Brigham Young University
BYU ScholarsArchive

Theses and Dissertations

2011-09-20

Phospholipid Transport in Silicon Hydrogel Contact Lenses

Yibei Zhao
Brigham Young University - Provo

Follow this and additional works at: https://scholarsarchive.byu.edu/etd

 Part of the Chemical Engineering Commons

BYU ScholarsArchive Citation

Zhao, Yibei, "Phospholipid Transport in Silicon Hydrogel Contact Lenses" (2011). *Theses and Dissertations*. 3083.
https://scholarsarchive.byu.edu/etd/3083

This Thesis is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact scholarsarchive@byu.edu, ellen_amatangelo@byu.edu.

Phospholipid Transport in Silicon Hydrogel Contact Lenses

Yibei Zhao

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

William G. Pitt
Kenneth A. Solen
Morris D. Argyle

Department of Chemical Engineering

Brigham Young University

Dec 2011

Copyright © 2011 Yibei Zhao

All Rights Reserved

ABSTRACT

Phospholipid Transport in Silicon Hydrogel
Contact Lenses

Yibei Zhao
Department of Chemical Engineering, BYU
Master of Science

Dry eye syndrome has been associated with the lack of phospholipids in the tear film, leading to disruption of the tear film and subsequent irritation. Characterization of the transport and release of phospholipids from a silicone hydrogel contact lens is required to assess the possible use of these lenses for phospholipid delivery to increase patient comfort. This thesis examines the use of silicone hydrogel contact lenses as phospholipid delivery devices.

Contact lenses of silicone hydrogel composition were loaded with varying amounts of radiolabeled 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) from a solution of n-propanol. These lenses were eluted at 35°C into artificial tear fluid (ATF) or ATFcontaining varying amounts of DMPC. The amount of DMPC loaded into a lens is a linear function of the time of exposure to the DMPC/propanol solution. The initial rate of elution into ATF appears to be diffusion controlled for at least 10 hrs and is proportional to the amount of DMPC loaded. The ease of loading and the controllable release of DMPC from silicone hydrogels present the possibility of using such lenses to counter eye discomfort caused by inherently low levels of phospholipid in tears.

To reduce manufacturing steps and concern for residual n-propanol in the lens, it is beneficial to incorporate the DMPC into the monomer formulation and then photopolymerize the lens. Results showed that using this process, DMPC can be placed in the lens and then eluted at faster rates than when it was loaded from n-propanol.

Key words: silicone hydrogel contact lens; dry eye discomfort; phospholipid delivery; artificial tear fluid; elution; drug transport

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

Contact lenses are used most commonly to improve vision. Given that the majority of ocular therapeutics delivered to the eyesis in the form of liquid solutions, contact lenses could also be used to deliver some comfort agents to the eye. While the use of a contact lens as an ophthalmic delivery device has been proposed for decades**[1-3]**, the enthusiasm for and clinical application of therapeutic lenses has expanded in recent years**[4-7]**.

Tear fluid, the liquid surrounding the ocular surface, contains phospholipids that stabilize the tear film and promote the health of the eye. Insufficient phospholipids in tears may upset the stability of a smooth tear film, allowing it to break down rapidly and create dry spots on the cornea.In addition, decreased availability of phospholipids is sometimes attributed to dysfunction of the phospholipid-secreting Meibomian gland**[8, 9]**, or possibly to the wearing of contact lenses, which are shown to sorb a small amount of phospholipids**[10-12]**. Therefore, it would be beneficial to deliver a phospholipid as a comfort agent to the eye for persons whose tears might be deficient in phospholipid**[8-12]**.

Since silicone polymer has very high oxygen permeability, hydrogel lenses made with silicone can deliver higher oxygen flux to the eye by using both the water phase and the siliconepolymer molecules to transport oxygen. These lenses promote better ocular health than conventional soft lenses because they allow up to 6 times more oxygen flux. Other advantages of modern silicone hydrogel lenses over conventional soft lenses include resistance to protein

deposits, less drying of the lenses, lower risk of eye infection, easier handling due to increased rigidity of material, and much lower incidence of complications during extended wear use. In fact, properly formulated silicone hydrogels provide enough transmission so that during sleep oxygen diffuses from the capillaries in the eyelid through the contact lens and to the corneal cells in sufficient quantities to keep the eye healthy.

Returning to the need of eye comfort, phospholipids can be loaded into the lens prior to lens insertion, and then the phospholipid can slowly diffuse into the eye during the course of the daily wearing period, providing a constant source of supplemental phospholipid. This study verifies that 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) can be loaded into silicone hydrogel contact lenses and then is slowly released from the lens into water and an artificial tear solution. This is the first step in developing a future daily-wear therapeutic contact lens to treat some types of dry eye syndrome or contact-lens-related dry eye symptoms. To determine if this lens has potential for commercialization, it is necessary to determine if the autoclaving process adversely reduces the DMPC concentration or reduces the release rate.Loading DMPC into lenses before polymerizationis also considered to be beneficial.

2 LITERATURE REVIEW

2.1 Silicon hydrogels

Silicone hydrogels are the latest development in soft contact lens materials. The hydrogel polymers in conventional soft contact lenses use water as a transport medium for oxygen to the surface of the eye. When covered by the contact lens, oxygen diffusion through the lens is the only source of oxygen to the cells of the cornea. However, the amount of oxygen the eye receives is restricted by the amount of water in conventional contact lenses. Although the oxygen permeability of water is greater than that of acrylates, even a hypothetical "pure water" contact lens has barely sufficient oxygen permeability to keep the eye healthy during sleep. Since silicone polymer has very high oxygen permeability, hydrogel lenses made with silicone polymers can carry oxygen to the eye both by using both the water and the siliconepolymer molecules to transport oxygen. These lenses promote better ocular health than conventional soft lenses because they allow up to 6 times more oxygen to pass through them**[13]**. The advantages of silicone hydrogel lenses over conventional soft lenses include more resistance to protein deposits, less drying of the lenses, lower risk of eye infection, easier handling due to increased rigidity of material, and much lower incidence of complications during extended wear use**[13]**. In fact, properly formulated silicone hydrogels provide enough transmission so that during sleep oxygen diffuses from the capillaries in the eyelid through the contact lens and to the corneal cells in sufficient quantities to keep the eye healthy.

2.2 **Dry eye syndrome**

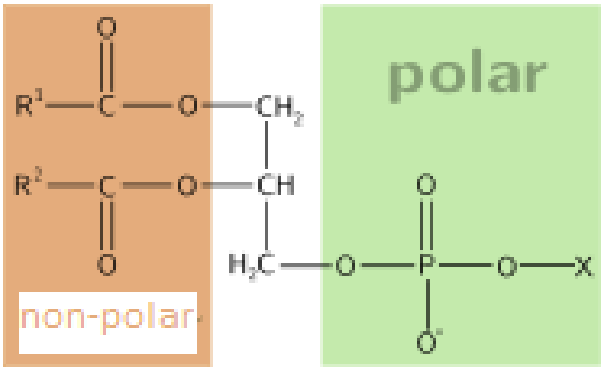
Tears perform a number of functions. Working together with the eyelids, tears protect, cleanse, and nourish the eye. In one way like miniature wipers, the eyelids distribute tears over the eyes, clearing off and flushing away dust, dirt, everyday debris, and— most important— bacteria and other potential disease-causing agents. In addition, they transport proteins, vitamins, and other important nutrients onto the eyes, where they are absorbed into the cornea. Tears also lubricate the eyeballs and prevent dehydration of conjunctival mucus membranes and other tissues associated with the eyes. Finally, when all is working well, tears create a smooth optical surface on the cornea and ensure that a high-quality image can be projected onto the retina**[14]**.

Dry eye syndrome is manifest by a tear film that is pathologically unstable. This can upset the stability of a smooth tear film, allowing it to break down rapidly and create dry spots on the cornea, the clear front surface of the eye.Dry eye syndrome develops when systems in the eyelids and the various tear glands do not function normally. The causes of dry eye syndrome are numerous and varied. In general, dry eye syndrome is caused by a number of very disparate problems,including behavioral disturbances to the normal healthy operation of the eye, certain environmental conditions, aging,and various diseases and disorders**[15]**.

Behavioral disturbances are thosethat disrupt the blink process or numb the surface of the eye. These disturbances in turn interfere with the production and distribution of tears. They are ‘‘behavioral’’ in nature; in other words, they result from activities we choose to do, such as spending long hours in front of a computer or wearing contact lenses. One common way healthy blinking can be disrupted is by the simple act of concentration— staring or gazing at a particular object, be it a computer screen, a long highway, or a good book— for long periods. We tend to blink less frequently when we focus in this way, because the brain in effect overrides the

involuntary blink mechanism to allow maximum concentration. When our blink rate slows down, evaporation on the surface of the eye speeds up, and symptoms of dry eye can appear.**[14]**

2.3 **Phospholipids**



Phospholipids are a class of lipids that contain a diglyceride, a phosphate group, and a simple organic molecule. The 'head' of a phospholipid is hydrophilic, while the hydrophobic 'tails' repel water.

In tears, the phospholipids arecritical for maintaining a stable tear film because the polar head group is able to interact with the aqueous tear film while the non-polar tail regions interact with the wax and cholesterol esters at the surface of the tear film that interact with the air. Such lipids, along with proteins and their hydrogen bonding to water are considered as a major contributor to tear film stability. **[16]**

If the phospholipids in tear film are insufficient, the temporalstability of a smooth tear film decreases, which allows the tear film to break down rapidly and create dry spots on the cornea. Thus low amounts of phospholipid in tear film can lead to dry eye syndrome.

2.4 **C-14 chemistry**

C-14 is the only radioactive material that will be used in my experiment. The half-life of C-14 is about 5730 years, meaning that the amount of carbon-14 in a sample decays to half its initial value over the course of 5730 years.Phospholipids can be labeled with C-14 on the terminal methyl of the lipid groups. Measurements are traditionally made by counting the radioactive decay of individual carbon atoms by liquid scintillation counting and accelerator mass spectrometry. In my experiments, liquid scintillation counting is the only method used.

In liquid scintillation counting, samples are dissolved or suspended in a "cocktail" containing an aromatic solvent. Beta particles emitted from the C-14 decay in the sample transfer energy to the solvent molecules, which in turn transfer their energy to the fluors; the excited fluor molecules dissipate the energy by emitting light. In this way, each beta emission should result in a pulse of light.

The radioactive samples are placed in small glass vials that are loaded into an instrument known as a liquid scintillation counter. The counter has two photomultiplier tubes connected in a coincidence circuit. The coincidence circuit assures that genuine light pulses, which reach both photomultiplier tubes, are counted, while spurious pulses, which would only affect one of the tubes, are ignored.

2.5 **Autoclaving**

Autoclaving is a procedure to sterilize equipment and supplies by subjecting them to the high temperature associated with high-pressure steam in a certain amount of time. Most contact lenses are sterilized by autoclaving before they are shipped for distribution. Our concern is that autoclaving may cause the phospholipids in contact lenses to leach out during the long time at high temperature

3 EXPERIMENTAL METHODS

3.1 Materials

Radiolabeled DMPC. ^{14}C-labeled DMPC was purchased from New England Nuclear (Waltham, MA) and received in a dry crystalline form. The specific activity was 171.4 mCi/mmol. Crystalline DMPC was dissolved in *n*-propanol (Mallinckrodt, Phillipsburg, NJ) to make a master batch solution of 1.5% w/v DMPC and stored in a sealed container at -20°C. As needed, aliquots of this were diluted in *n*-propanol to make a 0.15% w/v solution (called hot solution), which was also stored at -20°C. Dry powdered (non-labeled) DMPC was purchased from Lipoid (Ludwigshafen, Germany) and dissolved in *n*-propanol to form a 0.15% w/v solution (called cold DMPC), which was stored at -20°C.

Lenses. CIBA VISION kindly provided experimental daily-wear lenses in foil "blister packs" that contained water instead of saline. These lenses were not previously autoclaved. Lenses were stored at room temperature until use. There was never any evidence of bacterial or other contamination in the lens packages. Three types of lenses were received. The first is a daily wear lens, and was called "daily wear" in this thesis. The other 2 lenses were formulated as extended wear lenses, and are of different chemistry. They were called EWA (extend wear A) and EWB in this thesis.

Non-labeled monomer. Monomer formulation was provided by CIBA VISION in a glass container and was stored in a refrigerator at 4°C until use. While the composition of the formulation was not divulged, it was stated to be similar to that used to polymerize the experimental silicone hydrogels provided by CIBA VISION.**[1, 3, 17]**.

Solutions. Phosphate buffered saline was (PBS) formulated as 7.805 grams of sodium chloride, 0.7733 grams of sodium dihydrogen phosphate mono hydrate, and4.759 grams of disodium hydrogen phosphate in distilled deionized water. Artificial tear fluid was kindly provided by CIBA VISION. It was called ATF or ATF+. A second solution from CIBA is called ATF-. The compositions of both are given in Table 3.1. The only difference is that ATF- has no phospholipids. The ATF solutions were stored at -20°C. Distilled deionized water (DDH_2O) was also used for elution in some experiments.

Table 3-1. Composition of ATF

Component	ATF+ [mg/mL]	ATF- [mg/mL]
Lysozyme	2.2	2.2
Lactoferrin	2.3	2.3
Albumin	0.0286	0.0286
Mucin	0.1	0.1
Lipocalin (TSP) / β-lactoglobulin	1.5	1.5
Cholesterol*	0.00175	0.00175
Cholesterol oleate (esters)	0.0186	0.0186
Phosphatidylethanolamine	0.0005	0
Phosphatidylcholine	0.0011	0
Sodium dihydrogen phosphate[#]	0.285	0.285
Sodium phosphate[#]	2.128	2.128
Sodium chloride	8.0	8.0

*The cholesterol was complexed with β-cyclodextrin to solubilize the cholesterol in the aqueous solution. The concentration above is for the cholesterol portion.
[#]The pH was adjusted to 7.5

Radiolabeled monomer solution. To make the lenses radioactive enough for adequate scintillation counting, about 6.75 milligrams of dry powdered (non-labeled) DMPC, 0.05 ml of secondary hot standard DMPC solution (0.15% w/v), 0.95 ml of monomer and 0.04 ml of primary hot standard DMPC solution (1.5% w/v) were mixed. This resulted in a radiolabeled monomer solution containing 7.11 milligrams DMPC per milliliter of monomer solution.

3.2 **Procedures**

Loading the lens. Loading solution was prepared by mixing a calculated volume of hot solution (0.15% w/v) with a calculated volume of cold solution (0.15% w/v) to produce a 0.15% w/v of desired ^{14}C activity. The contact lenses, ^{14}C-DMPC loading solution, and ATF solutions were warmed to 35°C for at least 30 minutes prior to use.

A lens was removed from the blister pack, lightly blotted dry on a lint-free tissue (KimWipes, Kimberly-Clark, Neenah, WI), and immersed in 5 mL of loading solution for 30, 60, or 120 seconds. The lens was immediately removed and placed in a beaker of 35°C DD H_2O for 5 seconds, transferred to another beaker of DD H_2O for at least 5 seconds, rinsed with DD H_2O, and stored in a capped vial of DD H_2O for less than 2 minutes. A small portion (0.01 mL) of the loading solution was mixed with 0.09 mL DD H_2O and mixed with 10 mL of scintillation fluid (ScintiVerse™ BD, Fisher Scientific, Fair Lawn, NJ) to prepare a standard for subsequent quantitation of the activity of the loading solution.

Elution of DMPC. A lens was placed in a glass vial (with plastic snap cap) containing 3.00 mL of elution fluid (ATF+, ATF- or DDH_2O). The capped vial was placed on a rotary

shaker (60 rpm) in a 35°C incubator. Samples from the vial were collected at 0, 2, 4, 10, 24, 48 and 72 hrs by pipetting 0.1 mL from the vial into a scintillation vial containing 10 mL of scintillation fluid. Because of scheduling conflicts, some samples were collected at 26 instead of 24 hours.

Extraction of DMPC. To quantify the amount of DMPC loaded into the lenses, the lenses were extracted with *n*-propanol as follows. A lens was removed from aqueous solution (either at completion of loading or elution), rinsed with DDH_2O, and carefully blotted dry. It was placed in 2 mL of *n*-propanol in a capped glass vial and swirled gently on a rotary shaker in a 35°C incubator for 1 hour. It was then rinsed by pipetting 1 mL of n-propanol over the lens and into the same vial. Then it was transferred to another fresh vial of *n*-propanol and further eluted at 35°C for another hour. Lenses that were loaded only but not eluted were extracted similarly a third time. 10 mL of scintillation fluid were added to the 3 mL of n-propanol to count the amount of DMPC extracted.

Autoclaving. A lens (usually loaded with DMPC) was placed in a glass vial containing 2 mL of PBS, and the vial was capped with a aluminum cap. The vials were processed in a commercial medical autoclave. In these experiments, the autoclaving temperatures were at 121°C and 129°C, and the autoclaving times were 30, 60 and 90 minutes. This range includes typical autoclaving processes.

After autoclaving, the lenses in the vial were cooled with running water at room temperature. Then the container was opened and 0.1mL of solution was pipetted into scintillation fluid to quantitate the amount of DMPC extracted during the autoclaving. The lenses were

subjected to the normal elution and/or extraction processes described above. Four replicates were tested at each condition.

Quantitation. Calibrated pipettes were used for all procedures. Scintillation vials were stored in the dark at 4°C until they were counted on a Beckman Coulter LS6500 (Fullerton, CA) scintillation counter for 1 minute. Background counts of blank vials (containing appropriate amounts of ATF, water or *n*-propanol) were 29 – 34 cpm, and a mean background value was subtracted from all sample counts. The activity of each preparation of loading solution was counted and used as a standard since each loading solution was exactly 0.15% w/v DMPC. From this calibration and the background counts, the mass of DMPC eluted or extracted from a lens was computed.

Contact angle. Underwater contact angles of air and octane were measured using a modification of the method of Lelah et al.**[11, 12]**.Contact angles were sectioned into 3 pieces using 2 parallel cuts with a clean razor blade. The central curved section was super-glued on the anterior surface upside down to a polypropylene lens mole (female half) also cut in thirds and immersed in distilled deionized water (DD H_2O). In separate experiments, 2 µL volumes of air or 4 µL volumes of octane were injected with a Hamilton syringe and captured by posterior side of the curved lens. The contact angle was measured at 22°C using a Rame-Hart goniometer (Netcong, NJ). At least 6 angles were measured on each lens, and the average and 95% confidence intervals were calculated.

Modulus. These experiments were performed by personnel at CIBA VISION. The modulus of thecontact lens material before and after DMPC loading was measured by cutting a 2.90 mm-wide strip from the center of the lens. Each end of the lens strip was placed in each of the sample grips of a Vitrodyne V1000 mechanical tester (Liveco Inc, Burlington, VT). The sample was submerged in phosphate buffered saline (PBS) and stretched at a rate of 200 mm/sec. Stress versus strain was measured, from which the modulus was calculated.

Clarity. These experiments were also performed by personnel at CIBA VISION. Light transmissionat 610 nm was used to measure the visual clarity of the lenses. Two matching cuvettes containing phosphate buffered saline (PBS) were placed in both the reference and sample positions of a VARIAN Cary UV-Vis spectrophotometer. After the instrument was zeroed, the sample lens was placed in the cuvette in the sample position, ensuring consistent placement and orientation of the lens within the cuvette. The percent transmission was measured.

Commercial extraction. To eliminate the monomer and n-propanol residual during the polymerization process, a lens was extracted in a series of solutions for a certain amount of time. At the end, 100 μL of each extraction solution were added to the 10 mL of scintillation fluid to count the amount of DMPC extracted.

.

4 LOADING AND RELEASE OF DMPC

4.1 Rationale and objectives

Tear film disruption has been directly linked to deficiencies in the phospholipid portion of the lipid layer **[19-22]**. Phospholipids reside at the interface between the hydrophobic lipid layer and the aqueous layer, and they stabilize this interface via their amphiphilic nature.Without sufficient phospholipid, the oily lipid layer cannot spread evenly over the aqueous layer, leading to tear film disruption.

