

Measurement of Particle-Bound Reactive Oxygen Species in Rubidoux Aerosols

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Abstract. The diurnal variations in the concentrations of reactive oxygen species (ROS) in various size fractions were determined for ambient particles in Rubidoux, CA. Sampling of particles was conducted at 3 h intervals using a cascade impactor. The collected particles were reacted with dichlorofluorescein (DCFH), a non-fluorescent probe that fluoresces when oxidized. The factors affecting the diurnal variation in ROS concentration were also investigated with special emphasis on the relation between ROS concentration and the intensity of photochemical reactions where the ozone concentration was taken as an index. The intensity of photochemical reactions was found to be a moderate factor affecting the daytime ROS concentration. The concentration of ROS was found to be higher on the smaller particles of the ambient aerosol.

Key words: peroxides, particles, fluorescence, free radicals, photochemistry, ROS.

1. Introduction

Reactive oxygen species (ROS), encompassing oxygen-containing species with strong oxidizing ability, include molecules like H₂O₂, ions like hypochlorite ion (OCl⁻), radicals like hydroxyl (OH) radical and superoxide anion (O₂⁻). ROS have been found to be generated from photochemical reactions in polluted air containing two main classes of precursor pollutants, volatile organic compounds (VOCs) and NO_x (Seinfeld and Pandis, 1998). ROS are generated endogenously by biological systems as a defense against foreign organisms and other environmental challenges, an example being diesel exhaust particles (Kuo *et al.*, 1998).

Combustion of organic materials such as cigarette and wood can also generate ROS (Kao and Wang, 2002). The ROS have different lifetimes in the atmosphere depending on their sources and appear both in the gas and particulate phase. Age of particles has also been found to be a factor affecting the concentration of ROS in them. For example, it has been found that freshly fractured silica dust has a high concentration of free radicals that have been shown to decrease exponentially with a half-life of ~30 h (Vallyathan *et al.*, 1988; Shi and Dalal, 1988). In contrast, ROS

from welding fumes have also been shown to decrease with time exponentially with a half-life of ~ 10 days (Antonini *et al.*, 1998). It has been found that freshly generated ROS have a greater toxicological effect than aged ROS (Antonini *et al.*, 1998; Vallyathan *et al.*, 1995).

ROS has been implicated in the pathogenesis of many pulmonary diseases including asthma (Kehrer, 1993) and has also been known to play a role in tumor promotional stage of carcinogenesis (Cerutti, 1985). It is surprising that although studies of ROS in the gas phase and in rain and cloud droplets have been reported (e.g., Olszyna *et al.*, 1988; Sakugawa and Kaplan, 1990; Tsai *et al.*, 1991), concentration data on ROS in the particle phase is very limited (Hung and Wang, 2001).

The measurement of free radical concentrations in the atmosphere is a challenging problem today because of the low steady state concentrations involved. Quantitative analysis of the total ROS can be performed with a fluorogenic probe such as dichlorofluorescein (DCFH). DCFH is a non-fluorescent reagent that becomes fluorescent dichlorofluorescein (DCF) when oxidized in the presence of ROS. In fact, this fluorometric assay method has been proved to have a high sensitivity that is capable of detecting picomole levels of hydroperoxides (Cathcart *et al.*, 1983). It has been widely used in biological systems as an indicator of oxidative stress in toxicological phenomena (LaBel *et al.*, 1992). Only recently DCFH₂ has been used in the assay of ambient particles (Antonini *et al.*, 1998).

The primary objective of this study is to determine the concentration of ROS in the ambient particles sampled at Rubidoux, CA. The pattern of diurnal variation of ROS concentration was investigated. The relationship between the ROS concentration and ozone concentration as an indicator of the intensity of photochemical reaction was also explored.

