Light Assisted Drying (LAD) of Chlorophyll

NaKreshia Cox[a], Audrey Dorante [b], Heather Nash [b], Madison A. Young [c], Susan R. Trammell [c]
[a] Sedgefield Middle School, [b] East Mecklenburg High School,
[c] University of North Carolina at Charlotte, Dept. of Physics & Optical Science

Introduction

The overall goal of this project is to find an effective way to preserve drugs and vaccines without refrigeration. This purpose of this study was to determine if a novel biopreservation technique, Light-Assisted Drying (LAD), degraded an embedded molecule, chlorophyll.

- According to the World Energy Outlook, in 2016 an estimated 1.2 billion people (approximately 16% of the world's population) are without electricity.[1] A lack of reliable electricity can have a significant negative impact on healthcare. Many medications and vaccines require refrigeration to maintain potency.[2] Freeze-drying is the gold standard for processing these items. Unfortunately freeze-drying is expensive and not effective for all drugs and vaccines.[3]

- Anhydrous, or dry state, preservation in a trehalose matrix may be an alternative to freeze drying for the preservation of biological samples.[5] Disaccharide trehalose can form an amorphous solid (see Fig. 1) at room temperature and can also act as a bioprotectant, making trehalose an attractive option as a preservation matrix for embedded biologics.[7] Removing water from a trehalose solution forms an amorphous solid preservation matrix. This can then be stored at or below the associated glass transition temperature (T_g) (see Fig. 2). Samples must be dehydrated to low water contents for storage at room temperatures without degradation.[8]

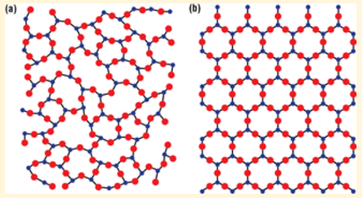

Figure 1: An amorphous solid is a non-crystalline solid in which the atoms and molecules are organized such that there is no long-range order.[6] The regular lattice of a crystalline solid can damage embedded biologics, limiting the usefulness of these solids as preservation matrices.

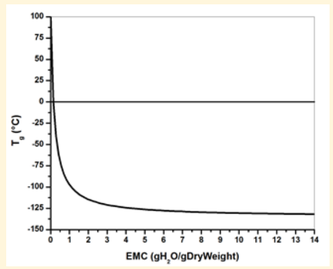

Figure 2: Glass transition temperature (T_g) of binary trehalose/ water solution as a function of moisture content. T_g is the storage temperature of the sample.

- LAD is a processing method to create an amorphous trehalose solid for the stabilization of drugs and vaccines.[9] The drug or vaccine is suspended in a trehalose (sugar) solution and then the sample is dehydrated using near-infrared laser light. The laser radiation speeds drying and as water is removed the sugar forms a protective matrix.

- Chlorophyll normally fluorescence under UV light. However, if LAD processing damages the chlorophyll, the molecule will no longer fluorescence. In this study, samples containing chlorophyll were processed using LAD and the degradation of the chlorophyll fluorescence was evaluated.

Methods

- Sample solution consisted of water soluble chlorophyll (MP Biomedical, LLC) dissolved in a 0.2M disaccharide trehalose in 0.33 x phosphate buffer solution (PBS) at a concentration of 0.55mg/ml. Chlorophyll was used as a test molecule in these studies. The molecule fluoresces at 635 nm when illuminated with UV light.
- All samples consisted of a 40 µL droplet of the chlorophyll solution deposited onto a glass substrate. All masses were recorded using 0.01 mg readability balance (RADWAG AS 82/220.R2), to calculate end moisture content (EMC).

$$EMC = \frac{[M_{df+cs} - M_{cs}] - (0.07 * M_{do}) - (0.55 * 0.04)}{(0.07 * M_{do}) - (0.55 * 0.04)}$$

- EMC is a measure of how wet a sample is.M_{df+cs} is the mass of the final droplet and the mass of the coverslip. M_{cs} is the mass of the cover slip. M_{do} is the initial mass of the droplet
- Samples were illuminated with a 405 nm UV LED (ThorLabs M405L2-C2). Fluorescence intensity specta were recorded before and after processing with an Ocean Optics spectrometer USB4000 (optical resolution- 1.5-2.3 nm FWHM and wavelength range 350-1000 nm). A change in the fluorescence spectrum indicates damage to the chlorophyll molecule during processing. An image of this setup is shown in Fig. 3.

