Cystinosis Research Foundation Final Report

Title: Liposome cysteamine carriers for eye drop formulation with long-term stability and smart release ability

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Mentor: Morgan DiLeo, PhD

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OVERVIEW

We have built up a solid foundation to make liposome cysteamine carriers through this fellowship. Preparation method of liposomes has been established. HPLC method and Ellman's assay have been developed for the evaluation of cysteamine concentration and stability. HPLC method could provide simultaneous detection of cysteamine and cystamine in the same run. Ellman's assay could provide a quick analysis of cysteamine concentration in drug uptake and release studies. These preliminary results lay a solid foundation for the future experiment plans to testing more types of liposomes cysteamine carriers.

PROGRESS

1. Preparation of liposomes

Thin-film hydration method was tested to produce liposomes. We tested different shape (pear shape vs round shape) of volume (10 ml vs 25 ml) of flasks to check how well the thin film formed and rehydrated.

For baseline liposome formulation:

After rotovap and drying, all the flasks formed uniformed thin form except the 25 ml pear shaped flask.

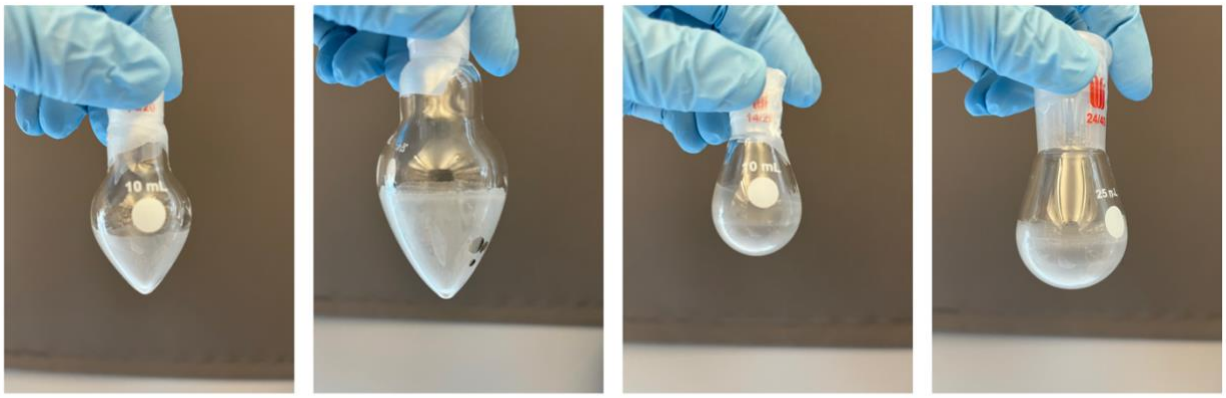

Figure 1. Dried lipid film formation after thin-film evaporation.

For lipid rehydration, 25 ml round shaped flask was the easiest.

