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Electrospray Ionization Mass Spectrometry for
Analysis of Organic Peroxides - an Application
to Atmospheric Secondary Organic Aerosol

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Abstract

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Organic peroxides comprise a significant fraction of atmospheric secondary organic aerosol (SOA). Detection and quantification of particle-phase organic peroxides are highly challenging, and current efforts rely significantly on filter extraction and offline mass spectrometry (MS). Here, a novel technique, iodometry-assisted liquid chromatography electrospray ionization mass spectrometry (iodometry-assisted LC-ESI-MS) is developed and evaluated with a class of atmospherically relevant organic peroxides, α-acyloxyalkyl hydroperoxides (αAAHPs), synthesized via liquid ozonolysis. Iodometry-assisted LC-ESI-MS unambiguously distinguishes organic peroxides, compensating for the lack of functional group information obtainable with MS. This technique can be versatile for a wide spectrum of environmental analytical applications where a molecular-level identification of organic peroxide is required. Here, Iodometry-assisted LC-ESI-MS is applied to the water soluble organic carbon (WSOC) of α-pinene SOA. Unexpectedly, a limited number of detectable compounds in WSOC appear to be organic peroxides, despite that spectroscopy-based iodometry indicates 15% of WSOC mass is associated with organic peroxides. This observation would be consistent with decomposition of multifunctional organic peroxides to small peroxides that are quantifiable by the spectroscopy-based iodometry but not by LC-ESI-MS. Overall, this study raises concerns regarding filter extraction-based studies, showing that assignment of organic peroxides based solely on MS signatures can be misleading.

Introduction

Organic peroxides are ubiquitous in the atmospheric environment, participating in the oxidation of SO₂ to form acid rain,¹ serving as a reservoir for atmospheric oxidants,² and potentially contributing to the adverse health effects of air pollution.³ Recent studies have revealed a critical role that organic peroxides play in the formation of secondary organic aerosol (SOA), submicrometer particulate matter that forms in the atmosphere via condensation of oxidation products of volatile organic compounds (VOCs).⁴ Despite the prominent
role that SOA plays in air quality and global climate, our understanding of the reaction mechanisms and products of VOC oxidation still remains incomplete. Particularly, the identity and chemistry of organic peroxides represent important missing aspects.

At the global scale, biogenic monoterpenes ($C_{10}H_{16}$) are important precursors to SOA. Estimated global SOA production from mono- and sesqui-terpenes varies from 14 to 246 Tg yr$^{-1}$.\textsuperscript{5,6} $\alpha$-pinene is the dominant monoterpene by mass\textsuperscript{7} and is readily oxidized in the atmosphere by the major oxidants, O$_3$ and the OH radical. It has been established that SOA arising from $\alpha$-pinene contains a substantial amount of total organic peroxides.\textsuperscript{8,9} Quantification of organic peroxides in SOA extracts has been successful using spectroscopic techniques, such as iodometry.\textsuperscript{8,10–12} Iodometry proceeds as $R_1OOR_2 + 2I^- + 2H^+ \rightarrow R_1OH + R_2OH + I_2$, followed by $I_2 + I^- \rightarrow I_3^-$,\textsuperscript{13} where $R_1$ and $R_2$ represent any alkyl group or H. With acid catalysis, $I^-$ reduces an organic peroxide molecule to the corresponding alcohol, liberating $I_2$ which subsequently forms $I_3^-$ in an excess amount of $I^-$. The characteristic absorption of $I_3^-$ reaches a peak at 350 nm and can be measured by UV-Vis spectrometry. As $I^-$ reacts with essentially all types of organic peroxides,\textsuperscript{14} iodometry determines the total organic peroxide content. Molecular-level identification of particle-phase organic peroxides is more challenging, due to the chemical complexity of SOA components, a lack of authentic organic peroxide chemical standards, and their chemical instability. A number of recent studies have reported decomposition of SOA organic peroxides in the particle phase\textsuperscript{12,15} and the aqueous phase.\textsuperscript{16}

Recent application of liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) on extracted SOA components has significantly advanced our understanding of particle-phase organic compounds, including both monomers and dimers,\textsuperscript{17–29} a number of which have been proposed to be organic peroxides. In particular, it is proposed that the stabilized Criegee intermediate (SCI) formed during ozonolysis can react with organic acids and form a class of hydroperoxy dimer esters, $\alpha$-acyloxyalkyl hydroperoxides ($\alpha$AAHPs).\textsuperscript{15,17,18,30,31} The importance of $\alpha$-AAHPs in the ambient atmosphere remains un-
clear, but a contribution up to 16% by mass to laboratory-generated SOA has been reported. Additionally, gas-phase measurements using chemical ionization mass spectrometry have detected highly oxidized multifunctional organic compounds (HOMs), which bear multiple hydroperoxy functional groups and arise from repeated intra-molecular hydrogen-abstraction reactions. HOMs exhibit extremely low volatility, and their presence in the particle phase has been reported. These studies have highlighted novel SOA formation pathways in which organic peroxides play a pivotal role. Determination of such organic peroxides at the molecular level is critical and is the only means to reveal the underlying formation mechanisms of SOA.

