Evaluation of Mucoadhesive Polymers in Ocular Drug Delivery. II. Polymer-Coated Vesicles

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Association of Carbopol 934P and Carbopol 1342 (a hydrophobic modified Carbopol resin) with phospholipid vesicles was assessed by photon correlation spectroscopy and microelectrophoresis at pH 7.4 and 5. The precorneal clearance of the polymer-coated vesicles was compared to that of uncoated vesicles by lacrimal dacryoscintigraphy in the rabbit. The mucoadhesive polymer-coated vesicles demonstrated significantly enhanced precorneal retention compared to noncoated vesicles only at pH 5 ($P < 0.005$). The entrapment and subsequent release of tropicamide from Carbopol 1342-coated and uncoated liposomes were determined *in vitro* together with an *in vivo* evaluation of the vesicles formulated at the lower pH. Mucoadhesive polymer-coated vesicles failed to increase significantly the bioavailability of the entrapped tropicamide compared to uncoated vesicles and aqueous solution.

KEY WORDS: ocular drug delivery; mucoadhesion; poly(acrylic acid); ocular retention; liposomes; tropicamide.

INTRODUCTION

Mucoadhesive poly(acrylic acid)-based polymer solutions exhibit enhanced precorneal retention compared to equiviscous nonmucoadhesive polymer solutions (1). However, only minor improvements of drug (pilocarpine) bioavailability occurred, an observation attributed to the absence of drug–polymer association. The present study evaluates a sustained-release liposomal delivery system which exhibits enhanced precorneal retention.

The potential of liposomes in ocular drug delivery has been investigated previously, with conflicting reports as to efficacy (2–8). A prerequisite for the use of liposomes in ocular drug delivery would be an enhanced precorneal retention. Schaeffer and Krohn (3) examined the corneal uptake of ^{14}C-phosphatidylcholine from labeled liposomes and concluded that the degree of association of liposomes with the corneal surface decreased in the order $MLV^+ > SUV^+ > MLV^- > SUV^- > MLV$, SUV (where the superscript indicates the charge carried by the vesicles), an observation which they attributed to the negative charge present on the corneal epithelium at physiological pH. Fitzgerald (9) examined the *in vivo* clearance of radiolabeled liposome formulations by gamma scintigraphy and reported that MLVs had a prolonged precorneal retention compared to SUVs of the same lipid composition. Positively charged liposomes were also shown to have a prolonged residence as compared to negatively charged or neutral liposomes. The reduced drainage rate of the liposomes was attributed to their affinity for the conjunctival membrane, and not to the corneal epithelium, a phenomenon also noted in the work of Singh and Mezei (5).

Singh and Mezei (5) suggested that the initial electrostatic interaction between vesicles and the corneal epithelium as demonstrated by the *in vitro* work of Schaeffer and Krohn (3) was not substantiated *in vivo* due to the precorneal tear fluid.

Thus, a practical limitation of liposomes in topical ocular drug delivery would appear to be the lack of specificity of the vesicles for the cornea. In this study, coating of liposomes with a mucoadhesive polymer was undertaken to investigate the influence of the polymer coating on the precorneal drainage rate of liposomes and subsequently on the bioavailability of encapsulated tropicamide.

MATERIALS AND METHODS

Materials

The following materials were used as received: Carbopol 934P and 1342 (B. F. Goodrich & Co., Ltd.); tropicamide (Smith & Nephew Research Ltd., Harlow, UK); technetium-99m as pertechnetate (200–400 MBq; Department of Medical Physics, UHW, Cardiff); egg lecithin, approx. 90%, sodium hydroxide (A.R.), Amberlite resin (IRA-400Cl), chloroform, and sodium chloride (A.R.; BDH Chemicals Ltd.); and stannous chloride (A.R.; Sigma Chemical Co., Ltd.). All water was glass distilled.

Methods

Preparation of Homogeneously Dispersed Multilamellar Vesicles

Egg phosphatidylcholine (EPC) was purified from 90% lecithin by the method of Martin *et al.* (10). An amount of pure EPC was dissolved in a small quantity of chloroform and rotary evaporated at 40°C (Büchi, Switzerland) to leave a thin lipid film. The "dry" film was flushed with N_2 to ensure complete removal of the solvent. The required amount of aqueous phase (0.9%, w/v, NaCl titrated to pH 7.4 with 0.1 *M* sodium hydroxide) was added, and the film allowed to hydrate by gentle shaking at 37°C. The resulting MLV dispersion was transferred to an extruder (Lipex Biomembranes Inc., Canada) fitted with standard 25-mm polycarbonate filters of 0.4-μm pore size (Nuclepore Corp., Pleasanton, CA) and operating at nitrogen pressures of up to 5000 kPa. The vesicles were extruded through the two stacked filters 10 times, employing flow rates of approximately 10 ml/min, to produce a homogeneously sized liposome suspension.