2. Atmospheric Chemistry of ROS Formation

Relating the concentrations of secondary pollutants such as ROS impacting a given geographical location to the emissions of primary pollutant precursors for a given set of meteorological conditions, is a major goal of atmospheric science. The ROS in the atmosphere is mainly formed in the presence of VOCs, NO_x, and ozone. The chemistry driving the formation of different ROS varies with the time of day. In the presence of sunlight, the phenomenon leading to ROS formation is predominantly photochemical. In the absence of sunlight, the mechanism of the formation of ROS is distinctly influenced by the chemistry of the NO₃ radical (Finlayson-Pitts and Pitts, 2000; Wayne *et al.*, 1991). The OH radical formation from the reactions of O₃ with alkenes (including Biogenic VOCs) could also be a significant source of ROS during nighttime (Paulson and Orlando, 1996).

3. Instrumentation, Method and Materials

3.1. AIR SAMPLING

Sampling of the aerosols was done using a Micro-Orifice Uniform Deposit Impactor® (MOUDI™, MSP, Minneapolis, U.S.A.) (Marple *et al.*, 1991) and a Nano-MOUDI™ (MSP, Minneapolis, U.S.A.) and the particles were collected on Nucleopore® polycarbonate membrane filters (Whatman, 1 μm pore size, U.S.A.). The MOUDI™ is a 10-stage cascade impactor with a sampling flow rate of 30 L/min and rotating stages so as to allow near uniform collection of particles on the substrate. In order to classify nanometer sized particles, the three-stage Nano-MOUDI™ is connected downstream of the MOUDI™ through a critical orifice, so that the flow rate of aerosols through the Nano-MOUDI™ is 10 L/min. The cut-sizes of the MOUDI™ are 18, 10, 5.6, 2.5, 1.8, 1, 0.56, 0.32, 0.18, 0.1 and 0.056 μm . The Nano-MOUDI™ facilitates collection at cut-sizes of 0.032, 0.018 and 0.01 μm .

Sampling of particles was carried out at the South Coast Air Quality Management District (SCAQMD) site in Rubidoux, CA during July 2003 when LA basin inversion conditions prevail. Rubidoux, in the eastern side of the basin, is in close proximity to highway traffic, and has high sunlight intensity that ensured high PM concentration and high ozone production rates. The sampling duration was 3 h and in order to determine the pattern of diurnal variation of ROS concentration, sampling was done at 3 h intervals in the early morning, early afternoon, late afternoon and night periods. Since the relative humidity of the region was generally below 60% during sampling, the effect of water on particle mass concentration can be neglected (Hung and Wang, 2001). The extent of adsorption of gas phase ROS on filters was assumed to be inconsequential. The SCAQMD routinely measures ozone using standard photometric ozone monitors. The resulting ozone data was obtained from SCAQMD.

3.2. MEASUREMENT OF ROS

3.2.1. *Preparation of Dichlorofluorescin*

As mentioned earlier, dichlorofluorescin (DCFH) was used as the probe for ROS. DCFH was prepared from 2', 7'-dichlorofluorescin diacetate (DCFH-DA) (Calbiochem, CA, U.S.A.) according to the procedure provided by Cathcart *et al.* (1983). Using 0.5 mL of 1 mM DCFH-DA in ethyl alcohol mixed with 2 mL of 0.01 N sodium hydroxide, the hydrolyzate was allowed to stand at room temperature for 30 min and then neutralized with 10 mL sodium phosphate buffer (pH = 7.2). The solution was kept on ice without exposing it to the light until use. Prior to analysis, the 1 μM DCFH in sodium phosphate buffer was mixed with Immunopure® Horseradish Peroxidase (HRP) (Pierce Chemical, IL, U.S.A.) in a ratio such that the working reagent of DCFH-HRP contained 2.2 units of HRP/mL of the reagent.

