X-Ray Mapping of Mineral Phases Incorporated into Liposomes

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Introduction

A liposome is a spherical particle formed by a lipid bilayer enclosing an aqueous compartment at its center. These particles can be comprised of a variety of lipids, particularly phospholipids. They have been long considered as potential delivery devices in the medical and pharmaceutical industries because of their ability to encapsulate different compounds as the lipids form into liposomes. The biocompatibility of many liposomes has been widely studied, and this has led to the development of a number of drug formulations [1]. The ability of liposomes to be stored in the body and to be taken up by cells makes them ideal for drug delivery.

Our research work aims to explore the variations in mineral deposits that can form inside liposomes by preparing liposomes in modified calcifying buffers. The overall aim of the work is to prepare calcium-loaded liposomes. This article deals with the characterization of liposomes, the investigation of the types of minerals that develop within liposomes, and the identification via x-ray mapping (XRM) of different phases forming within liposomes.

Methods

The liposomes were produced using L-α-phosphatidycholine (PC) and cholesterol, both obtained from Sigma-Aldrich (#61755 and #C3045, respectively). PC is a phospholipid with a polar head and non-polar tail. Cholesterol is used to strengthen the membrane of the liposomes and reduce the possibility of rupturing of the liposomes during processing and storage.

The liposomes were prepared in three different calcified aqueous solutions, one containing a $CaCl_2$-based solution (First Solution), the second containing $CaCl_2$ in addition to $NaHCO_3$ (Second solution), and the third containing $CaCl_2$ in addition to K_2HPO_4-based solution (Third Solution).

Encapsulation of calcium or mineral is believed to occur either because a preformed mineral deposit in the solution gets trapped as the liposome lamellar are forming or because the ions, which have an attraction to the phosphate of the lipid layers, build up on the surface of the liposomes before the second layer is placed on top. Portions of samples were collected and imaged via TEM to ensure the formation of liposomes. The remaining samples were washed in triplicate by re-suspending in 200 µl of Milli Q water and spun for one hour at 1200 rpm. The liposomes were re-suspended in 50 µl of Milli Q water. The ion concentration differences between the inside and outside of the liposome cause rupturing at this point, and the liposomes can no longer be visualized. However, because of the wash steps, it can be determined that all remaining mineral was bound inside the liposome. The solution was placed onto a silicon wafer to concentrate and dry. The samples were carbon coated and characterized using a Philips XL-30 ESEM and a JEOL 35CF with a Moran Scientific x-ray microanalysis mapping system.

Results

Quantitative x-ray mapping (QXRM) is able to show subtle changes in the distribution of elements, suggesting the phases present, which can then be positively identified using x-ray diffraction [2]. This makes QXRM particularly useful for identifying the location of individual elements and chemical phases within liposomes. Figures 1A–1C show primary-color pseudocoloring x-ray maps and reveal the types of phases produced. With primary coloring, each of three elements selected from the QXRM is assigned a color of red, green, or blue [3, 4]. In this example, for all three primary-color images in Figures 1A–1C, red codes for P, green is Ca, and blue is Si. When comparing these primary-color maps, it can be seen in Figure 1C that there is a concentration of yellow, indicating precipitates of a Ca-P phase, which was confirmed through further analysis by x-ray diffraction. Whereas, Figure 1B shows no mixing of colors (that is, no yellow structure), and, therefore, Ca and P have little association with each other. By rotating the color coding for various elements, features that could be missed, such as hairline cracks, fine precipitates, and small boundary interfaces, can be seen.