Coating of Liposomes

EPC vesicles (50 mg/ml) in 0.9% (w/v) NaCl, pH 7.4, were dispersed in a 0.05% (w/v) Carbopol solution which had been adjusted to either pH 7.4 or pH 5 by the addition of 1 *M* sodium hydroxide to give a final lipid concentration of 5

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mg/ml. The preparations were incubated at room temperature for 30 min.

Characterization of Liposome Suspensions

Size and Zeta Potential Measurements. The mean size and zeta potential of MLVs were determined by photon correlation spectroscopy and microelectrophoresis using a Zetasizer III Particle Electrophoresis and Multi Angle Particle Size Analyser (Malvern Instruments, UK) employing a standard AZ4 cell.

Measurements were conducted on coated/uncoated liposome suspensions at a lipid concentration of 0.1 mg/ml after dilution with 0.9% (w/v) NaCl which had been adjusted to a pH equivalent to that of the original suspending medium. All measurements were conducted at 25°C employing an experiment time of 60 sec for size determination and 20 sec for zeta potential analysis. Determination of the viscosity of the continuous phase was by U-tube viscometry (Table I), and the refractive index determined in accordance with the British Pharmacopoiea (1988).

Transmission Electron Microscopy (TEM). A small aliquot (1 ml) of the coated/uncoated liposome suspension was mixed with a similar volume of a 2% aqueous ammonium molybdate solution. A small drop of the sample was subsequently placed on a celloidin–carbon-coated copper grid. After allowing it to air-dry for 10 sec, the sample was blotted dry with filter paper. The samples were examined using an electron microscope (Phillips E.M. 400T) employing an accelerating voltage of 80 keV.

^{99m}Technetium-Liposome Radiolabeling

A ^{99m}Tc-liposome radiolabeling technique based on the method of Farr *et al.* (11) was used with preformed liposomes. Briefly, 0.5 ml of the liposome suspension (50 mg/ml), 50 MBq of ^{99m}Tc in 0.9% saline, and 40 μl of a 6.7 mg/ml solution of $SnCl_2$ in deoxygenated water were mixed simultaneously and vortexed. The mixture was then allowed to stand for 30 min at room temperature before any free ^{99m}Tc was removed from the liposome preparation by shaking with an ion-exchange resin [IRA-400(Cl), 1 g/100 MBq ^{99m}Tc] for 3 min. The suspension was then decanted from the resin and the resin washed with two 1-ml vol of 0.9% (w/v) saline to ensure removal of loosely adhering liposomes. Labeling efficiency was calculated by comparing the activity within an aliquot of the preresin liposome suspension to an equal volume of the postresin solution. The stability of the radiolabel was assessed by equilibrium dialysis (using a pore size of 10 kDa; Medicell Int., UK) and by high-performance liquid chromatography (12). The efficiency of labeling was found to be >80% and of suitable stability in pH 5.0 and 7.4 buffers over 3 hr (<10% dissociation).

Table I. Dynamic Viscosities of Carbopol Solutions[a]

Solution	Dynamic viscosity (mPa · sec) at Polymer conc. 0.001%, w/v (25°C)	Polymer conc. 0.05%, w/v (33°C)
Water	0.8904	0.7491
Saline	0.9000	0.7640
Carbopol 1342, pH 7.4	0.9091	0.9854
Carbopol 1342, pH 5.0	0.9090	0.9144
Carbopol 934P, pH 7.4	0.9090	0.9323
Carbopol 934P, pH 5.0	0.9089	0.9051

[a] 0.001%, viscosity of medium for liposome characterization; 0.05%, viscosity of solutions for *in vivo* clearance study. Each value represents a mean of three determinations.

Assessment of the Precorneal Clearance of Liposome Suspensions

The *in vivo* precorneal drainage of both coated and uncoated liposomes was assessed by gamma-scintigraphy in a group of five male NZW rabbits (3.5–4.5 kg) by the method of Davies *et al.* (1) using a 20-μl dose of each preparation.

Preparation of Tropicamide-Containing Liposomes

Incorporation of tropicamide into liposomes was achieved by mixing a solution of the drug in chloroform (1%, w/v) to the EPC/chloroform mixture prior to evaporation. Preparation of the liposome suspension was as described previously to provide a final lipid concentration of 50 mg/ml and drug concentration of 5 mg/ml. An encapsulation efficiency of 33.8 ± 0.55% was observed. Separation of entrapped from free drug was undertaken by centrifugation at 140,000*g* (4°C) for 1 hr (Europa 65M, MSE, ultracentrifuge) followed by removal of the supernatant. The lipid plug was stored inverted at 4°C for a time period not exceeding 24 hr prior to resuspension.

