

Development and evaluation of a particle-bound reactive oxygen species generator

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Abstract

Identifying the most important sources and variability of source impact for particulate matter is essential to assessing their health impacts. A central hypothetical mechanism of how particles affect human health involves the generation of reactive oxygen radicals at target sites *in situ*. These could be generated via activation of cells by cell–particle interactions and by oxidative species present in particulate matter. However, prior studies have shown that there are reactive oxygen species (ROS) associated with ambient respirable particles. In order to assess the health effects of particle-bound oxidative species, a generator was developed to produce ROS-bearing model particles and deliver known, reproducible, and stable exposures to these model particles that could be used in toxicological studies. The generator was found to be capable of providing a stable throughput of these particles with an average ROS generation capability of 6.5 nmol of equivalent $\text{H}_2\text{O}_2/\text{m}^3$ of (aerosol + ozone) flow sampled. The development, characterization, and evaluation of the ROS-bearing particle generator is described.

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1. Introduction

Over the last decade, fine particulate matter ($\text{PM}_{2.5}$) have been linked to a range of respiratory and cardiovascular health problems because of their long lifetimes in the ambient air and respiratory deposition characteristics (ICRP, 1994; Seinfeld, 2004). Numerous epidemiological studies conducted over this period have shown that $\text{PM}_{2.5}$ is correlated with severe health effects, including enhanced mortality (Bernstein et al., 2004 and references therein). Moreover, toxicological investigations have demonstrated substantial pulmonary toxicity of model and real environmental particles, and have postulated particle size, surface area, and composition as potential parameters and components relevant to the observed health effects (Donaldson & Tran, 2002). However, the mechanisms and processes that cause the observed adverse health effects have not yet been resolved.

A number of mechanisms have been proposed to explain the adverse health effects of PM. Recent studies have reported a major role for the generation of reactive oxygen species (ROS) and pulmonary and systemic oxidative stress in the initiation and progression of dysfunction associated with PM exposure (Gurgueira, Lawrence, Coull, Murthy, & Gonzalez-Flecha, 2002; Nel, Diaz-Sanchez, Ng, Hiura, & Saxon, 1998). ROS has been defined to include families of oxygen-centered or related free radicals, ions, and molecules. The free radical family includes hydroxyl, hydroperoxyl

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and organic peroxy radicals. Ions such as the superoxide, hypochlorite, and peroxyxynitrite ions, and molecules such as hydrogen peroxide, organic and inorganic peroxides also come under the umbrella of 'Reactive Oxygen Species'. Much of the attention has focused on the formation of these ROS *in situ* after particle deposition in the respiratory tract generally through the interaction with transition metal ions (Stohs, Bagchi, & Bagchi, 1997), organic hydrocarbons, such as polycyclic aromatic hydrocarbons and quinones (Squadrito, Cueto, Dellinger, & Pryor, 2001), and ultrafine particle surfaces (Li et al., 2003). However, recent work has shown that ROS is present in the atmosphere on respirable particles to which we are exposed (Hasson & Paulson, 2003; Hung & Wang, 2001; Venkatchari, Hopke, Grover, & Eatough, 2005; Venkatchari et al., 2007). The hypothesis that the ROS present on particles could cause the same kind of systemic dysfunction as endogenously generated ROS has merit and requires further investigation. It can also be hypothesized that the overall oxidative capacity of PM is a predictor for their toxicological effects.

In order to assess the health effects of particle-bound oxidative species, it is essential to generate ROS-bearing model particles and deliver known, constant, and reproducible exposures of these particles to animal models for toxicological study. The particle-bound ROS generator system must provide a stable throughput of particle-bound ROS. The output of the generator is characterized in terms of particle size distribution, concentrations, and formed products (described elsewhere by Venkatchari, 2007), such that the results obtained could be viewed in the perspective of a model particle providing atmospherically relevant doses of ROS.