Although ESI-MS is a versatile technique for a wide spectrum of organic compounds, unambiguous identification of organic peroxides using MS-based techniques is challenging, given that MS provides limited information on functional groups. A number of studies have used ESI-MS to detect synthesized organic peroxides, including peracids, alkylhydroperoxides, peroxy esters, diacyl peroxides, and αAAHPs. Despite the capability of ESI-MS for organic peroxide detection, detection of organic peroxide is highly sensitive to specific conditions employed in each ESI-MS instrument.

The primary objective of the current study is to develop and demonstrate the applicability of a novel technique, iodometry-assisted LC-ESI-MS, to unambiguously distinguish organic peroxides present in a complex chemical matrix. The method is evaluated with αAAHPs synthesized via liquid-phase ozonolysis. For the first time, iodometry is employed not only to determine the total peroxide content, but also to provide molecular-resolved information by coupling to LC-ESI-MS. We have applied iodometry-assisted LC-ESI-MS to investigate organic peroxides present in the water-soluble fraction of α-pinene SOA. Measurements of water-soluble organic carbon (WSOC) in SOA can be carried out using filter extraction and/or the particle-into-liquid sampler (PILS). WSOC has gained attention as a proxy for the oxygenated fraction of SOA that can dissolve in cloudwater and undergo multiphase chemistry.
Experimental

Chemicals

All chemicals were used without further purification. Chemicals were purchased from Sigma Aldrich: adipic acid (99%), α-pinene (> 99%), benzoyl peroxide (Luperox®, 75%), cis-pinonic acid (98%), D-sorbitol (> 98%), hydrogen peroxide (H₂O₂, 50% in water), lauroyl peroxide (Luperox®, 97%), leucine enkephalin (> 95%, HPLC), meso-erythritol (> 99%), pinic acid (custom-synthesized), potassium hydrogen phthalate (>99.95%), potassium iodide (KI, 99%), t-butyl hydroperoxide (Luperox®, 50% in water), as well as from other sources: acetonitrile (EMD), ammonium sulfate ((NH₄)₂SO₄, Mallinckrodt Chemicals), formic acid (Fluka, HPLC grade, 50% in water), glacial acetic acid (Macron Fine Chemicals), methyl-hydroperoxide (synthesized).

SOA generation and collection

SOA was generated in the steady state Caltech PhotoOxidation flow Tube (CPOT) reactor, details of which are given in Supporting Information (SI) Section S1. Briefly, α-pinene (175 ppb) and O₃ (1 ppm) reacted in the CPOT to generate SOA at room temperature without light and nitrogen oxides. No OH scavenger was added; therefore, α-pinene is oxidized primarily by O₃ with a contribution from the OH radical generated in ozonolysis. Total gas flow rate through the CPOT was 12.5 lpm, giving rise to an average residence time of 3.5 min. Polydisperse (NH₄)₂SO₄ seed aerosol was generated by aerosolizing an aqueous solution (0.01 M) with a custom-built atomizer, followed by a diffuser dryer and a neutralizer. Relative humidity (RH) in the CPOT was approximately 10%.

Approximately 10 lpm of flow from the CPOT was introduced through a Teflon filter (Pall Life Sciences, 47 mm diameter and 2 µm pore size) to collect SOA samples. A diffuser packed with activated charcoal was employed before the filter to remove O₃ and gas-phase species to prevent continuous on-filter reactions and further partitioning of gas-phase species.
to the collected particles. One filter sample was collected per experiment, with collection time of 15 to 18 h. The mass of collected particle samples was typically 1 to 2 mg. Filters were frozen at -16 °C immediately after collection. Note that we employed collection times longer than those in previous studies (0.6 to 4 h)\(^8,11,51,52\) to overcome the detection limits of offline analyses and to maximize detectable compounds by LC-ESI-MS.