A novel approach to dry eye is to use the contact lens itself as the depot for the required phospholipid. Phospholipids can be loaded into the lens prior to insertion, and then the phospholipid can slowly diffuse into the eye during the course of the daily wearing period, providing a constant source of supplemental phospholipid. In this study, we loaded DMPC for 30, 60 and 120 seconds, eluted lenses in ATF+ or ATF- solution, and extracted them in n-propanolby following the procedures of Section 3.1 to verify that 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) can be loaded into silicone hydrogel contact lenses, and then slowly released from the lens into water or an ATF. The light transmission, contact angles and moduli were also measured.

4.2 **Results**

Table 4.1 shows the mean average amount of DMPC that was loaded into the lenses during a 60 second dip in 0.15% w/v of DMPC in *n*-propanol. Four lenses were loaded and then immediately extracted to determine the total loading. These lenses were extracted 3 times, and the amounts from each extraction summed to obtain a total of around 33 µg/lens (see Table 4.1). The amount extracted in the third extraction was near zero and in the noise of the background counts, so in subsequent extractions of eluted lenses, only 2 extractions were performed.

Table 4-1 DMPC Loading Data

	Mean Loading	n	95% CI
Extraction only	33.1	4	±0.9
Elution then extraction	32.8	7	±0.7
All lenses	32.9	11	±0.5

Seven lenses that were eluted in ATF or DDH_2O for 72 hours were extracted (twice) at the conclusion of elution. The amounts of DMPC extracted were added to the amounts of DMPC eluted, and the totals summed to near 33 µg/lens. Because there was no statistical difference ($p > 0.2$, 2-sided t-test), the data from both sets of lenses were combined to obtain an average loading of 32.9 ± 0.5 µg/lens (mean and 95% confidence interval) for 60 seconds of loading at 35°C.

Figure 4.1 shows the mass of DMPC loaded at 30, 60 and 120 seconds of sorption, the mean average mass values being 23.7, 33.7 and 55.2 µg per lens, respectively. Surprisingly these data have a linear distribution within the range studied; however, an extrapolation of the line does not pass through zero, so it is not recommended to extrapolate outside this range.

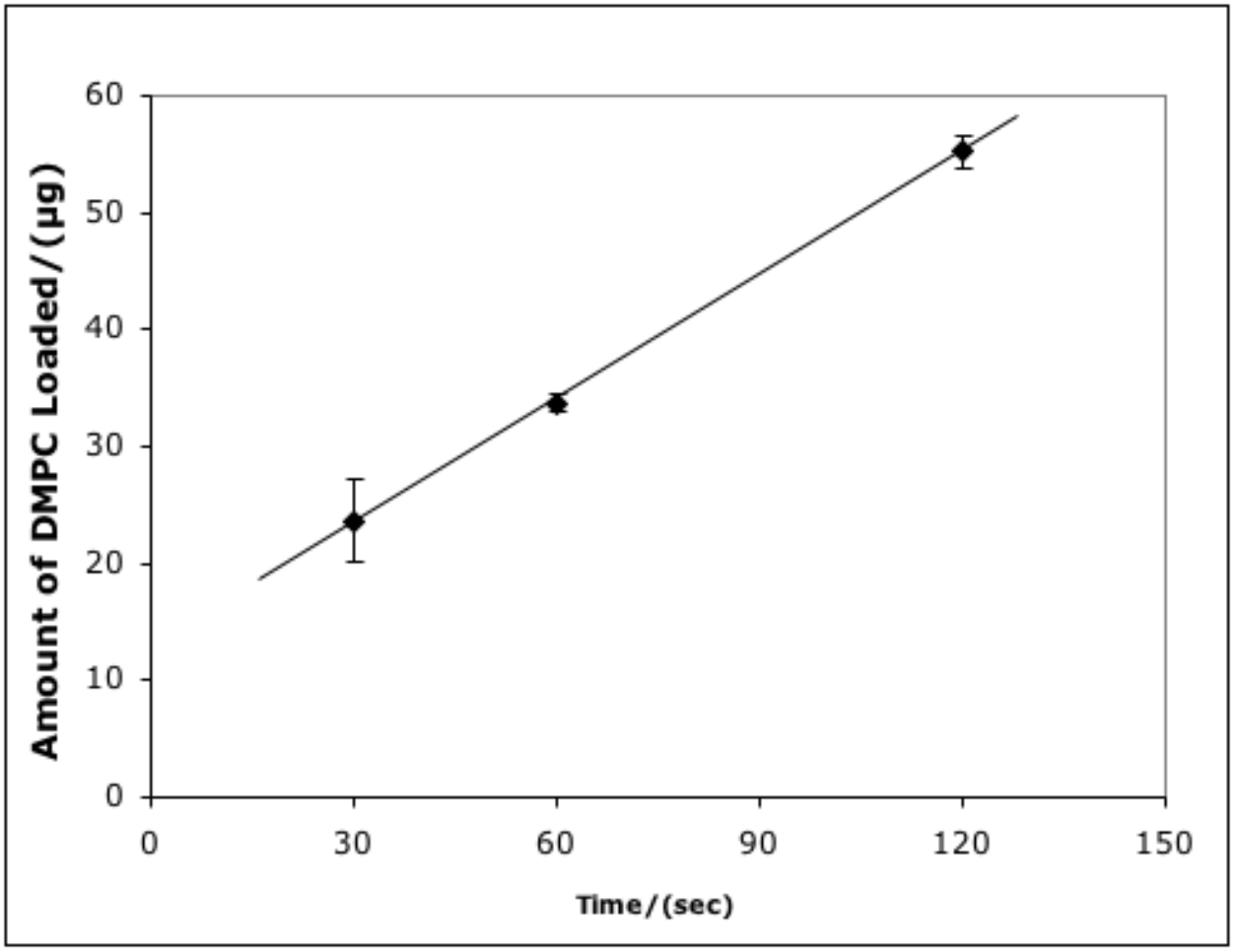


Figure 4-1. Amount of DMPC loaded per lens vs loading time from a solution of 0.15% DMPC in n-propanol. Data are the mean average and 95% confidence intervals.

Contact angle. Table 4.2 shows the underwater air and octane contact angles, and the calculated values of the polar and dispersive components of the surface energy using the geometric mean approximation of Fowkes **[23]**.Although there is some difference in the contact angles as DMPC was added, the polar components of surface energy (γ_p) remain high and the dispersive components (γ_d) remain low, indicating that the loaded lenses still have excellent wettability. There is no trend toward decreasing wettability as the loading increases; in fact the

lens loaded for 120 seconds has the highest polar and least dispersive surface energy components by this analysis.

Table 4-2 Some Properties of DMPC-Loaded Contact Lenses

Loading Time (sec)	Amount DMPC Loaded (μg)	Light Transmission[1] (%)	Modulus[2] (MPa)	Air C.A.[3] (°)	Octane C.A.[3] (°)	γ_d (dyn/cm)	γ_p (dyn/cm)
0	0	95.74 ± 0.21	0.71 ± 0.03	40.3 ± 1.4	38.7 ± 1.2	6.3	38.2
30	23.7	95.82 ± 0.26	0.72 ± 0.03	42.2 ± 1.7	38.3 ± 2.0	5.6	38.4
60	33.7	95.89 ± 0.19	0.70 ± 0.02	41.7 ± 2.1	38.0 ± 1.7	5.7	38.6
120	55.2	95.79 ± 0.28	0.70 ± 0.03	44.3 ± 1.2	36.7 ± 1.2	4.6	39.4

1 Mean and standard deviation, $n = 20$.

2 Mean and standard deviation, $n = 10$.

3 Mean and standard deviation, $n \geq 6$.

Elution into ATF. Figure 4.2A shows the elution of DMPC from lenses loaded at various amounts into pure ATF+. As expected, lenses loaded with more DMPC eluted more DMPC into the ATF+. A statistical analysis (paired comparison) shows that elution of lenses loaded for 120 sec is greater than that of the other two loadings ($p < 0.05$).

A

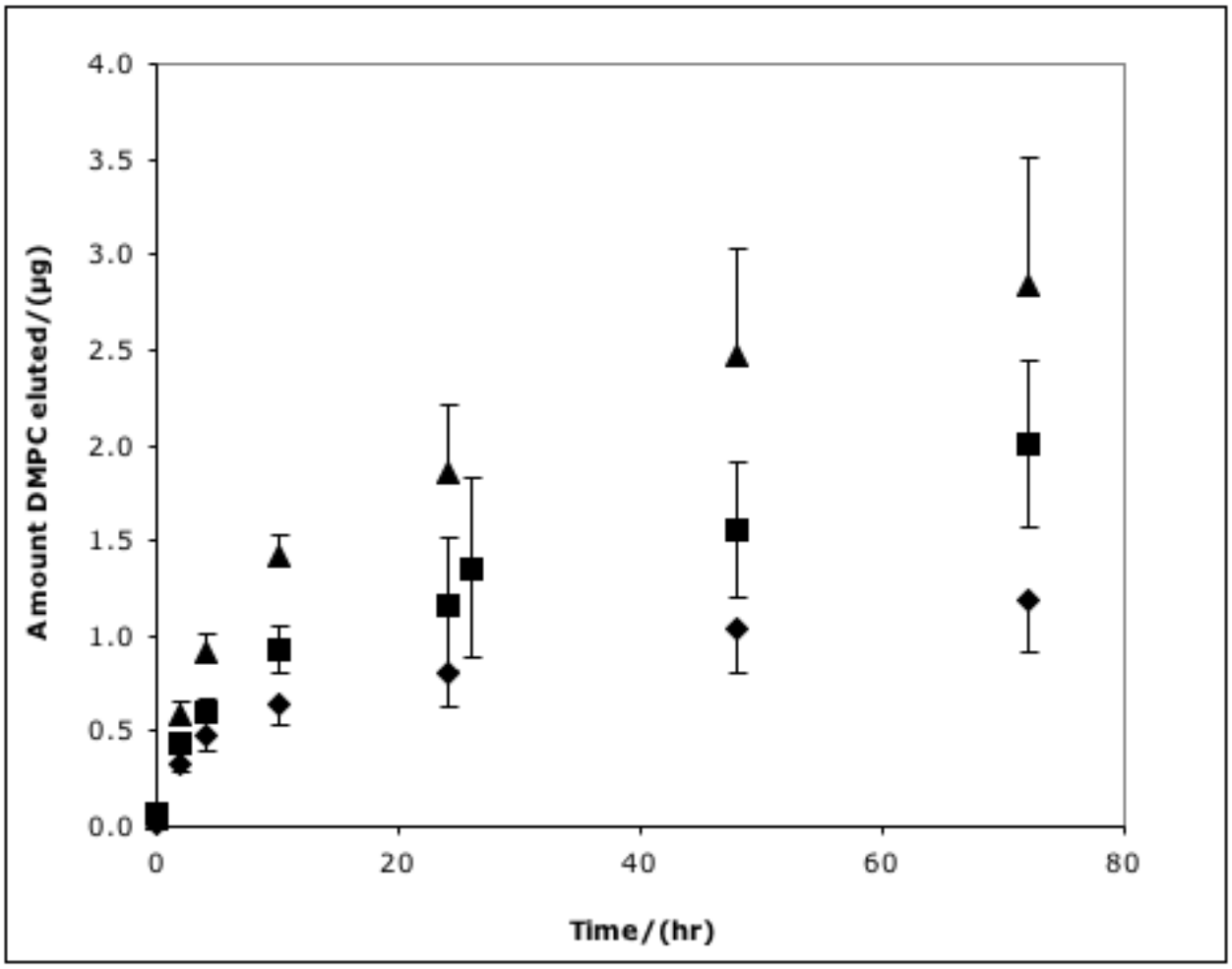


Figure 4-2. Amount of DMPC eluted from contact lenses loaded for varying times. (♦) Lenses loaded for 30 s with 23.7 µg DMPC. (■) Lenses loaded for 60 s with 33.7 µg DMPC. (▲) Lenses loaded for 120 s with 55.2 µg DMPC. Panel A: elution vs time. Panel B. Elution vs the square root of time (in hours). Panel C: fraction of elution vs time. Error bars are shown on one side only for clarity.

B

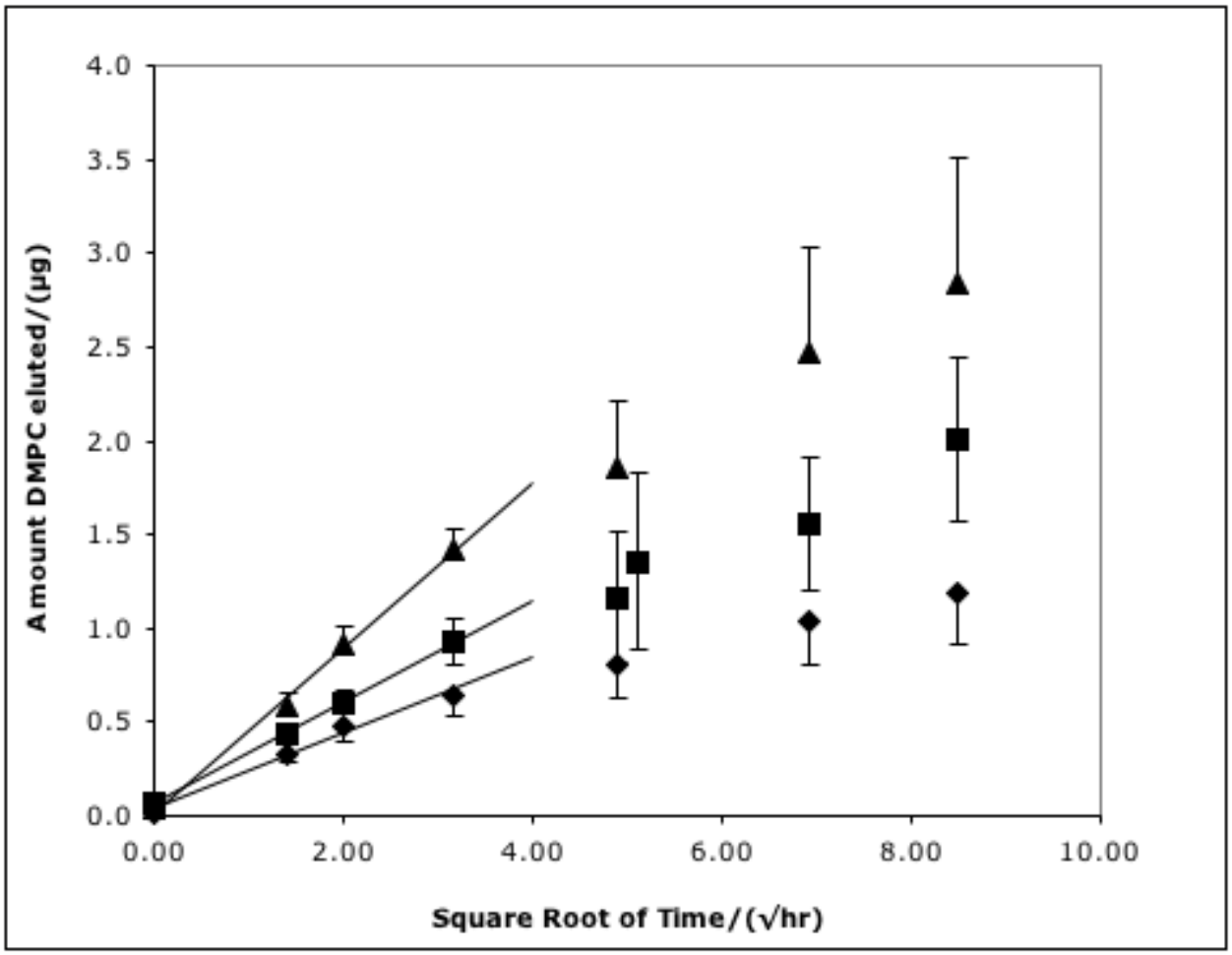


Figure 4-2 continued

C

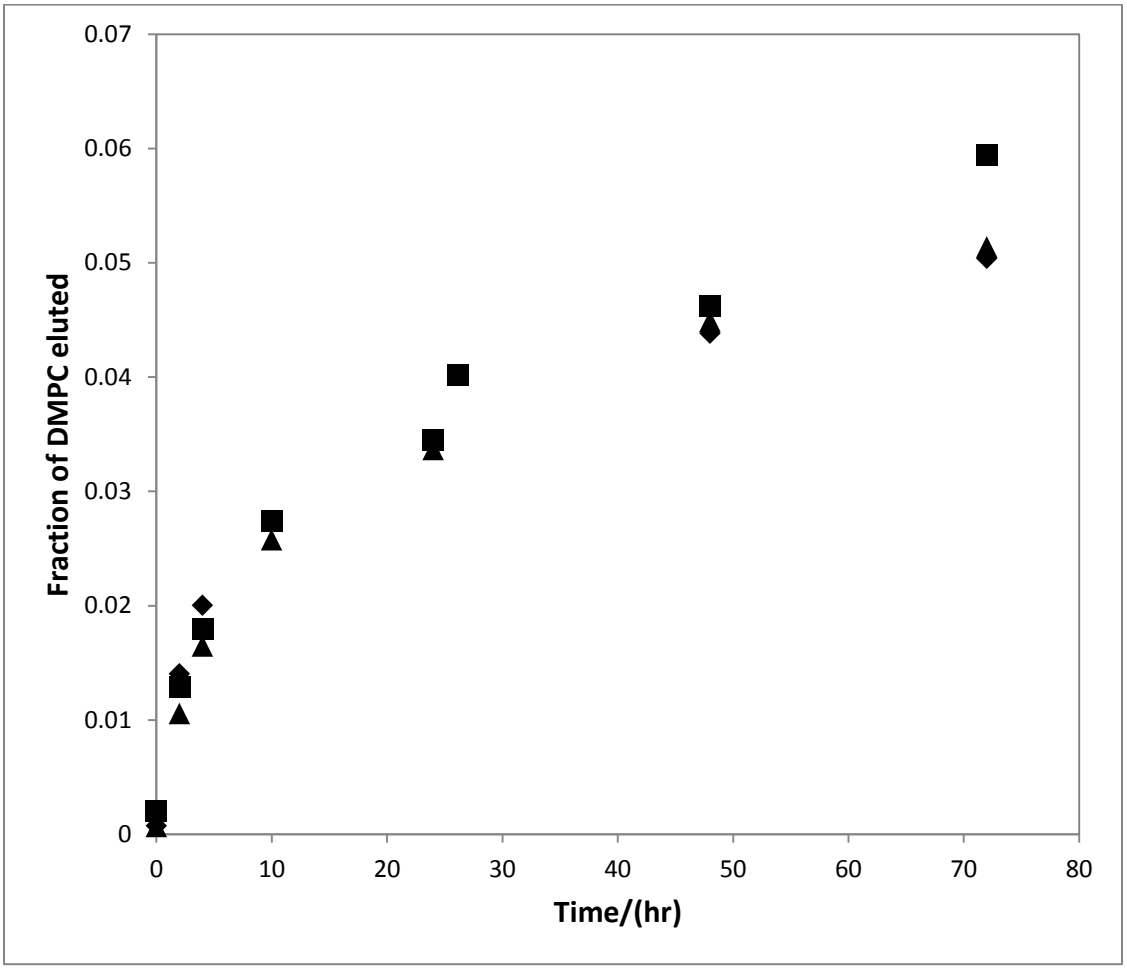


Figure 4-2 continued

The initial release of the drug is characterized by a very slight "burst" manifested by a non-zero amount of DMPC when the elution solution was sampled immediately (2 to 5 seconds) after the lens was placed in the elution vial. The subsequent elution is linear when plotted against the square root of time for about the first 10 hours (see Fig 4.2B.)