2. Experimental

2.1. α -Pinene–ozone chemical system

Terpenes such as α -pinene, are important biogenic volatile organic compounds (BVOCs) that are released by terrestrial vegetation to the atmosphere. α -Pinene is highly reactive toward ozone, with a reaction rate constant of 9.0×10^{-17} cm³/molecule/s at 298 K (Atkinson et al., 2004) and a calculated lower tropospheric lifetime of ~ 4.7 h with respect to reaction with ozone (Atkinson & Arey, 2003). The alkene–ozone reaction is initiated by addition of ozone across the carbon–carbon double bond to form a primary ozonide that rapidly decomposes to two Criegee intermediates. The two Criegee intermediates can decompose or collisionally stabilize, yielding a variety of multi-functional oxygenated products (Hoffmann et al., 1997). The oxidation products of α -pinene can have very low vapor pressures, condensing on existing aerosols to form secondary organic aerosol (SOA), or possibly, nucleating to form fresh particles. Experimental evidence proves that the ozonolysis of terpenes is an efficient source of SOA (Griffin, Cocker, Flagan, Seinfeld, & Blanchard, 1999; Hatakeyama, Izumi, Fukuyama, & Akimoto, 1989). In particular, the reaction of monoterpenes such as α -pinene with ozone has been found to perhaps provide the most effective routes to the formation of condensable products. Docherty, Wu, Lim, and Ziemann (2005) probed the identity of SOA formed from reactions of gaseous monoterpenes such as α -pinene with ozone, and concluded that the SOA was predominantly organic peroxides. They estimated that organic peroxides contributed to almost half the total SOA mass formed from the α -pinene–ozone reaction. Venkatchari (2007) performed LC/MSⁿ studies on the products of this generator system and found oxygenated species, predominantly containing hydroperoxide and peroxide functional groups. Since, most of the atmospheric particle-bound ROS is expected to be in the form of organic peroxides and peroxy radicals, the α -pinene–ozone system was the ideal basis for a particle-bound ROS generator.

2.2. Description of the ROS-bearing particle generator

The experimental layout of the particulate ROS generator is shown in Fig. 1.

The reaction of α -pinene and ozone was carried out in a 7.7 L (120 cm long, 90 mm internal diameter) Pyrex[®] flow reactor. In order to prevent any effect of ambient light or temperature fluctuations from affecting the reactions proceeding in the glass reactor, the reactor was wrapped in insulating wrap-on adhesive aluminum foil. Observing SOA in dark terpene–ozone systems is a widely accepted practice to limit interference from oxidation via reaction with photochemically generated OH radicals. A Collision nebulizer was used to generate α -pinene particles by nebulizing a solution of α -pinene–hexane. The hexane employed here, aside from serving as a solvent for the α -pinene also scavenges OH radicals that might be generated from dark reactions of α -pinene with ozone, especially if the reaction occurs in the gas phase. Both α -pinene (98%) and hexane ($\geq 99\%$, ACS grade) were purchased from Sigma Aldrich (Milwaukee, WI) in their purest available forms, and were used without further purification. The nebulizer was fed with house air

