Stability of various types of nanolipoprotein particles (NLPs) upon lyophilization

Purna Venkataraman[1], Craig D. Blanchette[2], Nicholas O. Fischer[2]

[1]San Lorenzo High School, San Lorenzo, CA; [2]BBTD, Lawrence Livermore National Laboratory, Livermore, CA

ABSTRACT: Nanolipoprotein particles (NLPS) have many potential uses in modern medicine, from cancer therapeutics to vaccine alternatives. As with all pharmaceuticals, the ease of storage and adequate stability of a compound is always a question. It has been found that NLPs can be very stable upon lyophilization, a freeze-drying technique in which all of the water in a sample is removed, if the initial conditions are suitable. In these experiments the stability of NLPs prepared with different combinations of lipids were tested in order to determine the optimum NLP conditions. NLPs composed of different types of lipids were constructed and then lyophilized in a buffered solution containing the sugar trehalose as an excipient. Then the samples were rehydrated and analyzed using size-exclusion chromatography (SEC) to determine if the NLP remained intact. Results indicate that the lipid composition of the NLP plays an important role in the particle stability upon lyophilization. NLPs prepared with the saturated DMPC lipid are more stable upon lyophilization than those prepared with unsaturated lipids (DOPC and/or DOGS-NTA-Ni), and require less excipient (i.e. trehalose) upon lyophilization to retain structure. These studies will have implications on NLP and storage formulations for vaccine and therapeutic applications.

Background Information

What are NLPs?

Nanolipoprotein particles or NLPs are composed of lipids and protein (See Figure A, to right). NLPs are useful because they can be used as vaccines or to carry drugs. We can make NLPs using a variety of different lipids depending on what the function of the NLP will be.

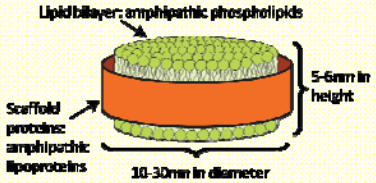

Figure A: NLPs are discoidal particles comprised of a lipid bilayer encircled by amphipathic scaffold proteins.

The reaction used to create NLPs is very simple. The lipids are mixed together in an aqueous buffer using a detergent called cholate. After all of the lipids are in solution, the protein is added. The solution is then dialyzed to remove the detergent and allow NLPs to form. The particles spontaneously form if the correct concentrations of lipids and proteins are mixed.

What is lyophilization?

Lyophilization is a way of quickly removing all of the water in a solution. Prior to lyophilization, samples are frozen using dry ice. Then the samples are placed in a container and all of the air in the container is removed which creates a vacuum. Under a vacuum, the frozen water sublimates; it turns from solid water to water vapor which effectively removes all of the water in the sample.

Varieties of NLPs

NLPs are most commonly made using these three lipids: DMPC, DOPC and DOGS-NTA-Ni.

DMPC and DOPC are both amphipathic lipids made up of long chains of hydrogen and carbon with a polar head group composed mostly of oxygen, phosphorus and nitrogen. DOGS NTA-Ni is also an amphipathic lipid but the polar part contains mostly oxygen and nitrogen in addition to a nickel atom.

Unsaturated lipid (DOPC & DOGS-NTA-Ni) Saturated lipid (DMPC)

Figure B: The double bonds in the unsaturated lipids do not let the lipid tails to get as close to one another as the saturated lipids.

Even though these two lipids are similar, they can make a variety of different sized NLPs. DOPC is an unsaturated lipid which means that some of the carbons are attached to each other by double bonds. The double bonds in the hydrocarbon chain create kinks which creates spaces when the lipids pack into a bilayer. DMPC is a saturated lipid which means that all of the carbons are connected by only single bonds. Since there are no double bonds these lipids can pack very close together when they assemble(see Figure B, above).

DOGS NTA-Ni is similar to DOPC, but the nickel atom gives it a different function. The nickel atom on the polar head can be used to attach other molecules that make the NLP useful in targeting drugs to specific parts of the body or making vaccines.

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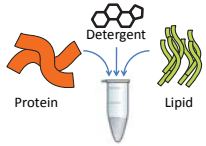
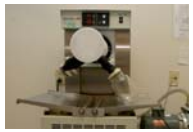
Methods





Assemble NLPs
- Mix lipids, detergent and protein
- Purify using SEC
- Analyze fractions using gel electrophoresis to decide which fractions contain the highest [NLP]

Lyophilize Samples
- Each sample contains a different type of NLP and a different [sugar] to [NLP] ratio

Analyze stability after rehydration using SEC
- Depending on the traces produced the amount of NLP that is intact can be calculated.

What is size exclusion chromatography?