Such behavior is consistent with, but not absolute proof of diffusion controlled release from the lens. A simplified model for diffusion-controlled release of DMPC is given by

$$M(t) = 2A\sqrt{\frac{Dt}{\pi}}\left(C_O - C_S\right) + M_O \qquad (4.1)$$

where $M(t)$ is the amount of DMPC eluted at time t, A is the surface area of the lens (both sides), D is the diffusivity of DMPC in the lens, M_O is any amount released in a burst at time zero, and C_S is the concentration of the component at the surface of the monolith. The data of Figure 4.2B indicate that elution intoATF+ adequately fit the model given by Eqn (4.1) for the first 10 hours of elution. The slope of each linear regression line in Figure 4.2B equals $2A\sqrt{\frac{D}{\pi}}\left(C_O - C_S\right)$, which value of the slope will be identified as $S_{t½}$. Employing Eqn (4.1) and assuming that the diffusivity of DMPC within the lens is independent of concentration, the slope of the data when plotted as in Figure 4.2B is proportional to the driving force $\left(C_O - C_S\right)$. Thus Figure 4.3presents the data as a plot of the slope $S_{t½}$ versus the amount of DMPC loaded, which correlation appears to be directly proportional to C_O. The linearity of the data in Figure 4.3 suggests that Eqn (4.1) is valid and that the values of C_S are close to zero at short times. Indeed, the ATF+ formulation contains very little DMPC and no radiolabeled DMPC.

Also plotted in Figure 4.3 is the average amount of elution during the first 10 hours. Again, this data is very linear and suggests that the average rate of elution is proportional to the amount of DMPC loaded into the lens.

Beyond 10 hours, the elution rate slows to values less than predicted by Eqn (4.1). Since this equation is only valid at short times, it could be that the deviation in the data from the "short time" model is caused by the inadequacy of the model at longer times.

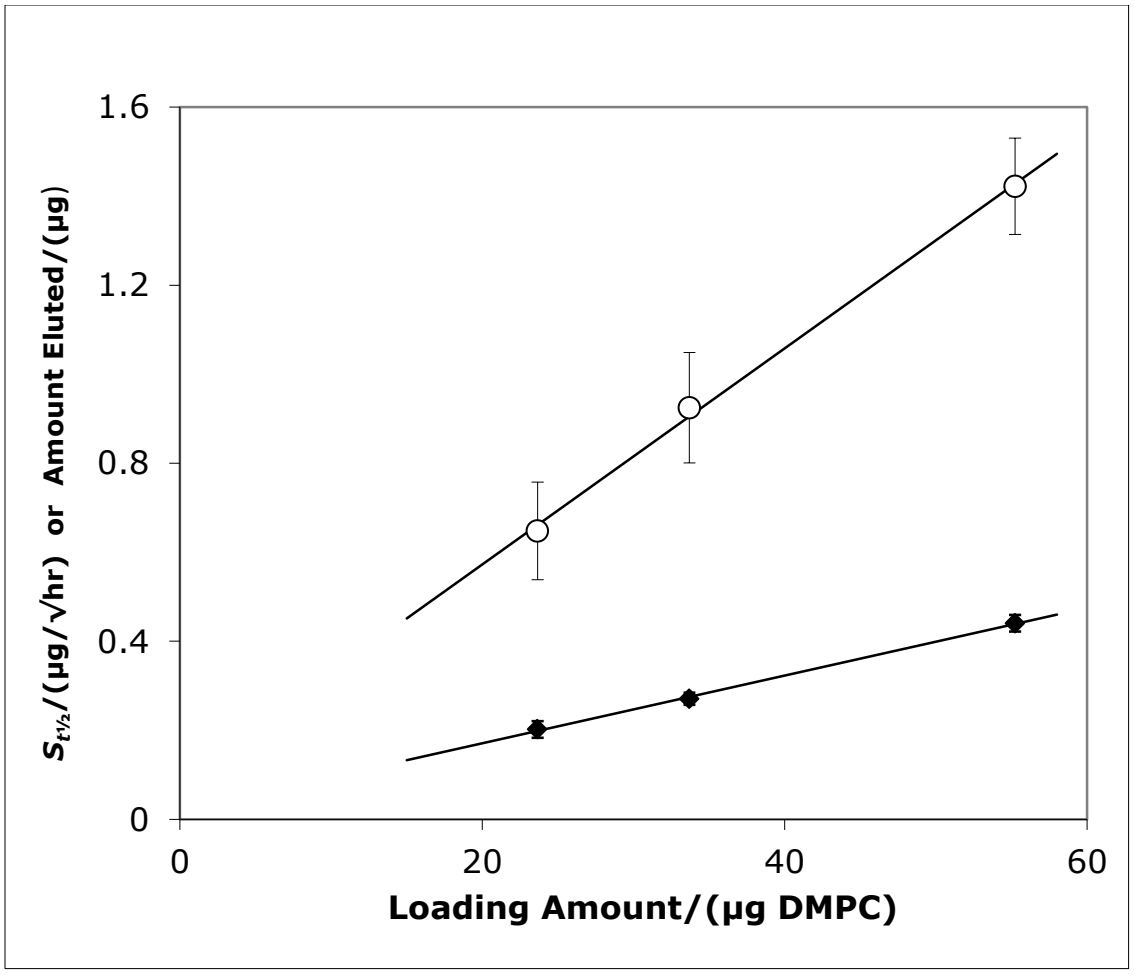


Figure 4-3 Estimates of elution rates from lenses loaded with various amounts of DMPC. (○) Estimates of St½. (♦) Average amount of DMPC elution during the first 10 hours. Error bars represent the 95% confidence intervals.

To investigate this deviation, and to determine if the release of DMPC remains diffusion-controlled, we modeled the diffusion of DMPC as classic 1-D diffusion from a finite slab;the concentration profile inside the slab is given by

$$\frac{C_S - C}{C_S - C_0} = 2 * \sum_0^\infty \frac{(-1)^n}{(n+0.5)\pi} exp\left(\frac{-(n+0.5)^2\pi^2 D_{AB} t}{b^2}\right) cos((n+0.5)\frac{\pi y}{b} \qquad (4.2)$$

Where C_S is the concentration of the component at the surface of the monolith, C_0 is the initial concentration of the DMPC, b is the half- thickness of the lens, D_{AB} is the diffusivity of DMPC in the lens, t is the releasing time, and y is the position of diffusion occurs. We took the derivative of C with respect to the diffusion direction "y" and evaluated the flux at the surface of the lens, y=b, which is as follows

$$Flux = \frac{dC}{dy}\Big|_{y=b} = 2(C_s - C_0)\sum_0^\infty \frac{(-1)^n}{\text{b}} exp\left(\frac{-(n+0.5)^2\pi^2 D_{AB}t}{b^2}\right) sin((n+0.5)\frac{\pi y}{b})\Big|_{y=b} \quad (4.3)$$

In order to find the mass of DMPC released by diffusion, we integrated the flux of Equation 4.3 with respect to time, and multiplied by the contact lens surface area A, which is shown as follows

$$Release = \int_0^t flux\, dt = \int_0^t \frac{dC}{dy}\Big|_{y=b} dt = 2(C_0 - C_s)\sum_0^\infty \frac{(-1)^n}{\text{b}} exp\left(\frac{-(n+0.5)^2\pi^2 D_{AB}t}{b^2}\right) sin((n+$$

$$0.5)\pi)\frac{b^2}{(n+0.5)^2\pi^2 D_{AB}} - \frac{(-1)^n}{\text{b}}(sin((n+0.5)\pi)\frac{b^2}{(n+0.5)^2\pi^2 D_{AB}} \quad (4.4)$$

$$Mass\ of\ DMPC\ diffused = A\int_0^t \frac{dC}{dy}\Big|_{y=b} dt \quad (4.5)$$

We calculated and verified that the result would converge before n=20, so we generated Figure 4.4 using Equation 4.5 with n=20, which compares the amount of DMPC released in the experiment to the mathematical model.

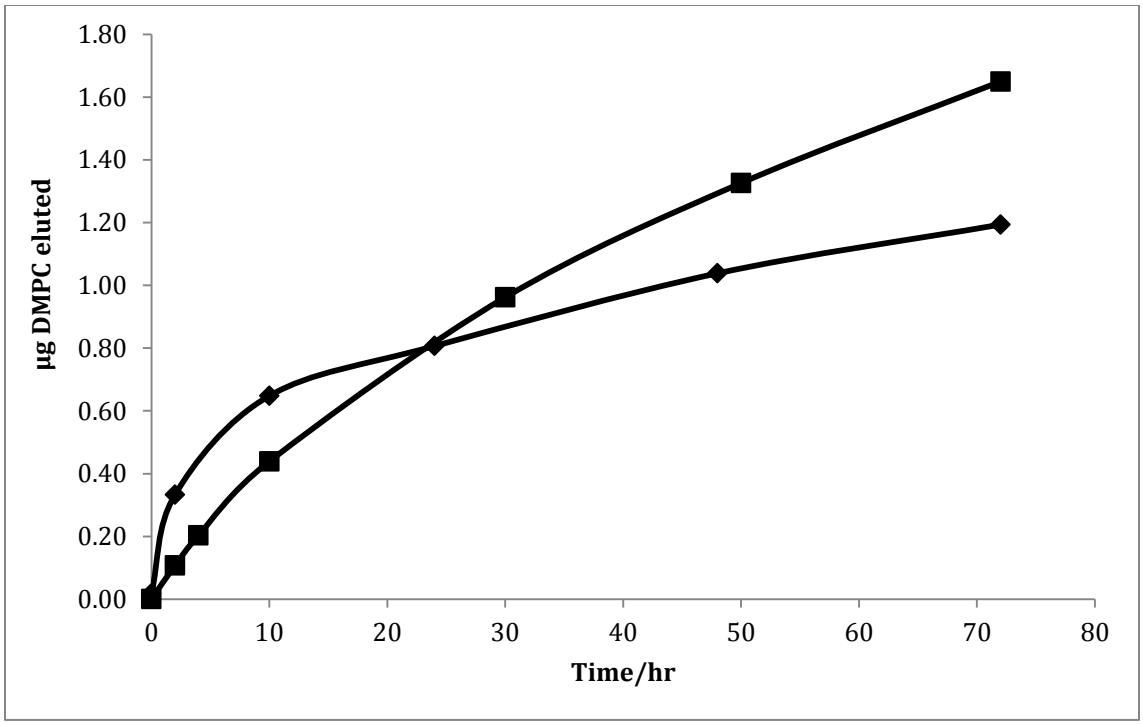


Figure 4-4. Elution of DMPC from lenses into ATF. (■) elution based on finite slab model, (♦) elution based on lenses loaded for 30s with 23.7 µg DMPC.

Elution into ATF spiked with DMPC. The poor agreement of the experimental data with the theoretical diffusion model of Eqn (4.5) suggests that something is causing the elution to slow down, as if there was not an infinite sink at the surface. Perhaps the parameter C_S, the concentration of DMPC at the lens surface, is in equilibrium with DMPC in the eluent, which increases with time. Therefore we conducted several experiments in which the amount of radiolabeled DMPC in solution was varied in order to vary the parameterC_S of the model of Eqn 4.1. Figure 4.5 shows the results of the elution when each lens was loaded for 60 seconds to an average amount of 33.7 µg, and then eluted in ATF- containing (spiked with) various amounts of radiolabeled DMPC at time zero. We have also included in this plot the elution into ATF+, which contains generic hen egg phospholipids, phosphatidylethanolamines and phosphatidylcholines at concentrations of 0.5 and 1.1 µg/mL respectively, although none were

radiolabeled. Figure 4.5B shows that the elution appears to follow a diffusion-controlled process over the first 10 hours, as observed previously. Release is faster into solutions with little or no DMPC than into ATF+ or ATF- spiked with 1.33 µg/mL DMPC.

A

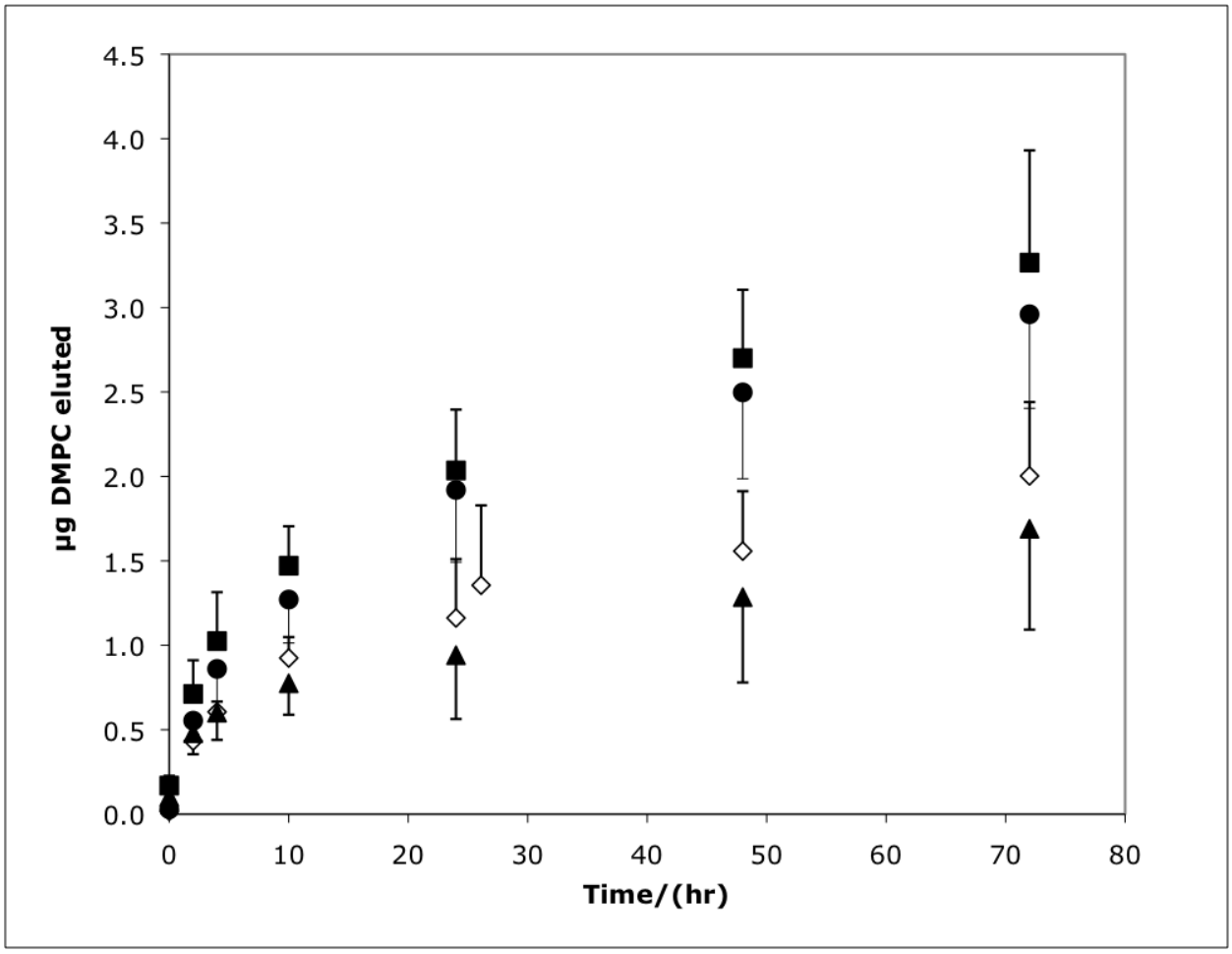


Figure 4-5. Elution of DMPC from lenses into ATF that is preloaded with various amounts of DMPC. (◊) Elution into regular ATF+ initially containing no radiolabled DMPC, but that contains 1.6 µg/mL other phospholipids. (●) Elution into ATF- initially containing no DMPC. (■) elution into ATF-intially containing 0.667 µg/mL DMPC. (▲) Elution into ATF-intially containing 1.333 µg/mL DMPC. Panel A: elution vs time. Panel B. Elution vs the square root of time (in hours). Error bars represent the magnitude of the 95% confidence intervals (n≥4).

B

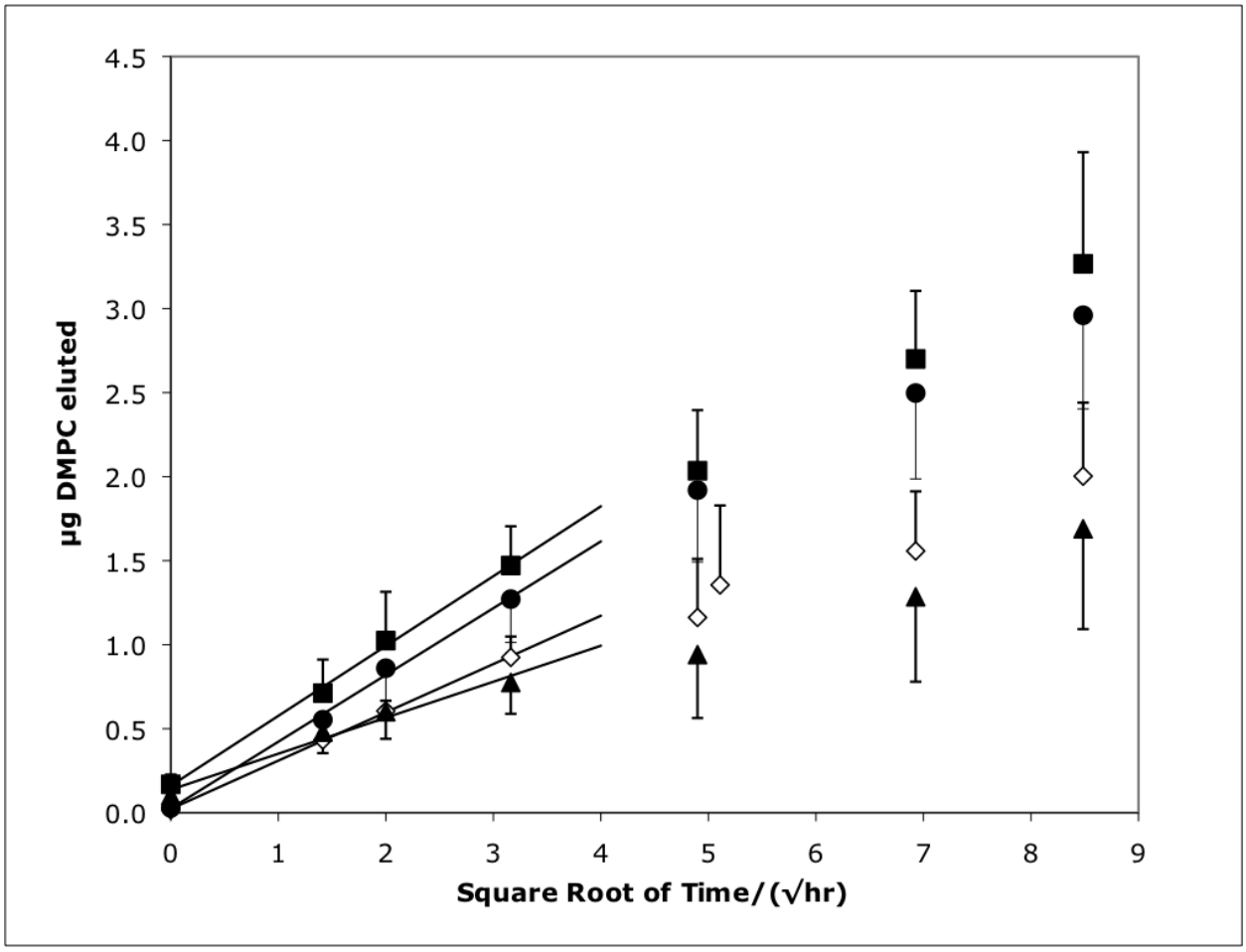


Figure 4-5 continued

4.3 **Discussion**

We have used ^{14}C-labeled DMPC to show that silicone hydrogels can absorb and deliver a phospholipid to an artificial tear solution over a wide range of loading conditions. Especially significant is the observation that adding the DMPC does not appear to change the physical properties of the lenses, including the clarity, modulus or wettability.