Assessment of Efflux Rates

Following resuspension of the lipid plug, the liposomes were incubated either in a 0.05% (w/v) Carbopol 1342 or in a saline solution, each having a pH of 5, for 5 min. The suspension was subsequently diluted 10-fold with pH 7.4 saline and maintained at 33°C in a shaking water bath (Grant Instruments Ltd., UK). Efflux was monitored by separation of the aqueous phase from the liposomes by ultrafiltration through a PH10 Diaflo membrane in a 10-ml ultrafiltration cell (Amicon UK) under N_2 (180 kPa) followed by the assay of free drug by UV spectrophotometry at 256 nm. Previously the ultrafilter was shown not to adsorb tropicamide from solutions within the concentration limits of the experiment and was of suitable small pore size (10 kDa) to prevent liposome permeation of the matrix.

Bioavailability Studies

From previously determined dose–response curves, a submaximal concentration of tropicamide (0.05%, w/v) was selected for the *in vivo* study.

On the day preceding the *in vivo* study, liposomes containing tropicamide were prepared to provide a final lipid concentration of 50 mg/ml and a drug concentration of 5 mg/ml. The liposome suspension was subsequently centrifuged at 140,000*g* (4°C) for 1 hr. The supernatant was completely removed and the lipid plug retained and stored, inverted, at 4°C. The lipid plug was resuspended to the previous volume with fresh pH 7.4 saline 10 min prior to the *in*

vivo study. Three-tenths milliliter of the resuspended liposome suspension was incubated for 5 min in 9.7 ml of a sterile solution of either 0.05% (w/v) Carbopol 1342 or 0.9% (w/v) NaCl, each of pH 5, to give a final liposome suspension containing 0.05% (w/v) tropicamide entrapped within the vesicles. An aqueous solution containing 0.05% (w/v) tropicamide was also formulated in pH 5, 0.9% (w/v) saline.

Tropicamide produces a rapid mydriatic response and therefore bioavailability was assessed by measuring pupil diameter photographically (1). All studies were conducted in a crossover manner on five unanesthetized preconditioned male NZW rabbits (3.5–4.5 kg), with a rest period of not less than 1 week allowed for each rabbit between successive studies. The test animals were positioned in restraining boxes in the normal upright position in a room with constant light intensity and devoid of distractions. All rabbits were acclimatized to the laboratory testing conditions for 30 min prior to initiating the study.

Baseline pupillary diameter measurements were taken each minute for 5 min prior to dosing. A 20-μl dose of each preparation was instilled into the lower fornix of the conjunctival sac and the eye manually blinked. Experiments in which there was evidence of spillage of solution onto the eyelashes or eyelids were repeated. Pupillary diameter measurements were made on the treated eyes at various time intervals postinstillation. The relative mydriatic response intensities at time t (IR_t) for each formulation was calculated by the equation $IR_t = (I_t - I_o)/I_o$, where I_o is the average baseline diameter and I_t the pupil diameter at time t. Activity parameters were calculated as described by Davies *et al.* (1) and subjected to statistical analysis employing Duncan's test for variability ($P < 0.05$).

RESULTS

The size and zeta potential measurements of liposomes and liposomes incubated in polymer solution (Table II) demonstrate an association between the polymer and the vesicles at both pH 7.4 and pH 5 as evidenced by an increase in size and a decrease in zeta potential. The zeta potential of the polymer-coated vesicles is lower than that of uncoated vesicles due to the presence of ionized carboxyl groupings, which increase as the pH is raised from 5 to 7.4, resulting in a decrease in zeta potential. Examination of the size increase suggests a thick polymer coating or even aggregation of the vesicles. Carbopol 934P and 1342 form a three-dimensional microgel structure when dispersed in water (13). Thus, this large increase in size due to incubation in the polymer solutions can now be better understood when it is realized that small microgel structures associate with the vesicles rather than a thin polymer coating. This microgel coating for 934P was visualized by transmission electron microscopy (TEM) (Fig. 1). Seki and Tirrell (14) studied the interaction of a range of poly(acrylic acid) derivatives with phospholipid membranes. Poly(acrylic acid) was shown to be able to complex with vesicle bilayers in a manner which was pH dependent as evidenced by the broadening of the lipid phase transition temperature, with an eventual disappearance of the MLVs when the pH was lowered to 4.6. EPC liposomes remained intact when incubated in solutions of Carbopol 934P and 1342 at pH 5.0 as evidenced by TEM.