**Synthesis of \(\alpha\)-acyloxyalkyl hydroperoxides (\(\alpha\)AAHPs)**

Two \(\alpha\)AAHP species were synthesized as surrogates for multifunctional organic peroxides.\(^{17,30,31}\) They were synthesized via liquid-phase ozonolysis with a method modified from a previous study.\(^{44}\) Briefly, \(\alpha\)-pinene (50 mM) and an organic acid (10 mM) were dissolved in acetonitrile. A 5 mL aliquot of this solution was bubbled with an air stream containing roughly 100 ppm of \(O_3\) at a flow rate of 120 sccm for 5 min. The ozonolysis solution was immersed in an ice bath throughout the synthesis and storage to minimize decomposition.

The proposed formation pathway for \(\alpha\)AAHPs, as well as their structures, are shown in Figure 1. Briefly, \(\alpha\)-pinene reacts with \(O_3\) to form a primary ozonide that decomposes to form two possible Criegee intermediates. Upon interaction with the surrounding solvent molecules, stabilized Criegee intermediates (SCIs) are formed. SCI reacts with the organic acid added to the solution in an excess amount, forming a \(\alpha\)AAHP species with two possible structural isomers. Two organic acids, pinonic acid and adipic acid, were chosen in this work to synthesize two different \(\alpha\)AAHPs. Pinonic acid was selected for its relevance in \(\alpha\)-pinene oxidation. Adipic acid, being a diacid, contains an additional carboxylic acid functional group for which \(\alpha\)AAHP-A is more easily detected by ESI\(^{-}\)-MS. These two species are hereafter referred to as \(\alpha\)AAHP-P and \(\alpha\)AAHP-A, respectively.

**Offline chemical analyses**

The frozen filter samples were thawed and extracted in 10 mL of milliQ water (18.2 mΩ) by mechanical shaking before in-depth chemical analyses were performed. Sonication was
avoided to prevent potential artifacts.\textsuperscript{10}

**Total organic carbon (TOC)**

The total organic carbon (TOC) content in the SOA extracts was quantified using a TOC analyzer (OI Analytical model 1030W). The total carbon (TC) method was employed, wherein all the carbon-containing species (i.e., both organic and inorganic) are oxidized to CO\textsubscript{2} by sodium persulfate with phosphoric acid at 100 °C, with the CO\textsubscript{2} detected by nondispersive infrared detection. TC content measured in a blank filter extract was subtracted as the background. The limit of detection is 0.6 ppmC, determined as $3\sigma + $ the mean of filter blank. The method was calibrated using potassium hydrogen phthalate, and the accuracy of the method was within 5%, tested by measuring meso-erythritol and d-sorbitol solutions at known concentrations.

**Iodometry**

Formic acid or acetic acid was added to an aliquot of WSOC sample to adjust the solution pH to 2 or 3, respectively. To this solution, a concentrated potassium iodide (KI) aqueous solution, made fresh daily and purged with N\textsubscript{2} gas, was added such that the concentration of I\textsuperscript{-} in the solution was 60 mM. Immediately after KI addition, the solution was gently purged with N\textsubscript{2} and placed in an air-tight vial in the dark for 1 h before the UV-Vis measurement was conducted with a spectrophotometer (Shimadzu, UV-1601). The method was calibrated prior to each experiment against a set of H\textsubscript{2}O\textsubscript{2} solutions, standardized with the molar absorptivity of H\textsubscript{2}O\textsubscript{2} at 254 nm. The calibration accounts for the reaction of I\textsuperscript{-} with dissolved O\textsubscript{2} and confirms the linearity of the method. The detection limit of the current method is 1.5 $\mu$M H\textsubscript{2}O\textsubscript{2}-equivalent, determined as $3\sigma$ of the water blank.
Iodometry-assisted LC-ESI-MS

The instrument and methods employed for the LC-ESI-MS analysis have been described previously. Briefly, the instrument is constituted of a Waters ACQUITY UPLC I-Class system, coupled to a Quadrupole Time-of-Flight MS (Xevo G2-S QToF). The LC separation was performed on an ACQUITY BEH C\textsubscript{18} column (2.1 × 50 mm) held at 30 °C. The total flow rate was 0.3 mL min\textsuperscript{-1}, and the injection volume was 10 µL. The LC uses two eluents: A (0.1% v/v formic acid in water) and B (100% acetonitrile). The gradient was programmed as: (0 to 2.0 min) 100% A; (2.0 to 10.2 min) 10% A and 90% B; and (10.2 to 12 min) 100% A. ESI settings are: capillary voltage 2.0 kV; sampling cone voltage 40 V; source offset 80 V; source temperature 120 °C; desolvation temperature 400 °C; cone gas 30 L h\textsuperscript{-1}; and desolvation gas 650 L h\textsuperscript{-1}. Leucine enkephalin was employed as the lock mass for accurate mass determination. The method stability is to within 5%, as determined by frequent consistency tests. Both the positive (LC-ESI\textsuperscript{+}-MS) and negative (LC-ESI\textsuperscript{-}-MS) modes were operated under the same settings, and data were acquired and process with MassLynx v.4.1 software.