The amount of DMPC that is loaded into the lens can be controlled by manipulating the time of exposure to *n*-propanol containing the phospholipid. Most interestingly, there is a linear increase in loading as the exposure time varies between 30 and 120 seconds, and yet extrapolation of the observed uptake to zero time does not predict a zero amount of absorption. Although this linear fit is convenient for designing the loading of DMPC, it would not be expected based on diffusive transport physics. If one assumes that uptake is based solely on diffusion of the DMPC into the lens, the rate of uptake should decrease as loading time increases, and the total adsorbed should not be linear with time. Furthermore we do not believe that the uptake is by diffusion alone since the contact lens swells for the first few seconds upon exposure to the *n*-propanol. Thus we expect that there is some convection of DMPC into the lens as the alcohol solution is imbibed into the polymer matrix. We postulate that the observed linearity between exposure time and mass uptake between 30 and 120 seconds is fortuitous, and do not recommend extrapolation beyond this range.

As described above, the elution of DMPC from the lenses into ATF+ appears to be consistent with diffusion controlled transport of DMPC within the lens matrix during the first 10 hrs of elution, as evidenced by the linear data of Fig 4.2B. Deviations from this model beyond about 10 hrs could be attributed to several possible factors, including a non-uniform initial distribution of DMPC in the lens, the non-constant diffusive driving force as DMPC builds up in the elution solution (C_S is not constant), or other factors. We examined the role of varying driving forces by spiking the elution solution with various amounts of radiolabeled DMPC.

By examining the first 10 hours of elution into ATF+, we tested the validity of Eqn (4.1). In the experiments of Fig 4.2, the amount of radiolabeled DMPC initially in the elution solution was zero, so we can assume that initially the value of C_S in Eqn 4.1 was zero, and remained low

during the first few hours of elution. Thus the values $S_{t½}$ (the initial slopes in Fib 4.2B) should be directly proportional to the initial DMPC concentration in the lens. If we calculate the average elution rate, averaged over the first 10 hours, these rates also are directly proportional to the lens loading. These observations further support the validity of Eqn (1) during the first 10 hrs of elution into ATF+. The average thickness of these lenses is 103 μm. From this value, Eq 4.1, and the slope of the lower line in Fig 4.2B (0.0076 $hr^{-½}$), we estimated the diffusivity of DMPC in these lenses to be 1.34 x 10^{-12} cm^2/s. This should be considered a lower limit and is based on the assumption that a sink condition exists during the experiment ($C_s = 0$). If this assumption were not correct, then the actual diffusivity would be a larger value.

The finite slab model used to verify the validation of assuming diffusion control shows that it is valid to make such an assumption only at very short times. Even though there are deviations between the two curves shown in Fig. 4.4, the trend of both curves is the same, Also, in setting up the theoretical model, we assumed the shape of the contact lens is a slab, and ignored the curvature effect. Such assumption is valid only when the thickness is very small compare to the lens diameter. In these contact lenses, the thickness-diameter ratio is 0.00069, so this would have introduced only a small error, probably less that 5%. Thus we conclude that the main deviation of the experimental data from a theoretical diffusion model is that the value of C_s (surface concentration) probably does not remain at zero during the experiment.

5 AUTOCLAVING AND TEMPERATURE EFFECT

5.1 Rationale and objectives

In the last chapter we have shown that an experimental silicone hydrogel contact lens can be easily loaded with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) to various concentrations. The DMPC can be slowly released into an artificial tear solution at a rate proportional to the amount loaded. To determine if this lens has potential for commercialization, it is necessary to determine if the autoclaving process adversely reduces the DMPC concentration or reduces the release rate. In these experiments we investigated the effect of autoclave processing time and temperature on DMPC elution in a study employing using 2 autoclaving temperatures (122°C and 129°C) and three processing times (30 mins, 60 mins and 90 mins). We also conducted related experiments to examine the effect of temperature on the elution process.

The procedures used were basically the same as in the last chapter, and are described in section 3.1. After loading, the lenses were autoclaved under various temperatures, and then they were eluted and extracted by the standard procedure. The liquid in the vials following autoclaving was sampled to determine the amount of DMPC lost from lenses during the autoclaving process.

5.2 **Results**

Autoclave effect. The saline solution surrounding the lens was sampled immediately following autoclaving and cooling to measure the amount of DMPC released during this procedure. Figure 5.1 shows that the amount of DMPC released from lenses at 122°C increases almost linearly with the autoclaving time. Since the lenses are loaded with 33 µg DMPC, a 30 minute processing time extracted about 3% of the DMPC while a 90 minute process extracted nearly 10%. When the temperature was increased to 129°C, the amount extracted was greater than at 122°C, but was nearly independent of processing time. More than 15% of the loaded DMPC was extracted at this higher temperature.

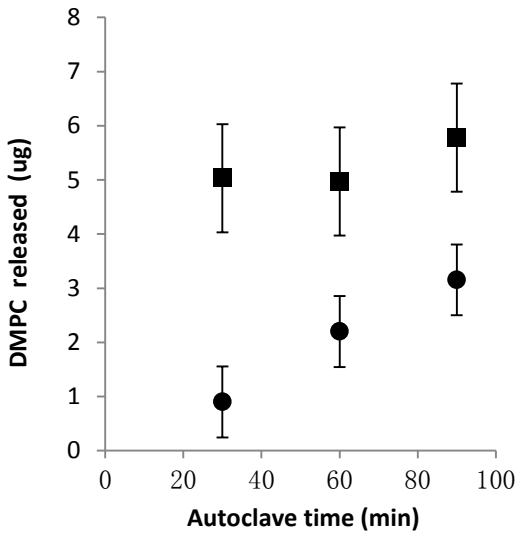


Figure 5-1. Mass of DMPC released during autoclaving as a function of time at 2 temperatures. (●) 122°C, (■) 129°C. Error bars indicate the standard deviation of the DMPC released(n=4).

Subsequent Elution of DMPC. The amount of DMPC released during 72 hours of elution is shown in Figure 5.2, and is plotted versus the square root of time. When plotted in this

manner, a linear correlation of the data suggests that the elution is diffusion controlled, as discussed in the previous chapter**[18]**.

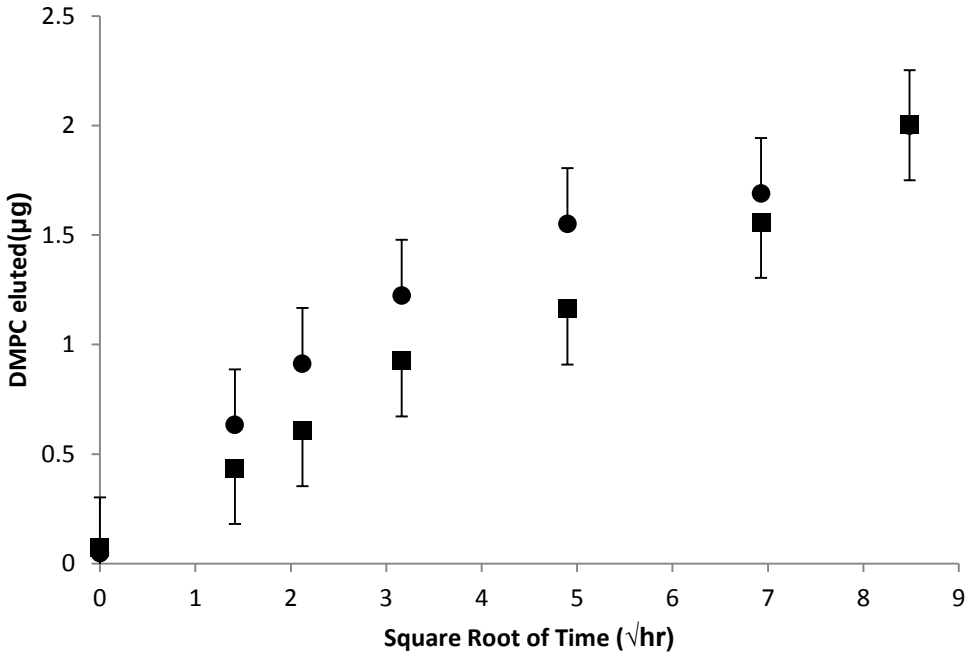


Figure 5-2. Average mass (n≥4) of DMPC eluted after 72 hours as function of time at 35°C under two conditions, (●) with autoclaving at 129°C, 90 min, (■) without autoclaving. Error bars represent the magnitude of the standard deviation. They are placed on one side only for clarity of presentation.

Note that this figure shows the DMPC kinetics under two conditions; one is with autoclaving at 129°C with 90 minutes processing time, the other one is without any autoclaving. Among all 6 autoclaving conditions, the conditions associated with Figure 5.2 were expected to have the maximum influence on DMPC release, if there is any. Therefore, we chose this one as an illustrative comparison; the data indicate that the DMPC elution of the maximally autoclaved lenses is very similar to the elution of non-autoclaved lenses, even though there was less DMPC in the lens following autoclaving. The data from autoclaving at other conditions also showed

similar results in that there was barely a significant statistical difference from their slopes (p=0.032) in elution from autoclaved lenses; another example is shown in Figure 5.3. In these experiments and those reported previously, the data during the first 10 hrs of elution appear to be linear when plotted against the square root of time**[18]**.

Figure 5.3 compares the DMPC elution under minimal autoclaving (122°C, 30 min) to that with no autoclaving. By doing the T-test on their slope and compare their P-value, the elutions are almost the same (p=0.037). This data suggests that autoclaving does not significantly decrease the rate of elution of DMPC from the lenses, even though there may be slightly less DMPC remaining inside the lens following autoclaving. In fact, it appears that autoclaving slightly increases the elution rate at short times.

Figure 5.4 shows a statistical comparison of the data of initial DMPC release. Figure 5.4A presents the $S_{t½}$values (initial slopes when plotted versus the square root of time), and Figure 5.4B presents the amount of DMPC eluted in the first 10 hours. Again, with the statistical t-tests of the slopes and the comparison of their p-values, it shows that there is faster (5.4A) and more (5.4B) elution than the control without autoclaving. However there are no statistically significant differences between the three sets of autoclaved lenses.

Table 5.1 was created to summarize the slopes when plotted versus the square root of time at each condition

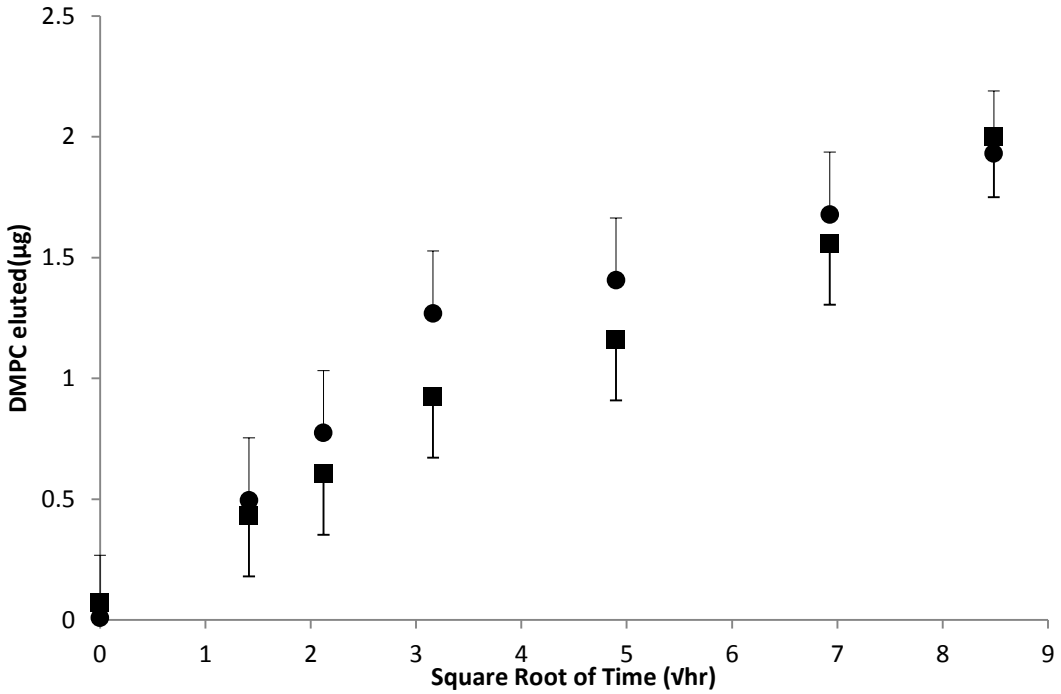


Figure 5-3. Average mass (n≥4) of DMPC eluted after 72 hours as function of square root of time at 35°C under two conditions, (●) with autoclaving at 122°C, 30 min, (■)without autoclaving. Error bar indicates the standard deviation. They are placed on one side only for clarity of presentation.

.

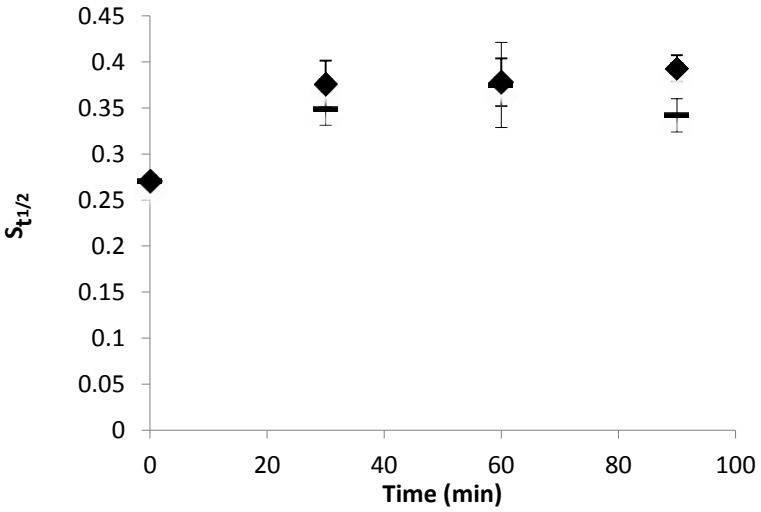


Figure 5-4A. Slope of DMPC released during the first 10 hours of elution as a function of the square root of time at 2 temperatures. (—) 122°C, (♦) 129°C. Error bars indicate the standard deviation of the slope(n=4).

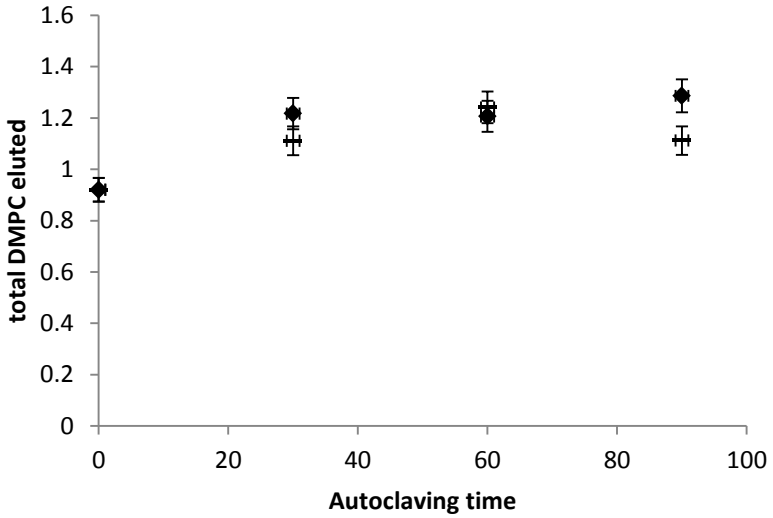


Figure 5-4B. Total Average mass (n≥4) of DMPC eluted during the first 10 hours of elution as a function of time at 2 temperatures. (—) 122°C, (♦) 129°C. Error bars indicate the 95% confidence interval (n=4).

Table 5-1. Values of $S_{t½}$ Having Units of $\frac{\mu g}{\sqrt{hr}}$

Time (mins)	122°C	129°C
0	0.271	0.271
30	0.349	0.376
60	0.375	0.378
90	0.342	0.393

5.3 **Temperature dependence of DMPC release**

As was mentioned, the initial rate of elution is fairly linear when plotted against the square root of time for about the first 10 hours. In the previous chapter we proposed that when plotted this way, the slope was related to the diffusivity of DMPC in the contact lens **[24].** In a phospholipid bilayer, DMPC has a solid-liquid-like transition temperature, T_m, of 24°C **[8].** which is below eye temperature. Above this T_m,the self-diffusivity of DMPC in a lipid layer is much higher than below the T_m**[24]**. We hypothesized that elution at a temperature below 24°C might produce a much slower rate of elution if DMPC has long-range self-association in the lens elution because the rate of DMPC diffusion would be slower at lower temperature. To test this hypothesis we loaded lenses with 33µg DMPC and eluted into ATF at temperatures of 20°C, 10°C, 5°C and 1°C. These data sets all had linear slopes in the first 10 hrs when plotted versus the square root of time, and these slopes decreased with decreasing temperature, indicating that indeed, diffusional transport was slowing, as a function of the lower elution temperature. The values of $S_{t½}$are plotted in Figure 5.5.

This data shows that there is a major difference in diffusive transport between 35° and 20°C, and another large decrease between 1° and 5°C. This suggests that there is a major transition near the Tm of DMPC in phospholipid bilayers, and that the DMPC may exist in bilayers in the silicone hydrogel contact lenses of this study. However, there is much more than this occurring as indicated by the even larger change in transport rate between 1° and 5°C.

An analysis of the data of $S_{t½}$ versus temperature can also reveal the activation energy for diffusion within the contact lens. A simplified model of the temperature dependence of binary diffusion is commonly accepted to be $S_{t½}=A\exp(-E/RT)$, where A is a constant, R is the gas constant, and E is the activation energy for diffusion. From the experimental data between 5°C

and 20°C, this activation energy was calculated from the $S_{t½}$values and was found to be 11.9 kilojoules per mole.

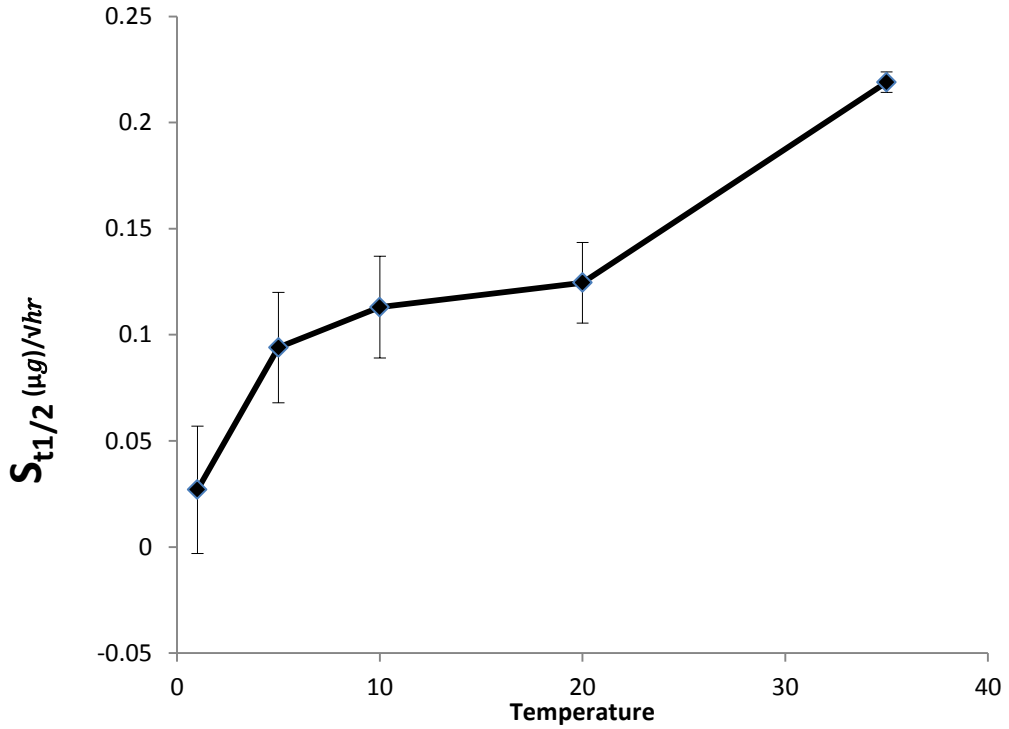


Figure 5-5. Values of $S_{t½}$ at 1°C, 5°C, 10°C, 20°C and 35°C. Error bars indicate the standard deviation of the slope (the $S_{t½}$ value)(n=4).