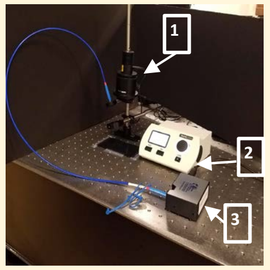

Figure 3: Spectrometer set-up: (1) ThorLab 405 nm UV LED, (2) LED driver, (3) Ocean Optics USB4000.

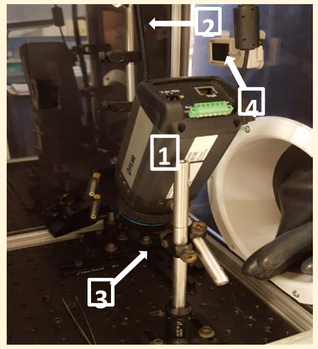

Figure 4: Light assisted drying set-up: (1) Thermal camera, (2) Fiber laser, (3) Sample location, (4) Relative humidity meter.

- Drops were processed with LAD for 60 minutes with a continuous wave Nd:YAG laser source at 1064 nm (IPG Photonics YLR-5-1064). The maximum power output of the laser was 5 W and the laser spot size was 4.5 mm. A schematic of the experimental setup is shown in Fig. 4.
- A FLIR SC655 mid-IR camera was used to record the temperature of samples in all drying experiments. The camera has an array of 640 x 480 pixels and a maximum frame rate of 200 fps.
- All studies were performed in a humidity-controlled environment that was kept at approximately 22% relative humidity (RH). This was achieved by pumping dried air into a chamber containing the experimental setup and monitoring the RH with a temperature and RH logger (ONSET UX100-011). Maintaining a low relative humidity expedited the drying process.
- In addition, identical samples were allowed to air dry in the relative humidity chamber as a control.

Results

- Table 1 shows that LAD leads to lower EMC than air drying alone after 60 minutes of processing and that processing at higher temperatures gives the lowest EMC. The air drying samples show that an increase in RH of the processing environment yields higher EMC.

Table 1: EMC and T_g of chlorophyll samples.

Sample	EMC (gH_2O/gDryWeight)	T_m (°C)	T_g (-C)	% RH
Light Assisted Drying Samples				
1	0.11734	48.5	10.94	20.222 ±1.6
2	0.11093	55.13	14.49	19.956 ±1.7
3	0.14495	50.5	-1	22.752 ±1.6
4	0.09579	60.9	21.86	21.953 ±2.3
5	0.19304	51.4	-17.72	45.468 ±19.0
Air Dried Samples				
1	0.14817	N/A	-2.26	22.709 ±1.6
2	0.18553	N/A	-15.39	19.270 ±1.2
3	0.27966	N/A	-39.24	19.602 ±1.4
4	5.40363	N/A	-126.92	39.618 ±19.3
5	5.46521	N/A	-127.01	31.430 ±4.2
6	5.35011	N/A	-126.84	33.030 ±5.3

- Minor variations in EMC lead to large variations in T_g. LAD samples have a wide range of storage temperatures but most are above freezing compared to air drying alone which is consistently sub zero T_g

Table 2: EMC of chlorophyll and lysozyme.

