to size all the multilamellar vesicles. The electrolyte used was filtered Isoton II (Coulter Electronics).

Surface charge. The charges conferred on the MLVs by incorporating cationic and anionic molecules were determined by microelectrophoresis as described by Bangham et al (1958). The electrophoretic mobilities (Apparatus-Zeta sizer Rank Mark II, Rank Brothers, Bottisham, UK) were determined in a series of succinic acid and phosphate buffers (I = 0·01) covering a pH range of 4·8 to 7·7. From a knowledge of the direction and rate of migration of the particles, the sign and magnitude of the zeta potential was determined using the equation derived from Smoluchowski (1903).

Labelling techniques. Preformed MLVs were labelled with the high energy gamma emitter, indium-111, using the method of Hwang et al (1982). Charged and neutral liposomes were prepared in an aqueous medium of 1 mM nitrilotriacetic acid (NTA), a weak chelating agent, in isotonic phosphate buffer, pH 7·4. Unencapsulated chelate was removed by ultracentrifugation at 140 000*g* for 30 min (4 °C) and the pellet resuspended in 1·0 mL 0·9% NaCl in 5 mM sodium acetate, pH 5·5. A mixture composed of 100 μL 1·8% w/v NaCl in 20 mM sodium acetate, pH 5·5, was added to 100 μL [^{111}In]indium chloride (20–30 MBq), followed by 10 μL of 6·9 mM 8-hydroxyquinoline in ethanol previously mixed with 200 μL 1·8% NaCl in 20 mM sodium acetate pH 5·5, to form the lipophilic complex [^{111}In]-labelled oxine. 140–200 μL of the loading solution was then added dropwise to 1 mL of the liposome suspension whilst vortexing. The loading mixture was allowed to incubate above the transition temperature of the major lipid of the formulation for 60 min. Loading was terminated by passage of the suspension through an Ag 1-X8 (1 × 10 cm) ion-exchange column. The elutions were pooled and concentrated to a final volume of 0·5 mL by ultracentrifugation. A final activity of 1–2 MBq per 25 μL suspension was required for satisfactory imaging.

The efficiency of the loading method for egg lecithin MLVs was 55 ± 4% and for synthetic MLVs it was 84 ± 13%. The liposomes were used within 1 h of labelling. The release of label from egg lecithin MLVs, as determined by equilibrium dialysis at room temperature, was 0·15 ± 0·03% min^{-1}.

In the initial studies using egg lecithin MLVs, the suspending buffer was labelled with [^{99}Tc]sodium pertechnetate to enable a direct comparison to be made between the distributions of the liposomes and the suspending medium using a synchronous dual isotope counting technique. A control study of free [^{111}In]-labelled oxine and free [^{99m}Tc]-labelled DTPA was also carried out to measure the rates of drainage of the two radiopharmaceuticals.

Protocol. NZW rabbits were used as the test species, each preparation being tested in a group of six rabbits. 25 μL of the radiolabelled colloidal suspension was instilled directly onto the corneal surface (at the 12 o'clock position) of the eye, and the animal positioned in front of the pinhole collimator of the gamma camera. The head was placed on a neck support and the animal gently supported by hand. Sequential images were accumulated at 15 s intervals for 10 min and stored on computer for analysis.

Results

Characterization of MLVs. The geometric mean diameter (δg) and standard deviation of charged and uncharged egg lecithin and synthetic MLVs are summarized in Table 1, together with the mean zeta potentials (mV) at pH 7·4. The electrophoretic mobility profiles of egg lecithin MLVs are illustrated in Fig. 1.

Table 1. Geometric mean diameter (δg) ± s.d. of the liposomal formulations and mean zeta potentials of the liposomal formulations used in the ophthalmic studies.