3.2.2. Determination of ROS in Collected Aerosol Particles

Subsequent to the particle collection, 1 μ M DCFH-HRP reagent was added to each sample filter and field blank filter in a 50 mL beaker to suspend the particles uniformly in the reagent. The suspension was then ultrasonicated in a BransonTM Ultrasonic Cleaner for 15 min to extract the ROS in the particles collected on each filter and then incubated at 37 °C for 15 min. After incubation, each sample of the particle suspension in 3 mL aliquots was taken in a clear, four-sided cuvette (10 mm \times 10 mm) and placed in the sample chamber of a TurnerTM Quantech Digital Filter Fluorometer (model # FM109535) which was used in this study to measure the fluorogenic intensity of DCF in each sample and field blank. The emission wavelength was 530 nm and the excitation wavelength was 485 nm. The measured fluorescent intensity of the field blanks (approximately 22–56% of the fluorescence of the particle samples) was subtracted from those of the particle samples. The fluorescent intensity contributed by fluorescent organic substances found through analysis of particle samples with DCFH-free reagent solution, was found to be near the lower detection limit of the measuring instrument and thus considered negligible.

3.2.3. Calibration Assay

In order to correlate the fluorescent intensities and concentrations in terms of equivalent H_2O_2 concentrations, an assay for standard solutions of hydrogen peroxide was performed. Four solutions of H_2O_2 concentrations of approximately 1.0, 2.0, 3.0 and 4.0 $\times 10^{-7}$ M were taken in 0.1 mL aliquots each and were added to 3 mL of DCFH-HRP reagent in a glass test-tube. The procedure adopted was that described by Hung and Wang (2001). Blanks were prepared by mixing 3 mL DCFH-HRP reagent with 0.1 mL of distilled and deionized water. Standard solutions and blanks were incubated at 37 °C for 15 min in the same way as the assay for ROS determination. The resulting fluorescent intensity was measured by the TurnerTM Quantech Digital Filter Fluorometer.

4. Experimental Results and Discussion

4.1. CALIBRATION ASSAY

Least-squares analysis was performed on the experimental data obtained from the measurement of fluorescent intensities of standard H_2O_2 solutions. Figure 1 shows that the fluorescent intensity and the H_2O_2 concentration had a linear relationship with a high correlation ($r^2 = 0.998$). From the regression, the relationship between H_2O_2 concentration and fluorescent intensity can be expressed as

$$[\text{H}_2\text{O}_2] = \{0.1203 + 0.005097 \times (\text{Fluorescent Intensity})\} \times 10^{-7}\text{M}.$$

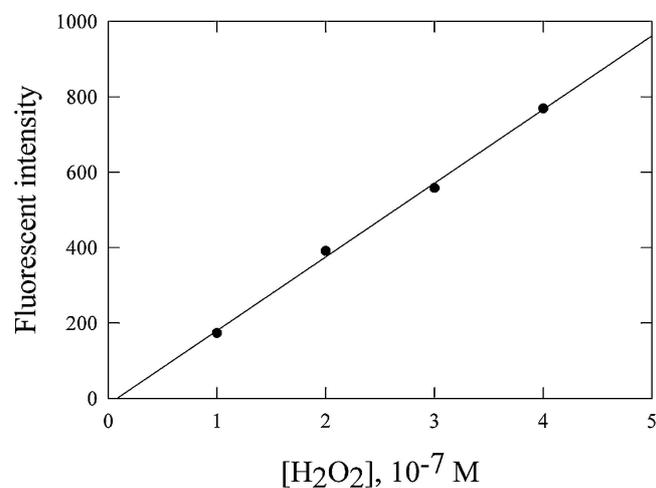


Figure 1. Fluorescence intensity of DCF obtained by reaction of DCFH with H₂O₂ solutions of indicated concentrations.

This equation can be used to convert the fluorescent intensity measured for the particle suspension to an equivalent H₂O₂ concentration. This concentration can be interpreted as an indicator of the reactivity of ROS in the particle sample which has oxidizing capability identical to the H₂O₂ solution of the calculated concentration. This concept of equivalent reactivity (Hung and Wang, 2001) was used to represent the ROS concentration in particles.