For a number of samples, the iodometry method described earlier was applied prior to the LC-ESI-MS measurement. Formic acid was used to adjust the solution pH to 2. To ensure the completion of iodometry, the iodometry solutions were allowed to react 5 to 7 h before the LC-ESI-MS measurement was conducted. As iodometry selectively reacts away organic peroxides, it is hypothesized that organic peroxide compounds can be elucidated by a comparison of iodometry-treated samples to non-treated samples. Four different conditions were examined to explore the effects of formic acid and iodide on WSOC: with neither formic acid nor KI (Condition I), with formic acid (Condition II), with KI (Condition III), and with both formic acid and KI (Condition IV). These four conditions maintain the same dilution ratio but with variable reagents added.
Results and discussion

Detection of Organic Peroxides

The LC-ESI-MS method separated and detected the two synthesized αAAHP species. Base peak intensity (BPI) chromatograms of an aqueous solution containing both of the αAAHPs are shown in Figure 2.

LC-ESI⁻-MS detected αAAHP-A as its deprotonated form ([M-H]⁻) due to the additional carboxylic acid functional group in the molecule (Figure 2a). Without a carboxylic acid functional group, αAAHP-P is not detected as the deprotonated form. Instead, a peak with a nominal mass of 183 Da appears at the retention time (Rt) corresponding to αAAHP-P, and its elemental composition is identical to that of the deprotonated pinonic acid (C₁₀H₁₅O₃). We propose that this peak is not pinonic acid, but instead a fragment of αAAHP-P because the Rt of pinonic acid is 4.7 min. In addition, as will be discussed shortly, this m/z 183 fragment disappears when iodometry is applied, while pinonic acid does not. This observation gives rise to an important implication for the detection of SOA components, as a fraction of organic acids commonly observed by LC-ESI⁻-MS may have been fragments of αAAHP or other high molecular weight compounds. LC-ESI⁺-MS has detected the αAAHP species predominantly as their ammonium clusters ([M+NH₄]⁺) but also as their sodium clusters ([M+Na]⁺). Note that the BPI chromatogram presents only the most intensive peak at each Rt.

To provide general guidance for future applications of ESI-MS in organic peroxide detection, we have also carefully evaluated the detection of organic peroxides using direct-infusion ESI-MS, which bypasses the LC component and directly injects the sample solution to ESI-MS (SI Section S2). Two commercially available organic peroxides, benzoyl peroxide and lauroryl peroxide, were chosen as representatives for ROOR species, while the synthesized αAAHP species were employed as those for multifunctional ROOH species. As expected, αAAHP-P is not detected by ESI⁻-MS as it does not contain any carboxylic functional
group. ESI−-MS detects αAAHP-A as its [M-H]− and [2M-H]− forms. ESI+−MS detects all
the four organic peroxides as their sodium clusters, as opposed to LC-ESI+−MS, in which
ammonium clusters dominate. As sodium formate is introduced to the infusion system reg-
ularly for calibration, there can be a potential source of Na+ in the system. On the other
hand, the amount of Na+ coeluting with analytes in LC-ESI-MS is likely much smaller. Our
results show that difference in the ionization environment can likely change the detection
mode of organic peroxides. In future studies, the detectability of organic peroxides should
be examined before any assumptions are made for their detection.

Characterization of Iodometry and the Total Peroxide Contents of
α-pinene SOA

Prior to applying iodometry to LC-ESI-MS, spectroscopy-based iodometry was performed
to determine the total organic peroxide content in the WSOC samples. We performed
measurements for 5 filters in replicate. The H2O2 equivalent concentration of total organic
peroxide ranged between 14 to 30 μM. Since the amount of SOA collected on each filter
and the extraction efficiency vary, the measured total peroxide concentration of each filter
extract was normalized to the measured TOC concentration.

