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Feasibility of a Selective Epoxidation Technique for Use in Quantification of Peracetic Acid in Air Samples Collected on Sorbent Tubes

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Abstract

Peracetic acid is a disinfection agent used in medical and food processing facilities, and occupational exposures have been documented. To facilitate characterization of daily occupational exposures, the current work describes the development of a personal sampling technique to quantify the peracetic acid concentration in air samples. Peracetic acid atmospheres were generated in 100 L Teflon chambers, and samples were collected on 350 mg XAD-7 solid sorbent tubes for 4 h at a flow rate of 250 mL/min using a personal sampling pump. Indirect measurement of peracetic acid was achieved by desorption from the sorbent and subsequent treatment with cyclohexene to initiate an epoxidation reaction, formally known as the Prilezhaev reaction. The epoxidation product, cyclohexene oxide, was quantified by gas chromatography–

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ASSOCIATED CONTENT
Supporting Information
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Additional data regarding sorbent capacity experiments as well as variable time gas measurements (PDF)

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.chas.2c00025

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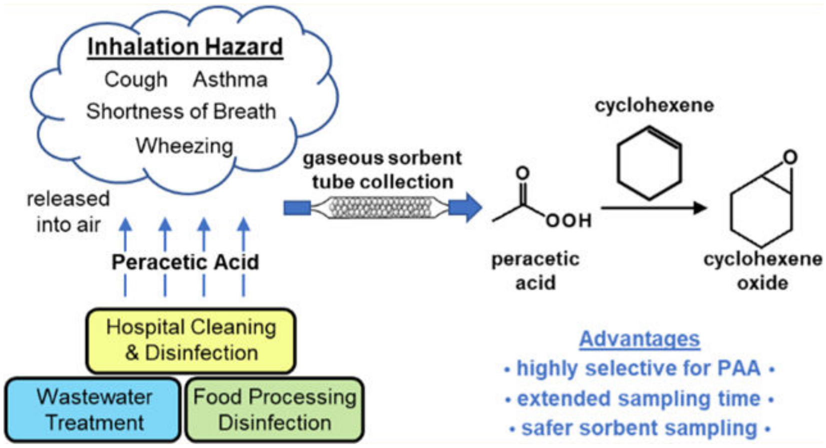
mass spectrometry. The reaction enabled quantification of peracetic acid with high specificity over the common co-contaminants hydrogen peroxide and acetic acid, which were introduced in 10-fold and 100-fold excess to challenge the reaction. The technique also demonstrated an overall estimate of bias and precision of 11 and 8%, respectively, and a limit of detection of 60 ppbv was estimated. Preliminary storage experiments indicate that unreacted peracetic acid is stable on the sorbent tubes for 72 h when stored at −20 °C following collection. Overall, the specificity of the reaction and capability to sample for longer time periods than current methods, in addition to the use of safer personal sampling materials, demonstrate the utility of this technique for peracetic acid measurement in air.

Graphical Abstract



Keywords

occupational exposure surveillance; peroxyacetic acid; sorbent; disinfection

INTRODUCTION

Peracetic acid (peroxyacetic acid, PAA) is a peracid that is synthesized from the reaction between acetic acid (AA) and hydrogen peroxide (HP) in the presence of an acid catalyst. PAA is widely used as a disinfectant, sterilant, biocide, and sporicide across several industry settings including healthcare facilities, poultry and meat processing plants, dairy sanitation, and wastewater treatment centers.[1–11] It is a strong oxidant, highly reactive upon contact with organic material. In solutions ranging from 5 to 35 wt % PAA, higher vapor pressures of 1.7–2.1 kPa indicate that PAA can exist as a vapor at ambient temperatures and has also been shown to be an irritant to the skin, eyes, and mucous membranes of the respiratory tract.[12,13]

Efforts are ongoing to determine the health effects of PAA and evaluate exposure limits in the workplace. Use of products containing PAA is associated with cough, wheezing, watery eyes, shortness of breath, and asthma in medical settings.[14–18] A threshold limit value–short term exposure limit (TLV-STEL) of 0.4 ppm PAA over 15 min was recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) in 2013.[19] However, official full work shift time-weighted average (TWA) occupational exposure limits have not been established for PAA, although the recommendation of 0.1–0.2 ppm for a full-shift TWA has been asserted by research groups.[20–22] A recent occupational air sampling study was conducted to measure the exposure levels of mixtures of PAA, HP, and AA over a full shift for hospital workers. Despite the measured concentrations of the chemicals falling below the established and/or suggested occupational limits, the exposures were correlated with eye and respiratory symptoms in workers.[15,16] These studies suggest that exposure to even lower concentrations of PAA over long time periods may pose risks to workers, and additional studies to determine full-shift TWA exposures to PAA are warranted.

