On-Line Measurement of Particle-Bound Reactive Oxygen Species (ROS)

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1) Introduction

• The adverse health effects associated with ambient aerosol particles have been well documented in epidemiological studies and further supported with biological cell culture/in-vivo studies. There is a widely accepted association between ambient aerosol particle levels and increases in hospital admissions/mortality due to respiratory and cardiovascular disease.

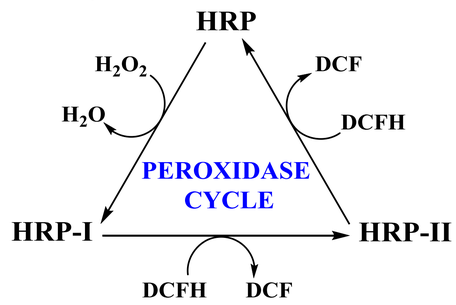
• Previous studies suggest the property of particulate matter most detrimental to human health is its oxidising capacity i.e. its ability to cause oxidative stress in the lung. Reactive oxygen species (ROS) found in organic particulate matter is often highlighted as a potential major cause for the negative health impact of air pollution.[1] ROS is defined as including families of oxygen-centred or -related free radicals ($HO^{\cdot}$, $HOO^{\cdot}$ or $ROO^{\cdot}$), ions (HOO-) and molecules (H_2O_2, organic peroxides, inorganic peroxides).

• A number of previous studies attempting to quantify levels of particle-bound ROS have used the fluorescence probe 2',7'-dichlorofluorescein (DCFH) in combination with catalytic enzyme horseradish peroxidase (HRP).[2] However, such previous studies have only been achieved in a non-continuous capacity. This study describes the development of a fully continuous ("on-line") instrument for ROS quantification.

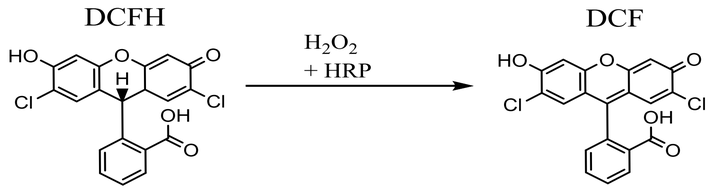
Particulate Matter
↓
ROS in Lung Lining Fluid
↓
Antioxidant Depletion/ Oxidative Stress
↓
Inflammation/Cell Damage
↓
COPD, Asthma, CVD, Cancer

2) Assay Chemistry: DCFH and HRP

• It has been suggested that HRP interacts with DCFH via two alternative cycles: the peroxidase cycle and the oxidase cycle.[3]

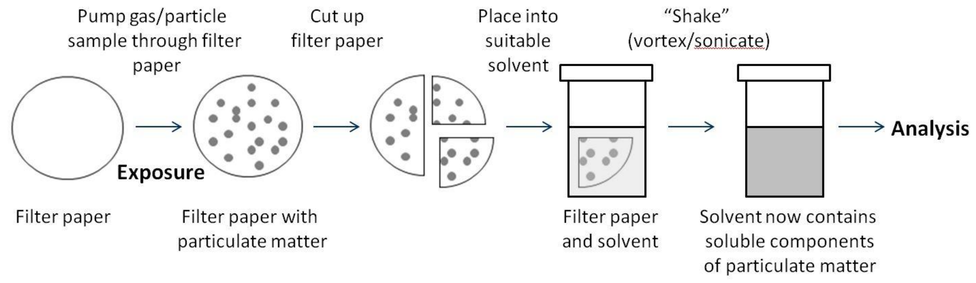


• The peroxidase cycle is sensitive to hydrogen peroxide (H_2O_2) and related ROS, with one mole of H_2O_2 yielding two moles of fluorescent product DCF. The H_2O_2 concentration of a sample can thus be determined via fluorescence spectroscopy of the DCF product. The oxidase cycle is dependant on dissolved oxygen in the reaction solvent and provides background fluorescence.



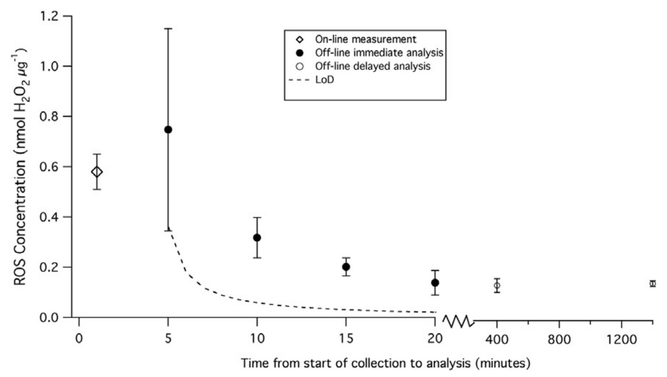
3) Typical Off-line Methodology

• Sample collected on filter for hours/days and subsequently analysed in lab
• Poor time resolution
• Labour intensive and requires large resources (equipment/man-power)
• Exposure→Analysis: **hours – days**



4) Comparison of On-line and Off-line Methods to Quantify Reactive Oxygen Species (ROS) in Atmospheric Aerosols[4]

Fuller, S.J., Wragg, F.P.H., Nutter, J., Kalberer, M., *Atmospheric Environment* **92**, 97-103 (2014).