Size exclusion chromatography (SEC) separates molecules based on size. Samples containing particles of different sizes are injected into a column containing porous beads. These beads have pores and channels of different sizes that accommodate small particles easier than larger particles (see Figure C). In this manner, large particles cannot fit into the channels and quickly pass around the beads. Small particle easily enter the channels, and then require a longer time to flow out of the column. This technique is used to identify the sizes of the NLPs and also to separate out the particular sizes of NLPs that will be needed in an experiment.

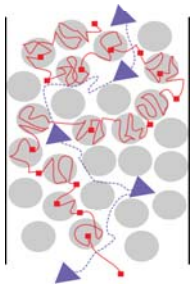
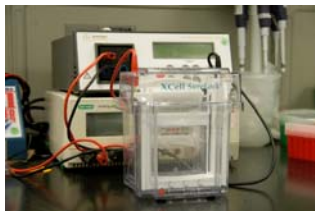


Figure C: The red molecules are small enough travel through the porous beads. The blue molecules are too big and move around the beads



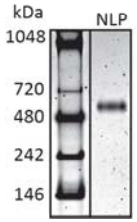


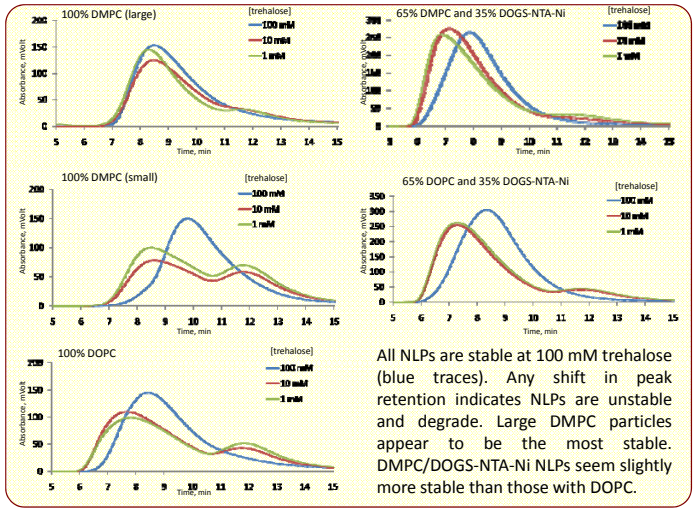
Figure D: The gel apparatus is connected to a power supply that sends current through the buffer chambers and gel (left). Native gels can be used to assess the purity of NLPs. A single band indicates that the NLPs are all a single size (right).

What is native gel electrophoresis?

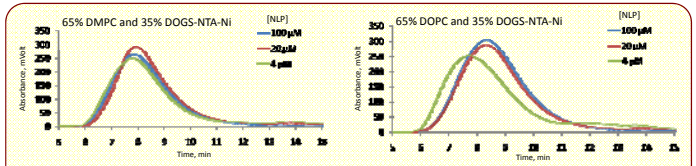
Native gel electrophoresis is another method used to separate particles based on size. In this technique, samples are placed on a gelatin-like substance composed of cross-linked polymers. This gel is placed in a salt solution (buffer) and a current is run through the gel. The flowing current pulls the particles in the sample along the gel. Large particles do not move very far and small particles move a longer distance down the gel.

Data

SEC chromatograms of the various types of NLPs tested demonstrate the differences in NLP stability depending on composition, trehalose concentration, and NLP concentration.



All NLPs are stable at 100 mM trehalose (blue traces). Any shift in peak retention indicates NLPs are unstable and degrade. Large DMPC particles appear to be the most stable. DMPC/DOGS-NTA-Ni NLPs seem slightly more stable than those with DOPC.



The two most commonly used NLPs for vaccine applications are those containing 35% DOGS-NTA-Ni and 65% of either DMPC or DOPC. These graphs represent tests of different concentrations of these two NLPs at a constant trehalose concentration (100 mM). For the DMPC-based NLPs, any NLP concentration is significantly stable as long as the sugar concentration is 100 mM. For NLPs composed of DOPC and DOGS-NTA-Ni, the NLPs start to fall apart at a low concentration (4 µM). These results suggest that NLPs composed of both saturated and unsaturated lipids are more stable upon lyophilization than NLPs composed only of unsaturated lipids.

Conclusion/Future Research

Based on the data above, it is reasonable to say that NLPs containing DMPC are most stable. This is because the saturated DMPC lipids are able to pack more closely together, providing a more ordered and stable lipid bilayer. Therefore, they do not fall apart as easily at NLPs containing the unsaturated DOPC or DOGS-NTA-Ni lipids.

The next step to this project would include tests using NLPs containing adjuvants. Adjuvants are molecules that stimulate the immune system. NLPs containing adjuvants have greatly improved the potency of certain vaccine candidates and are the focus of ongoing research at LLNL.

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