Time-resolved and Online Determination of Reactive Oxygen Species (ROS) in Ambient Air

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Overview

• Oxidative stress - a possible mechanism of particle-induced health effects

• A new instrument measuring reactive oxygen species (ROS) concentration on-line: design and instrument characterization

• First applications of the instrument
Ambient particle cause negative biological/health effects as observed in epidemiological and laboratory studies.

Six cities study, Dockery et al. 1993

- **Deaths**
- **SO$_2$**
- Aerosol particles (dust)
What particle properties are relevant for biological effects?

Particles e.g., from combustion sources

- potential damaging properties
  - organic components
  - metals
  - mass, size, surface properties

Effects in the cell
- ROS?
- oxidative stress?
- inflammation
Oxidative stress in the lung

- Oxidative stress = disturbance of prooxidant-antioxidant balance leading to potential biomolecular damage
- Oxidative stress can be induced by ROS in the lung lining layer
- Antioxidant depletion may cause damage to cell membranes, proteins & DNA
PM induced ROS in the lung

Formation routes of ROS from aerosol particles:

1. Particle bound ROS – e.g. $\text{H}_2\text{O}_2$, ROOH, radicals = reactive and short-lived

2. (Catalytic) ROS production in the lung lining fluid by redox active components; e.g., quinones and metals

3. Metabolism of organics (e.g. PAHs) may lead to ROS formation
Measuring ROS concentrations in solution - the reaction system

- DCFH is oxidised by ROS in the presence of Horseradish Peroxidase to the fluorescent product DCF; excited at 470 nm and emitting at 520 nm.

- DCFH reacts with almost all ROS
Measuring ROS concentrations in solution - the reaction system

(HRP = Horesradish Peroxidase)

\[ I \propto [DCF] \propto [H_2O_2] \]

- The fluorescence is directly proportional to the concentration of DCF.
- While the DCFH assay gives a linear relationship with H\(_2\)O\(_2\), organic peroxides react much slower with HRP.
- This assay gives an indication of ROS activity related to an equivalent H\(_2\)O\(_2\) concentration. NOT an absolute ROS concentration.
Measuring ROS concentrations *online*

- Many current aerosol studies measuring composition use *off-line filter collection techniques*

- The time delay between collection and analysis possibly allows for *degradation and loss of reactive components*

- *Online* analysis allows for fast analysis that *minimizes loss of reactive components* and allows for higher time resolution

- *Online* analysis allows for *high time resolution*
Fast Online Quantification of Oxidizing Particle Components
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- Continuous collection and solvent extraction
- Hydrophobic filter
- Aerosol sampling
- 5 slpm
- Aerosol inlet
- 15 cm
- HRP solution
- To vacuum pump
- To mix with DCFH solution
Particle into liquid sampling

Collection Efficiency

Collection Efficiency

Aerodynamic Diameter / nm
ROS quantification

Fan cooled 5 W 470 nm LED

Spectrometer

Connected to LabView program

470 nm LED Light Source

Solution (chilled)

Aqueous DCFH

Flow Cell

10 minute Reaction Coil

Water Bath 37° C

Waste
Experimental set up

Fan cooled 5 W 470 nm LED

Spectrometer
Sensitivity calibration with $\text{H}_2\text{O}_2$

Fluorescence intensity after reaction has gone to completion after <10min
Sensitivity calibration with $\text{H}_2\text{O}_2$

Normalised to equivalent fluorescein fluorescence to account for non linear spectrometer behaviour at higher concentrations.
Sensitivity calibration with H₂O₂

Dynamic range:
ca. 2 orders of magnitude

Detection limit:
in liquid H₂O₂: ~ 10 nM
ambient air (5lpm): 4 nmol / m³
Smog Chamber Experiments

• Experiments at Paul Scherrer Institut (PSI), Switzerland
• Primary moped emissions photochemically aged in smog chamber
• Mopeds conforming to both Euro 1 and Euro 2 emissions regulations

UV + NOx + O₃

27 m³ Chamber
Smog Chamber Experiments

Secondary Organic Aerosol Formation

**Primary**
- Primary organic aerosol (POA)
- Soot
- Gaseous volatile organic compounds (VOCs)

**Processing**
- Oxidation by ozone and OH in the presence of UV light and NOx

**Secondary**
- Oxidised VOCs condense to form secondary organic aerosol (SOA)
Smog Chamber Experiments

Remove gaseous oxidants

Charcoal Denuder

Online ROS Instrument
Smog Chamber Experiments

With photochemical SOA formation: increase of ROS

ROS = ca. 1 nmol μg⁻¹ SOA

ROS concentration / nMoles m⁻³

SOA mass / µg m⁻³

Time after lights on / hours
Smog Chamber Experiments: POA vs. SOA

ROS concentration in POA (before photochemical aging): negligible
Smog Chamber Experiments: Euro1 vs. Euro2

ROS / \( \mu g \) POA large differences between Euro 1 and Euro 2
Ambient measurements

ROS in ambient urban air (Cambridge)

Fluorescence signal

- clean air
- ambient air
- clean air

Time

11:50 12:50 13:50 14:50 15:50
Conclusions

• Design of online instrument to quantify ROS
• ROS “scavenged” within seconds: reactive ROS are *not lost*
• Time resolution: ca. 10min
• Detection limit: 10nmol (H$_2$O$_2$), 4 nmol / m$^3$
• ROS only observed in SOA from moped emissions
  ROS concentrations of ca. 1 nmol μg$^{-1}$ SOA
• No ROS in primary moped emissions
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