Experimental report

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Proposal:	**9-10-1517**	**Council:**	4/2017
Title:	Oxidation of bilayer of labelled DPPC by aqueous hydroxyl radical; elucidating a mechanism for atmospheric science		
Research area:	Other...		

This proposal is a new proposal

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Samples: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine DPPC

Instrument	Requested days	Allocated days	From	To
D17	3	2	20/04/2018	22/04/2018

Abstract:

Oxidation of atmospheric aerosol can produce cloud condensation nuclei capable of changing the reflectivity and lifetime of clouds. The oxidation of organic film on atmospheric mineral dust may activate mineral dust into cloud droplets. To assess the atmospheric importance the chemical lifetime of the organic material to a common atmospheric oxidant (OH radical) must be measured and compared to deposition lifetime of the mineral aerosol (4-10 days). The chemical lifetime will be assessed by measuring the rate of oxidation of a bilayer of a lipid DPPC from the solid-liquid interface of a silica mineral window, using neutron reflection. Selective deuteration, the ability to measure film thickness and amount of material at the solid-liquid interface will allow chemical mechanism to be deduced. Preliminary studies show the experiment viable, and these data are needed for publication.

Experimental report

No. 9-10-1517

Oxidation of bilayer of labelled DPPC by aqueous hydroxyl radical – elucidating a mechanism for atmospheric science

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This was an extremely successful experiment that very successfully demonstrated that we can follow film thickness and kinetics of the chemical attack of a bilayer film (representative of a cell wall) by a reactive oxygen species, the hydroxyl radical. Enough data was collected for a publication of a high quality peer-reviewed article.

Introduction

The atmosphere of the Earth is an oxidising medium and effectively acts as a low temperature, dilute fuel, combustion system, oxidizing complex compounds to simple molecules and returning them to the surface of the Earth via cloud water and dry deposition. Cloud processing of atmospheric contaminants changes the optical properties of clouds.[1] The chemical composition of particulate matter affects climate directly, by scattering and absorbing solar radiation, and indirectly, owing to its ability to act as cloud condensation nuclei, leading to an increase in cloud formation and changing rainfall patterns.

A large contribution to atmospheric aerosol is non-soluble solid particles produced as Aeolian dust by mechanical means often comprising mineral or soil dust. Mineral dust is a significant contributor to atmospheric aerosol loading because its source strength is between 1000 to 5000 Mt/year [3]. Oxides such as silica, aluminum, titanium and iron are often used a proxies for mineral dust.[2] Single particle atmospheric studies have shown that mineral dust contains organic films that effect the reactivity of the particle and its potential to act as cloud condensation.[2] Organic films on aqueous atmospheric aerosol may (a) reduce the rate of evaporation from the particles, (b) inhibit the transport of molecules and radicals from the gas to the liquid phase (c) reduce the scavenging of the particles by larger cloud and rain droplets.[3] We have previously shown that chemical reactions at the surface of water droplets can have climatic effects.[4,5] The work has been STFC highlights (2004 and 2009) and reported in New Scientist. In this proposal we wished to use POLREF to study the mechanism of the oxidant *penetrating* the water droplet and *reacting* within the film on the silica.

The experiment unexpectedly demonstrated the removal of the material from the air-water interface was following mixed kinetics (stepwise degradation and first-order loss) depending on which part of the molecule was being followed. ***Thus we wish to repeat the experiments with selective deuteration of the DPPC (tail only, head only) to answer the following questions: (1) does the reaction process differ for tail region and head region? (2) Does the radical enter the tail region first and react? (3) Is there material remaining at the interface after oxidation?***

The few studies of the oxidation of lipid bilayers (as a proxy for biological membranes), generate mixtures and unknown amounts of ROS and describe typically what happens to either the morphology of the bilayer/lipid (e.g [4]) or report the resultant products (e.g [5]). We wished to use our skills of chemical mechanism and chemical oxidation gained on the oxidation of amphiles (lipids and insoluble monolayers) at the air-water interface[6] to study the oxidation of lipid bilayers. We generated known amounts of a hydroxyl radical and conduct experiments that will simultaneously (a) determined the rate constants of the bilayer attack and (b) in real time record the change in bilayer morphology (film thickness) by neutron reflection. We started studies using neutron reflection with a well-behaved lipid bilayer, DPPC that can be part or fully deuterated and a radical from the ROS family that we have copious experience with, the hydroxyl radical (the cloud water radical oxidant).