Liposome type		δg (mm)	Mean zeta potential (mV)
Egg lecithin	MLVNeu	1·95 ± 1·88	−8·6
	MLVPos	1·75 ± 2·20	+30·0
	MLV Neg	1·48 ± 2·05	−44·5
DPPC	MLVNeu	3·95 ± 2·20	
	MLVPos	3·90 ± 1·95	
	MLVNeg	3·20 ± 1·98	

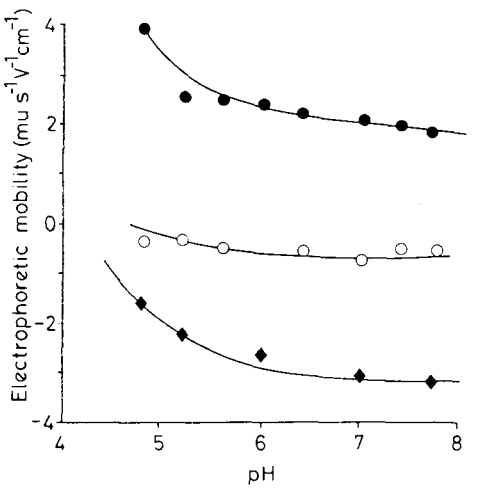

FIG. 1. Electrophoretic mobilities of liposomal preparations. Key: (●) positively charged multilamellar liposomes; (○) neutral multilamellar liposomes and (◆) negatively charged multilamellar liposomes.

Lacrimal scintigraphy. Individual 15 s frames were summed to produce an overall picture of label distribution (Fig. 2). Three anatomical regions of interest (ROIs) were defined, the cornea, inner canthus and lacrimal duct. A fourth region was drawn for background correction. In the dual isotope regimen, a correction was made for 30% overlap of indium-111 in the ^{99m}Tc window and radioactive decay.

Ocular drainage is a multiphasic phenomenon. On instillation of each preparation, an immediate distribution occurred in the corneal and inner canthal regions. More than 70% of the initial activity was lost from the cornea within the first 15 s of imaging. This initial distribution was too rapid to be imaged and analysed. A

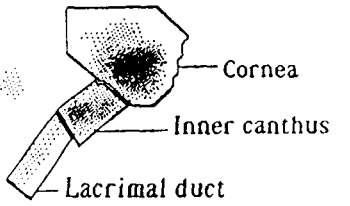

FIG. 2. Division of scintigraphic image into regions of interest.

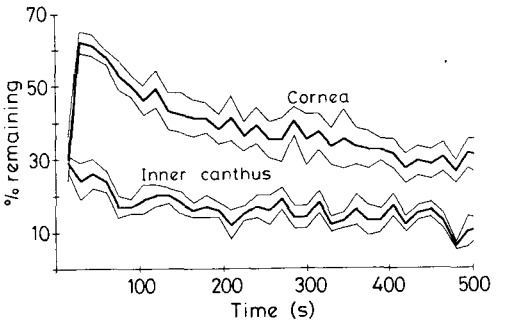

FIG. 3. Drainage of positively charged multilamellar liposomes from the cornea and inner canthus. (mean ± s.d., $n = 6$).

second, less rapid, phase of corneal drainage occurred from 15 to 150 s of imaging, followed by a much slower basal phase. After the initial rise in activity in the inner canthus, the preparation drained into the nasolacrimal duct slowly and monophasically (Fig. 3).

The mean maximum activities occurring in the corneal and inner canthal regions were summed and the activity remaining in each region for sequential time points (or frames) was calculated as a percentage of their combined maximum. Graphs of percentage activity remaining in each region as a function of time were plotted for each study on linear and logarithmic scales.

From the logarithmic plots of percentage remaining against time, it was possible to determine the drainage rate constants (k_d) in the corneal and inner canthal regions. Statistical comparisons between preparations were made by analysis of covariance. The results are summarized in Table 2.

Statistically significant differences ($P < 0·05$, paired t-test) in precorneal drainage rate (k_{d1}) occurred between positively charged egg lecithin MLVs and suspending buffer, but not between MLVNeu or MLNeg and suspending buffer. Statistical differences ($P < 0·05$, analysis of covariance) were found between all the DPPC MLVs and solutions of either [^{99m}Tc]DTPA or [^{111}In]indium oxine. In the inner canthal region, both charged and neutral egg lecithin and DPPC MLVs drained at a significantly slower rate ($P < 0·05$, analysis of covariance) than the suspending buffer or solutions of the radiopharmaceuticals.