5.4 **Discussion**

Silicone hydrogel contact lenses were loaded with DMPC and then autoclaved to determine if the autoclave procedure causes loss of DMPC or inhibition of release of DMPC. We observed that during autoclaving, up to 30% of the loaded DMPC was eluted. When autoclaving at 122°C, the amount eluted increased with processing time, as would be expected. When processing at a higher temperature (129°C), the amount of elution was greater than at 122°C, but appeared to be independent of the processing time. About 6 µg of DMPC was released at the

higher temperature. I do not have any clear evidence of why this might occur, but I will speculate that about 6 µg DMPC might represent the equilibrium partitioning of DMPC between the silicone hydrogel and the PBS solution in the vial under autoclaving conditions. Given that the volume of water in the vial is 2 mL, and the volume of the contact len is about 0.031 mL, such an equilibrium would represent a partition coefficient of 0.01 at 129°C. Autoclaving at 122°C might eventually achieve the same level of elution, given enough elution time. These issues remain to be addressed in future research.

However, even with the loss of some loaded DMPC during autoclaving, the subsequent rate of elution into ATF at 35°C was not decreased compared to non-autoclaved lenses. In fact, the initial rate of elution was actually increased in the first 10 hrs; but by 72 hrs, the total amount eluted was about the same, with or without autoclaving. This observation has great positive impact for the potential of these contact lenses to be employed commercially. The data suggest that they can be loaded with DMPC, packaged and sterilized without loss of any "comfort" effect due to the autoclaving procedure. DMPC will still elute, and at short times in even greater amounts than without autoclaving.

In the study of non-autoclaved lenses eluted at various temperature, we observed a transition in elution rate bewteen 20° and 35° and between 1° and 5°. Initially in these sudies, we selected the phospholipid DMPC because it had a diffusion transition temperature in a lipid bilayer of 24°C. This temperature is lower than eye temperature but is greater than typical storage temperatures in a warehouse, clinic or home. Thus elution would be slow or negligible during storage and faster once the user inserted the contact lens into the eye at 35°C. Apparently the transition temperature within the lens is close to the transition temperature DMPC bilayers from literature.

This study further promotes the feasibility of loading a phospholipid into a silicone hydrogel contact lenses. The necessary step of lens sterilization can be accompolished without the loss of more than 15% of the loaded DMPC. Even with this much DMPC loss, the subsequent elution into ATF is not compromized by the autoclaving procedure.The DMPC is still available and elutable to provide additional phospholipds that may provide relief of eye irritation caused by lack of phospholipid.

6 EXTENDED ELUTION OF PHOSPHOLIPID FROM SILICON HYDROGEL CONTACT LENSES

6.1 Rationale and objectives

We have shown that the lenses can be easily loaded and that delivery of the DMPC to artificial tears *in vitro* is apparently governed at short times by diffusion of phospholipids from the lens. The experiments of previous chapters only examined 72 hrs of delivery in lenses that were designed for single use daily wear. About 1 μg of DCPC was delivered from those lenses during the first 10 hours of simulated wear. Thus a further objective in our research was to determine if a similar release of DMPC can be accomplished from an extended wear lens, and if the release can be sustained for up to 30 days. Obviously the lens would need to be loaded with more DMPC, and thus a corollary question arises of whether a lens can be loaded sufficiently high to provide 30 days of sustained phospholipid release without compromising visual clarity.

The lenses in this study were designed to be evaluated as extended wear lenses, and were designated as experimental lenses only. Two formulations of extended wear lenses were used, and they were namely EWA and EWB. The EWA lenses were used in 30-day experiments from Feb 23rd to Mar 24th 2010. Following that study, CIBA sent another set of lenses that were studied from Oct 20th to Nov 18th 2010.

The lenses were loaded with DMPC for 85 seconds in 0.9% (w/v) solution of DMPC in *n*-propanol at room temperature. This DMPC solution had a ^{14}C activity of 21 µCi/mL. Following loading, the lenses were eluted in a simulation of daily wear followed by nightly cleaning for 30 days.The lenses were placed in ATF solution at 35°C with shaking for 16 hrs daily. The solution was sampled for eluted DMPC at the end of each 16-hr period. Each lens was rinsed and transferred to one of 3 cleaning solutions for 8 hrs at room temperature, without shaking. The solutions were ReNu (Bausch & Lomb), RepleniSH (Alcon) and Clear Care (CIBA Vision). When using Clear Care, 2 lenses were placed in each container. The solution was sampled for eluted DMPC at the end of each 8-hr period. This sequence, repeated daily for 30 days, represented 16 hrs of wear followed by 8 hrs of cleaning.

6.2 **Results**

Loading amount. 0.9% (w/v) DMPC solution was prepared bypipetting about 3.85 mL of cold 0.90% DMPC (in n-propanol) and 0.15 mL of Secondary Master DMPC(labled with C-14) into a vial. Exposure of EWA lenses to 0.9% (w/v) DMPC solution in n-propanol for 85 s loaded 200.6 $\pm$ 1 µg (mean and standard error) per lens as measured by the standard extraction procedure. This is a much greater loading than previous research reported in chapters 4 and 5 **[18, 24]**. As shown in Figure 6.1, the amount loaded appears to be linear with exposure time (R^2 = 0.981), but extrapolation of this line indicates that the intercept does not pass through zero. Although the DMPC concentration in this loading solution was 6 times greater (0.9% compared to 0.15%), the amount loaded was not 6-fold greater for the same loading times as the previous lenses. This might be attributed to the fact that these experimental lenses are reported to have a

different chemistry (designed for extended wear) than the daily wear lenses of the previous studies.

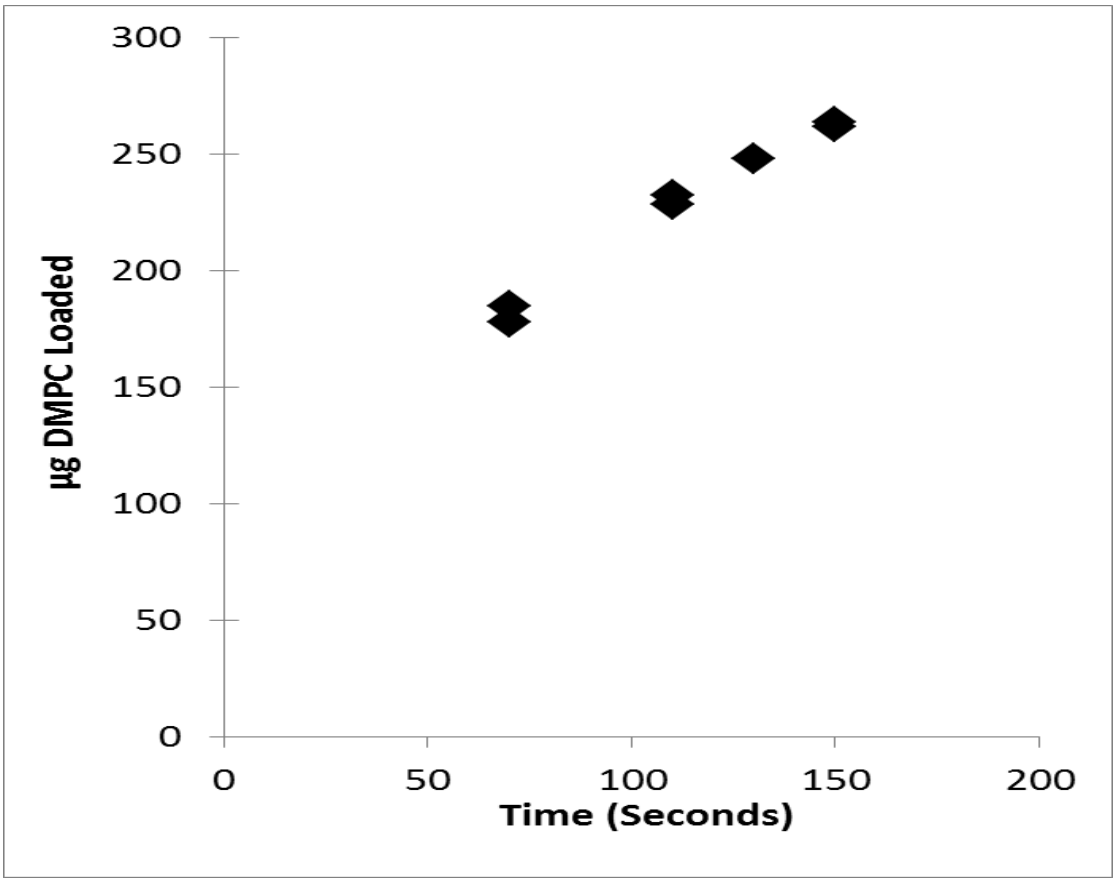


Figure 6-1. Mass of DMPC loaded per contact lens from 0.9% DMPC in n-propanol as a function of the loading time.

Elution in ATF. We attempted to simulate elution during daily wear by soaking in ATF at 35°C with shaking for 16 hrs. The amount eluted per day for EWA lensesand EWB lenses (16-hr period) are shown in Figure 6.2 and 6.3. For EWA lenses, there was a very large burst effect on day 1. On days 2 through 15, the lenses cleaned in the ReNu, RepleniSH and Clear Care systems (but eluted in ATF) for 16 hours had accumulative elution of 2.61, 6.15 and 2.21

µg/lens respectively. On days 16 through 30, these 16-hr total elution rates slowed to 2.13, 3.74 and 1.47 µg/lens respectively. Note that the elution in ATF of those lenses cleaned in RepleniSH was nearly twice as high as lenses cleaned in the other cleaners. Lenses cleaned in ReNu and Clear Care systems showed a nearly zero-order elution rate from 15 to 30 days, while the lenses cleaned in RepleniSH showed a slightly decreasing elution rate.

For EWB lenses, there was also a very large burst effect on day 1. On days 2 through 15 the lenses cleaned in the ReNu, RepleniSH and Clear Care systems (but eluted in ATF) for 16 hours had accumulative elution means of 1.98, 3.58 and 2.36 µg/lens respectively. On days 16 through 30, these 16-hr accumulative elution rates slowed to 1.40, 2.42 and 1.57 µg/lens respectively. Note that the elution in ATF of those lenses cleaned in RepleniSH was nearly twice as high as lenses cleaned in the other cleaners. As before, lenses cleaned in ReNu and Clear Care systems showed a nearly zero-order elution rate from 15 to 30 days, while the lenses cleaned in RepleniSH showed a slightly decreasing elution rate. Although the general trend is similar, the obvious difference between EWA and EWB lenses is that the elution amounts from EWB lenses were much greater than from EWA lenses. While we don't know what change in lens formulation occurred, it obviously increased elution.

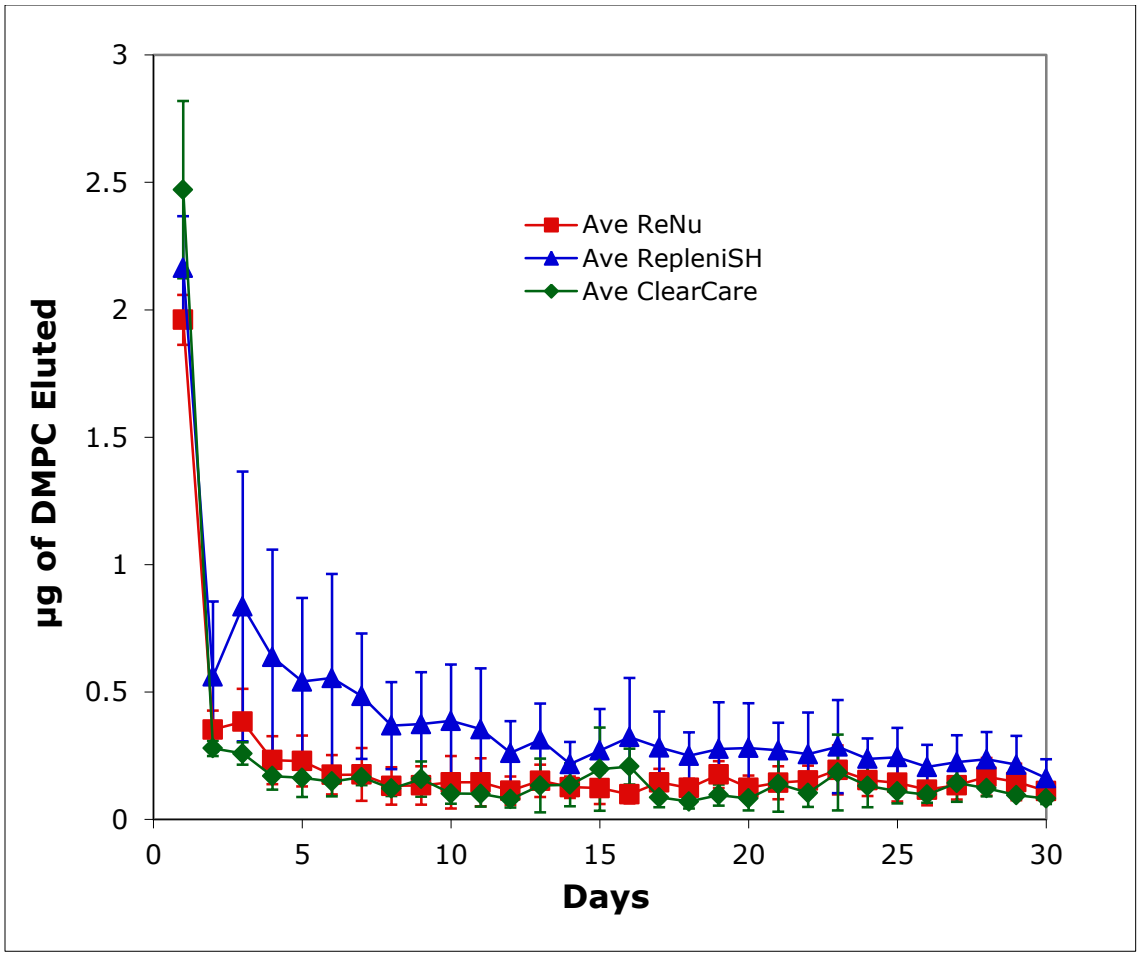


Figure 6-2. Mean mass of DMPC eluted per 16 hr period in ATF for EWA. (■) lenses cleaned in ReNu. (▲) lenses cleaned in RepleniSH. (♦) lenses cleaned in Clear Care. Error bars represent the standard deviation(n=4).

Elution in cleaning solutions. Figures 6.4 and 6.5 present the data for elution of the EWAand EWB lenses into each of the 3 cleaning systems during the 8 hrs of cleaning. For EWA lenses, there appears to be not much of a burst effect on the first day for all systems, but this would be expected because these lenses had already been exposed to ATF for 16 hrs. The ReNu produced very low amounts of elution. The average and standard deviation in ReNu over days 1 to 30 is 0.424± 0.013 µg/lens per day (8 hrs). This is less than 1/8 of the elution in ATF. The RepleniSHpromoted much more elution with an average and standard deviation over days 1

to 30 of 1.29± 0.023 µg/lens. The Clear Care showed even more elution with an average and standard deviation over the 30 days of 2.0± 0.042 µg/lens. The elution rate in Clear Care was 5times the elution rates in ReNu.

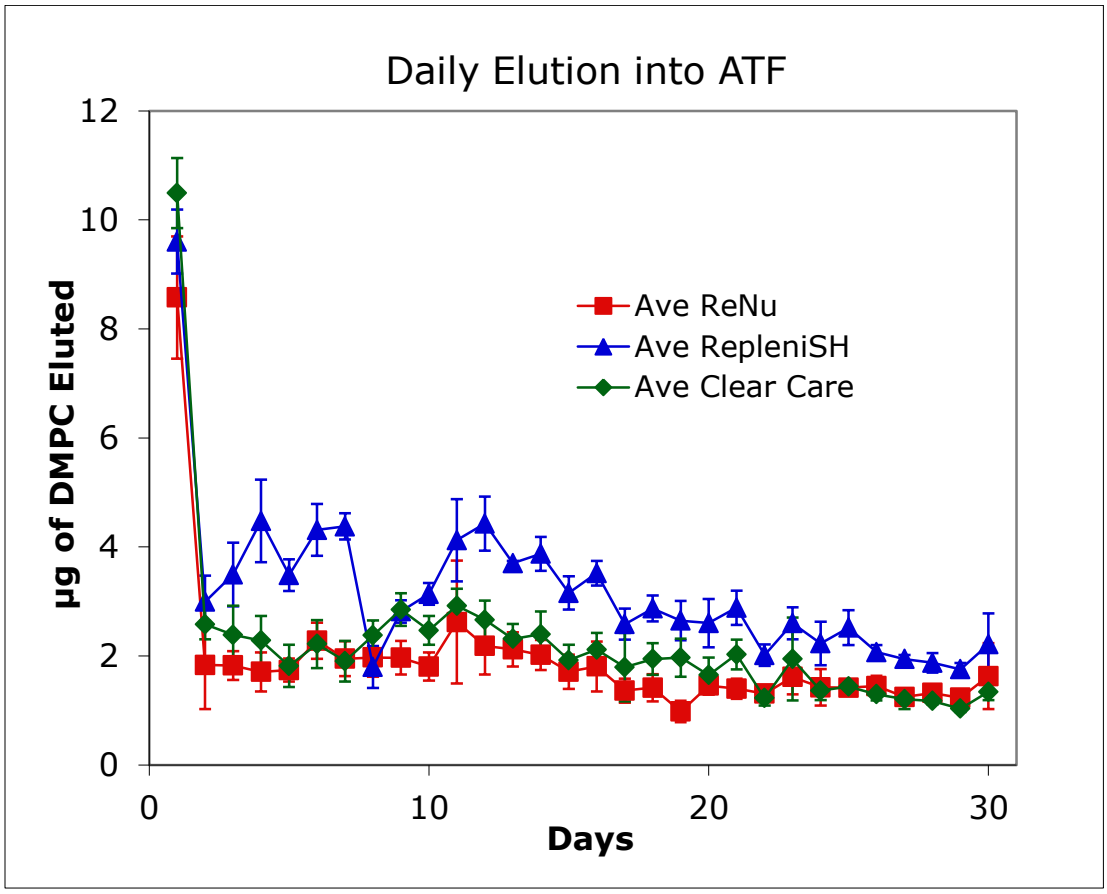


Figure 6-3. Mean mass of DMPC eluted per 16 hr period in ATF for EWB. (■) lenses cleaned in ReNu. (▲) lenses cleaned in ReplenISH. (♦)lenses cleaned in Clear Care. Error bars represent the standard deviation (n=4).

For EWB lenses, similar trends appear. The ReNu and Clear Care solutions produced very low amounts of elution. The average and standard deviation in ReNu over days 1 to 30 is 0.053 ± 0.031 µg/lens per day (8 hrs). This is less than 1/4 of the elution in ATF. The ReplenISH showed much more elution with an average and standard deviation over days 1 to 30 of 0.365 ±

0.195 µg/lens. The Clear Care showed much less elution with an average and standard deviation over the 30 days of 0.031 ± 0.016 µg/lens. Again, the elution rate in RepleniSH was 7 to 10 times the elution rates in ReNu and Clear Care

Lenses cleaned in ReNu and Clear Care showed a fairly constant (zero-order) elution rate over days 6 to 30, while lenses cleaned in RepleniSH displayed a decreasing elution during the first 20 days for both types of lenses.