4.2. ROS REACTIVITY IN PARTICLES AND DIURNAL VARIATION

Sampling of ambient aerosols in Rubidoux was conducted in 3 h intervals between 8–11 a.m. (early morning), 12–3 p.m. (early afternoon), 4–7 p.m. (late afternoon) and 9 p.m.–midnight (night) to determine the diurnal variation of ROS concentration, to explore the extent of influence that photochemistry has on ROS formation and the night-time chemistry of ROS formation. The results are presented in Table I. High concentrations of ROS were obtained throughout the study that were at least an order of magnitude higher than the concentrations of ROS obtained in a similar study in Taipei (Hung and Wang, 2001). This result can be attributed to the geographical location of Rubidoux. There are topographical obstructions with mountains to the north and the east. Large scale subsidence inversions occur during the summer when the study was performed and the marine layer (advective) dispersion produce retention of pollutants. Hence there is increased photochemistry in the atmosphere and higher concentrations of free radicals. As seen from Table I, there were comparable concentrations of ROS found during the night-time as well when no photochemistry is going on. The ozone levels during the night-time periods were consistently low as seen in Figure 2. Although it has been

Table I. ROS concentrations over the various size fractions, during the four sampling periods

Size interval (μm)	Equivalent H_2O_2 concentrations in 10^{-7} M/m^3											
	8-11 a.m.			12-3 p.m.			4-7 p.m.			9 p.m.-12 a.m		
	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.
>18	0.21-0.85	0.41	0.16	0.16-0.59	0.37	0.09	0.15-0.42	0.29	0.08	0.11-0.60	0.40	0.15
10-18	0.02-0.65	0.34	0.14	0.05-0.39	0.28	0.10	0.12-0.40	0.26	0.09	0.05-0.23	0.14	0.06
5.6-10	0.06-0.92	0.31	0.21	0.09-0.70	0.24	0.15	0.11-0.36	0.18	0.07	0.04-0.36	0.16	0.08
2.5-5.6	0.04-0.845	0.30	0.18	0.14-0.39	0.28	0.07	0.05-0.37	0.24	0.07	0.04-0.17	0.11	0.04
1.8-2.5	0.13-0.40	0.27	0.08	0.11-0.55	0.32	0.11	0.13-0.38	0.27	0.09	0.20-0.27	0.24	0.03
1.0-1.8	0.15-0.42	0.29	0.07	0.20-0.53	0.31	0.10	0.10-0.41	0.25	0.09	0.12-0.27	0.20	0.05
0.56-1.0	0.08-0.36	0.16	0.08	0.04-0.44	0.24	0.09	0.05-0.35	0.21	0.09	0.13-0.27	0.20	0.05
0.32-0.56	0.06-0.46	0.23	0.09	0.15-0.40	0.23	0.07	0.03-0.27	0.16	0.07	0.05-0.19	0.12	0.04
0.18-0.32	0.06-0.81	0.22	0.19	0.08-0.26	0.14	0.05	0.05-0.27	0.18	0.05	0.03-0.12	0.08	0.03
0.10-0.18	0.10-0.47	0.28	0.10	0.05-0.50	0.18	0.14	0.11-0.50	0.32	0.09	0.10-0.29	0.17	0.06
0.056-0.10	0.13-0.42	0.30	0.10	0.12-0.41	0.18	0.07	0.01-0.36	0.25	0.08	0.05-0.30	0.15	0.08
0.032-0.056	0.25-1.15	0.82	0.26	0.71-1.76	1.32	0.38	0.45-2.19	1.28	0.36	0.65-1.48	1.10	0.26
0.018-0.032	0.71-1.62	1.04	0.26	0.39-1.75	0.91	0.48	0.29-2.10	1.04	0.48	0.35-1.83	0.99	0.46
0.010-0.018	0.31-1.56	0.94	0.39	0.62-2.12	1.12	0.41	0.76-1.63	1.02	0.24	0.79-1.64	1.12	0.28
Sample size	15		14	15		15	12					
Average total ROS conc.	5.70		6.11	5.96		5.19						
S.D.	0.96		1.39	1.16		0.83						
Range	3.53-7.41		3.96-8.91	2.96-8.23		4.38-6.35						