Table 2. Initial (k_{d1}) and basal phase (k_{d2}) kinetic parameters of drainage from the cornea and inner canthal region (k_{d3}).

Liposome type		k_{d_1} (min^{-1})	k_{d_2} (min^{-1})	k_{d_3} (min^{-1})
Egg lecithin	MLVNeu	0·12	0·01	0·03
	Buffer	0·16	0·02	0·15
	MLVPos	0·09	0·04	0·04
	Buffer	0·24	0·09	0·18
	MLVNeg	0·20	0·06	0·04
	Buffer	0·24	0·11	0·19
DPPC	MLVNeu	0·20	0·07	0·07
	MLVPos	0·12	0·01	N.D.
	MLVNeg	0·15	0·04	0·02
[^{111}In]Oxine		0·54	0·12	0·14
[^{99m}Tc]DTPA		0·51	0·15	0·14

Note: N.D. = No detectable drainage in the period of the experiment.

Discussion

Previous workers have determined values for the precorneal first order drainage rate constants, following instillation of 25 μL of solution in the rabbit eye, using various sampling techniques. Chrai et al (1973) obtained a value of 0·55 min^{-1} for [^{99m}Tc]-labelled sulphur colloid which was similar to a value of 0·57 min^{-1} obtained by Makoid & Robinson (1979), using tritiated pilocarpine nitrate. Lee & Robinson (1979) obtained a k_d value of 0·54 min^{-1} for pilocarpine and more recently, Lee et al (1983) using labelled sodium cromoglycate, obtained a value of 0·37 min^{-1}. The above results correlate well with the first phase rate constants determined by gamma scintigraphy using [^{111}In]oxine and [^{99m}Tc]DTPA solutions (0·54 and 0·51 min^{-1}, respectively).

Unlike the techniques used by previous workers, gamma scintigraphy allows the separate determination of elimination rate constants of the basal phase of corneal drainage and inner canthal drainage. The rate constants obtained in these regions are approximations of the real elimination rates as contributions from tear turnover, variable tear flow and reflux between compartments (Hilditch et al 1983) are not corrected for in the calculation. The latter phase of drainage is a complex process and difficult to analyse. However, as the object of this study was to compare the in-vivo distribution of microparticulate systems with solutions, the use of hybrid rate constants did not affect the overall conclusions. The k_d values for the initial drainage from the cornea illustrate that surface charge influences particle residence significantly. The positively charged egg lecithin MLVs were retained significantly longer in this region compared with the suspending buffer. There was no difference between the drainage of the negatively charged or neutral MLVs and solution. Similarly, positively charged DPPC MLVs were retained longer than negatively charged or neutral MLVs.

The second, or basal phase of corneal drainage depends on several processes, largely tear turnover, conjunctival absorption, and reflux between compartments. Tear turnover in the rabbit is low (7% min^{-1}) and the average blink rate is only four times per hour. Therefore, loss of radioactive liposomes and solutions from this region is very slow. It can be seen however, that there is a significant difference between the drainage of particulate material and free solution. This might be caused by interaction of liposomes with the conjunctival membranes.

Kinetic data obtained from the inner canthal region illustrate that all particulate systems are retained for prolonged periods compared with solution. The dual isotope studies demonstrate this directly. The suspending medium drains away independently of the liposomes as described by Sieg & Triplett (1980). However, obstruction to flow does occur in the presence of liposomes, illustrated by the discrepancy in k_d values of free solution and suspending buffer.

Although liposomes are retained in the region of the eye, they are poorly adsorbed to the corneal surface. They may prove useful in the topical delivery of antibiotics and anti-inflammatory drugs owing to their prolonged association with the external ocular tissues. However, their effectiveness in intraocular delivery via the cornea appears to be limited.

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