

X-Ray Mapping of Minerals Incorporated into Liposomes

Kanthi Lewis^{*}, Richard Wuhler^{**}, B. Ben-Nissan^{*}, S. M. Valenzuela^{***} and K. Moran^{****}

^{*} Department of Chemistry and Forensic Sciences, University of Technology, Sydney.

^{**} Microstructural Analysis Unit, University of Technology, Sydney

^{***} Department of Medical and Molecular Biosciences, University of Technology, Sydney.

^{****} Moran Scientific Pty Ltd, Goulburn.

Liposomes are spherical particles in an aqueous medium formed by a lipid bilayer enclosing an aqueous compartment. They have been long considered as a potential delivery device in the medical and pharmaceutical industries due to their ability to encapsulate different compounds, as the lipids form into liposomes. The ability of these liposomes to be stored in the body, and to be taken up by cells, makes them ideal for drug delivery. They can also potentially increase the efficiency of supplements, particularly those with no accumulation toxicity.

Our research work aims to explore the variations in the mineral deposits formed, by preparing liposomes in modified calcifying buffers. The overall aim of the work is to prepare calcium loaded liposomes which can then be functionalised to target the bone, and due to the localised delivery increase the efficiency of calcium supplementation. This is of particular significance for patients with a low bone mineral density such as those with osteoporosis, where calcium supplementation is still the first line of treatment. This paper will deal with the characterisation of the liposomes and the investigation of the type of minerals that develop within the liposomes.

Three solutions have been produced with varying mineral compositions. The first is a calcium chloride solution. Any calcium contained in the liposomes (after preparation) should only be that bound to the liposome, or free ions remaining in the aqueous centre of the liposome, as the wash steps remove any surrounding material. The second solution is prepared by adding calcium chloride and sodium carbonate. This solution not only allows the calcium to adhere to the liposome membrane (as determined from solution 1), but also looks at increasing the amount of calcium encapsulated by allowing calcium carbonate mineral to form, thus building up the calcium trapped as the crystals grow larger. Since the amount of phosphate remains constant in the two solutions due to the unchanging quantity of lipids, the calcium to phosphate ratio is an indicator of the amount of calcium retained. Equal quantities of calcium have been added to each solution; however some will remain unbound in aqueous solution and is removed via the wash steps. The amount which is bound to or trapped inside the lipid (and therefore remaining after washing) in the presence or absence of carbonate, is the only variable for solution one and two. The third solution is a mixture of calcium chloride, magnesium sulphate and potassium phosphate. This solution was included in order to examine whether other ions will incorporate into the liposome, and whether changing the carbonate ions in solution to phosphate ions will still cause calcium to be retained in the liposomes, rather than being unbound and being removed in the wash steps. This solution also aims to explore the possibility of the magnesium and sulphate ions being retained. Calcium sulfate and magnesium phosphate are both biocompatible, and have greater solubility than that of calcium phosphate mineral. If apatite mineral is forming, the presence of magnesium may also produce a substituted apatite.

Once the liposomes were produced, with various mineral solutions, they were then washed by resuspending in 200µl of Milli Q water and spun for a further hour at 1200 rpm. This wash step was repeated 3 times in order to remove any remaining ions which are unbound or unincorporated in the liposome. The liposomes were then resuspended in 50µl of Milli Q water and placed onto a silicon

wafer to dry. The samples were then carbon coated and characterised using a Philips XL30ESEM and a Jeol 35CF with a Moran Scientific x-ray microanalysis mapping system.

Through the use of quantitative x-ray mapping (QXRM) subtle changes in the distribution of elements and subsequent phases in biological materials can be determined and thus provide an image related to the distribution and relative abundance of elements within a given specimen [1]. This makes QXRM particularly useful for identifying the location of individual elements within liposomes and x-ray mapping the spatial distribution of specific elements and phases within these liposome samples. Figure 1a-c shows the pseudo colouring of the x-ray maps and reveals the type of phases produced. Figures 1d-f show the scatter diagrams produced from the selection of three elements (Ca, P and Si). From these scatter diagrams we observe clusters, also referred to as nodes, which correspond to different chemical phases. As can be seen from Figure 1 d-f, the scatter diagrams reveal a difference with the type of solution preparation technique used. The CaCl_2 and CaCl_2 with K_2HPO_4 and MgSO_4 have correlation with either increasing Ca or P. However, Figure 1e shows no-correlation between Ca and P for the solution 2 which was prepared with CaCl_2 plus NaHCO_3 solution. X-ray diffraction analysis revealed results consistent with XRM results, where solution 2 produced calcium carbonate in the liposome samples and solution 3 produced a brushite phase ($\text{CaPO}_3\text{OH}\cdot 2\text{H}_2\text{O}$ and $\text{CaHPO}_4\cdot 2\text{H}_2\text{O}$).