The precorneal clearance studies (Fig. 2) demonstrated that uncoated liposome suspensions behave in a similar manner to solutions, the precorneal drainage being a biphasic phenomenon with an initial rapid phase (30–60 s) followed by a much slower basal drainage phase. Kinetic analysis of the corneal drainage phase by least sum of squared errors gives rise to two drainage rate constants of the same magnitude as those for solution clearance with an initial drainage constant two orders of magnitude greater than the basal drainage rate constant (Tables III and IV). The corneal clearance profiles (Fig. 2) show that 50 and 58% of the uncoated liposomes are cleared within the first 30 sec at both pH 7.4 and pH 5, respectively, while 11 and 12% remain after 30 min, thus demonstrating the biphasic nature of the clearance which is unaffected by the pH of instillation.

The coating of liposomes with Carbopol 934P and 1342 at pH 7.4 (Fig. 2a) reduces the effect of the initial rapid drainage phase, resulting in an increase in the percentage of instilled activity remaining associated with the cornea at the termination of the dynamic study (300 sec), being 30 and 28%, respectively, for Carbopol 934P and 1342, compared to 18% for uncoated vesicles. Despite this enhanced initial residence of coated vesicles when instilled at pH 7.4, the percentage remaining at 1800 sec, corresponding to the termination of the study, is similar to that of uncoated vesicles. Analysis of the AUC (0–1800 sec) also indicates that the enhanced corneal retention of polymer-coated liposomes is of short duration, and no statistically significant increase in corneal retention is afforded by polymer coating when determined at 30 min.

Decreasing the coating solution pH to 5 dramatically

Table II. The Size and Zeta Potential of Coated and Uncoated Liposomes ($n = 10$)

	Hydrodynamic diameter (nm ± SD)	Polydispersity index (±SD)	Zeta potential (mV ± SD)
pH 7.4			
MLV	259.1 ± 4.7	0.141 ± 0.038	−6.15 ± 0.56
MLV (Carbopol 934P)	681.3 ± 65.9	0.150 ± 0.079	−37.12 ± 0.62
MLV (Carbopol 1342)	1297.0 ± 66.3	0.381 ± 0.080	−50.00 ± 2.20
pH 5			
MLV	260.0 ± 3.7	0.171 ± 0.040	−6.24 ± 0.51
MLV (Carbopol 934P)	784.1 ± 27.7	0.311 ± 0.044	−32.79 ± 0.46
MLV (Carbopol 1342)	1061.0 ± 98.4	0.371 ± 0.048	−42.20 ± 1.64

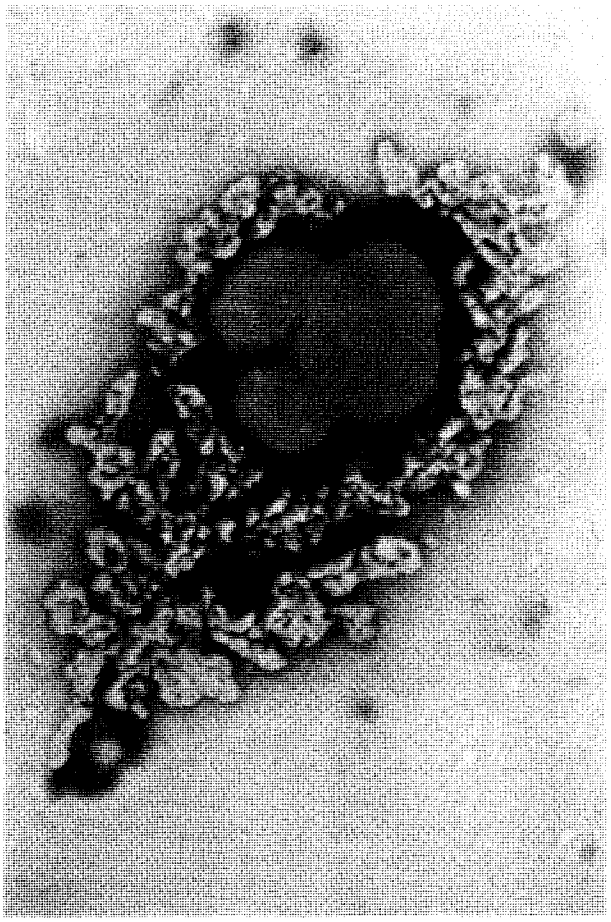

Fig. 1. TEM of Carbopol 934P-coated liposomes. ×80,000; reduced to 75% for reproduction.

