Preparation of Ag_2O Crystallites within Phospholipid Vesicles and their use in Nucleation Studies

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Summary Ag_2O crystallites have been prepared within phosphatidylcholine vesicles and characterised by high resolution electron microscopy; the potential of the approach for nucleation studies generally is described.

THE possibility of locating biological compartments through the combined use of electron microscopy (E.M.) and high resolution n.m.r. spectroscopy has been described.[1,2] The procedure relies on the incorporation of a paramagnetic probe within the internal compartment of phospholipid vesicles of diameter *ca.* 30 nm. The precipitation of the probe as an inorganic solid within this compartment permits easy location of the vesicles in the electron microscope.

The chemistry in such small volumes (*ca.* 10^{-17} cm^3) has not been previously studied. It is well known that biology makes some hard materials such as $CaCO_3$ (shells), $Ca_2(OH)PO_4$ (bones), SiO_2 (diatoms), and Fe_3O_4 (in bees, pigeons, and bacteria) and these are often initially concentrated in small biological compartments. The study of the formation of inorganic solids within synthetic vesicles allows us to model these biological processes.

Another use of this system could be in the study of nucleation. Elsewhere we have described the preparation of CoS[1] and Fe_3O_4[2] particles within phosphatidylcholine vesicles. The CoS is precipitated *in situ* and is amorphous. The Fe_3O_4 is encapsulated within the vesicles as small crystallites during sonication. We now report the *in situ* preparation of a crystalline Ag_2O, within vesicles.

Phosphatidylcholine (17 mM) was sonicated in the dark at 4 °C in the presence of $AgNO_3$ solution (250 mM) for 10 min. The clear solution was then passed down a cation ion-exchange column (sodium form) to remove the Ag^I ions from the external phase of the vesicles. Addition of 1 drop of sodium hydroxide solution (1 M) turned the solution progressively darker as shown by absorption spectroscopy. The precipitation of Ag_2O inside the vesicle was complete within 10 min. ^1H N.m.r. spectra were recorded before and after precipitation and showed that the vesicles remained intact during the chemical reaction.

A drop of the solution was allowed to dry in the air on a carbon-coated copper grid before introduction into a JEOL JEM 100CX electron microscope operating at 100 keV. Uniform spheres of average diameter *ca.* 20 nm were observed. *X*-Ray microprobe analysis on these spheres over areas of 100 nm^2 detected only phosphorus and silver (Table). Electron diffraction patterns were taken over

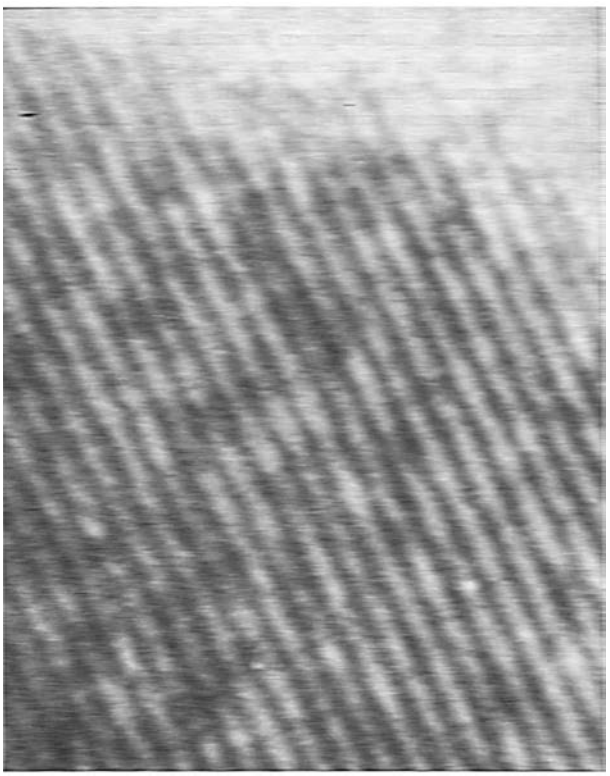

FIGURE E. M Micrograph showing 111-lattice spacings on Ag_2O crystallite inside a vesicle. Fringe distance is 2·7 Å.

TABLE. *X*-Ray microprobe analysis for Ag_2O vesicles.

Sample	Elements, counts above background/100 s		Ag/P atom ratio
	P	Ag	
1	239	2160	9·05
2	340	2600	7·65
3	1240	7290	5·88

small areas of the spheres and identified the particles as crystalline, cubic, Ag_2O. Lattice spacings of 2·7 Å could be resolved on many of the Ag_2O crystallites (Figure) using a JEOL 200CX THG2 electron microscope operating at 200 keV. This distance corresponds to the 111-lattice plane of the cubic Ag_2O crystal. Most crystallites appeared to be single domain although some multi-domain particles were observed.

The formation of Ag_2O crystallites within phosphatidylcholine vesicles could be used as a method for studying nucleation since we can vary the concentration of the Ag^I ions in the vesicle and then image and analyse the reaction product. We estimate that at an internal concentration of 250 mM $AgNO_3$ there are *ca.* 600 Ag^I ions trapped within the vesicle membrane. The rate of precipitation is dependent on the rate of diffusion of the hydroxide ions through the membrane. Since it takes 10 min for the reaction to come to completion we have a slow, well controlled chemical system.

Similarly we could introduce different head groups into the lipid bilayers and observe their effect on the nucleation process, and known inhibitors of nucleation could be located at selective sites in the membrane.

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[2] S. Mann, A. J. Skarnulis, and R. J. P. Williams, *J. Chem. Soc., Chem. Commun.*, 1979, 1067.