Experiment

We used a well-established vesicle fusion mechanism to place a bilayer of DPPC at the solid-liquid interface of a quartz-water cell[7]. The cell was a sandwich of one quartz (silica) window and one plexi-glass backer separated by a PTFE spacer. An aqueous solution of 1% (m/m) hydrogen peroxide will be gently flowed through the cell. The solution will be illuminated with UV light (254 nm) through the quartz window. Illumination of the quartz window will prevent attenuation of the UV radiation by the water. Experiments with DPPC at the air-water interface demonstrate that DPPC has a very slow insignificant, degradation when illuminated with UV light at a wavelength 254nm. The photolysis generated HO_2 and OH radicals in aqueous solution in known quantities from Haber-Weiss chemistry. With careful modelling of the neutron reflection as a function of momentum transfer, the loss of neutron reflection can be attributed to the loss of the lipid molecule. Our methodology was a combination of fast kinetic measurements and structural measurements of film thickness. With careful modelling of the reflectivity as a function of momentum transfer, the changes can be attributed to the loss of the lipid molecule. Our methodology gave structural evolution and amount of lipid at the solid-water interface. The experimental set-up is in figure 1.

Preliminary experiments.

This was an extremely successful experiment that very successfully demonstrated that we can follow film thickness and kinetics of the chemical attack of a bilayer film (representative of a cell wall) by a reactive oxygen species, the hydroxyl radical. Enough data was collected for a publication of a high quality peer-reviewed article. The successful experiment demonstrated we can (a) record the structure (thicknesses and volumes) of lipid bilayer as it oxidized and (b) follow the loss of the DPPC, suggesting that with a deuterated contrast we could follow the loss of different parts of the molecule. The decay is surprising with different parts of the molecule following very different kinetics, e.g. Multi-step degradation or first-order loss: not an exponential decay, but loss of material from the “buried” tail region. Figure 2 demonstrates the surface coverage of the DPPC bilayer (as determined by neutron reflection) as a function of time during oxidation by OH radicals. The figure demonstrates the raw data from that experiment. The surface coverage shows a gain of scattering length from increased oxygen in the surface layer and then a subsequent loss of deuterated material with time. There is a reorganization of surface followed by an almost exponential decay and almost complete removal of the bilayer. The film thickness also changed, providing tentative evidence of hydroxyl radicals entering the bilayer and oxidizing the tails.

References

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Figure 1

The Solid-liquid cell mounted on the D17 beamline before (LHS) and after (RHS) addition of the UV photolysis lamp.

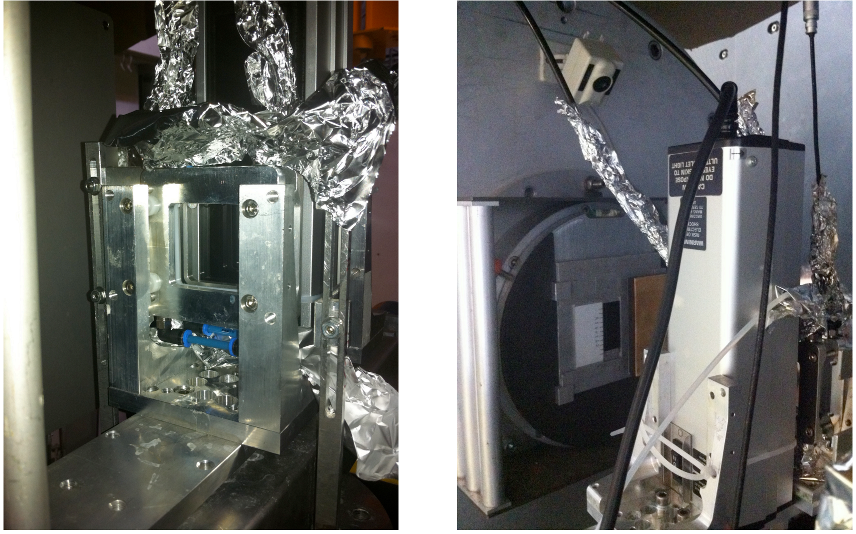

Figure 2

Preliminary results from the experiment. The surface coverage of the lipid molecules are plotted as a function of time, as determined from neutron reflection. Tentatively we can suggest there appears to be a period of re-arrangement followed by complete oxidation and loss of nearly all material from the interface.