Characterisation of an analysis system used to quantify health-relevant reactive oxygen species (ROS) in atmospheric aerosol particles

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The adverse health effects associated with ambient aerosol particles have been well documented in epidemiological studies, and further supported with biological cell culture studies. There is a widely accepted association between ambient aerosol particle levels and cardiovascular or respiratory based hospital admission/mortality rates. However, due to the large variability in ambient particulate matter, it is still unknown which physical or chemical properties are responsible for these negative health effects.

Previous studies highlight reactive oxygen species (ROS) in organic particulate matter as being a potential major cause. Many of these studies have used the fluorescence probe 2-7 dichlorofluorescein (DCFH), in combination with horseradish peroxidase (HRP), to quantify particle-bound ROS, but the reaction mechanism and kinetics of the assay itself has not been clarified to a great degree of detail (Venkatachari & Hopke 2008). This lack of detailed understanding leads to various assumptions having to be made in order to interpret the results obtained.

To characterize the DCFH/HRP assay with model compounds for atmospheric particle components, varying concentrations (0.9 ml, 0.0 - 1.0 μM) of different ROS were combined with the DCFH (1 ml, 10 μM, 20 % PBS) and HRP (0.1 ml, 10 units ml^{-1}) solution. The fluorescence measurements were taken during a 15 minute reaction time using a 4-way cuvette holder maintained at 41^{o}C.

HRP interacts with DCFH via two alternative cycles: the peroxidase cycle – whereby the HRP ground state is oxidized by peroxide and subsequently reduced by DCFH to give the fluorescent product DCF; and the oxidase cycle – HRP is reduced by DCFH, then oxidized back to its ground state by oxygen (Berglund et al. 2002). The peroxidase cycle provides two moles of fluorescent product DCF per mole of HRP used, and provides a steeper gradient of fluorescence intensity over time.

Figure 1 shows the increase in fluorescence over time of the DCFH/HRP assay both with, and without, a ROS present. Two distinct phases are clearly visible in the reactivity of the assay. The oxidase-cycle phase, causing a background fluorescence gradient in both runs, is due to exposure of the assay to ambient oxygen levels within the lab air. The steeper peroxidase-cycle phase is unique to the sample with ROS (peracetic acid) present. Peracetic acid reacts in the aqueous system to give H_2O_2, as shown in Figure 2.

This data suggests that the DCFH/HRP assay is specific to H_2O_2. When considering the oxidative ability of different reactants, rather than measuring reactivity of each individual species, it measures the sum conversion into H_2O_2/reactive radicals. This further strengthens the validity of using DCFH/HRP to determine potential health impacts of particle-bound ROS, because its reactivity is specific to the very species suspected of causing oxidative stress/inflammation within an aqueous human lung environment.

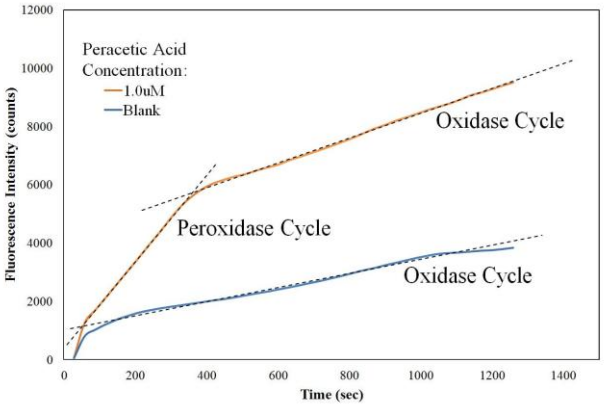

Figure 1. Fluorescence vs time plot of the DCFH/HRP assay with and without peracetic acid present.

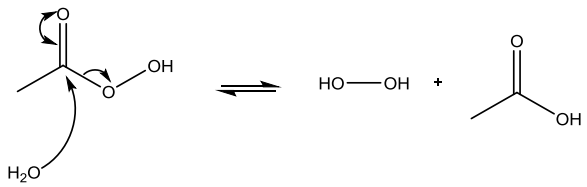

Figure 2. Equilibrium between peracetic acid and H_2O_2 in an aqueous environment.

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