• Direct comparison between on-line and off-line methods for detection of particle-bound ROS. The above plot considers measurement of ROS in oxidised oleic acid particles

• This study suggests ROS concentration in organic particulates decreases by a factor of 5–10 within 15 minutes of collection of a sample on a filter

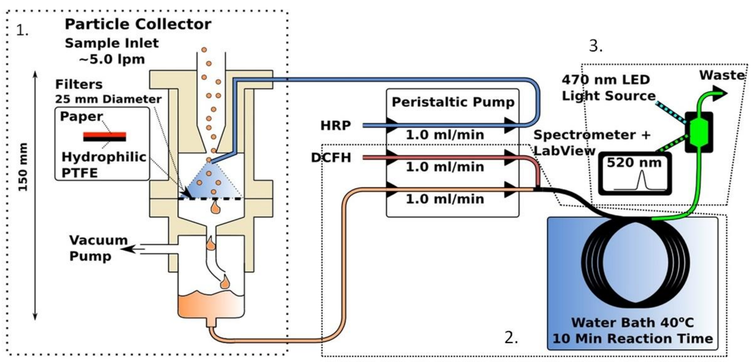
• ROS concentrations quantified with the on-line instrument are comparable with the off-line method only for samples with the shortest collection time (1 minute)

• Therefore, off-line ROS measurements are potentially a drastic underestimate of reality. In order to obtain reliable and accurate measurements of particle-bound ROS, a relatively fast on-line technique should be used

5) On-line ROS Instrument as used by Fuller et al (2014)

Exposure→Analysis:
seconds – minutes

Detection Limit:
4 nmol H_2O_2 equiv. per m^3 air



1. Particle collector:
Converts aerosol sample from gas phase to liquid phase

2. Liquid phase reaction system:
Provides suitable time and temperature to allow reaction of DCFH/HRP assay with soluble ROS components

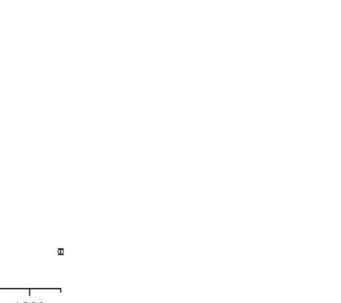
3. Flow cell:
Allows detection and quantification of ROS levels in the aerosol sample via fluorescence spectroscopy

6) Construction of a 2nd Generation On-Line ROS Instrument

• Increased stability and sensitivity of the system

• Instrument is in a more compact/portable form, allowing increased deployment and application possibilities

• Measurement process could be automated/un-manned for 12–24 hour periods, increasing measurement possibilities and reducing human error

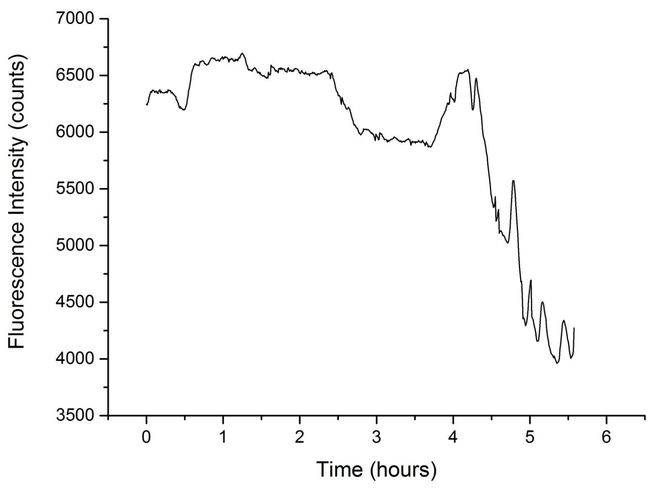


7) Example Data from Functionality Tests of 2nd Generation Instrument

• Plot shows data from a biogenic VOC dark ozonolysis experiment in the Cambridge 5.4 m^3 smog chamber

• Temporal resolution is greatly enhanced compared to that available with traditional off-line methods

• Combination with H_2O_2 calibration plots allows fluorescence intensity to be given in conventional units: nmol H_2O_2 equivalents per m^3 air sample.



8) Conclusions

• The described on-line ROS instrument has a greatly improved temporal resolution compared to that seen in previous ROS studies

• The 2nd generation design is more compact, portable, and can be used to take continuous automated measurements from ambient or smog chamber environments for a large number of hours

• Such instrument capabilities allow for a huge variety of potential future studies

1. Brunekreef, B. & Holgate, S. T., *Lancet* **360**, 1233-1242, (2002);
2. Wang, Y., Hopke, P. K., Sun, L., Chalupa, D. C. & Utell, M. J., *Journal of toxicology* **2011**, 419476, (2011);
3. Berglund, G. I. *et al. Nature* **417**, 463–8 (2002);
4. Fuller, S.J., Wragg, F.P.H., Nutter, J., Kalberer, M., *Atmospheric Environment* **92**, 97-103 (2014).