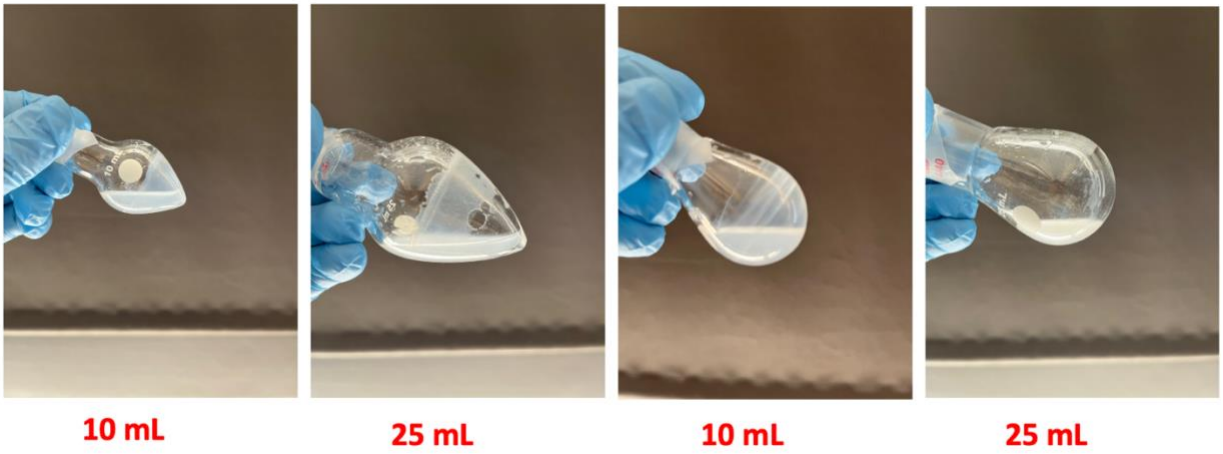

Figure 2. Dried lipid rehydration by vortex.

Therefore, 25 ml round shaped flask was chosen for liposome formulation.

Alginate/chitosan coated liposomes were obtained by layer-by-layer self-assembly technology as shown in figure 3.

Figure 3. Alginate/chitosan coating for liposomes.

2. Characterization of liposomes

Baseline liposome (no vitamin E and chitosan/alginate coating) was characterized by DLS for particles size. Liposomes had a diameter of 568 nm before sonication and 411 nm after sonication.

Table 1. Z-average diameter and PDI of liposomes

	Before sonication	**After sonication**
Z-average diameter (nm)	568 ± 49	411 ± 3
PDI	0.17 ± 0.12	0.43 ± 0.10

Average hydrodynamic diameter, particle size distribution (PDI), and surface charge (ζ-potential) of liposomes (with vitamin E and chitosan/alginate coating) will be determined once I finished the current training on Malvern Zetasizer Nano ZS90.

3. Evaluation of cysteamine stability by HPLC

HPLC method was used to simultaneously detect cysteamine and cystamine in their mixture solution. As shown in figure 4, the cysteamine standard curves for cysteamines only and cysteamine mixed with 50µl/ml cystamine are similar, which means that the present of cystamine wound not affect the detection of cysteamine.