Wavelength (nm)	Temperature (°C)	EMC (gH_2O/gDryWeight)
Chlorophyll Sample		
1064	53.2°	0.13241 ±0.03
Lysozyme Sample		
1064	35.2°	0.1880 ±0.02
	43.0°	0.1682 ±0.04
1850	35.0°	0.2200 ±0.03
	42.6°	0.1654 ±0.03
	77.6°	0.1373 ±0.02

- Chlorophyll samples lead to lower EMCs than lysozyme samples even when lysozyme was processed at higher T_m (Table 2). Potentially because the 1064 nm laser couples strongly into chlorophyll and maintains a higher temperature for a longer processing time than lysozyme at other processing parameters (see Fig. 5).

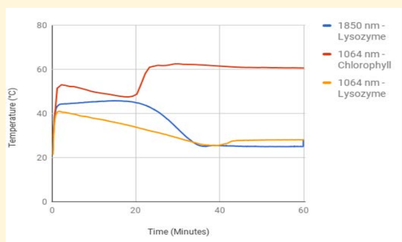

Figure 5: Thermal histories of samples from Table 2.

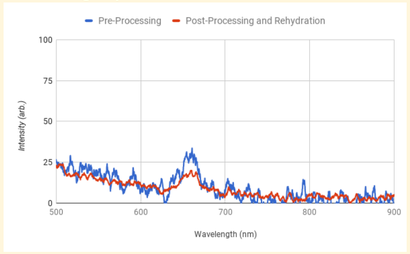

Figure 6: Fluorescence spectra of chlorophyll sample.

- Fluorescence intensity of a sample of chlorophyll in solution was analyzed before processing and again after it was processed and rehydrated.
- Fluorescence of chlorophyll decreased by about 37%. (See Fig. 6) This may indicate that LAD processing degrades the sample, possibly due to temperature.

Future Work

- Polarized Light Microscopy to determine whether or not there is crystallization in an amorphous solid.
- Raman Spectroscopy to determine how the sugar content is distributed across a sample.
- White Light Interferometry to determine the topography of a sample.

Acknowledgements:

This research was supported by Charlotte Teachers Institute and the Dept. of Physics and Opt. Science at UNC Charlotte

References:

[1] Energy access and resilience." World Health Organization. Accessed June 21, 2017. http://www.who.int/sustainable-development/health-sector/health-risks/energy-access/en/.
[2] Vaccine Storage and Handling Kit, US Department of Health and Human Services: Centers of Disease Control and Prevention, June 2016, Looking to find a way to preserve structure/function without refrigeration for protein-based drugs, vaccines, etc.
[3] Young, Madison A., Andrew T. Antczak, Gloria D. Elliott, and Susan R. Trammell. "Light assisted drying (LAD) for protein stabilization: optimization of laser processing parameters." In *Proc. of SPIE Vol*, vol. 10081, pp. 1008104-1. 2017
[4] "Protein stability and storage," Pierce Biotechnology, Inc., Rockford (2005) TR0043.1,
[5] Elliott, G.D., Chakraborty, N., and Bilswas, D., "Anhydrous preservation of Mammalian cells: cumulative osmotic stress analysis.," Biopreservation and biobanking 6(4), 253–60 (2008).
[6] Yu, L., "Amorphous pharmaceutical solids: preparation, characterization and stabilization," Advanced Drug Delivery Reviews 48(1), 27–42 (2001).
[7] Crowe, J.H., and Crowe, L.M., "Preservation of mammalian cells-learning nature's tricks.," Nature biotechnology 18(2), 145–6 (2000).
[8] Champion, D., Le Meste, M., and Simatos, D., "Towards an improved understanding of glass transition and relaxations in foods: molecular mobility in the glass transition range," Trends in Food Science & Technology 11(2), 41–55 (2000).
[9] Young, Madison A., Andrew T. Antczak, Gloria D. Elliott, and Susan R. Trammell. "Light assisted drying (LAD) for protein stabilization: optimization of laser processing parameters." In *Proc. of SPIE Vol*, vol. 10081, pp. 1008108-1. 2017.