Porous Inorganic Supported Lipid Membranes

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Introduction

Inorganic materials can be used to support lipid membranes in both planar and spherical geometries. These supported lipid membranes are of great interest because they mimic biomembranes and provide an ideal environment to host proteins which can be used in ion exchange. The inorganic supports are important and novel for two reasons: they provide mechanical support which allows the membrane increased stability, and the porous support allows a large capacity to store materials for ion exchange.

Experimental

The membranes used in this research combine properties of the liposomes which are spherical shells formed by a bilayer of lipid molecules [1], as well as the supported lipid bilayers. The particle is spherical in shape and has the ability to separate intracellular liquid from the extracellular bulk liquid, but also provides support for the lipid. Yang has studied the lipid membranes on spherical gel beads, but lipid membranes on powder supports has never before been studied.[2,3] The inorganic support also aids in the stability of the lipid membrane. Typically lipid membranes have a lifetime of less than eight hours.[4] The support particle, shown in Figure 1, consists of a mesoporous γ-alumina core, followed by a microporous silica layer, and finally the surface is modified by the addition of C18. The C18 then interacts with the lipid molecules to create a bilayer.

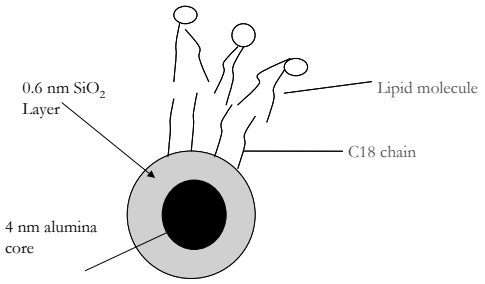

Figure 1: Porous Oxide support.

The inner core is made up of a mesoporous γ-alumina. The alumina is made by the PICA (Polymer Induced Colloid Aggregation) method. Boehmite Sol, made from alumina precursor ATSB, is mixed with Urea and Formaldehyde at a pH of 2. The mixture becomes milky white, and it settles into a clear liquid and spherical particles. The particles are washed several times with de-ionized water and ethanol. The particles are then collected by filtration, dried and calcined in order to burn off the organic materials. The γ-alumina core has pore diameters of approximately 4nm and around 50% porosity. Figure 2 shows the appearance of the alumina powders. The purpose of the porous alumina core is to provide support, but more importantly in provides large reservoirs to store a variety of material used in ion channel transfer.

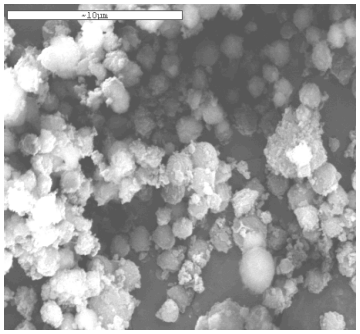
Figure 2: γ-alumina particles

The γ-alumina core is surrounded by a microporous silica layer. The silica layer is prepared by the sol-gel method using silica precursor TEOS (tetraethylorthosilicate). The silica layer has pore sizes of approximately 0.7 nm with around 40% porosity. The purpose of the

microporous silica layer is to provide a surface to attach the C18 functional groups. If the C18 was attached directly onto the alumina core, the C18 may infiltrate into the pores and not remain on the spherical surface.

A C18 group is added to the surface of the γ-alumina particle covered with the silica layer. The C18 is added by a surface silyation reaction, as shown in Figure 3. In this reaction the chlorine in the C18 group reacts with the hydroxyl group on the silica surface, releasing hydrochloric acid. It is also assumed the all three chlorines on the C18 group interact with a hydroxyl group, causing the C18 to be even further stabilized on the surface. The purpose of the silica layer is to modify the hydrophilic silica surface into a hydrophobic surface. The hydrophobic C18 then interacts with the hydrophobic tail end of the lipid molecule to create a bilayer.

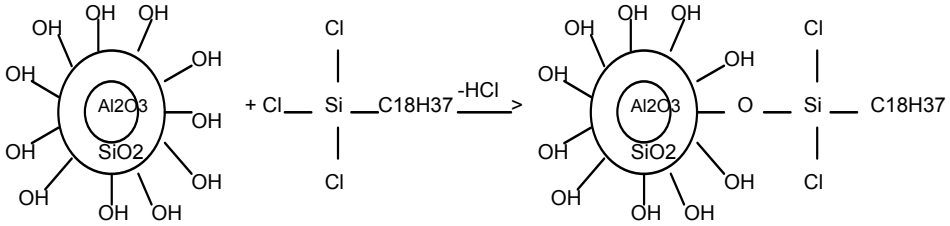

Figure 3: Surface silyation reaction between C18 and the silica surface

The FTIR curve shown in Figure 4 shows evidence of the presence of C18 on the surface of the silica. The top curve of the composite alumina/silica/C18 sphere shows clear C-H adsorption bands around 2900-3000 cm^{-1}. This is evidence that the surface silyation reaction did take place and the C18 is bound to the surface.