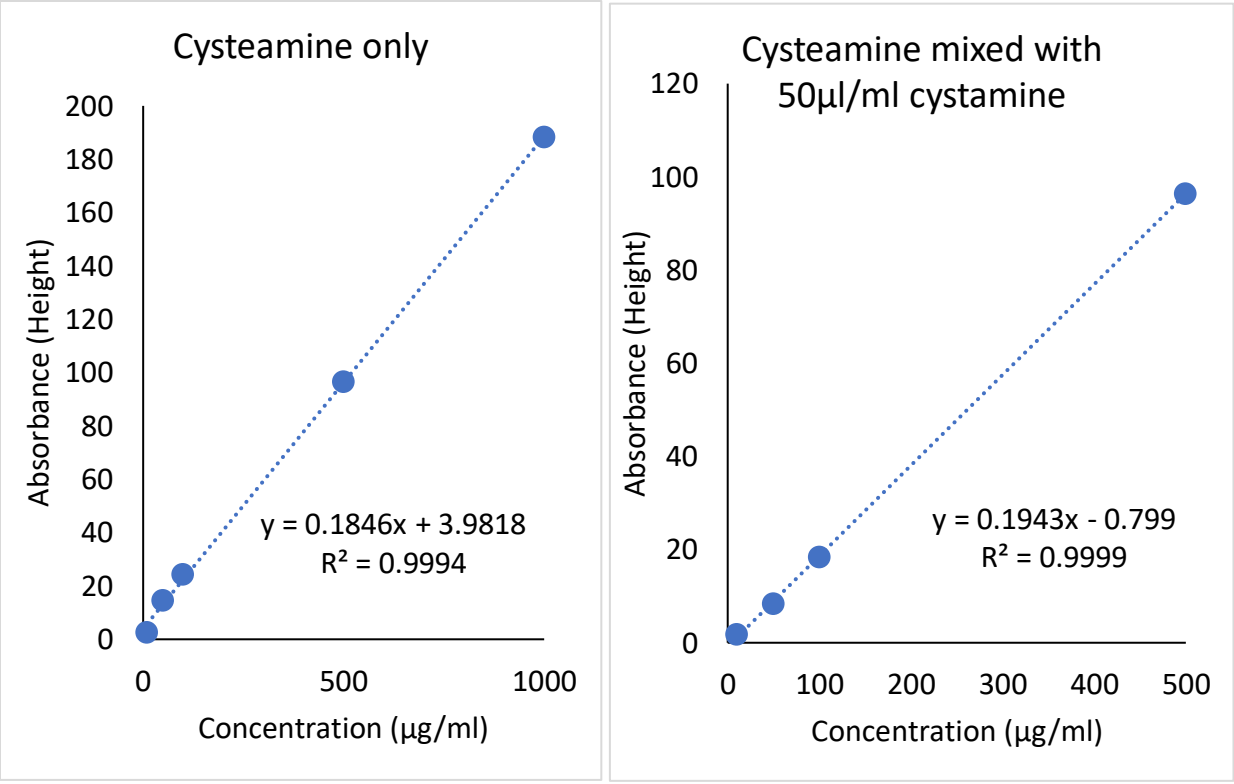

Figure 4. Cysteamine standard curves by HPLC.

Therefore, we used this metohd to test how the compositation of cysteamine solution changed overtime. As shown in figure 5, the concentration of cysteamine changed from 500μg/ml as Day 0 to 134μg/ml at Day 19. So, this results showed that there is a possibility to use HPLC for the evaluation of cysteamine stability besides NMR spectroscopy technique. As we have HPLC in our lab, so using HPLC is more convient for us to generate more testing data.

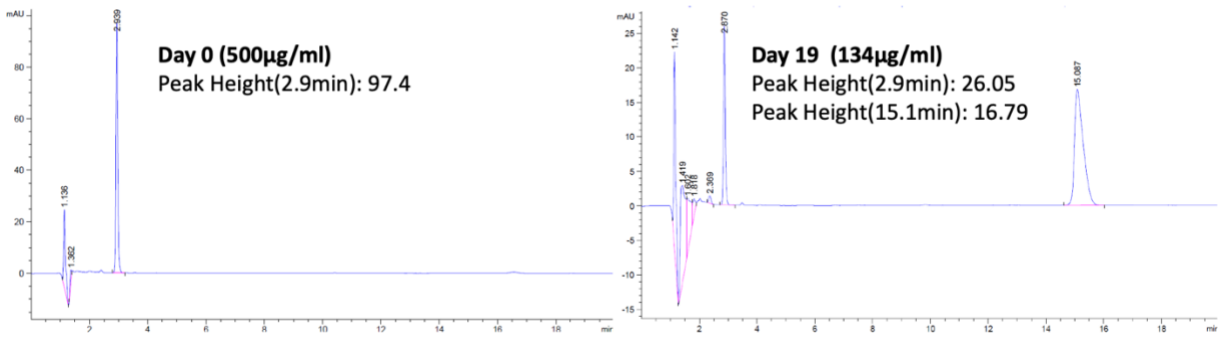

Figure 5. HPLC results of cysteamine solution over time.

4. Quantification of cysteamine in aqueous solution by Ellman's assay

Ellman's assay is a useful tool that can be used to determine the thiol group concentration of in cysteamine solutions. As shown in Figure 6, the calibration curves are almost identical for cysteamine in reaction buffer, PBS, and water ranging from 5 to 500 μg/mL. So, Ellman's assay can be a rapid method to detect cysteamine in liposome loading solution and in *in vitro* release samples.