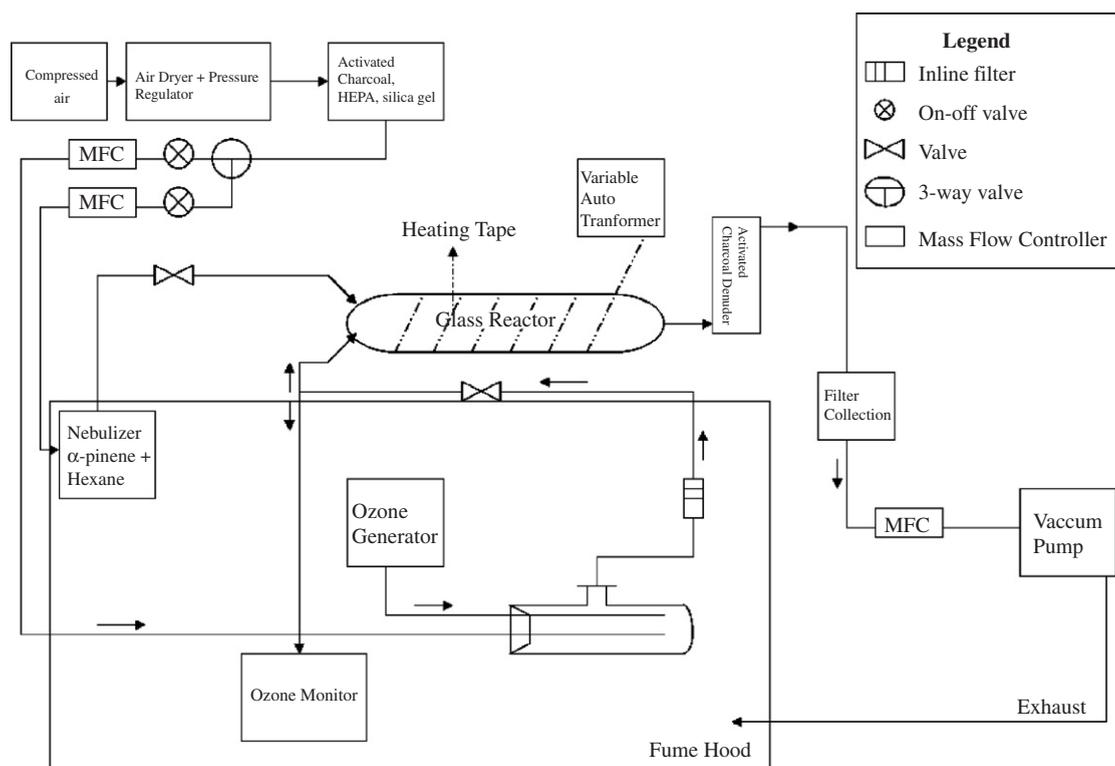


Fig. 1. Schematic of the experimental setup of the ROS-bearing particle generator.

subjected to sequential stages of HEPA-filtration, activated charcoal treatment, and silica-gel drying, so as to provide the nebulizer with particle-free, dry, de-ozonized air. The air was supplied to the nebulizer at a pressure of 15 psi within the normal operating range of this nebulizer (May, 1973). The air flow rate was approximately 2.1 L/min at this supplied air pressure. The nebulizer was found to provide a stable particle output of $\sim 8 \times 10^7 \text{ cm}^{-3}$. The particle number concentration of the dry, filtered air was found to be less than 20 cm^{-3} . The ozone used in the experiment was generated by a commercially available 'Ozone Purification' system (Air Zone Inc., VA, USA). The ozone concentrations were measured with an EnviroNics™ Series 300 Computerized Ozone analyzer (EnviroNics Inc., Tolland, CT). Anti-static Tygon tubing was used for all aerosol flow connections between the glass components, and teflon tubing was used for the ozone carrier flow.

The particle size distributions were monitored with a differential mobility analyzer (Model 3071, TSI Inc., Shoreview, MN) linked to a TSI ultrafine Condensation Particle Counter (CPC Model 3025, TSI Inc.) configured as a scanning mobility particle sizer (SMPS). The SMPS aerosol sampling, and sheath flows were set at 0.3 and 1.5 L/min, respectively. The selected sample flow rate influenced the available particle size range and the residence time (t_f) of the aerosol through the internal plumbing of the SMPS classifier. The particle size distributions were measured over the size range of 14.9–750 nm, and the scanning time and t_f in the SMPS were 120 and 7.5 s, respectively. The aerosol was passed through a Kr-85 neutralizer before entering the DMA–CPC, in order to impart an equilibrium charge distribution to the aerosol. Also, in order to ensure that the measured size distributions were correct, the length of the sampling line from the flow reactor to the SMPS was increased to double the residence time in the SMPS system. This increase resulted in no measurable change in the size distributions, and this consistency suggested that the measured size distributions were indeed correct.

Experiments were conducted with α -pinene:hexane mixing ratios ranging from about 0.1–0.5 M. The ozone concentrations were varied, but kept high enough to ensure pseudo-first order conditions (2–3 ppm). The flow reactor was maintained at a temperature above ambient by use of heating tape in order to aid evaporation of the solvent from the generated particles. Terpenes are known to react with ozone even in the adsorbed state during sampling (Hemlig, 1997),