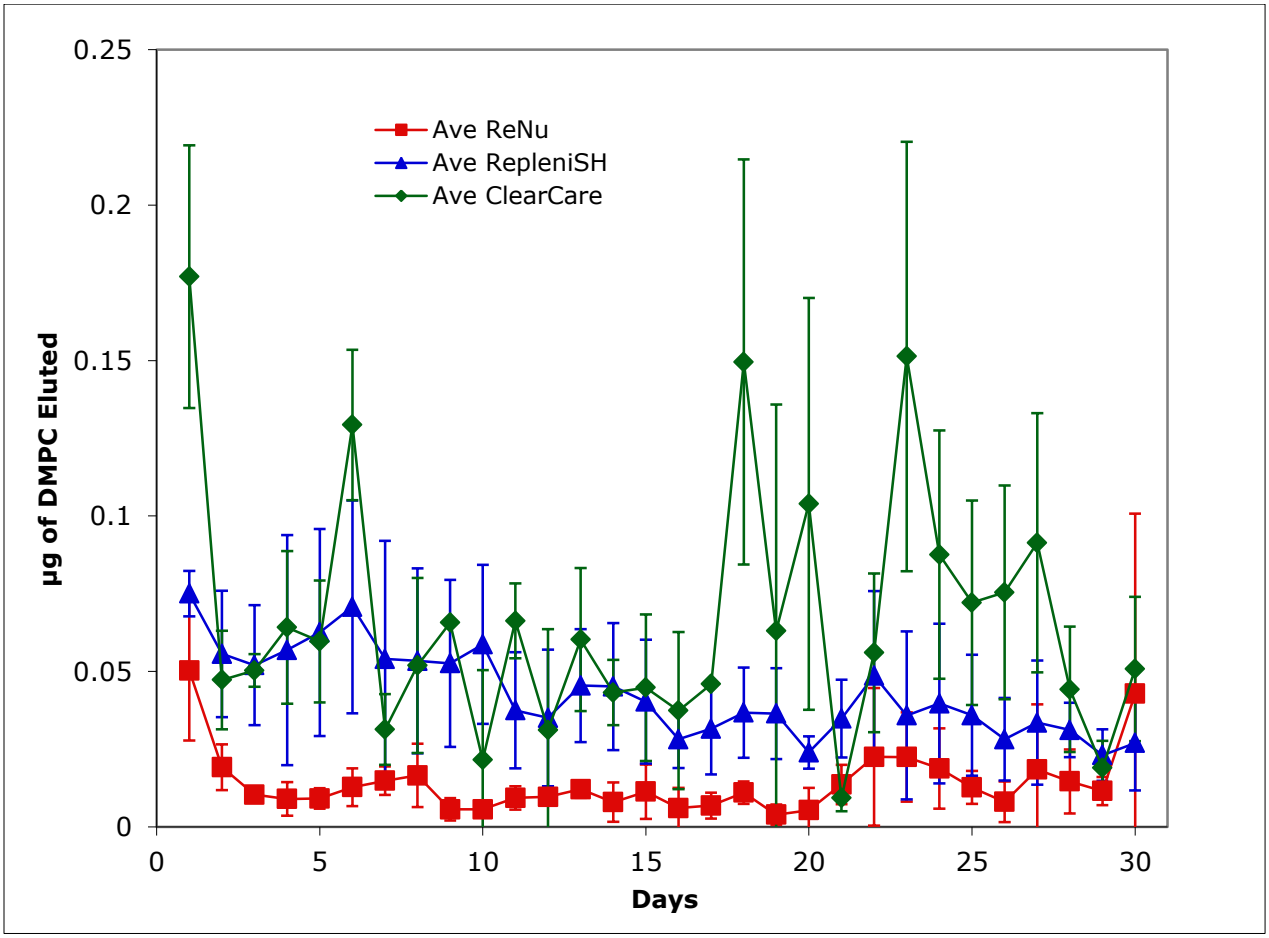


Figure 6-4. Mean mass of DMPC eluted per 8 hr period in a cleaning solution for EWA. (■) lenses cleaned in ReNu. (▲) lenses cleaned in RepleniSH. (♦) lenses cleaned in Clear Care. Error bars represent the standard deviation. The data is corrected for area of lost lens fragments (n=4).

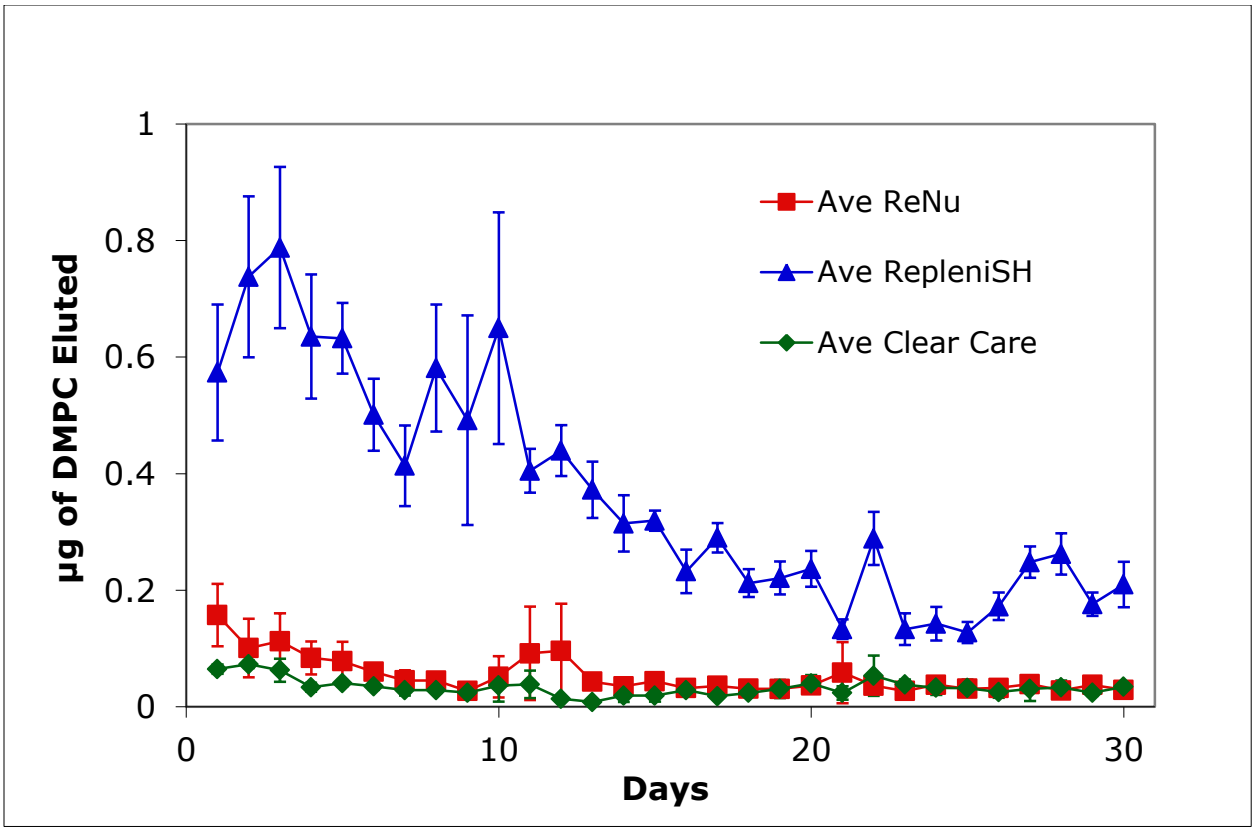


Figure 6-5. Mean mass of DMPC eluted per 8 hr period in a cleaning solution for EWB. (■) lenses cleaned in ReNu. (▲) lenses cleaned in RepleniSH. (♦) lenses cleaned in Clear Care. Error bars represent the standard deviation (n=4).

Cumulative Elution. Figure 6.6 and 6.7 show the cumulative DMPC elution comprising DMPC released during both the simulated wear (ATF) and simulated cleaning periods for EWA and EWB lenses. Elution from EWA lenses in the RepleniSH system shows the greatest cumulative elution. The fairly linear slope of the data indicates fairly constant release for all 3 systems. From day 6 and later, the cumulative release from lenses cleaned in RepleniSH was statistically greater than from lenses cleaned in the other 2 cleaners. Regarding the elution from lenses cleaned in ReNu and Clear Care, there is no statistically significant difference at any single day, even at the last day. However, a paired comparison (comparing ReNu and Clear

Care) of daily averages over the entire 30-day period indicates that elution from lenses cleaned in Clear Care is greater than from lenses cleaned in ReNu ($p<0.000$, $n=30$).

Elution from EWB lenses in the RepleniSH system shows the greatest cumulative elution. The fairly linear slope of the data indicates fairly constant release for all 3 systems. From day 4 and later, the cumulative release from lenses cleaned in RepleniSH is statistically greater than from lenses cleaned in the other 2 cleaners. Regarding the elution from lenses cleaned in ReNu and Clear Care, there is no statistically significant difference at any single day, even at the last day. However, a paired comparison of daily averages over the entire 30-day period indicates that elution from lenses cleaned in Clear Care is greater than from lenses cleaned in ReNu ($p<0.0001$, $n=30$).

6.3 **Discussion**

This study showed that 200 µg of DMPC could be easily loaded into both types of extended wear silicone hydrogel contact lenses by a simple incubation in an n-propanol solution of the phospholipid. This loading is much higher than shown in previous chapters, but the loading solution had a greater concentration of DMPC.

We were able to simulate 30 days of elution without risk of bacterial colonization by exposing the lenses to 16 hrs of ATF and then 8 hrs of the cleaning solutions, which each contained an antimicrobial agent. In the first study, the results indicated that elution during the cleaning stage was a function of the cleaning solution used. A statistical evaluation indicated that RepleniSH was associated with the highest elution during exposure to the cleaning solution ($p<0.0001$), regardless of the fluctuation that occurred with the Clear Care, and also the highest elution during the daily exposure to ATF ($p<0.0001$). The reason that large fluctuations occurred

on the ClearCare data was due to the fragmentation problems. Several days after the experiment started, the EWA lenses cleaned in Clear Care began to tear apart, and we started losing broken lens pieces during elution. At the end of the whole experiment, we recovered the remaining pieces and estimated the results based on what was left. The first study gives us an idea of what happened to the lenses after eluting in different cleaning solutions. Since the fragmentation problem affected the accuracy of the results, CIBA had us perform another study with the same clearners and improved lenses.

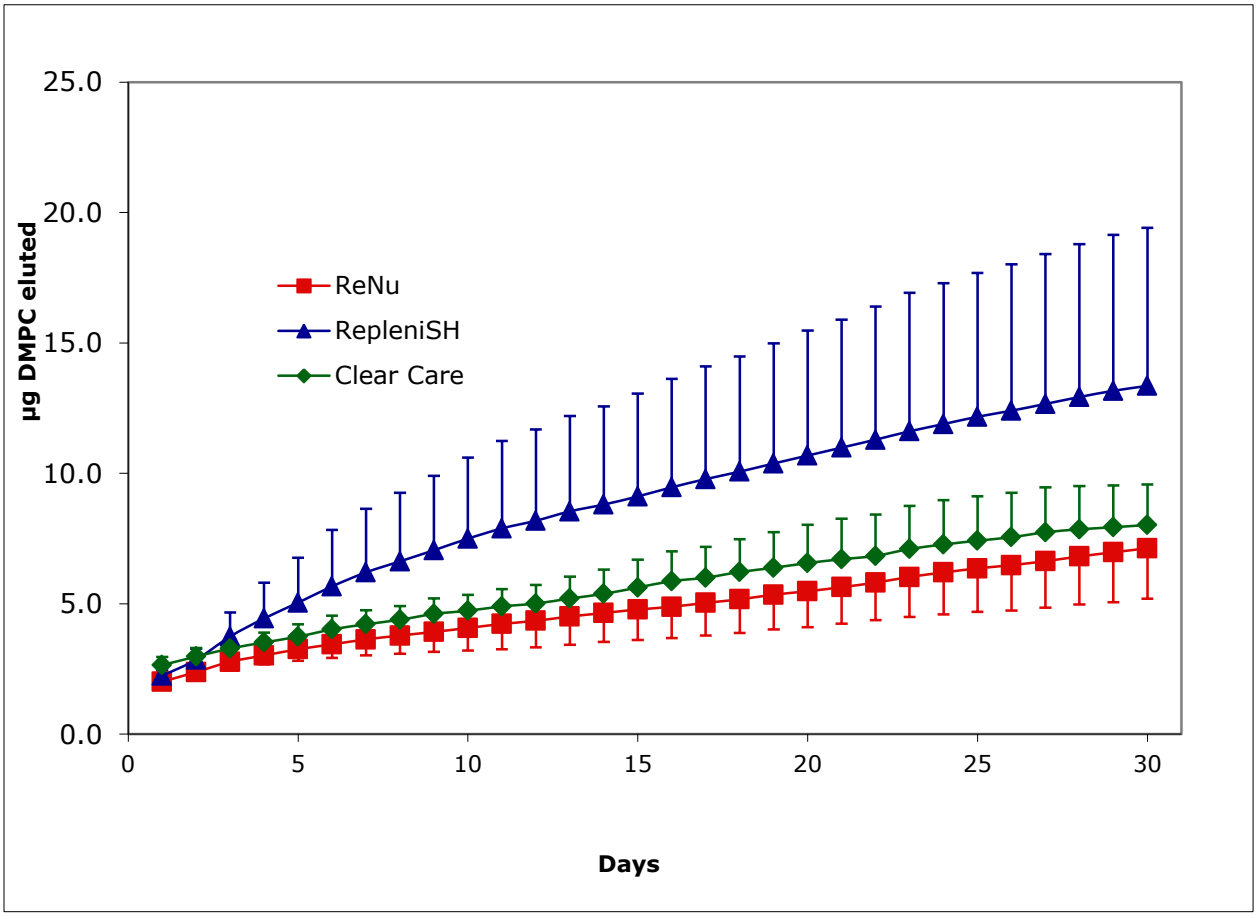


Figure 6-6. Average cumulative elution for EWA over 30 days. The data includes release during elution in ATF and elution in cleaning solution. (■) lenses cleaned in ReNu. (▲) lenses cleaned in ReplenishSH. (♦) lenses cleaned in Clear Care. Error bars represent the standard deviation (n=4).

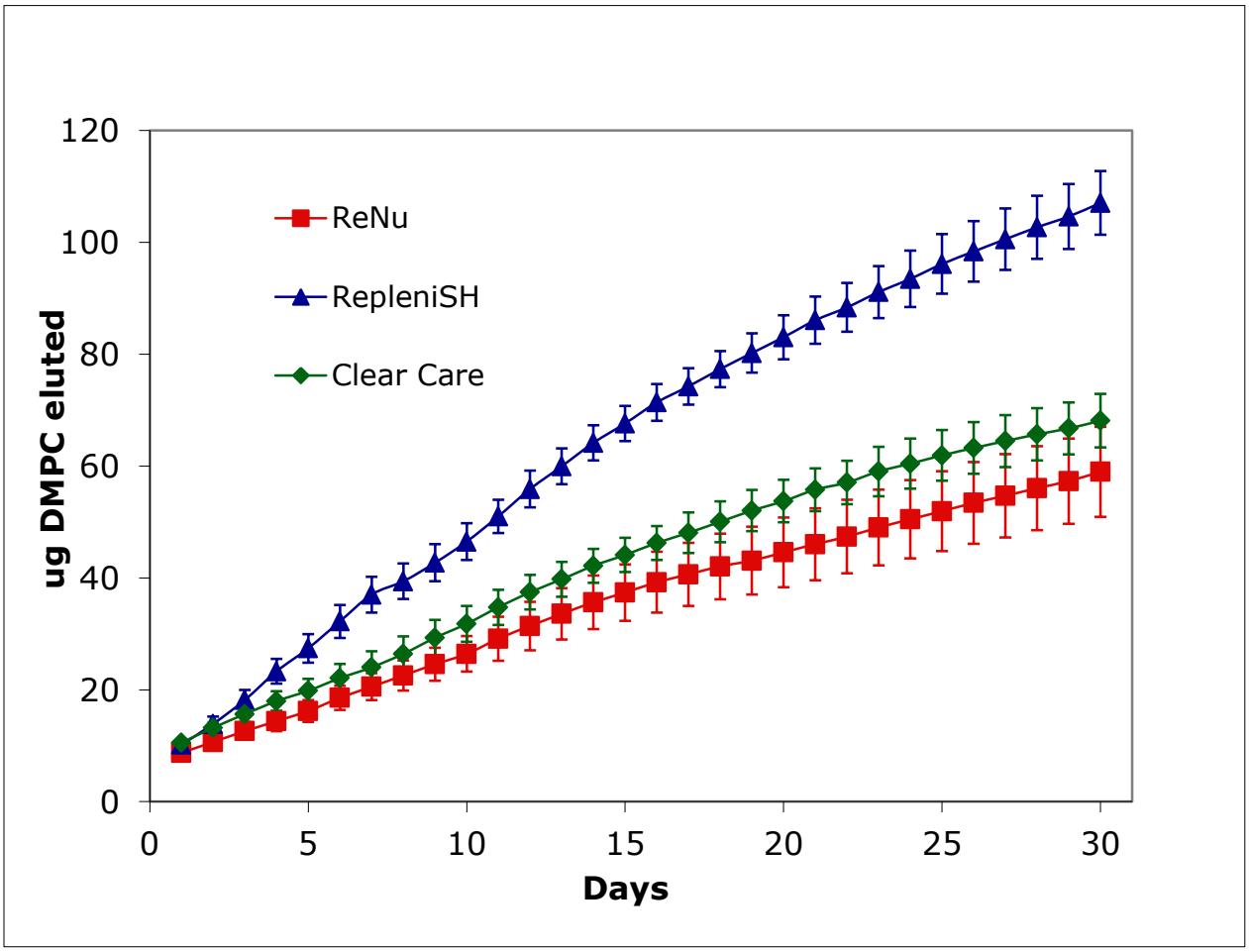


Figure 6-7. Average cumulative elution for EWB over 30 days. The data includes release during elution in ATF and elution in cleaning solution. (■) lenses cleaned in ReNu. (▲) lenses cleaned in RepleniSH. (♦) lenses cleaned in Clear Care.Error bars represent the standard deviation(n=4).

In the second study, the results indicated that elution during the cleaning stage was a function of the cleaning solution used. A statistical evaluation indicated that RepleniSH was associated with the highest elution during exposure to the cleaning solution ($p<0.0001$), and also the highest elution during the daily exposure to ATF ($p<0.0001$). Because higher elution occurs during ATF exposure, we postulate that some component of the OPTI-FREE RepleniSH diffuses

into the contact lens and remains present and active during elution in ATF. To further support this conclusion, we note that on the first day of elution into ATF, before any exposure to any cleaning solution, the amount of DMPC eluted from each of the 3 sets of lenses was not statistically different between sets, as can be seen from Figure 6.3. However, on the third day of elution into ATF, after two 8-hr exposures to the cleaning solutions, the lenses exposed to RepleniSH start to distinguish themselves as having greater elution into ATF than lenses cleaned in the other 2 solutions. We postulate that there is something in the RepleniSH solution that enhances elution, and that it apparently adsorbs or absorbs on the lenses and effectuates an increased elution in ATF even after the lenses are removed and rinsed from the RepleniSH solution.

Components of the OPTI-FREE RepleniSH that are not found in the other cleaning solutions are sodium citrate, propylene glycol, Tetronic 1304, nonanoyl ethylenediaminetriacetic acid, polyquaternium-1, and myristamidopropyl dimethylamine. At this time it is unknown if any of these are responsible for the higher elution rates. Alternatively it could be that something in the ReNu and Clear Care reduces the elution rates, but we suspect that is less likely, particularly since the components of Clear Care have very little in common with those of ReNu. The components of all the cleaning solutions used herein are listed in Table 6.1, as taken from the containers and/or their packaging.

Table 6-1. Components of Cleaning Solutions (water based)

Brand Name	Components
ReNu MultiPlus® Multi-purpose solution	Hydranate® (hydroxyalkylphosphonate), boric acid, edetate sodium, Poloxamine, sodium borate, sodium chloride, Dymed® (polyaminopropyl biguanide) 0.0001%
Alcon Opti-Free RepleniSH Multi-purpose disinfection solution	Sodium citrate, sodium chloride, sodium borate, propylene glycol, TEARGLYDE® (Tetronic 1304, nonanoyl ethylenediaminetriacetic acid), POLYQUAD® (polyquaternium-1) 0.001%, ALDOX® (myristamidopropyl dimethylamine) 0.0005%
Clear Care	Hydrogen peroxide, sodium chloride, phosphonic acid, phosphate buffer, Pluronic 17R4

We speculated that faster elution might be due to the solubility of DMPC in the elution solution. The ATF we employ contains several components, including proteins and phospholipids. It might be that there are already micelles, liposomes, or proteins in the ATF solution that can easily absorb DMPC as these structures collide with the contact lens surface. No such structures exist in pure water, so transport off of the lens surface would be slower in water. As for the differences in these cleaning solutions, they all contain various salts and surfactants that might be involved in removing DMPC from the lens surface into solution. Perhaps more of this happens with RepleniSH than with the other two cleaners.