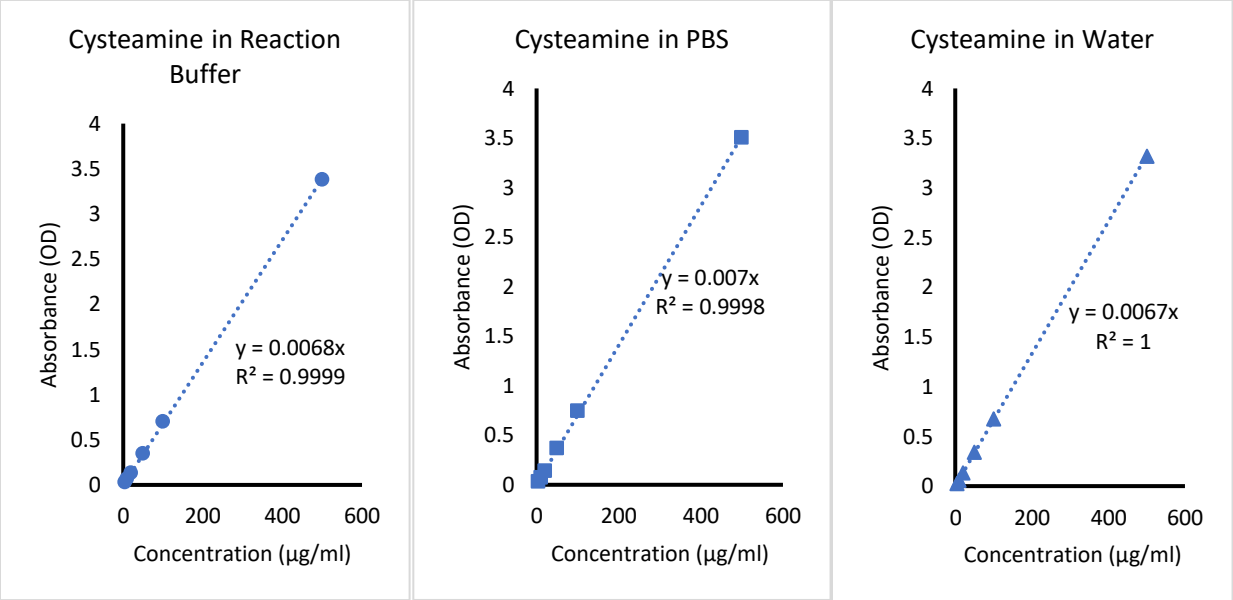

Figure 6. Calibration curves of cysteamine in different solvents by Ellman's assay.

5. Cysteamine uptake by liposomes

First, conventional hydration method was tested to load cysteamine in liposomes. In this method, cysteamine solution was used for rehydration lipid thin film following by size reduction with tip sonication. However, the drug loading efficiency was only 5% for two types of lipids (egg PC and DOPC) tested as listed in Table 2.

To increase the drug loading efficiency, Dehydration–Rehydration Vesicles (DRV) method was tested. In this method, a freeze-drying cycle is applied to disrupt the "empty" small unilamellar liposomes, then controlled rehydration is carried out in the presence of high concentration of cysteamine (achieved by using low amount of solvent). As shown in Table 2, DRV method increases the cysteamine loading efficiency to 38% when 1mg/ml of cysteamine was used, while only 5% loading was achieved by conventional hydration method with same cysteamine concentration. A higher cysteamine loading concentration (10 mg/ml) was also tested, and a 25% loading efficiency was resulted. This loading condition was used for the in vitro release testing.

Table 2. Cysteamine uptake by liposomes with different methods

Lipid	**Lipid Amount**	**Cysteamine Loading Concentration (mg/ml)**	**Drug Loading Method**	**Loading Efficiency**
Egg PC	20µmol	1	Conventional hydration	5%
DOPC	20µmol	1	Conventional hydration	5%
DOPC	20µmol	1	Dehydration–Rehydration Vesicles (DRV)	38%
DOPC	20µmol	10	Dehydration–Rehydration Vesicles (DRV)	25%