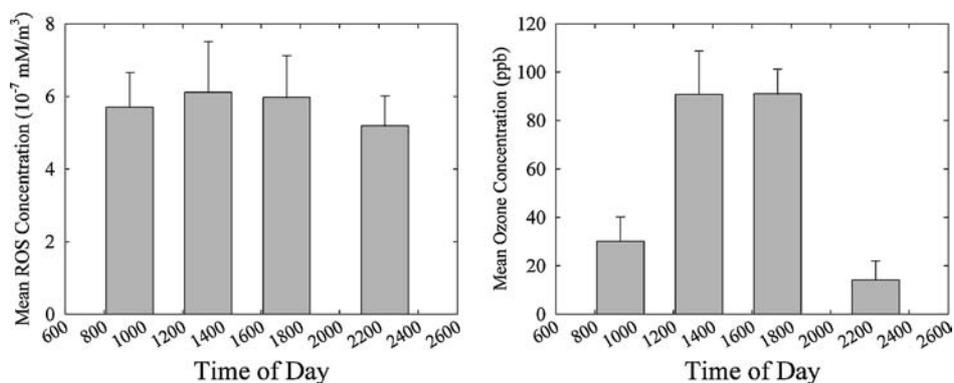


Figure 2. Comparison of diurnal variations of (a) average ROS concentrations and (b) average ozone concentrations.

postulated that the nitrate radical plays an important role in the night-time tropospheric chemistry, the fact that the night-time ROS concentrations are comparable to the daytime values seem to suggest that the NO_3 radical has considerable oxidation potential at night. It appears that it may be roughly equal to that of ozone during the day. This potential along with the oxidation of alkenes by the available ozone to produce free radicals, leads to concentrations of ROS that are only slightly lower than the daytime concentrations. The trend in the average ROS concentrations as shown in Figure 2 suggests the highest average total ROS concentrations, occurring during the daytime sampling intervals, at $6.11 \pm 1.39 \times 10^{-7} \text{ M/m}^3$ during the early afternoon sampling interval between 12–3 p.m., at $5.96 \pm 1.16 \times 10^{-7} \text{ M/m}^3$ during the late afternoon sampling interval between 4–7 p.m., and at $5.70 \pm 0.96 \times 10^{-7} \text{ M/m}^3$ during the early morning sampling interval of 8–11 a.m. The stage-by-stage measurements of the concentrations of ROS are also shown in Table I.

The much higher concentrations in the ultrafine size range stages might be explained by the conclusions of the study by Hung and Wang (2001), where it was suggested that particles produced by photochemical reactions were predominantly in the submicron range. However, the inordinately high concentration of ROS in the Nano-MOUDITM stages could also be attributed to condensation of vapor phase onto the particles as a result of their adiabatic expansion at the low pressures encountered in the Nano-MOUDITM stages. This phenomenon would be favored because of the high ambient temperatures in Rubidoux during July that were of the order of 35–40 °C. Hence, the ROS concentration in these stages might have been over-estimated. Also, it is pertinent to mention at this point that since 3 h samples were collected, short-lived ROS having lifetimes less than 3 h cannot be estimated and hence the concentration of ROS might also be underestimated in this study.