The total peroxide content is commonly reported as molar yield (moles of organic perox-
ide per SOA mass) and mass yield (mass of organic peroxide per mass of SOA). Obtaining
these values requires the average organic matter to organic carbon ratio (OM/OC) of SOA
components and the average molecular weight of organic peroxides. Here, we assume the
average OM/OC to be equivalent to that of pinic acid (i.e., 1.7) which is one of the most
abundant compounds in α-pinene SOA,7 while the average molecular weight of 300 g mol−1
for organic peroxides is adapted from previous studies.8,52 With these assumptions, an av-
average molar yield of (4.8 ± 1.2) × 10−10 mol μg−1 and an average mass yield of 15 ± 3.7%
were obtained from this study. As discussed with details in SI Section S3.1, this mass yield
is lower but is comparable to those reported in the literature.8,11,51–55
To better understand the current iodometry method, we have investigated the reaction kinetics of the iodometry reaction by monitoring the solution absorbance at 350 nm. We performed this experiment on WSOC as well as a number of individual peroxide solutions, including H$_2$O$_2$, t-butyl hydroperoxide, and methyl hydroperoxide. The detailed results are presented in SI Section S3.2 and Figure S3. Our results show that the iodometry proceeds with different organic peroxides at different rates, with H$_2$O$_2$ reacting with iodide the most rapidly. The reaction with WSOC may not have reached completion after 1 h, at which point the UV measurement is taken in the current and a number of past studies, giving rise to a potential underestimation of the total organic peroxide content (see SI Section S.2).

**Iodometry-assisted LC-ESI-MS**

**Iodometry Performed on Non-peroxide Species**

Iodometry was first performed on an aqueous solution containing 5 µM each of three non-peroxide organic acids: adipic acid, pinonic acid, and pinic acid. These three organic acids can be readily detected by LC-ESI$^-$-MS. An aliquot treated with iodometry (Condition IV described in Experimental Section) was compared with a control (Condition II), and the BPI chromatograms are shown in SI Section S4. The peak intensities of the three organic acids treated with and without iodometry are essentially identical, confirming that iodide does not react with non-peroxide compounds. Another important observation is that the $Rt$ of the three organic acids is not affected by iodometry. For the iodometry-treated sample, a large peak of iodide (127 Da) emerges at the beginning of the chromatogram ($Rt < 2$ min) but is directed to waste.

**Iodometry Performed on αAAHP Species**

Iodometry was performed on an acetonitrile solution containing both of the synthesized αAAHP species. Acetonitrile is used here to minimize hydrolysis of αAAHPS and to ensure that the spectral changes are induced only by iodometry. The solution was allowed to react
for 2 h before LC-ESI-MS measurement. To the best of our knowledge, this is the first investigation of the iodometry reaction at the molecular level; therefore, detailed results are presented in Figure 3. Figure 3a to d shows the results obtained with LC-ESI⁺-MS. When formic acid was added to the solution, several additional peaks appeared on the chromatogram, but the αAAHP peaks were unaffected (Figure 3b). When KI was added to the solution, either with or without formic acid, only the αAAHP peaks disappeared (Figure 3c and d). The excess amount of organic acid added to the solution for the synthesis likely made the solution sufficiently acidic, and iodometry proceeds without additional formic acid. A similar observation was obtained using LC-ESI⁻-MS (Figure 3e to h). In particular, the m/z 183 peak at a Rt of 7 min disappears with iodometry, confirming that it is likely a fragment of αAAHP-P and unrelated to pinonic acid. These results illustrate that iodometry selectively reacts away organic peroxides with negligible impact on non-peroxide species.

The Effect of Iodometry on SOA Extract: Negative Mode (LC-ESI⁻-MS)

Analysis of α-pinene SOA components using LC-ESI⁻-MS has been reported by a number of studies, including our previous work. The chromatogram and mass spectra recorded in this work are provided in SI Section S5. The BPI chromatogram obtained in the current study (SI Figure S5a) reproduces well those of our previous study, confirming the reproducibility of the LC-ESI-MS method. SI Figure S5b shows the reconstructed mass spectrum, defined to be the sum of all mass spectra from Rt of 2 to 9 min with a peak height of 2 × 10³ counts per second (cps) or higher. The mass spectrum demonstrates a bimodal form, attributed to monomers and dimers in WSOC. In SI Table S2, we provide a list of major peaks detected in the current work. We did not conduct a detailed structural analysis, as this has already been done by a number of other studies. Instead, we have annotated peaks that have been previously proposed as candidates of organic peroxides. We performed a comparison of samples treated with and without iodometry, and the results for LC-ESI⁻-MS are shown in Figure 4, focusing on the comparison between the
sample treated with only formic acid (Condition II) and that treated with both formic acid
and KI (Condition IV), as this comparison excludes any mass spectral changes induced by
formic acid alone and best reflects changes induced by iodometry.