6. Cysteamine in vitro release experiment

The *in vitro* release profiles of cysteamine loaded DOPC liposomes (fresh made ones vs store 7 days ones) were tested in PBS at 37°C. Fresh made cysteamine loaded liposomes exhibited a sustained release profile over 24 hours. After store the same batch of liposome for 7 days under 4°C. A similar release profile is observed as shown in Figure 7, especially in the first 2 hours. Liposomes store for 7 days showed 92% of total cysteamine released compared to the fresh made liposomes at 4 hour and 6 hour. Significantly variations were observed at 24 hours for the release of liposomes store for 7 days. Further in vitro release experiment will be tested to check the cysteamine stability and release after different storage conditions for drug loaded liposomes.

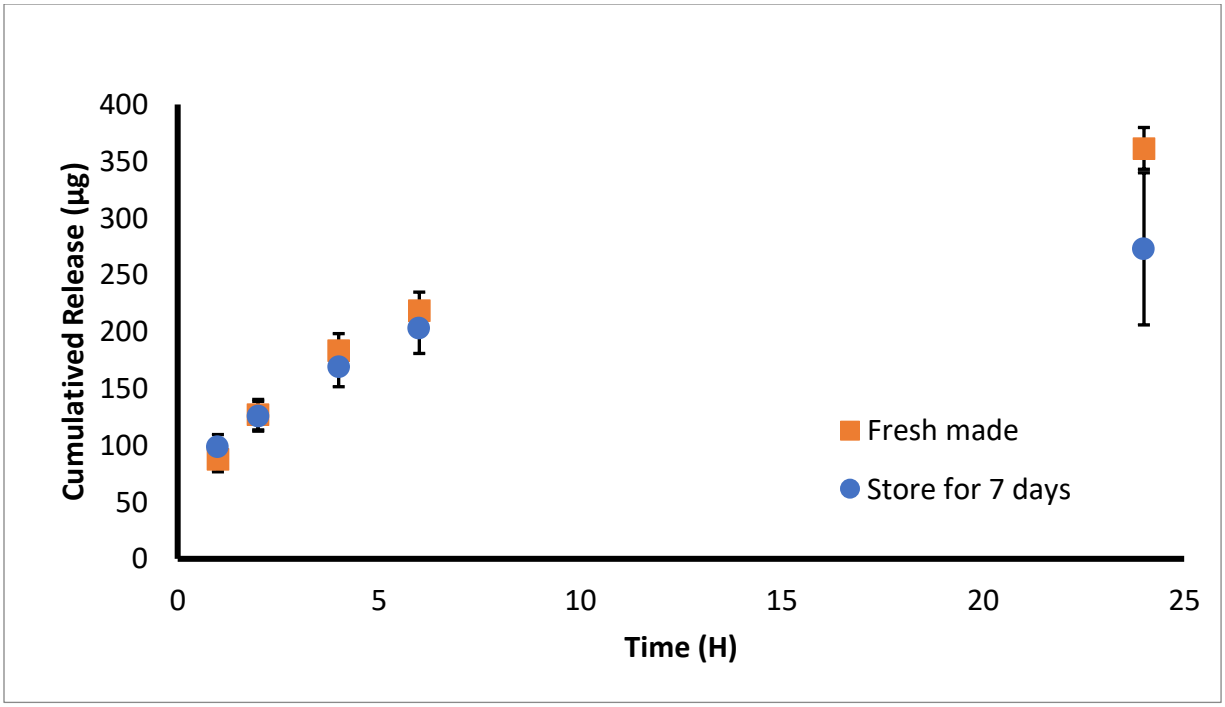

Figure 7. Cumulative release of cysteamine from different liposomes.

CONCLUSION

Through this fellowship, we have developed cysteamine loaded liposomes with sustained drug release ability last for more than 24 hours. In addition, the baseline liposome formulation can maintain 92% of the cysteamine after 7-day storage. More testes will be studied moving forward with additional chitosan/alginate layers applied to the liposomes. In addition, mucoadhesive test and pH-responsive ability will also be tested. The goal is to publish at least one manuscript by the support of this fellowship.

