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Liposomes - Frequently Asked Questions

How should I store my liposomes?
Once lipid particles have been formed, maintaining the physical properties of the particles can be difficult. Size distribution can change on storage due to degradation of the components. Permeabilization of the membrane can lead to leakage of encapsulated material. Lipid suspensions should be stored at close to pH 7 as possible. Lipids containing ester-linked hydrocarbon chains are susceptible to acid and base hydrolysis. Hydrolysis rate is dramatically affected by temperature, therefore lipid suspensions should be kept refrigerated during storage. Lipid suspensions should not be frozen as the freezing process could fracture or rupture the vesicles leading to a change in size distribution and loss of internal contents. The use of cryoprotectants such as dextrose, sucrose, and trehalose may increase stability from hydrolysis. Also, samples may experience oxidation upon storage. The addition of small amounts of antioxidants during processing may stabilize the suspension and limit oxidation of the product.

How long can I store liposomes?
Storage time depends on a number of factors including temperature, pH, medium, etc. For example, liposomes stored in a buffer at pH 7.4 and at ~4°C did not display membrane structural changes for 5-7 days as demonstrated by retention of a trapped fluorescent marker. Beyond that time the fluorescent marker began to leak out of the liposome indicating the presence of membrane destabilizing components, presumably lyso lipid and free fatty acid generated by hydrolysis of the lipid. Liposome suspensions should not be frozen as the freezing process could fracture or rupture the vesicles leading to a change in size distribution and loss of internal contents. Depending on the application for the liposome, changes in particle size can have a dramatic effect on functionality. If membrane structure is not a critical parameter in your experiments, vesicles may be stored for 1-2 months with minimal (<10%) hydrolytic degradation.

What are the differences between liposomes and micelles?
Liposomes are composed of a lipid bilayer separating an aqueous internal compartment. Micelles are closed lipid monolayers with a fatty acid core and polar surface.

How do I concentrate a liposome?
There is not a good general procedure for concentration of liposomes. Large liposomal particles can be concentrated using centrifugation. The lowest speed possible to achieve pelleting is best since higher speeds could induce deformation and/or fusion of particles. Centrifugation is only possible with larger particles (>100nm). Small, sonicated (SUV) particles will not readily pellet due to their small size. Other methods of concentration may be possible but could lead to particle shape deformation depending on the sample and technique.

What issues do I need to consider when selecting lipids?
PHASE TRANSITION TEMPERATURE: The phase transition temperature is defined as the temperature required to induce a change in the lipid physical state from the ordered gel phase, where the hydrocarbon chains are fully extended and closely packed, to the disordered liquid crystalline phase, where the hydrocarbon chains are randomly oriented and fluid.[1] There are several factors which directly affect the phase transition temperature including hydrocarbon length, unsaturation, charge, and headgroup species. As the hydrocarbon length is increased, van der Waals interactions become stronger requiring more energy to disrupt the ordered packing, thus the phase transition temperature increases. Likewise, introducing a double bond into the acyl group puts a kink in the chain, which requires much lower temperatures to induce an ordered packing arrangement. Echelon's diC16 lipids, are saturated and therefore, have a phase transition temperature of approximately 48°C

STABILITY: Stability issues due to hydrolytic degradation is a general problem with lipid products. This hydrolysis is dependant on several factors[6] including pH,[3] temperature,[3,5] buffer species,[5] ionic strength, acyl chain length and headgroup,[4] and the state of aggregation.4 Generally, the more unsaturated a compound, the easier its oxidized, and thus the shorter the shelf life of the product. Lipids from biological sources (e.g., egg, bovine, or soybean) typically contain significant levels of polyunsaturated fatty acids and therefore are inherently less stable than their synthetic counterparts. Saturated lipids offer the greatest stability in terms of oxidation and also have much higher transition temperatures. If unsaturation is a requirement, keep the degree of unsaturation as low as possible to improve stability.
Others have shown that the cause of this hydrolysis is possibly due to the penetration of water into the membrane. Cholesterol could play a role in stabilizing lipid membranes to hydrolysis[7] and more recently, lipid preparations have been stabilized using carbohydrates.[8,9]

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CHARGE: Many biological membranes carry a net negative charge on their surface. The charge is generally imparted by the presence of anionic phospholipid species in the membrane. The major naturally occuring anionic phospholipids are phosphatidylserine, phosphatidylinositol, phosphatidic acid, and cardiolipin. Some bacterial systems also contain phosphatidylglycerol. The charge may provide a special function for the membrane and therefore, in some systems, not only must the charge requirement be satisfied, the system specificity for a particular species must be satisfied.

SOURCE: There are two basic sources of phospholipids: synthetic and tissue-derived. Animal-derived products do not offer any advantage to synthetic lipids. They are inherently less stable due to the polyunsaturated fatty acids, and in most cases the synthetic counterpart cost the same or less than the tissue-derived product.

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