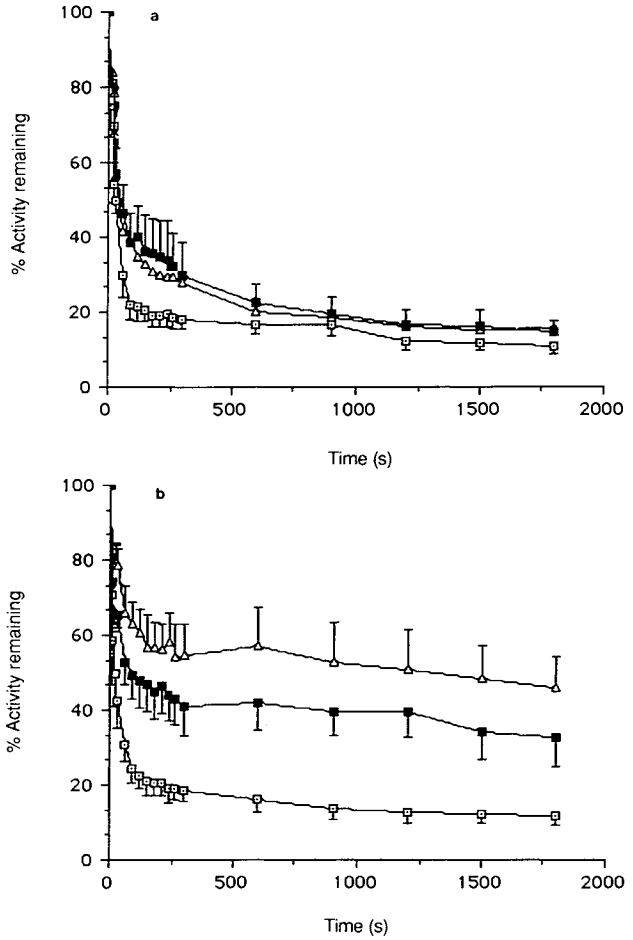

Fig. 2. Precorneal drainage profiles of liposome formulations at (a) pH 7.4 and (b) pH 5.0 (mean ± SEM). (□) No coating; (■) Carbopol 934P; (△) Carbopol 1342.

changes the corneal retention of both Carbopol 934P- and Carbopol 1342-coated vesicles (Fig. 2b). The presence of the polymer decreases the influence of the initial drainage phase, resulting in 55 and 41% of the initial activity remaining associated with the corneal region at the termination of the dynamic study for Carbopol 1342- and 934P-coated vesicles, respectively, compared to 18% for uncoated liposomes. The percentage of total residence associated with the initial rapid phase as calculated from the area under the percentage remaining–time curve (AUC) associated with both clearance phases was significantly decreased (Duncan's test, $P < 0.05$) when the liposomes were coated at pH 5. The basal drainage rate constants were also decreased for polymer-coated vesicles. The superiority of Carbopol 934P- and 1342-coated liposomes at pH 5 is further substantiated by analysis of the AUC (0–1800 sec). Analysis of variance of the AUC (Duncan's test, $P < 0.05$) indicates that the corneal retention of the Carbopol-coated vesicles is significantly greater than that of uncoated vesicles, with no statistically significant differences being observed between the two polymer formulations (Table IV). The latter was also true for the percentage remaining at 1800 sec.

Examination of the acquired gamma-camera images revealed that initially the coated liposome suspension was distributed over the entire precorneal area. However, the images at the termination of the study showed an uneven distribution of activity over the corneal region for the polymer-coated vesicles. The activity, which is representative of the liposome population, appeared to be concentrated in the conjunctival sac, together with the nictitating membrane, with a reduced activity associated with the corneal surface.

The entrapment of tropicamide within lipid vesicles was 33.8 ± 0.55% ($n = 5$). At pH 7.4, the drug (p*K*a, 5.25) will be essentially nonionized and is likely to be associated predominantly with the lipid (50 mg/ml) bilayers, which is confirmed by the partition coefficient of 10. The half-life of tropicamide release on exposure to sink conditions from coated and uncoated vesicles was found to be 29.6 hr ($r^2 = 0.995$) and 10.5 hr ($r^2 = 0.982$), respectively (Fig. 3).