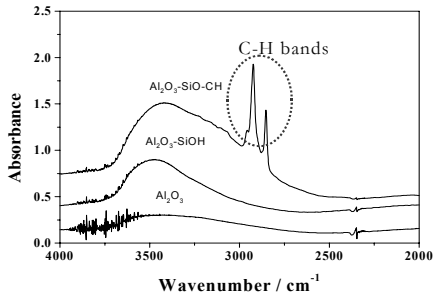

Figure 4: FTIR curve of composite sphere.

A TGA/DSC curve was also performed on the composite alumina/silica/C18 sphere. The peak at approximately 350°C shows the decomposition of the C18, further proving the presence of the C18 on the silica surface. The TGA curve also shows that the surface coverage of C18 is 2.1/nm^2, which is around half the theoretical coverage of 4.6/nm^2.

Because the spherical support used in this research has properties similar to liposomes, as well as supported bilayers, we must combine techniques to deposit lipid molecules on the surface of the particles. First, a water layer is placed in a beaker, then 500μL of lipid solution is placed on top of the water layer. The decane solvent is allowed to evaporate for 30 minutes and then 500mg powder containing the C18 group is added to the top of the lipid layer. An additional 500μL of lipid solution is then placed on the powder. The solvent is again allowed to evaporate for 30 minutes. The contents are then mixed with a stir bar for 1 hour and then sonicated for 2 hours. The lipid containing the protein is deposited in same fashion, except 550μL of lipid/protein solution is used instead of the 500μL lipid.

Results and Discussion

Because the spherical particles have an alumina center with around 50% porosity, it is able to hold a large amount of material. Therefore, material can exchange across the surface of the particle. The proteins in the lipid layer create very selective ion channels. In order to

determine the permeability and selectivity of various layers of the particle, we employ a pH change test. 300mg of powder was placed in 20mL of 0.01M KCl. The mixture was stirred overnight. The particles were collected by filtration and then immediately immersed into 20mL of 0.01M HCl. The pH change was measured with time. The results can be seen in Figure 5.

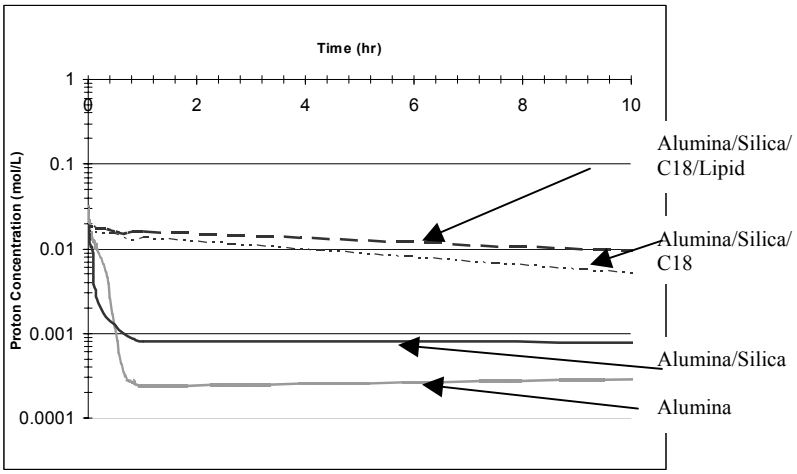

Figure 5: pH Change with Time.

We can see from the graph that the γ-alumina shows an immediate change in proton concentration. After the addition of the silica layer, the proton concentration change is reduced slightly. The addition of the C18 layer creates a large barrier for proton transfer and we can see a much slower proton transfer rate. The rate is even further decreased after the addition of the lipid layer.

The resistance to ion exchange of the sample with the C18/Lipid bilayer observed in the pH change tests shows the inorganic materials do provide a support for a lipid membrane. In addition to providing support, the pH test also shows the ability for the support to hold a large amount of material for ion exchange with the bulk solution. This supported membrane has the potential to host materials such as proteins for ion channeling, which will be the pathway to several applications such as biosensors and controlled drug delivery.

References

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[4] Tien, H. and Ottova, A. The lipid bilayer concept and its experimental realization: from soap bubbles, kitchen sick, to bilayer lipid membranes. *J. Membrane Science.* 2001; 189: 83-117.