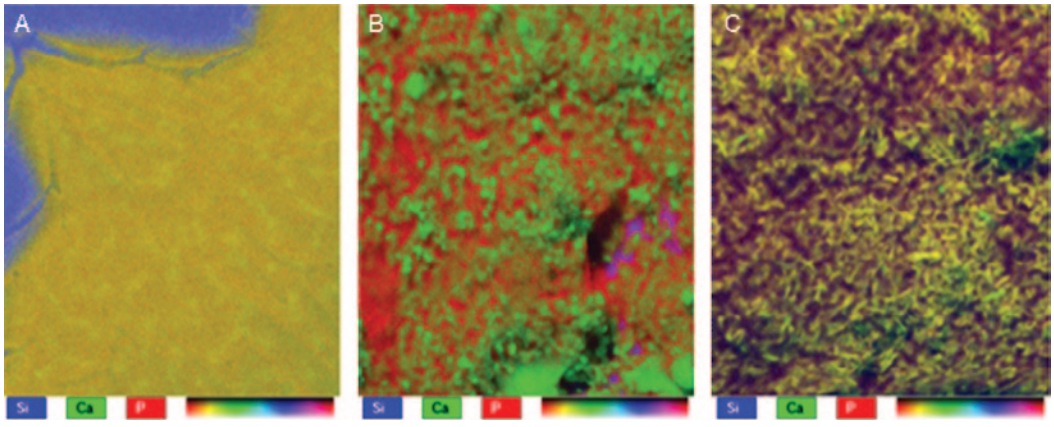

Figure 1: Primary color x-ray maps for (A) Ca solution (First Solution), (B) Ca with CO_3 solution (Second Solution), and (C) Ca with and PO_4 (Third Solution). X-ray maps were collected at 20 keV, 512 × 512 pixels, 100 msec/pixel, and 7 kcps. Width of field (WOF) = 300 µm.

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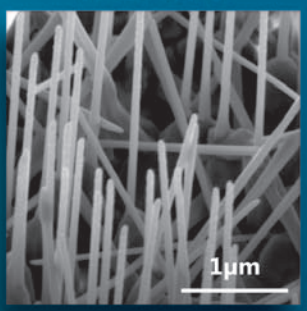

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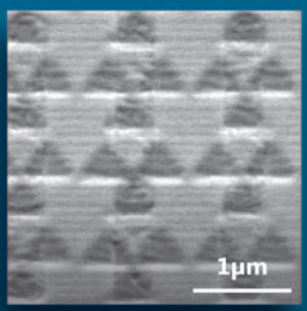

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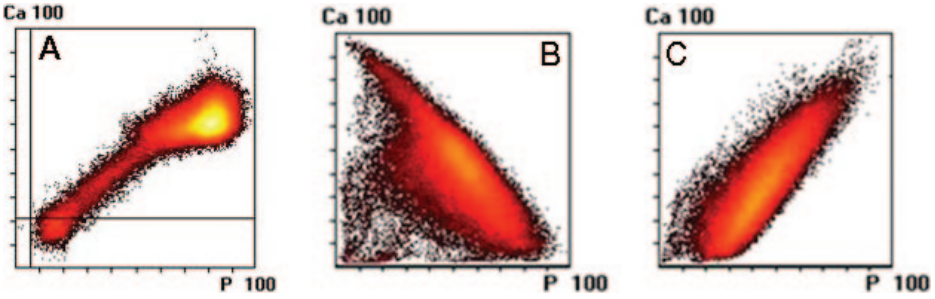

Figure 2: Ca-P scatter diagrams for (A) First Solution, (B) Second Solution, and (C) Third Solution.

Figures 2A–2C show scatter diagrams produced from the selection of two elements (Ca and P) from the X-ray maps. These two-dimensional scatter diagrams indicate pixel frequency where element concentration profiles are plotted against each other for selected elements within the sample. The scale 100 on the scatter diagrams represents 0 to 100 percent of the maximum element concentration recorded during the map process. The scatter diagram scale is qualitative, but the data taken from the map can be processed to be quantitative. These scatter diagrams were given an extra dimension by coloring the intensity of points on the diagrams with the thermal color scale. Thus, the intensity within the scatter diagram indicates the number of points in the image with similar concentration [4]. From these scatter diagrams we observe clusters, also referred to as nodes, which correspond to different chemical phases. As can be seen from Figures 2A–2C, the scatter diagrams reveal differences for the three types of solution. The Ca-containing and P-containing solutions exhibit association of these elements with both increasing Ca and increasing P (Figures 2A and 2C). However, Figure 2B shows little association between Ca and P for the Solution 2, which was prepared using Ca plus CO_3 mineral-containing solution.

Conclusion

This paper described elemental mapping and a number of post-processing methods such as pseudo coloring with primary colors, scatter diagram creation, and chemical phase mapping. All of these methods aid in obtaining a better understanding of the distribution of elements and phases within mineral-encapsulated liposome biological materials.

References

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