Figure 4 compares the pupil dilatory effect of an aqueous solution and polymer-coated and uncoated liposome suspension of tropicamide after topical instillation in the rabbit eye. The main activity parameters are summarized in Table V. Statistical analyses (Duncan's test for variability) failed to show any significant difference between any formulation. The mydriatic response intensity curves are of similar shape for the aqueous solution and vesicle preparation. However, for the Carbopol 1342-coated vesicles, the initial peak declines to a minimum IR_t value after 4 hr. Thereafter a linear

Table III. Clearance Parameters of Liposome Formulations at pH 7.4 (Mean ± SE)

	No coating	Carbopol 934P	Carbopol 1342
Cornea			
% remaining (1800 sec)	10.87 ± 1.78	14.66 ± 2.98	15.48 ± 6.31
AUC relative to no coating	1.00 ± 0.14	1.40 ± 0.31	1.30 ± 0.37
k_{d_1} (min)	1.04 ± 0.49	1.61 ± 0.23	1.45 ± 0.59
k_{d_2} (min)	0.015 ± 0.004	0.037 ± 0.005	0.030 ± 0.01
% total residence associated with initial clearance phase	5.55 ± 1.54	2.97 ± 0.91	4.25 ± 3.62
Inner canthus			
% remaining (1800 sec)	10.79 ± 3.78	12.55 ± 3.46	10.08 ± 2.53
AUC relative to no coating	1.00 ± 0.25	1.19 ± 0.28	0.83 ± 0.21

increase was observed to the termination of the study at 10 hr.

DISCUSSION

The potential of liposomes in the ocular delivery of both hydrophilic and hydrophobic compounds is limited by their rapid clearance from the precorneal region. It is well documented that the precorneal residence of ophthalmic solutions can be promoted by the inclusion of viscosity enhancing polymers. This approach may, therefore, prolong the precorneal retention of vesicle formulations. The influence of viscosity on precorneal clearance of vesicles in the current study will be minimal, as all polymer solutions employed were dilute (0.05%) and possessed similar viscosities (Table I). Fitzgerald (9) studied the *in vivo* precorneal clearance rates of SUVs and MLVs in the presence of 0.45% (w/v) hydroxypropylmethyl cellulose and 3.0% (w/v) poly(vinyl alcohol) solutions. Vesicles suspended in these polymer solutions were retained on the corneal surface for a significantly longer period than those suspended in buffer. It was also demonstrated that MLVs suspended in the polymer solutions were cleared from the corneal region at a rate similar to that for the suspending solutions.

In the present study, the precorneal drainage rate of liposomes suspended in a 0.05% (w/v) poly(acrylic acid)-based polymer solution at pH 7.4 is initially reduced, with an increase in the percentage remaining at the termination of the dynamic study as compared to liposomes suspended in saline. However, no benefit in precorneal retention was observed for polymer-coated vesicles over the 30-min experimental duration. Reducing the pH of instillation to 5.0, however, had a dramatic effect on the precorneal retention of polymer-coated liposomes. Many workers have investigated the effect of pH on the interaction of poly(acrylic acid) with mucus (15–18), noting that the adhesion of acrylic acid-based polymers to mucous membranes is greater at a pH <6. Protonation of the carboxyl groups (p*K*a, 4.75) permits H-bonding between the polymer and the mucin network, resulting in an enhanced precorneal retention of the polymer. Interaction between such mucoadhesive polymers and phospholipid vesicles has, in turn, resulted in the prolonged precorneal residence of phospholipid vesicles. Further evidence to support mucoadhesion as a mechanism of interaction for achieving prolonged residence of coated vesicles is provided by the drainage scintigrams. The mucoadhesive polymer-coated liposomes are observed to concentrate in the fornices, i.e., areas rich in mucin.

The entrapment of drugs within liposomes has an unpredictable effect on their ocular bioavailability. In the present study, the bioavailability of tropicamide is not enhanced when encapsulated within liposomes. A similar result was reported by Meisner *et al.* (8) for the ocular bioavailability of atropine, a structurally similar molecule to tropicamide. The instillation of [^{3}H]atropine base encapsulated within liposomes resulted in an increased pulsed entry, which was attributed to the enhanced association of the drug to the cornea provided by the inherent lipophilicity of the liposomal bilayers and the corneal epithelium.

Endocytosis is considered the dominant interaction be-

Table IV. Clearance Parameters of Liposome Formulations at pH 5.0 (Mean ± SE)

	No coating	Carbopol 934P	Carbopol 1342
Cornea			
% remaining (1800 sec)	11.59 ± 2.49	32.68 ± 7.57	45.67* ± 8.54
AUC relative to no coating	1.00 ± 0.18	2.48* ± 0.42	3.30* ± 0.53
k_{d_1} (min^{-1})	2.10 ± 1.47	1.42 ± 0.51	1.23 ± 0.27
k_{d_2} (min^{-1})	0.024 ± 0.006	0.011 ± 0.005	0.007 ± 0.003
% total residence associated with initial clearance phase	3.30 ± 1.66	0.65* ± 0.5	0.29* ± 0.26
Inner canthus			
% remaining (1800 sec)	10.47 ± 2.86	13.20 ± 2.58	15.42 ± 6.59
AUC relative to no coating	1.00 ± 0.21	1.15 ± 0.21	1.07 ± 0.38

* Values statistically significant with respect to uncoated liposomes ($P < 0.05$, Duncan's).