Iodometry did not induce significant changes to the BPI chromatograms and recon-
structed mass spectra. The top panel of Figure 4b shows the difference mass spectrum
between the control and iodometry samples. Given that the reactivity of $\alpha$AAHP species
has been exhibited (Figure 3), any organic peroxides present in the WSOC of $\alpha$-pinene SOA
should be depleted by the time of measurement. Therefore, only peaks exhibit a significant
change, i.e., either depleted by more than 70% or newly introduced by iodometry, are shown
in the difference mass spectrum.

The only major peak that is depleted by iodometry and showed consistency between
different filter samples is that of the deprotonated form of $C_8H_{14}O_6$ (205.071 Da, $Rt$ of 3.71
min). The effect of iodometry on other major peaks is discussed in SI Section S5. The
decay of $C_8H_{14}O_6$ on the BPI chromatogram is observable (Figure 4a). The EICs of this
compound are presented in Figure 5a, showing that $C_8H_{14}O_6$ is depleted only when both
formic acid and KI are present. As the WSOC does not contain an excess amount of organic
acids, compared to the synthesized $\alpha$AAHPs in which an organic acid is added in excess
for synthesis, it seems that additional formic acid is necessary for the iodometry reaction
to proceed. We have attempted to perform MS$^2$ measurement on $C_8H_{14}O_6$, but the signal
intensities of its fragments were too low to obtain structural information. $C_8H_{14}O_6$ has
been observed previously and has been tentatively defined as an unknown carboxylic acid.$^{17}$
Results from this study suggest that this compound contains a peroxide functional group.
Given the high oxygen to carbon ratio (O:C) of this compound, $C_8H_{14}O_6$ can potentially
be a HOMs species arising from intra-molecular hydrogen-abstraction (see Introduction).
Alternatively, it can also be a compound similar to 3-methyl-1,2,3-butanetricarboxylic acid
(MBTCA, $C_8H_{12}O_6$, $Rt = 3.73$ min), a well-established organic tracer for $\alpha$-pinene SOA.$^{60-62}$
The Effect of Iodometry on SOA Extract: Positive Mode (LC-ESI\textsuperscript{+}-MS)

Driven by the abundance of organic acids, the use of ESI\textsuperscript{-} has prevailed in molecular analyses of \(\alpha\)-pinene SOA components. Only a few studies have employed ESI\textsuperscript{+}.\textsuperscript{24,35,37,56,59,63} The BPI chromatogram recorded by LC-ESI\textsuperscript{+}-MS and its reconstructed mass spectrum are shown in SI Figure S5 a and c, respectively. A number of major compounds are detected by both the positive and negative modes, while some can be detected only by the positive mode, as clusters of H\textsuperscript{+}, Na\textsuperscript{+}, as well as NH\textsubscript{4}\textsuperscript{+}. Since the polarity of ESI detection does not affect the chromatographic \(R_t\), we have conducted peak assignment for the positive mode by comparing with peaks detected by the negative mode, and a list of major compounds are listed in SI Table S3.

Comparison of a sample treated with and without iodometry is shown in Figure 6 in the same manner as in Figure 4. The only noticeable change on the BPI chromatograms is that two major peaks, appearing at \(R_t\) of 7.3 and 8.1 min, disappear when iodometry is applied. Judging solely based on the comparison of Condition II vs. IV, which is the case for Figure 6, these two peaks appear to be organic peroxides. However, a detailed investigation of these peaks under all the four conditions, presented in SI Section S6.1, indicates that the addition of formic acid has introduced artifacts, and these two peaks are unlikely to be peroxides. Among all the peaks with a peak height of 2000 cps or higher, five appear to be candidates for organic peroxides: \([C_8H_{10}O_3+H]^+\) (155.07 Da), \([C_{10}H_{16}O_5+NH_4]^+\) (234.13.34 Da), \([C_{10}H_{18}O_6+NH_4]^+\) (252.15 Da), \([C_{20}H_{30}O_5+NH_4]^+\) (368.24 Da) and \([C_{19}H_{30}O_7+NH_4]^+\) (388.23 Da). The EICs of \([C_{20}H_{30}O_5+NH_4]^+\) under Condition I to IV are shown in Figure 5b, while those of the others are presented in SI Section S6.2. \([C_8H_{10}O_3+H]^+\) and \([C_{10}H_{16}O_5+NH_4]^+\) are detected as multiple peaks, and not all of them appear to be organic peroxides. This observation demonstrates the ability of iodometry-assist LC-ESI-MS to resolve organic peroxides from non-peroxide isomers. The consistency of these observed trends has been confirmed with a separate filter sample. All five candidates for organic peroxides are detected as minor peaks and do not belong to the 50 largest peaks summarized in Table
S3. Their peak areas are 2% to 5% that of pinyl-diaterpenyl ester, which is the largest peak detected by LC-ESI\(^+\)-MS. The effect of iodometry on other major peaks is presented in SI Section S5.