thereby raising the possibility of artifact reaction products on the collection substrate during sampling. Since ozone was used in excess, to minimize such a possibility, it was deemed necessary to remove the unreacted ozone from the flow exiting the reactor. A diffusion denuder, consisting of a metal jacket with a central tube of fine steel mesh surrounded by a dense packing of activated charcoal (8–12 mesh, EM Science, NJ, USA) was utilized for this purpose. Pöschl, Letzel, Schauer, and Niessner (2001) have shown that activated charcoal diffusion denuders are capable of removing reactive trace gases such as ozone with efficiencies > 90%. The activated charcoal denuder was determined to be successful in removing the unreacted ozone from the aerosol stream exiting the flow reactor. The particulate reaction products, exiting the charcoal denuder, were subsequently collected on Teflo[®] membrane filter collection substrates (PALL Life Sciences, 47 mm, 2 μm pore diameter) at a fixed flow rate of 18 L/min through the filter. The collected filters were used to measure the ROS concentrations on them, and to analyze the stability of the particulate ROS generation system.

3. Results and discussion

Fig. 2 shows the changes in the size distribution of the aerosol from the ROS generator as the conditions in the reactor changed. No significant changes were observed in the shape of the size distributions with the introduction of

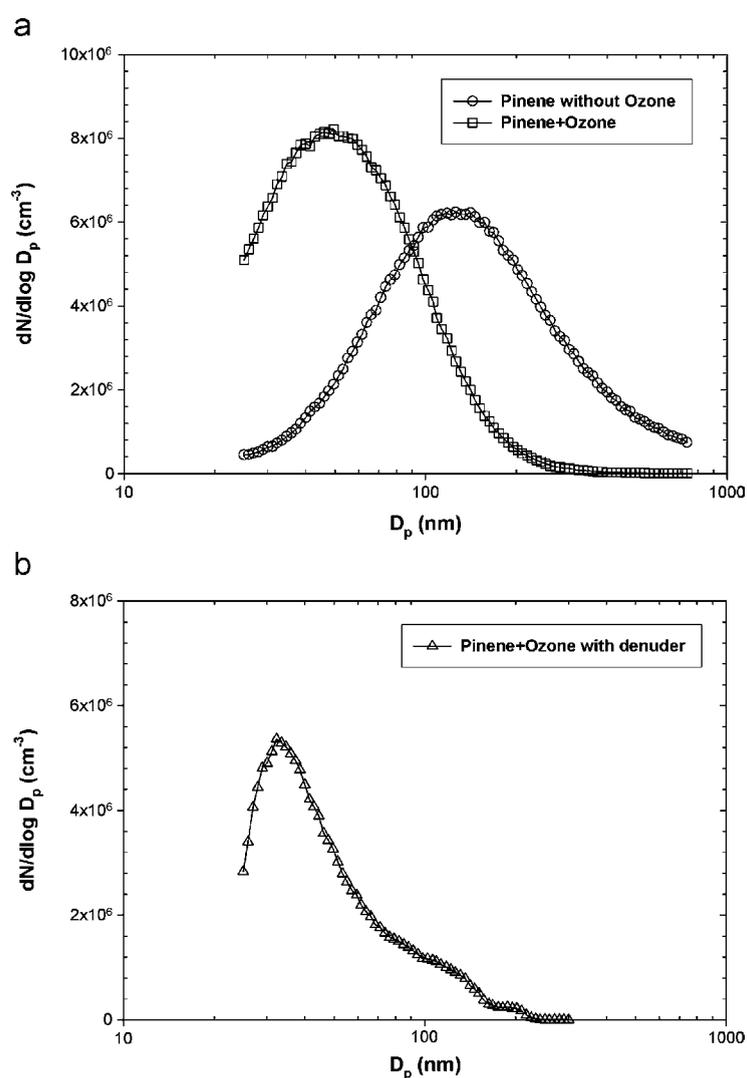


Fig. 2. The evolution of particle size distributions of the aerosol exiting (a) the flow reactor, and (b) the activated charcoal denuder.