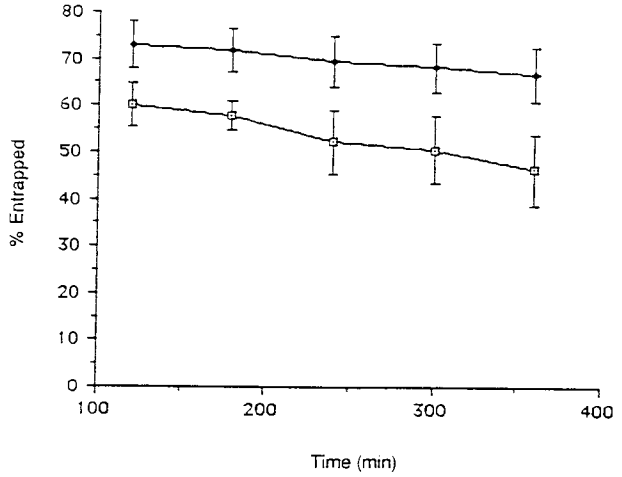

Fig. 3. *In vitro* release of tropicamide rom Carbopol 1342-coated vesicles compared with uncoated vesicles at pH 7.4. (□) Uncoated; (♦) Carbopol 1342 coated.

tween liposomes and cells. The cornea, however, has been demonstrated to exhibit poor phagocytic activity (19) and endocytosis is unlikely to be important in the interaction of vesicles with the corneal epithelium. In addition, Meisner *et al.* (8) demonstrated that the elimination profiles of atropine from various ocular tissues were similar when the drug was administered encapsulated within liposomes or in solution, an unexpected observation should the drug remain associated with the vesicle when it reached the internal structures of the eye. Fusion of phospholipid vesicles with cells normally requires special conditions of lipid fluidity and temperature and is more prominent in the presence of certain fusogenic agents. Therefore, the major mechanism of interaction between liposomes and the corneal epithelium in the absence of mucoadhesive agents is probably adsorption and/or lipid exchange (5).

Lee *et al.* (20) studied the adsorption of liposomes onto the rabbit cornea and reported that the predosing of the eye with empty liposomes 30 min prior to the instillation of liposomes containing insulin vitiated the beneficial effects afforded by the liposome preparation, and they therefore proposed liposome affinity for the corneal surface as a mode of action. These workers also speculated that the liposomes were merely in a loose association with the corneal surface, as evidenced by their low resistance to removal by rinsing the eye with saline.

Further evidence for adsorption being a mechanism of interaction between liposomes and the corneal surface is gained from the precorneal drainage of charged vesicles, where positively charged vesicles are found to exhibit a prolonged precorneal retention (3,21–22). At physiological pH, the corneal epithelium is negatively charged and thus electrostatic attraction may enhance adsorption. Studies have subsequently shown that positively charged vesicles can enhance the bioavailability of entrapped drugs over neutral or negatively charged vesicles (3,5,8).

The capacity of vesicles, particularly positively charged vesicles, to exhibit prolonged corneal retention by adsorption onto the corneal surface would be expected to result in a delayed ocular absorption of drugs which exhibit pro-