4.3. CORRELATION BETWEEN ROS CONCENTRATIONS AND O₃ CONCENTRATIONS

The intensity of photochemical reactions is an important factor in the production of ROS in the atmosphere, since photochemical reactions account for the majority of the free radicals in the daytime atmosphere. The average O₃ was used as an indicator of the intensity of photochemical reactions. Figure 3 shows the scatter plots for ROS concentrations and O₃ concentrations during the four diurnal sampling periods. The graph depicting the night-time ROS concentrations against the O₃ concentrations was also plotted, but with the purpose of determining the extent to which O₃ contributed to ROS formation at night. As can be seen, the concentration of ROS increased with the O₃ concentration, peaking during the afternoon, when the intensity of photochemical reaction was at its highest. The lower coefficients of determination (r^2) during the early morning (8–11 a.m.) and late afternoon (4–7 p.m.) periods may be because of the titration of the ozone from increased NO_x emissions from the increased automobile traffic during these rush hour periods. These results show that the intensity of photochemical reactions was a moderate factor in the formation of daytime ROS. This finding is consistent with the results of the prior study (Hung and Wang, 2001) that indicated a moderate correlation as well.

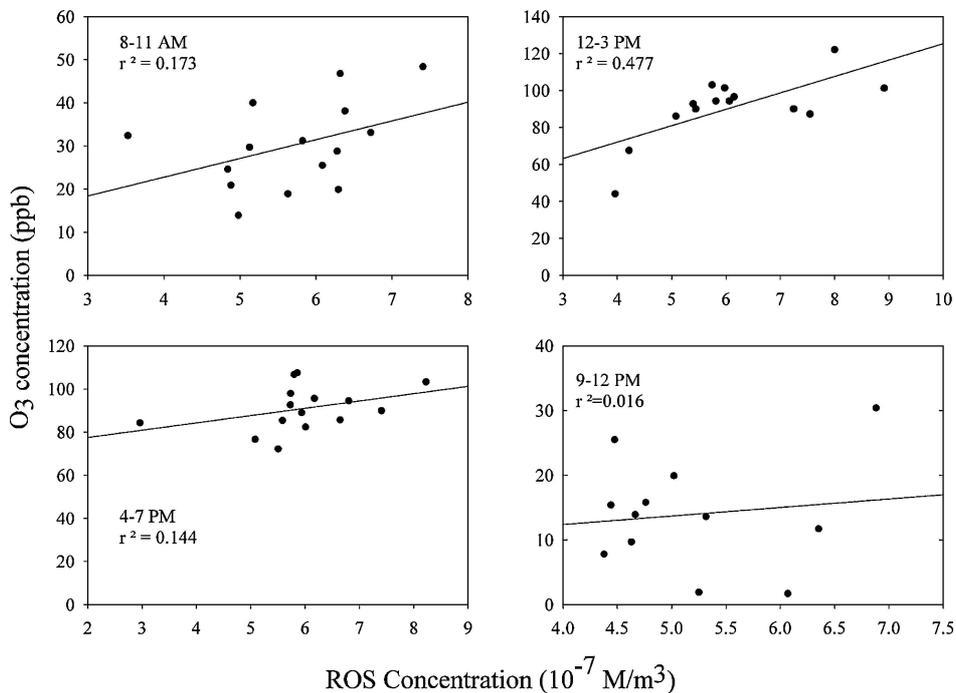


Figure 3. Regression graphs showing correlation between ozone concentrations and ROS concentrations during the four sampling period.

5. Summary and Conclusions

The ROS concentrations were measured over the period of one month in Rubidoux, CA as part of an effort to characterize particulate ROS concentrations. In summation of the results of this exercise, it was found that intensity of photochemical reactions, as indicated by the moderate correlation between ROS and O₃ concentrations, was a factor affecting the ROS concentrations. At night, it was found that the nitrate radical overshadowed the role of O₃ in the production of free radicals as seen from the poor correlation between ROS and O₃ concentrations.

The ROS production at night was found to be comparable to daytime production. Smaller particles were observed to have higher ROS concentrations, especially particles in the 10–56 nm range. The general magnitude of ROS concentrations was found to be at least an order of magnitude higher than estimated from studies in Taipei. Given the location of Rubidoux in a receptor region of the Los Angeles basin, the result is reasonable. The ROS concentrations are low, with values of the order of 10⁻⁷ M/m³. It is not possible to fully define the size distribution of the particle-bound ROS, but it is likely to be in the submicron range.

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