Table 1

Measured ROS concentrations (in nmol of equivalent H_2O_2) for different sampling intervals at 0.3 M α -pinene–hexane and ~ 2.4 ppm ozone

Duration of sample (h)	Measured ROS concentration (nmol)
1	7.6 ± 1.2
2	12.9 ± 2.2
3	18.2 ± 2.0

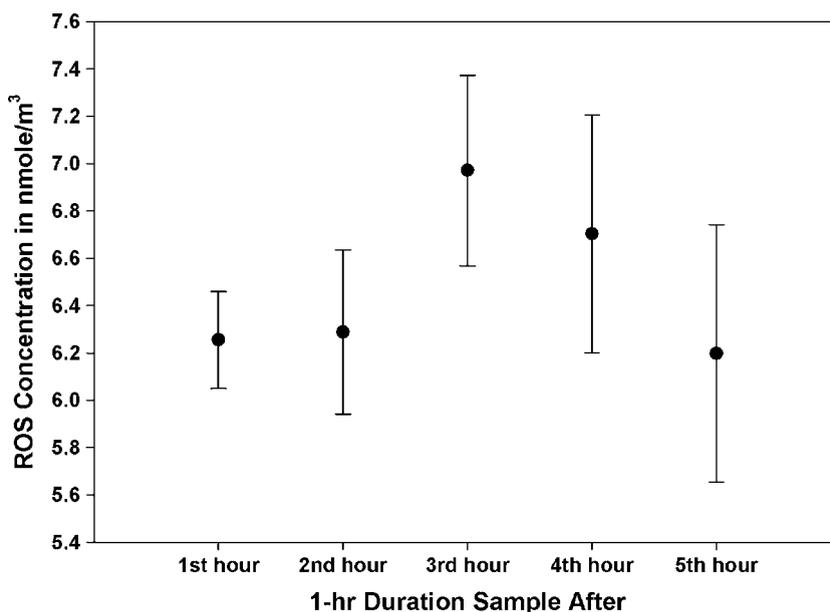


Fig. 3. Stability-time series plot of the ROS concentrations of 1-h duration samples collected in successive 1-h intervals.

ozone or post-treatment of the aerosol with a charcoal denuder. However, it is observed that the mode of the mobility diameter decreased with the introduction of ozone confirming the formation of secondary aerosol from the oxidation of α -pinene by the ozone. The ozone concentrations in the aerosol exiting the charcoal denuder were found to be negligible, illustrating that the charcoal denuder was successful in removing the unreacted ozone from the aerosol stream.

Filter samples were collected for durations ranging from 1 to 3 h, of the aerosol exiting the flow reactor after stripping it of any unreacted ozone. Quantitative determination of the filter extract solution for their ROS concentrations was performed with dichlorofluorescein, a non-fluorescent probe that fluoresces when oxidized by ROS. The same analysis procedure, used in our previous studies (Venkatachari et al., 2005, 2007), was followed to determine the ROS concentrations. In order to correlate the fluorescent intensities and concentrations in terms of equivalent H_2O_2 concentrations, calibration with standard H_2O_2 solutions was performed. Furthermore, to test the stability of the ROS generation system, 1 h samples were collected at the end of successive 1-h periods, and analyzed for their ROS concentrations. Flow through the filter for all these runs was regulated at 18 L/min. Table 1 and Fig. 3 show the results of these experiments. It can be seen that the system was stable with an average ROS generation capability of 6.5 nmol of equivalent $\text{H}_2\text{O}_2/\text{m}^3$ of (aerosol + ozone) flow sampled. However, it is to be noted that these ROS concentrations may have been underestimated due to the fact that some of the oxidative species formed in the reactor have decay times that are smaller than the sampling intervals employed. The generated ROS concentrations are of the same order of magnitude as the observed particle-bound ROS concentrations in the ambient air in Rubidoux, CA (Venkatachari et al., 2005) and almost an order of magnitude higher than those observed at Flushing, NY (Venkatachari et al., 2007) and in the ambient air in Taipei (Hung & Wang, 2001). The results obtained were found to be reproducible from day to day,

as observed from the low standard deviations, and suggest that the ROS generator can be used to provide a constant throughput of particle-bound ROS for exposure studies.

4. Conclusion

A ROS-bearing particle generator was developed, that could deliver known exposures of ROS. It was seen that the system was generally stable with an average ROS generation capability of 6.5 nmol of equivalent H₂O₂/m³ of (aerosol + ozone) flow sampled. This ROS generator could be utilized in toxicological studies to provide exposures comparable to measured ROS concentrations.

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