**Explanations for the Absence of Organic Peroxides in WSOC**

The absence of organic peroxides among the major products was unexpected, as \(\alpha\)-pinene SOA has been believed to contain a high organic peroxide content.\(^9,64\) In fact, our conventional iodometry measurement using UV-Vis detected a total organic peroxide content comparable to previous studies (SI Table S1). Many of the proposed organic peroxides from previous studies\(^17,18,23,59\) are detected in the current work with the corresponding elemental compositions (SI Table S2). However, none of these compounds decayed in response to the iodometry treatment. We have altered a number of experimental and instrumental conditions that can potentially affect iodometry and the detection of organic peroxides, including the temperature of iodometry reaction and the ESI settings. Detailed results of these experiments are presented in SI Section S7. However, varying these factors did not explain the absence of major organic peroxide peaks.

The current LC-ESI-MS method is not quantitative, as determination of electrospray ionization efficiency for compounds without definite structural information is difficult; therefore, the peak area of a compound does not directly reflect its concentration in the WSOC sample. With a computational approach, our previous study\(^23\) has found that the ionization efficiencies of the dimer esters are 3 to 10 times higher than those of the monomeric compounds. Currently, we cannot rule out the possibility that LC-ESI-MS fails to detect certain organic peroxide species due to inefficient ionization. In particular, the major peaks detected by the current method include very few HOMs compounds which typically exhibit \(O:C > 0.7\). It is likely that our method and/or instrument is less optimized towards HOMs compared to a number of previous studies.\(^34,35,37\)

Alternatively, decomposition of organic peroxides may play an important role in filter
extraction-based techniques. Decomposition can occur at different stages of the experiment. Highly labile organic peroxides may have decomposed in suspended particles before they can be collected.\textsuperscript{12,15,23,52} Decomposition may also occur on the filter, with longer collection times leading to loss of organic peroxide.\textsuperscript{52} In future applications, the filter collection duration can be shortened from that in the current work (15-18 h) to minimize decomposition and evaporation. Finally, decomposition may occur after the SOA component is extracted to condensed phases, forming the OH radical\textsuperscript{16} and H\textsubscript{2}O\textsubscript{2}.\textsuperscript{53,55,65} Small and polar peroxides, such as H\textsubscript{2}O\textsubscript{2}, can contribute to the total peroxide content measured by the conventional, spectroscopy-based iodometry but are not detected by our LC-ESI-MS method which is optimized for monomeric and dimeric oxidation products of $\alpha$-pinene.

Environmental Implications

With an emerging awareness of the role that organic peroxides play in SOA formation and the consequent health effects, identification of particle-phase organic peroxides has become a priority in atmospheric chemistry research. Employing advanced MS techniques, many recent studies have reported detection of particle-phase organic peroxides. However, structural assignment with MS is often based only on exact mass and/or fragmentation patterns, supported by feasible formation mechanisms, leaving room for potential misassignment due to lack of structural information on functional groups. In this work, we have developed a novel technique, iodometry-assisted LC-ESI-MS, to unambiguously identify organic peroxides present in extracted SOA components at the molecular level.

Owing to a lack of commercially available organic peroxides, characterization of our method was performed with synthesized $\alpha$-acyloxyalkyl hydroperoxides ($\alpha$AAHPs). Detection of $\alpha$AAHPs was successful, but our results reveal concerns regarding the use of ESI-MS for the detection of organic peroxides. In particular, even with the same ESI-MS instrument, a difference in the ionization mode was observed between direct-infusion and LC-ESI-MS,
likely due to different ionization conditions. In future studies, the utility of LC-ESI-MS for
organic peroxide identification should be thoroughly characterized.

The utility of iodometry-assisted LC-ESI-MS has been demonstrated with αAAHPs. Iodometry selectively reacts away organic peroxides with negligible interference to non-peroxide species. While iodometry-assisted LC-ESI-MS was applied in this work to study one specific issue related to atmospheric particulate matter, the versatility of this technique makes it applicable to a wide range of environmental applications that require the determination of organic peroxides at the molecular level.

Iodometry-assisted LC-ESI-MS was applied to the water soluble organic carbon (WSOC) of α-pinene SOA collected from a flow tube reactor, following a standard sample collection procedure. Unexpectedly, only a limited number of detectable compounds, C₈H₁₄O₆ from the negative mode and five minor peaks from the positive mode, appeared to be organic peroxides. This observation is inconsistent with conventional, spectroscopy-based iodometry which suggests that the average mass yield of organic peroxides is 15% in this system.