In addition, we also speculated that faster elution might be due to the DMPC adsorption to the polyethylene surface of thedifferent cleaning solution holders. After finishing up the 30-day experiment, we eluted more lenses loaded with the same amount of DMPC within ATF solution in the same containers. Then we rinsed the containers, and extracted them with n-propanol to test if there is any DMPC adsorbed by the containers. The data acquired from the counter shows that all of them have C-14 counts extremely close to the non-radioactive backgrounds value, which suggested that no DMPC was adsorbed on the surface of the containers.

An alternative hypothesis is that some component of RepleniSH is diffusing into the contact lens and interacting with the polymer itself to reduce the resistance of DMPC transport out of the lens. To date there is no solid evidence for either hypothesis, but future experiments should be done to examine these phenomena in more detail.

With respect to a practical application of this technology in a possible commercial contact lens, the most significant observation is that elution from DMPC does not dwindle to nothing after a few days, but continues at a fairly constant rate for 30 days in both lens types. Depending on the cleaning solution used, up to 110 µg of the 200 µg available was eluted at a fairly constant rate following the burst on the first day. Even with ReNu, nearly 60 µg (30% of the available DMPC) was released in 30 days. From EWA lenses, less DMPC was eluted, but elution was still occurring at 30 days.

The data in Chapter 4 showed that daily wear lenses eluted about 0.92 µg in the first 10 hours of exposure to ATF, or an average of 0.09 µg/hr. How do the EWB lenses compare? On the first day there was 9.56 µg eluted in 16 hrs. For comparison this is about 0.6 µg per hour, much more than in daily wear in the previous study; but the extended wear lenses were more highly loaded (200 µg instead of 33 µg) than the daily lenses, and the first day showed a large burst of elution from both EWA and EWB. The average elution into ATF over the entire study was 0.20 µg/hr for EWB lenses exposed to RepleniSH (30 days, 16 hr/day), 0.14 µg/hr for lenses exposed to Clear Care, and 0.12 µg/hr for lenses exposed to ReNu. Compared to the daily wear lenses, these are fairly high average release rates, but one should always consider that the loading is six-fold higher and the lens material is different.For commercial application, the question remains as to whether this amount is sufficient to provide any comfort effect, which can only be answered by clinical trials.

7 POLYMERIZATION OF A SILICON HYDROGEL CONTACT LENS CONTAINING ELUTABLE PHOSPHOLIPIDS

7.1 Rationale and objectives

In previous chapters we have shown that an experimental silicone hydrogel contact lens can be easily loaded with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) from n-propanol to various concentrations, and the DMPC can be slowly released into ATF or water.Furthermore autoclaving the lenses does not interfere with the release of DMPC. Although this type of lens has great potential, in a commercial production line, loading a lens after polymerization and extraction of residual n-propanol would increase the number of process steps and would also generate large volumes of solvent waste. Therefore, to reduce environmental impact and simplify the production process, I investigated the alternative procedure of incorporating the DMPC to the monomer mixture before polymerization of an experimental contact lens. In this study, we polymerized lenses after incorporating radiolabeled DMPC into the monomer, and then I performed various extractions and elutions to track the fate of the DMPC, including final release of DMPC into an artificial tear solution.

The experimental details of the monomer formulation and polymerization are found in Chapter 3.2.

7.2 **Results and discussion**

The n-propanol solution of DMPC mixed very well with the monomer formulation. There was no evidence of phase separation or precipitation. After polymerization, the lenses were easily removed from the molds; they appeared to be very uniform in composition and clarity.

Loading. Table 7.1 shows the mean average amount of DMPC that was loaded into each lens as evaluated in two different ways. Four lenses were polymerized and then immediately extracted following the lab extraction procedure to determine the total loading after polymerization but before commercial extraction. These lenses were extracted 3 times, and the amounts from each extraction vial summed to obtain a total of around 223.2 μg/lens (see Table 7.1). For comparison, this is even higher loading than the extended wear lenses of chapter 6.

Another set of 4 lenses were polymerized and then subjected to commercial extraction before the DMPC remaining in the lenses was removed by lab extraction.

Table 7-1. DMPC Loading in Lenses

	Commercial extraction (μg)	Elution(μg)	Lab extraction(μg)	Total Loading (μg)
Set 1, n=4	N/A	N/A	223.2±6.4*	223.2±6.4*
Set 2, n=4	186**	6.8±1.6*	37.0±5.4*	229.8±7.0*

* Mean and standard deviation
** Only one measurement

Table 7.1 shows the results of these extractions. The first set of lenses, extracted by lab extraction only, contained 223.2 ± 6.4 μg/lens. In the second set, about 186 μg were extracted by commercial extraction, 6.8 ± 1.6 μg were eluted and 37.0 ± 5.4 μg were extracted by lab extraction, which totals 229.8 ± 7.0 μg. These values are not statistically different (p= 0.44), and

so they are combined to estimate that there was 226.5±6.7 μg/ lens (mean and standard deviation, n=8).

By weighing several polymerized lenses, I estimated that the mold volume was about 33 μL, and I would have expected 230 μg/lens of DMPC to be extracted based on that volume. Since the average was 226.5μg/lens, our extraction is only 98% efficient, or the mold volume is slightly (2%) smaller than estimated. These results indicate that mixing DMPC with monomer and then processing lens polymerization is an alternative way to loadover 200 μg of DMPC per lens. During commercial extraction, 82% of the DMPC is removed, but the remaining 18% is available for delivery to the eye.

It should be noted that the initial amount of DMPC loaded in this method is very high compare to the loadings of 33 μg/lens in the daily wear lenses**[18]**,and the amount after commercial extraction, 43.8 μg, is slightly more than used in those studies. In previous chapters, lenses were immersed into solutions with certain concentration of DMPC dissolved in n-propanol, and anywhere from 22μg to 55μg were easily loaded. Thus, mixing DMPC with monomer before the polymerization allows one to adjust the amount of DMPC per lens, simplifies the manufacturing steps, reduces manufacturing time, and eliminates the use of n-propanol.

Elution. Four polymerized lenses loaded with an average of 226.5 μg DMPC were eluted in ATF for 72 hours at 35°Conce the commercial extraction process was completed. At this point, about 44 μg of DMPC were left in each lens. The amount eluted vs. time is shown in Figure 7.1.

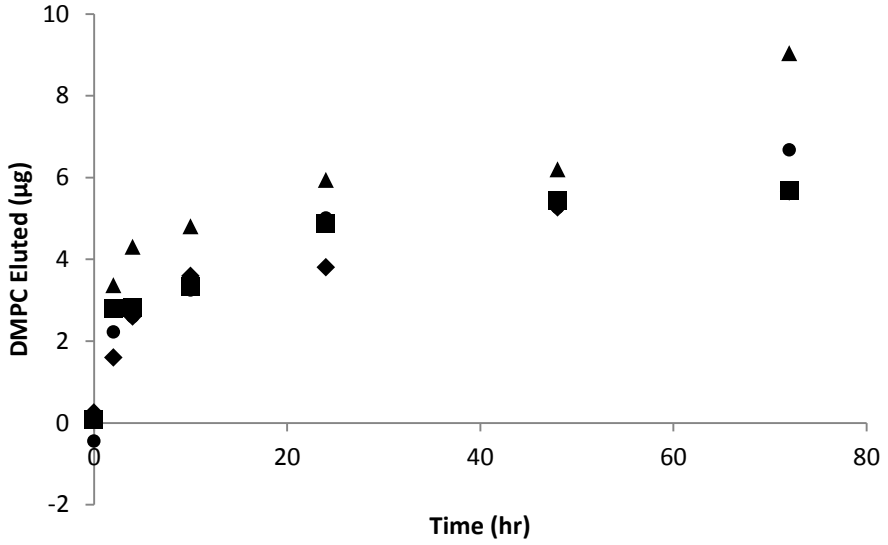


Figure 7-1. Mass of DMPC eluted over 72 hours in ATF as function of time at 35°C. (●), (■), (▲) and (♦) represent different lenses.

An average of 3.7 µg of DMPC was eluted at 10 hrs and 6.8µg per lens was eluted at 72 hours. To compare this data to elution from the previous studies, the amount eluted is presented as a fraction of the initial amount of DMPC in the lens at zero time (see Figure 7.2). One can see that when the data are normalized in this manner, the rate of elution appears to be faster when the DMPC is added to the monomer formulation, than when absorbed into the lens after polymerization.

To summarize, this study shows that it is possible to polymerize a silicone hydrogel with DMPC in the monomer formulation. These data also supportthe proposition of designing a new loading method to simplify the steps, save chemical agents and use a more environmentally friendly process. Elution into ATF appears to be greater using this process than when loading the DMPC from n-propanol.

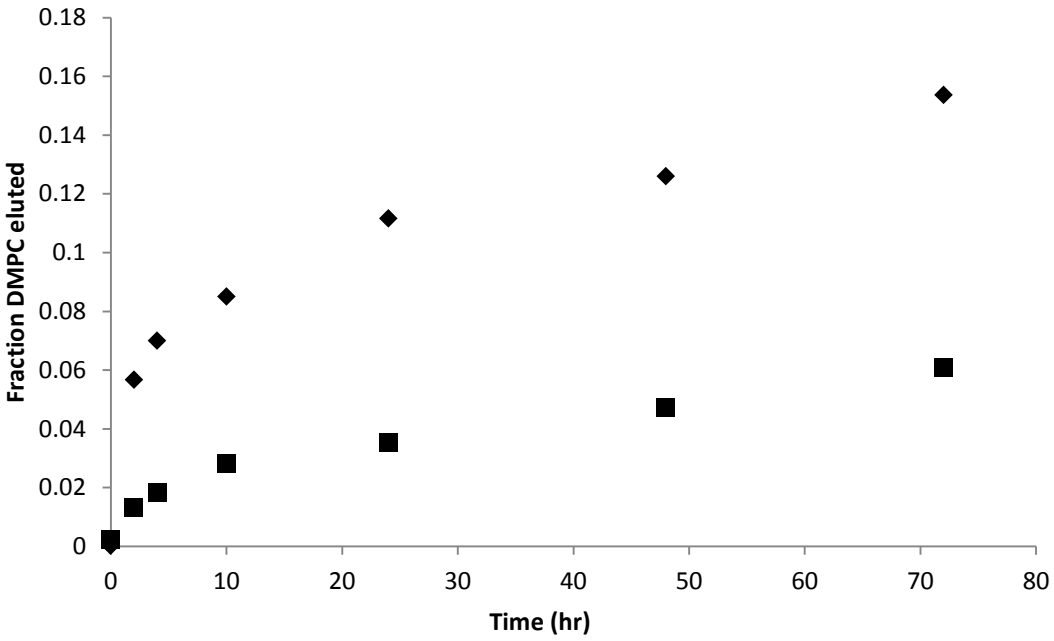


Figure 7-2. Mean value of the fraction of DMPC eluted after 72 hours in ATF as function of time at 35°C. (♦) represents lenses of this study, which the DMPC is in the monomer formulation, and (■) represents data from a previous study in which lenses that are loaded with DMPC from n-propanol after polymerization (n=4).

8 CONCLUSIONS AND RECOMMENDATIONS

Our data shows that it is possible to load DMPC into silicone hydrogel contact lenses, and to increase the loading of DMPC by increasing the sorption time during the loading process. The subsequent rate of elution into ATF appears to be diffusion controlled for about 10 hours, to be proportional to the amount loaded(as one would expect based on Fickian diffusion of DMPC within the contact lens) and not to benegatively affected by the autoclaving process. Also, similar loading can be performed in another type of extended wear lens, and elution from such lenses can persist for a longer time.

We can also conclude that loading before polymerization did appear to increase the elutable fraction of DMPC, reduce environmental impact, and simplify the production process.

We must emphasize that there are still many aspects that require further study. In our studies, we have been using silicone hydrogel contact lenses for all of our experiments. It is possible that other types of lenses may showsimilar or even better results on loading and releasing of phospholipids. Thus,we recommend performing the same experiments described in Chapter 4 with other types of lenses to verify the possibilities of using other types of lenses.

Among all of our experiments, we did not load any lipids other than DMPC. So we do not know the feasibility of loading and releasing other lipids in the same solution. Since other phospholipids may have some clinical potential to provide eye comfort, it is important to perform further research on loading and releasing of other lipids, or even the mixture of lipids, to

show the efficiency of loading and release, and therefore to explore the wider use of phospholipids in contact lenses for eye care.

In our 30-day study, we investigated the elution of DMPC in different cleaning solutions, and saw the feasibility of DMPC releasing over an extended time. Although we were able to identify the composition of each solution, we still have no idea which chemicalswould enhance the elution happened or hinder it. So it may be useful to do further analysis with different solutions that contain one or more components to find possible alternative solutions to maximize the elution efficiency and reduce the potential cost of buying chemical agents.

To load DMPC into the lenses, we used quite a large amount of n-propanol as solvent,and we realized that there may be a way of loading without solvents to reduce environmental impact and simplify the production process. Besides the study described in Chapter 8, there are still many aspects that deserve further research. It is recommended to investigate the feasibility of loading by placingDMPC liposomesin the buffer solution during autoclaving, and thus combining theloading and autoclaving procedure.

REFERENCES

1. Jain, M.R., *Drug delivery through soft contact lenses.* British Journal of Ophthalmology, 1988. **72**(2): p. 150-154.

2. Lesher, G.A. and G.G. Gunderson, *Continuous Drug Delivery Through the Use of Disposable Contact Lenses.* Optometry & Vision Science, 1993. **70**(12): p. 1012-1018.

3. Xinming, L.,Yingde, C.,Lloyd, Andrew W., Mikhalovsky, Sergey V., Sandeman, Susan R., Howel, Carol A., and Liewen, L.,*Polymeric hydrogels for novel contact lens-based ophthalmic drug delivery systems: A review.* Contact Lens and Anterior Eye, 2008. **31**(2): p. 57-64.

4. Ciolino, J.B., Hoare, Todd R., Iwata, Naomi G., Behlau, I., Dohlman, Claes H., Langer, R., and Kohane, Daniel S., *A Drug-Eluting Contact Lens.* Investigative Ophthalmology & Visual Science, 2009. **50**(7): p. 3346-3352.

5. Hui, A., A. Boone, and L. Jones, *Uptake and Release of Ciprofloxacin-HCl From Conventional and Silicone Hydrogel Contact Lens Materials.* Eye & Contact Lens, 2008. **34**(5): p. 266-271 10.1097/ICL.0b013e3181812ba2.

6. Kapoor, Y., Thomas, Justin C., Tan, G., John, Vijay T., and Chauhan, A., *Surfactant-laden soft contact lenses for extended delivery of ophthalmic drugs.* Biomaterials, 2009. **30**(5): p. 867-878.

7. Schultz, C.L., T.R. Poling, and J.O. Mint, *A medical device/drug delivery system for treatment of glaucoma.* Clinical and Experimental Optometry, 2009. **92**(4): p. 343-348.

8. Butovich, I.A., T.J. Millar, and B.M. Ham, *Understanding and Analyzing Meibomian Lipids—A Review.* Current Eye Research, 2008. **33**(5-6): p. 405-420.

9. McCulley, J.P. and W.E. Shine, *The lipid layer of tears: dependent on meibomian gland function.* Experimental Eye Research, 2004. **78**(3): p. 361-365.

10. Carney, F.P., W.L. Nash, and K.B. Sentell, *The Adsorption of Major Tear Film Lipids In Vitro to Various Silicone Hydrogels over Time.* Investigative Ophthalmology & Visual Science, 2008. **49**(1): p. 120-124.

11. Mochizuki, H., Yamada, M.,Hatou, S., Kawashima, M., and Hata, S.,*Deposition of Lipid, Protein, and Secretory Phospholipase A2 on Hydrophilic Contact Lenses.* Eye & Contact Lens, 2008. **34**(1): p. 46-49 10.1097/ICL.0b013e3180676d5d.

12. Prager, M.D. and R.P. Quintana, *Radiochemical studies on contact lens soilation. II. Lens uptake of cholesteryl oleate and dioleoyl phosphatidylcholine.* Journal of Biomedical Materials Research, 1997. **37**(2): p. 207-211.

13. Nicolson, P.C. and J. Vogt, *Soft contact lens polymers: an evolution.* Biomaterials, 2001. **22**(24): p. 3273-3283.

14. McNamara, N.A., Polse, K.A.,Brand, R.J., Graham, A.D., Chan, J.S., and McKenney, C,D.,*Tear mixing under a soft contact lens: effects of lens diameter.* American Journal of Ophthalmology, 1999. **127**(6): p. 659-665.

15. Holly, F.J. and M.A. Lemp, *Tear physiology and dry eyes.* Survey of Ophthalmology. **22**(2): p. 69-87.

16. Peters, K. and T.J. Millar, *The role of different phospholipids on tear break-up time using a model eye.* Current Eye Research, 2002. **25**(1): p. 55.

17. Lorentz, H. and L. Jones, *Lipid Deposition on Hydrogel Contact Lenses: How History Can Help Us Today.* Optometry & Vision Science, 2007. **84**(4): p. 286-295 10.1097/OPX.0b013e3180485d4b.

18. Pitt, W.G., Jack, D.R., Zhao, Y., Nelson J.L., and Pruitt, J.D.,*Loading and Release of DMPC from Contact Lenses.* Optometry and Vision Science, 2011. **88**(4): p. 502-506.

19. Glasson, M.J., F. Stapleton, and M.D.P. Willcox, *Lipid, lipase and lipocalin differences between tolerant and intolerant contact lens wearers.* Current Eye Research, 2002. **25**(4): p. 227-235.

20. Ham, B.M., R.B. Cole, and J.T. Jacob, *Identification and Comparison of the Polar Phospholipids in Normal and Dry Eye Rabbit Tears by MALDI-TOF Mass Spectrometry.* Investigative Ophthalmology & Visual Science, 2006. **47**(8): p. 3330-3338.

21. Peters, K. and T.J. Millar, *The role of different phospholipids on tear break-up time using a model eye.* Current Eye Research, 2002. **25**(1): p. 55-60.

22. Yamada, M., Mochizuki, H., Kawashima, M., andHata, S.,*Phospholipids and Their Degrading Enzyme in the Tears of Soft Contact Lens Wearers.* Cornea, 2006. **25**: p. S68-S72 10.1097/01.ico.0000247217.16510.f2.

23. Walpole RE, M.R., *Probability and Statistics for Engineers and Scientists*. 1978, Macmillan: New York.

24. Pitt, W.G., Jack, D.R., Zhao, Y., Nelson J.L., and Pruitt, J.D., *Transport of Phospholipid in Silicone Hydrogel Contact Lenses.* J Biomat Sci Polym Ed, 2011. **in press**.

APPENDIX 1. PROTOCOLS

A. **Protocol for polymerizing contact lenses loaded with *DMPC.**

Preparing the monomer

1. Remove from fridge and warm the monomer solution (blue) from CIBA in a 40°C water bath for 30 min. Then stir on stirring plate (bar is already inside container).
2. Remove the Secondary Standard (*DMPC) from the freezer and warm to 20°C. This standard should be 0.15% w/v *DMPC with an activity of about 90 uCi/mL and 112 uCi/gm.
3. Weigh about 6.75 mg of cold DMPC onto a weigh paper using the Mettler microbalance.
4. Transfer (without spilling anything) the DMPC to a 1.5 mL microfuge tube. Weigh the weigh paper again to obtain by difference the amount of DMPC transferred to the tube.
5. From the weight of DMPC in the tube, calculate the amount of hot Secondary Standard to add to the tube. Multiply the mass by 7.4832 uL solution/mg DMPC.
6. Carefully pipette this volume of Secondary Standard into the microfuge tube. Try to shake the DMPC dust down into the liquid.
7. Multiply the volume of Secondary Standard by 19 uL monomer/uL Secondary Standard.
8. Carefully pipette this amount of warm stirred monomer into the microfuge tube. Vortex.
9. Label the tube as hot monomer, date and name. Put radioactive yellow tape on it. Store in fridge until use.