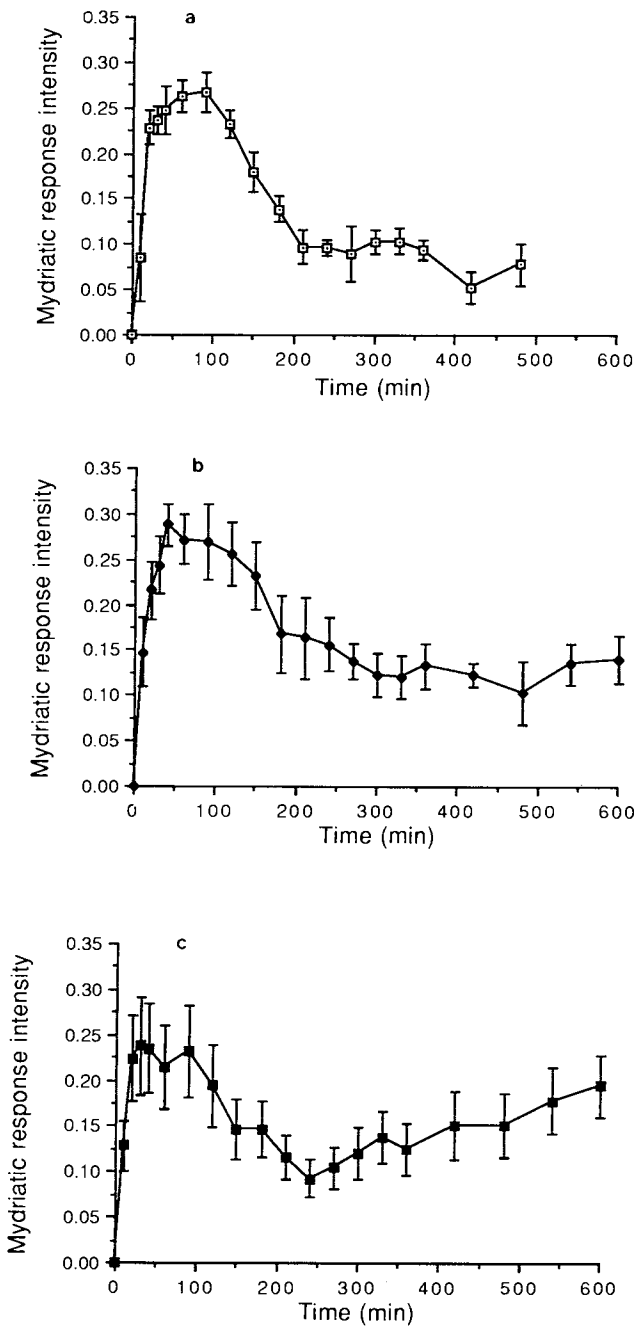

Fig. 4. Mydriatic response intensity elicited by tropicamide formulations (mean ± SEM): (a) aqueous solution; (b) liposome suspension; (c) coated liposome suspension.

longed efflux rates. To date, however, no delay in time to elicit maximum response or any sustained-release effect, has been reported for drugs encapsulated within liposomes. Literature suggests that ocular delivery via liposomes may benefit lipophilic compounds to a greater extent than hydrophilic compounds (3–8,20,23), which seems anomalous in view of the fact that such compounds are only slowly released from vesicles (24). Taniguchi *et al.* (22) studied the release of dexamethasone valerate from phospholipid vesicles and showed that less than 5% of the steroid was released

Table V. Mean Activity Parameters of Tropicamide Formulations (Mean ± SE)

	Aqueous solution	Liposome suspension	Coated liposome suspension (Carbopol 1342)
IR_t (max)[a]	0.267 ± 0.022	0.274 ± 0.023	0.238 ± 0.053
IR_t (8 hr)[b]	0.078 ± 0.051	0.103 ± 0.077	0.174 ± 0.065
AUC (0–8 hr)[c]	64.70 ± 9.1	82.30 ± 27.0	85.00 ± 15.1
AUC (4–8 hr)[d]	20.01 ± 5.4	30.10 ± 10.9	34.80 ± 11.5
t (max)[e]	60	40	40

[a] Maximum mydriatic response intensity.
[b] Mydriatic response intensity at 8 hr postinstillation.
[c] Area under mydriatic response intensity–time graph from 0 to 8 hr (min).
[d] Area under mydriatic response intensity–time graph from 4 to 8 hr (min).
[e] Time to reach maximum mydriatic response intensity (min).

from the vesicle over a 24-hr period. However, the bioavailability of the lipophilic drug was enhanced due to vesicle entrapment, with no delay in the delivery of the drug to the ocular tissues.

The equivalent bioavailability of tropicamide from liposomes as compared to solutions, however, may be due to the already efficient biphasic availability of tropicamide at pH 5, as a result of the interaction of the cationic drug to anionic binding sites in the corneal tissue (25), together with the small percentage of liposomes adsorbed onto the corneal surface (6). Increasing the drug to phospholipid ratio or selection of a bilayer-associated drug with a normally low bioavailability may result in an increased drug bioavailability from a liposomal formulation.

The behavior of the polymer-coated liposomes appears more complex and we may merely speculate on the mechanism of action. The pulsed entry of tropicamide is initially observed, together with a delayed or second-phase response initiated 4 hr postinstillation. Carbopol 1342 forms a three-dimensional microgel structure when dispersed in water which can interact with phospholipid vesicles, thus prolonging their precorneal residence via a mechanism of adhesion to the mucin network. The prolonged residence of these suspensions may subsequently allow for a delayed release of tropicamide, resulting in the second-phase mydriatic response. However, this delayed response would be expected to occur at a later time than that observed when the slow efflux rate of the drug is considered. Incubation of vesicles in rabbit tear fluid, however, has been shown to increase the rate of efflux of liposome-entrapped compounds (6,26) and thus the slow efflux of tropicamide from vesicles in saline may not be representative of the drug efflux *in vivo*.

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