This paper will discuss XRD results, elemental mapping and a number of post-processing methods such as pseudo colouring, scatter diagram creation, and chemical phase mapping all of which aid in obtaining a better understanding of the distribution of elements and phases within these mineral encapsulated liposome biological materials.

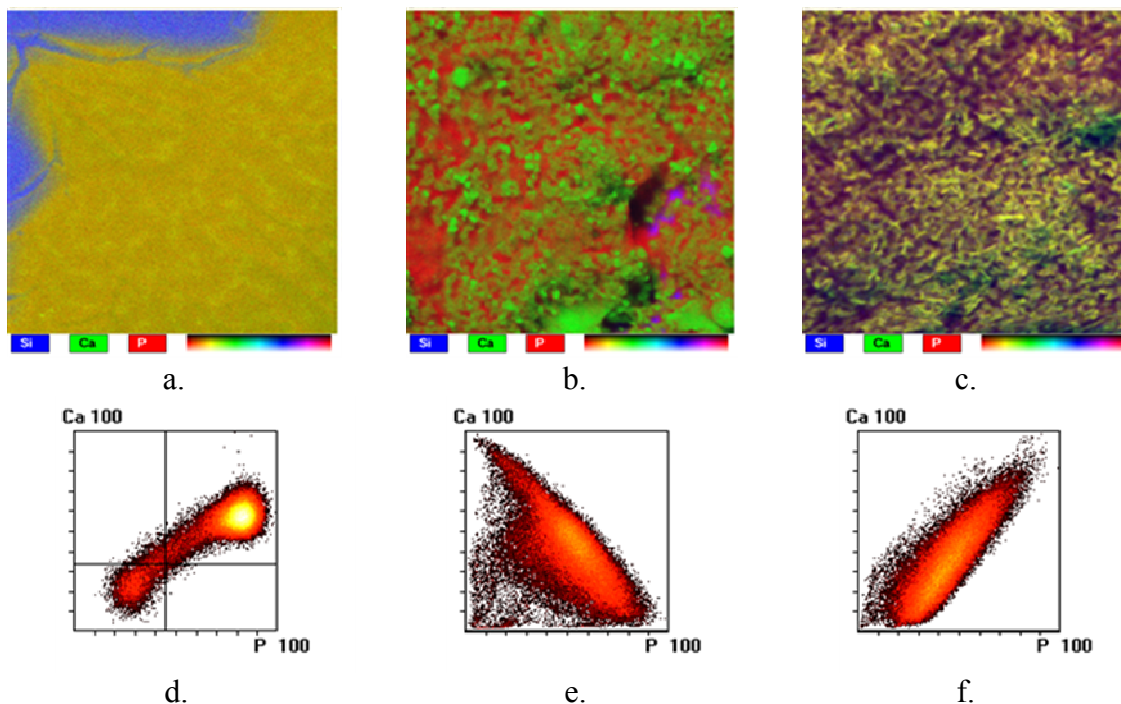


Figure 1: a-c) Pseudo coloured x-ray maps for a) CaCl_2 solution (First Solution), b) CaCl_2 with NaHCO_3 solution (Second Solution) and c) CaCl_2 with K_2HPO_4 and MgSO_4 (Third Solution). d-f) Ca and P scatter diagrams for d) CaCl_2 solution (First Solution), e) CaCl_2 with NaHCO_3 solution (Second Solution) and f) CaCl_2 with K_2HPO_4 and MgSO_4 (Third Solution). X-ray maps collected at 20keV, 512x512 pixel, 100msec/pixel and 7kcps. Width of field (WOF) = 300 μm .

[1] K. Moran and R. Wuhrer, "X-ray Mapping and Interpretation of Scatter Diagrams", *Mikrochimica* Vol. 155, pp. 209-217 (2006).