Polymerizing the lens

10. Warm the monomer in the microfuge tube to 40°C for 30 minutes. Vortex it.
11. Set up the UV polymerization apparatus. Turn on the lamp for at least 30 minutes before use.
12. Use fresh clean polymerization molds. Do not reuse them. Throw them away after 1 use.
13. Pipette 65 uL of warm monomer into bottom mold. Carefully cap upper mold without trapping bubbles.
14. Close the mode and put it directly under the light source.
15. Use the box with two holes for connection to cover the light source, and also use aluminum foil to cover the holes.
16. Press the “operate” button to start Polymerization for 224 seconds.
17. wait a few minutes before removing, but if you need to wait long time, seal in a ziplock in the fridge.

Commercial extraction and coating (proprietary information has been deleted)

1. Add a little water, use a tweezers with rubber covers covered on the two tips to slightly remove the lens from the mold. Shake it and then put it onto parafilm.
2. Dump the lens into xxx xxx for x min xx sec.Push them around with a forceps to keep them from stacking on each other.
3. Remove it and place in xxx for xx sec, push them around with a forceps to keep them from stacking on each other, like previously did.
4. Transfer to xxx solution for xxx sec.
5. Rinse in xxx for xx sec.

B. **Protocol for simulated commercial extraction of a contact lenses loaded with *DMPC.**

Reagents and solutions

1. xxx, HPLC grade
2. Distilled deionized water
3. xxx solution. Prepared as follows and stored in -20°C freezer
    a. Use a clean 250 mL volumetric flask
    b. Add x gm xxx in 250 mL volumetric flask
    c. Then filled with n-prop to mark (250mL)
    d. Add 1.4mL to account for stir bar volume
    e. Stir for 24 hours
4. PBS, 1X
    a. In the lab, we only have PBS 10X. Therefore, we use 4mL of PBS 1X and 36mL of ddH2O to make 40mL of PBS 1X.
5. 4 labeled beakers for the 4 solutions above

Setting up the experiment

1. Weigh 4 containers accurately, write their mass in the lab book.
2. For xxx, since it dissolves pipette, calculate the mass required based on its density and required column, 40mL, then pour xxx directly from its bottle to the container on the balance.
3. For xxx, use pipette, xx mL is required.
4. For xxx, use pipette, xx mL is required
5. For xxx, use pipette, xx mL is required
6. Weigh the mass of both containers and added liquids; write them on lab book.

Simulated Commercial Extraction Procedure

1. After lenses are packed, transfer them into xxx for x min xx sec, make sure all of them are merged.
2. Shake them well.
3. Place lenses in xxx for xx sec, after it, shake them well to reduce carry-over
4. Transferlenses in xxx for xx sec, after it, shake them well to reduce carry-over.

5. At last, put lenses in xx for x sec, after it, shake them well to reduce carry-over
6. Weigh the 4 containers again, write their mass on lab book
7. Take 0.1 mL of each, add them to scintillation vial.

C. **Protocol for 30-day lens elution**

Materials
12 sets of lens holder labeled with lens numbers at each holder, color coordinated.
ReNu white holder. OptiFree, green. ClearCare clear holder.
Squirt bottle full of normal saline
Rubber-tipped forceps

Each day:
12 mL of ATF thawed. This comes in 12 mL tubes, so thaw a full tube. VORTEX it!!
4 mL of each cleaning solution, 20 mL of ClearCare solution. 24 scint vials with 10 mL fluid.

1. Prepare lenses loaded with 200 ug DMPC (see loading protocol below).
2. On first day, start at about 5:00 pm. Weigh the empty containers; record.
3. In the afternoon, prepare 12 scintillation vials with 10 mL each.
4. Put 0.5 mL of ATF in bottom of each contact lens holder.
5. Dab dry and transfer a lens to the correctly numbered holder.
6. Add 0.5 mL of ATF on top of lens. Watch it sink, or sink it. Close lid. Weigh closed case.
7. Put all 12 lenses (6 cases) in the 35° incubator with slow swirling (60 rpm). Leave overnight.
8. Next morning at 9:00, prepare 12 scintillation vials, 10 mL each. Put 0.5 mL of ReNu in 4 holders (white), put 0.5 mL of OptiFree in 4 holders (green), pipette 9.0 mL of ClearCare into plastic vial, filling to mark.
9. Weigh the overnight cases. Open them, one at a time. Remove lens with forceps, rinse with saline solution by squirting into a beaker, dab dry, place in lens holder with cleaner.
10. Add 0.5 ml of cleaner to OptiFree and ReNu. For ClearCare, just insert the lens holder into the clear container. Set in sink in case of overflow.
11. Put 0.5 mL from each overnight case into 10 mL of scintillation fluid. Label vials. Wash out the holders and drip dry.
12. Wipe off any liquid & weigh containers. Leave on bench top during day in cleaning fluid.
13. At 5:00 put ATF from freezer into water beaker in incubator.
14. Lay out 12 holders, 12 scintillation vials, label vials.
15. When ATF is thawed, fill 12 holders with 0.5 mL each.

16. Open the cases, one at a time. Remove lens with forceps, rinse with saline solution by squirting into a beaker, dab dry, place in lens holder with cleaner.
17. Add 0.5 mL of ATF on top of lens. Watch it sink, or sink it. Close lid.Refreeze extra ATF by putting into partly filled frozen containers.
18. Put 0.5 mL from each overnight case into 10 mL of scintillation fluid. Label vials. Wash out the holders and drip dry.
19. Repeat steps 3 to 17 above.
20. After 30 days, extract any remaining DMPC from lenses using extraction procedure.

Lens Loading Procedure for 30-day Elution experiments

Materials:
Scintillation vials (2); 12 plastic storage vials (1 per lens); 12 glass extraction vials (1 per lens); cold 0.9% DMPC solution (~3.85 mL); hot 0.9% *DMPC solution (~0.15 mL); pipette tips; 2 of 50 mL plastic beakers ; tweezers; pure water, double distilled (~50 mL); 12 contact lenses; tape/markers (for labeling vials); Kim-wipes; scintillation fluid (10 mL); incubator (35°C); freezer (-20°C); micropipettors; 4-channel timer; mixer; hot liquid recycle container

1. Coat all vials with cold DMPC by swirling cold 0.15% DMPC solution and then label vials.
2. Pipette ~3.85 mL of cold 0.90% DMPC (in n-propanol) into labeled scintillation vial.
3. Pipette ~0.15 mL of Secondary Master *DMPC (0.90% ≈ 84 μCi) into vial.
4. Swirl to mix (solution should now have an activity of ~21 μCi/mL).
5. Place mixture in incubator at 35° for ~30 minutes.
6. Place additional materials in incubator: 12 extraction vials (4 mL) per lens; ATF (3 mL/lens); 12 storage vials (plastic) per lens w/~10 mL pure water; 2 plastic beakers w/ ~30 mL pure water; blister packs w/ lenses.
7. Wash tweezers thoroughly.
8. Remove materials from incubator: 1 blister pack; sorbtion solution; rinse beakers; 1 storage vial.
9. Set timer for 85 seconds (This should load about 200 ug into a lens.).
10. Remove lens from the pure water with forceps.
11. Blot dry on Kimwipes.
12. Introduce lens into *DMPC solution while starting the timer.
13. At 85 seconds, remove lens from *DMPC sorbent solution.
14. Transfer lens to first beaker of pure water for 5 seconds to stop sorption.
15. Return sorption solution to incubator.
16. Transfer lens to second vial of pure water for 5 seconds.
17. Transfer lens to a labeled plastic storage vial of pure water and place vial on shaker.
18. Dispose of rinsing solutions (not sorption solution) down the sink.
19. Repeat for all the lenses of that day.
20. For your 1st standard, pipette 10 μL of sorption solution into scintillation fluid; then add 90 μL pure water. Label vial as a 10 μL standard.
21. For your 2nd standard, pipette 100 μL of sorption solution into scintillation fluid. Label vial as a 100 μL standard.
22. At the end of the day, put sorption solution in storage container. Keep a log in the lab book of all hot waste transferred to storage.
23. Put hot recycle storage container in -20° freezer.

D. **Protocols for varies solution preparation**

Procedure for Hot Master Batch Preparation
Materials:
Weigh paper (1 piece); absorbent paper (1 sheet); scintillation vial with cap (1); pipette tip (1 large); hot DMPC (~20 mg); pure water, double distilled (~1-2 mL); spatula; tape/markers (for labeling vial); Geiger counter; micropipette (200 µL -1 mL); freezer (-20° C)

1. Put absorbent paper in the hood.
2. Put hot box in the hood.
3. Conduct a radiation survey on outside of box.
4. Open box; record contents of box in lab book.
5. Conduct a radiation survey on inside of box.
6. Open hot container.
7. Label scintillation vial.
8. Weigh out ~5.0 mg *DMPC onto folded weigh paper and add it to scintillation vial.
9. Weigh out ~15.0 mg of cold DMPC onto weigh paper and add it to vial.
10. Add n-propanol to approriate dilution: (mass / 20 mg) * 1.333 mL.
11. Swirl to mix.
12. Put the vial back in hot box. Put in -20° freezer.

This should make about 1.34 mL of solution with an activity of around 1.25 mCi, or 0.94 mCi/mL.

Procedure for Hot Secondary Master Preparation
Materials:
Scintillation vial w/cap (1); n-propanol (0.900 mL); pipette tips (1 large, 1 small); 1.5% *DMPC master solution (0.100 mL); micropipettors (200 µL-1 mL, 10 µL-100 µL); tape/markers (for labeling vial);

1. Label scintillation vial (name, date, contents, estimated µCi).
2. Wearing gloves and lab coat, retrieve Master Batch from the freezer.
3. Set in hood to warm to room temperature.
4. Pipette .100 mL of Master batch (1.5 % *DMPC ≈ .94 mCi/mL) into vial.
5. Pipette .900 mL of n-propanol into vial.
6. Swirl to mix.
7. Store in -20° freezer in working box.

This should make 1.000 mL of 0.15% w/v *DMPC solution with an activity of around .094 mCi, or 94 µCi.

Procedure for Cold Secondary Master Preparation
Materials:
Volumetric flask, glass (100 mL); cold DMPC (150.0 mg); weigh paper (1 piece); n-propanol (~100 mL); 20 mL serological pipette (1); pipette pump, thumbwheel; spatula; freezer (-20°)

1. Put on gloves and lab coat.
2. Label 100 mL volumetric flask.
3. Weigh 150 mg of cold DMPC on weigh paper.
4. Pour DMPC into volumetric flask.
5. Fill flask to 100 mL with n-propanol at room temperature using serological pipettes.
6. Store in freezer.

Makes 100 mL of 0.15% w/v cold DMPC solution

E. **Protocols for loading and release of DMPC**

Lens Sorption Procedure
Materials:
Scintillation vials (2); plastic storage vials (1 per lens); glass extraction vials (1 per lens); cold 0.15% DMPC solution (~4.7 mL); hot 0.15% *DMPC solution (~0.3 mL); pipette tips; 50 mL plastic beakers (2); tweezers; pure water, double distilled (~50 mL); contact lenses; tape/markers (for labeling vials); Kim-wipes; scintillation fluid (10 mL); incubator (35°C); freezer (-20°C); micropipettors; 4-channel timer; mixer; hot liquid recycle container

1. Coat all vials with cold DMPC by swirling cold 0.15% DMPC solution and then label vials.
2. Pipette ~4.7 mL of cold 0.15% DMPC (in n-propanol) into labeled scintillation vial.
3. Pipette ~0.3 mL of Secondary Master *DMPC (0.15% ≈ 94 µCi) into vial .
4. Swirl to mix (solution should now have an activity of ~5.64 µCi/mL).
5. Place mixture in incubator at 35° for ~30 minutes.
6. Place additional materials in incubator: 1 extraction vial (4 mL) per lens; ATF (3 mL/lens); 1 storage vial (plastic) per lens w/~10 mL pure water; 2 plastic beakers w/ ~30 mL pure water; blister packs w/ lenses.
7. Wash tweezers thoroughly.
8. Remove materials from incubator: 1 blister pack; sorbtion solution; rinse beakers; 1 storage vial.
9. Set timer for 60 seconds.
10. Remove lens from the pure water with forceps.
11. Blot dry on Kimwipes.
12. Introduce lens into *DMPC solution while starting the timer.

13. At 1 minute, remove lens from *DMPC sorbent solution.
14. Transfer lens to first beaker of pure water for 5 seconds to stop sorption.
15. Return sorption solution to incubator.
16. Transfer lens to second vial of pure water for 5 seconds.
17. Transfer lens to a labeled plastic storage vial of pure water and place vial on shaker.
18. Dispose of rinsing solutions (not sorption solution) down the sink.
19. Repeat for the lenses of that day.
20. Pipette 10 μL of sorption solution into scintillation fluid; then add 90 μL pure water. Label vial.
21. At the end of the day, put sorption solution in storage container. Keep a log in the lab book of all hot waste transferred to storage.
22. Put hot recycle storage container in -20° freezer.

Elution Procedure
Materials:
ATF or pure water (3.0 mL per lens); contact lenses; 4 mL glass elution vial (1 per lens); tweezers; micropipettor; pipette tip (1 large); timer; scintillation vials (8); scintillation fluid; incubator; mixer

1. Put ATF and pure water in 35° incubator.
2. Prepare scintillation vial: add 10 mL scintillation fluid and label the vial.
3. Pipette 3.00 mL elution fluid (either ATF or water) into small glass elution vial.
4. Pipette 0.100 mL elution fluid into scintillation fluid in a prepared scintillation vial.
5. Vortex the scintillation vial to mix and place vial in the refrigerator.
6. Remove lens from storage vial and dab it dry with a Kimwipe.
7. Transfer lens to elution vial.
8. Start timer.
9. Make sure lenses are totally immersed.
10. Pipette 0.100 mL of elution fluid into another scintillation vial; vortex, refridgerate.
11. Place elution vial on mixer in the incubator at 35°.
12. Repeat steps 4-5 after 2 hours, 4 hours, 10 hours, 24 hours, 48 hours, and 72 hours.

Control Preparation Procedure
Materials:
Micropipettor; scintillation vial (4); markers/tape (for labeling vial); pure water or ATF (90µL); scintillation fluid
ATF calibration:

1. Pipette 10 µL of hot sorption solution into 10 mL scintillation fluid.
2. Pipette 90 µL of ATF into scintillation fluid.
3. Label, shake, and put in fridge.
4. Repeat three more times to ensure accuracy of calibration.

n-propanol calibration:

1. Pipette 10 µL of hot sorption solution into 10 mL scintillation fluid.
2. Pipette 3.0 mL of n-propanol into scintillation fluid.
3. Label, shake, and put in fridge.
4. Repeat three more times to ensure accuracy of calibration.

Scintillation control:

1. Prepare a scintillation fluid vial.
2. Add 100 µL pure water or ATF to vial.
3. Label, shake, and put in fridge.

Extraction procedure
Materials:
Scintillation vials (2 per lens); n-propanol (6 mL per lens); scintillation fluid (20 mL per lens); tweezers; kimwipes; water

1. Fill 2 Sc Vials with 2.00 mL n-propanol.
2. Remove the extracted lens from the ATF or water, and rinse it into extraction vial 1 mL water to seal lens. (note: don't use n-propanol for this first rinsing, or the extraction will not be accurate).
3. Shake dry, blot dry.
4. Place lens in n-prop, shaking 1 hr.
5. With forceps, remove lens, rinse with 1.000 mL n-propanol into same vial. (note: this must be done with extreme care, as the lens very fragile at this point and will tear easily)
6. Place lens in second vial of n-propanol for 1 hr, shaking.
7. Add 10 mL Sc Fluid to the first vial; vortex, refridgerate.
8. Wrap extracted lens in kimwipe, put in dry hot waste.
9. With forceps, remove lens (carefully!), rinse with 1.000 mL n-propanol into same vial.
10. Add 10 mL Sc Fluid to this second vial; vortex, refridgerate.
11. Count the vials.

Spiking Procedure
All materials and procedures are the same as for a regular extraction procedure with the following exceptions:
Materials:
1 additional coated scintillation vial; ATF- instead of ATF (ATF- has all lipids removed)

1. After preparing the loading solution (.15% *DMPC), prepare a spiking solution as follows:
    a. Add (# μg DMPC desired in the elution solution)*(5 μL) of loading solution to a coated vial [ie if 2μg DMPC is desired, add 10μL loading solution].
    b. Add sufficient ATF- to bring the total fluid in the vial to 1 mL [ie if 10μL loading sltn is used, add 990μL ATF-].
2. Allow spiking solution to heat up to 35°C in the incubator.
3. Instead of initially adding 3mL ATF to each elution vial, add 2.867mL ATF- and .133mL spiking solution to each elution vial (for a total of 3mL solution).

All other procedures, controls, and amounts, and times are the same as in the elution protocol

APPENDIX 2. CODE TO CALCULATE ELUTION FROM A FINITE SLAB

Equation

$$C(c1, c0, n, D_{ab}, t, b, y) := c1 - 2\cdot(c1 - c0)\cdot \sum_{n=0}^{\infty} \left[\frac{(-1)^n}{(n + 0.5)\cdot\pi} \cdot \exp\left[\frac{-(0.5+ n)^2\cdot\pi^2\cdot D_{ab}\cdot t}{b^2} \right] \cdot \cos\left[\frac{(n + 0.5)\cdot\pi\cdot y}{b} \right] \right]$$

$$C(c1, c0, n, D_{ab}, t, b, y) := c1 - 2\cdot(c1 - c0)\cdot \left[\frac{(-1)^n}{(n + 0.5)\cdot\pi} \cdot \exp\left[\frac{-(0.5+ n)^2\cdot\pi^2\cdot D_{ab}\cdot t}{b^2} \right] \cdot \cos\left[\frac{(n + 0.5)\cdot\pi\cdot y}{b} \right] \right]$$

differentiate with respect of y

$$\frac{d}{dy} C(c1, c0, n, D_{ab}, t, b, y) \rightarrow -\frac{(-1)^n \cdot e^{-\frac{\pi^2\cdot D_{ab}\cdot t\cdot(n+0.5)^2}{b^2}} \cdot \sin\left[\frac{\pi\cdot y\cdot(n + 0.5)}{b} \right] \cdot (2\cdot c0 - 2\cdot c1)}{b}$$

Evaluate at y=b

$$C(c1, c0, n, D_{ab}, t, b) := \frac{(-1)^n \cdot e^{-\frac{\pi^2\cdot D_{ab}\cdot t\cdot(n+0.5)^2}{b^2}} \cdot \sin[\pi\cdot(n + 0.5)]\cdot(2\cdot c1 - 2\cdot c0)}{b}$$

Integration

$$\int C(c1, c0, n, D_{ab}, t, b)\, dt \rightarrow \frac{(-1)^n\cdot b\cdot \sin[\pi\cdot(n + 0.5)]\cdot 75 \cdot e^{-\frac{\pi^2\cdot D_{ab}\cdot t\cdot(n+0.5)^2}{b^2}} \cdot (2\cdot c0 - 2\cdot c1)}{\pi^2\cdot D_{ab}\cdot(n + 0.5)^2}$$

$$C(c1, c0, n, D_{ab}, t, b) := \frac{(-1)^n \cdot b \cdot \sin[\pi \cdot (n + 0.5)] \cdot e^{-\frac{\pi^2 \cdot D_{ab} \cdot t \cdot (n+0.5)^2}{b^2}} \cdot (2 \cdot c0 - 2 \cdot c1)}{\pi^2 \cdot D_{ab} \cdot (n + 0.5)^2} - \frac{(-1)^n \cdot b \cdot \sin[\pi \cdot (n + 0.5)] \cdot (2 \cdot c0 - 2 \cdot c1)}{\pi^2 \cdot D_{ab} \cdot (n + 0.5)^2}$$