APPENDIX

The work of this fellowship was presented in a poster at the 2023 Controlled Release Society Annual Meeting.

Develop a controlled release system of cysteamine for corneal cystinosis with improved drug stability

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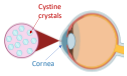
OCULAR CYSTINOSIS

Cystinosis in the Eye

- *Cystine crystals mainly accumulate in the cornea and cause severe ocular manifestations*



- *Photophobia is the most frequently reported ocular symptom in cystinosis*

Challenges in Current Treatments

- *Cystaran™ - one drop every waking hour*
- *Cystadrop™ - four drops per day*
- *Medication is only stable for 1 week*
- *Making adherence difficult for many patients*

PROPOSED DDS

Proposed Drug Delivery System(DDS)

➢ **Sustained drug release**

> 4X per day *< 1X per day*

➢ **Prolonged drug storage**

Stable 7 Days *Stable 30 Days*

RESULTS

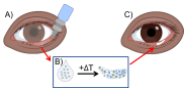
Spray-dried cysteamine microspheres/Gel (SD-CMS/Gel) Drug Delivery System

- Poly(lactic-co-glycolic acid) *PLGA* used in microsphere fabrication
- Poly(N-isopropylacrylamide) *PNIPAAm* and Poly(ethylene glycol) *PEG* used in thermoresponsive gel fabrication
- Cysteamine used as drug model

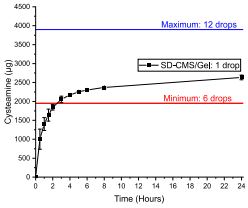
$T_1 < 34°C < T_2$

SD-CMS/Gel Application

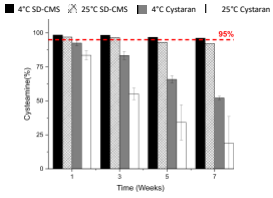
A. Topical application
B. Thermoresponsive
C. Locally sustained drug release



✓ **Sustained drug release**

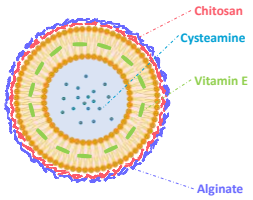


✓ **Prolonged drug storage**



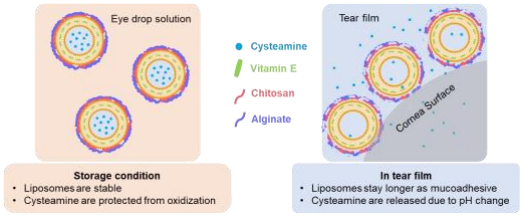
Liposome Drug Delivery System

- Cysteamine used as drug model
- Liposome as drug carriers
- Vitamin E offer anti-oxidization
- Alginate/chitosan coating offer mucoadhesion and pH-triggered release



DLS

Z-AVERAGE	PDI
568 ± 49 nm	0.17 ± 0.12



CONCLUSION

- *In vitro* drug release results showed SD-CMS/Gel could release cysteamine for at least 24 hours
- Encapsulation of cysteamine in SD-CMS/Gel maintained at least 95% drug stability for more than 7 weeks under 4°C

FUTURE WORKS

- Drug release and storage for liposomes
- Optical coherence tomography (OCT) analysis of cystine crystals in cornea

DISCLOSURES

- Morgan V. DiLeo: Code P (Patent) University of Pittsburgh

REFERENCES

- Jimenez J, Resnick J, Chaudhry A, Gertsman I, Nischal K, DiLeo, M. Int J Pharm 2022; (624):121992
- Jimenez J, Washington W, Resnick J, DiLeo, M. Drug Deliv Transl Res 2021; (11): 2224-2238

SUPPORT

CYSTINOSIS RESEARCH FOUNDATION

The Eye & Ear Foundation of Pittsburgh — Research to Prevent Blindness

National Institutes of Health — P30 EY0890

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