We propose that the multifunctional organic peroxides may have decomposed to smaller peroxides that cannot be detected with the current LC-ESI-MS technique. Future studies should investigate the stability and decomposition mechanisms of organic peroxides on filters and in extraction solutions. Although the current work focused only on the decay of organic peroxides during iodometry, an interesting direction would be the investigation of the corresponding alcohols arising from iodometry. Unlike organic peroxides, functionalized alcohols and polyols are commercially available, which may lead to new avenues for quantifying organic peroxides.

Our results raise significant concerns applicable to all filter extraction based studies for atmospheric SOA, showing that labile organic peroxides can be lost either during sample collection or after extraction. The use of MS prevails in the field of Atmospheric Chemistry, and the versatility of LC-ESI-MS has been proved by many studies. However, previously unrecognized considerations are required for the interpretation of MS data, particularly for
the assignment of organic peroxides.

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Supporting Information Available

Section S1 supplies additional information on the generation and collection of SOA. Section S2 presents the detection of organic peroxides with direct-infusion ESI-MS. Section S3 provides a comparison of the total organic peroxide content obtained in this work to those from previous studies and a detailed characterization of the iodometry method. Section S4 presents the results from iodometry performed on non-peroxide species. Section S5 provides the chromatograms and lists of major compounds detected by LC-ESI-MS. Section S6 provides additional information on the analyses of iodometry-assisted LC-ESI-MS. Section S7 provides additional examination of various instrumental conditions to iodometry-assisted LC-ESI-MS. This material is available free of charge via the Internet at http://pubs.acs.org/.
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Figure 1: Schematic of the mechanism underlying the synthesis of the αAAHP species. Reactions in a) illustrate the general reaction between a stabilized Criegee intermediate (SCI) and an organic acid, giving rise to αAAHP with two possible structural isomers. Reactions in b) show the specific cases employed in the current work to synthesize αAAHP-P and αAAHP-A. Only one structure isomer is shown for each of them.
Figure 2: Base peak intensity (BPI) chromatogram of an aqueous solution containing αAAHP-P and αAAHP-A detected by LC-ESI−MS (a) and LC-ESI+MS (b). Colored areas represent the extracted ion chromatograms (EICs) of these two αAAHP species.
Figure 3: Results of iodometry performed on a mixture of synthesized \( \alpha \)AAHP-P and \( \alpha \)AAHP-A dissolved in acetonitrile. Results of LC-ESI\(^+\)-MS and LC-ESI\(^-\)-MS are presented in panels a) to d) and e) to h), respectively.
Figure 4: Comparison of a sample treated with and without iodometry, measured by LC-ESI−MS. Comparison of the base peak intensity (BPI) chromatograms is shown in panel (a). The reconstructed mass spectra are shown in the bottom panel of (b) where the peak intensities have been normalized to that of pinic acid ([C₉H₁₄O₄-H]− at 185.08 Da). The top panel of (b) is a difference mass spectrum showing peaks that are depleted by more than 70% and those newly introduced by iodometry. Each BPI chromatogram and mass spectrum shown here is the average of triplicate. The Control sample refers to Condition II described in Experimental section, while the iodometry sample refers to Condition IV.
Figure 5: Extracted ion chromatogram (EIC) of two organic peroxide candidates: $[\text{C}_8\text{H}_{13}\text{O}_6]^-\text{ from }\text{LC-ESI}^{-}\text{-MS (a) and }[\text{C}_{20}\text{H}_{30}\text{O}_5+\text{NH}_4]^+\text{ from LC-ESI}^+\text{-MS (b). From the top to the bottom, the four traces represent iodometry Condition I to IV, respectively (please refer to the Experimental section).}
Figure 6: Comparison of a sample treated with and without iodometry, measured by LC-ESI$^+$-MS. Comparison of the base peak intensity (BPI) chromatograms is shown in panel (a). The reconstructed mass spectra are shown in the bottom panel of (b) where the peak intensities have been normalized to that of pinyl-diaterpenyl ester ([C$_{17}$H$_{26}$O$_8$+Na]$^+$ at 376.20 Da). The top panel of (b) is a difference mass spectrum showing peaks that are depleted by more than 70% and those newly introduced by iodometry. Each BPI chromatogram and mass spectrum shown here is the average of triplicates. The Control sample refers to Condition II described in the Experimental section, while the iodometry sample refers to Condition IV.
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