Air sampling methods for PAA quantitation have been developed, and many rely on the indirect measurement of PAA through its highly oxidative chemical nature. These strategies include active sampling of PAA atmospheres into liquid-filled impingers and onto sorbent tubes[23–27] as well as passive sampling methods.[28] Chemicals including methyl *p*-tolyl sulfide (MTS), methyl *p*-tolyl sulfoxide (MTSO), and 2-([3(2-[4-amino-2-(methylsulfanyl)phenyl]-1-diazenyl)phenyl]sulfonyl)-1-ethanol (ADS) are added to PAA sampled from air, and the products from oxidation by PAA are then measured by chromatography methods. As sampling for greater than 15 min has not been demonstrated for current published PAA methods, the development of PAA air measurement strategies that incorporate multi-hour sampling is necessary to facilitate full-shift TWA measurements.

PAA exists in equilibrium with AA, HP, and H_2O according to eq 1:[29]

$$CH_3CO_2H + H_2O_2 \rightleftharpoons CH_3CO_3H + H_2O \quad (1)$$

Its equilibrium complicates the sampling and analysis of PAA; as such, methods for accurate PAA measurement must account for the stabilization and selective detection of PAA without interference from AA and HP. In early work, problems were noted when MTS oxidation to MTSO was used to quantify PAA in solution. Under high HP concentrations or acidic conditions, the oxidation of MTS to MTSO continued past the point at which the initial amount of PAA had been consumed, leading to overestimation of the quantity of PAA.[30] Thus, more recent methods utilizing MTS and MTSO have sought to selectively remove HP. For example, addition of sodium thiosulfate to the impinger sample after PAA collection was used to react with any existing HP.[25] Additionally, the Hecht method incorporated a separate cassette containing titanium oxysulfate into the sampling train that captured HP prior to collection of PAA, which allowed for the separate quantification of HP.[27] It has been noted that a minimum pump rate of 1 L/min must be maintained using this cassette strategy; otherwise, the recovery of PAA will suffer.[31,32]

In this work, the feasibility of gas-phase PAA quantification using a new derivatization strategy based on the Prilezhaev reaction is presented. The Prilezhaev reaction involves the epoxidation of an alkene by a peracid (Scheme 1).[33] Because the Prilezhaev reaction is selective for peracids in the absence of a base, experiments herein were designed to determine its applicability for quantifying PAA concentrations in air samples.

The strategy described here employed active gas-phase sampling using XAD-7 sorbent, whose acrylic acid composition allows for the capture of polar compounds more readily than hydrophobic sorbents (i.e., XAD-2 and XAD-4).[34] This has been demonstrated with a number of organic acids and other highly oxygenated compounds.[35–38] PAA was quantified indirectly through cyclohexene oxide (CHO), the epoxide product generated from the Prilezhaev reaction following the introduction of cyclohexene. This technique was tested using 4 h sampling periods, which could be useful for assessing the longer-term personal exposure of workers to PAA.

METHODS AND MATERIALS

General Considerations.

Peracetic acid (PAA) (32% in acetic acid), cyclohexene (≥99.7%), anhydrous acetonitrile (ACN) (99%), *n*-hexane (98%), 1-bromohexane (98%), hydrogen peroxide (HP) (30% in H_2O), glacial acetic acid (AA), and cyclohexene oxide (CHO) (98%) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Preloaded XAD-7 glass sorbent tubes (350 mg (part no. 226–211) and 175 mg (part no. 226–97 C)) were purchased from SKC, Inc. (Eighty Four, PA). Water was distilled, deionized to a resistivity of 18 MΩ cm (DI H_2O), and filtered using a Milli-Q filter system (Billerica, MA). Helium and nitrogen gas (UHP grade) were supplied by Butler Gas (McKees Rocks, PA) and used as received.

PAA Liquid-Phase Experiments.

Liquid-phase PAA experiments were conducted for the determination of initial reaction conditions as well as for use in constructing calibration curves. PAA stock solutions with concentrations ranging from 0.041 to 3.27 M were prepared in DI H_2O. Freshly prepared PAA solution (2 μL) was added to a clean 7 mL glass septum-capped vial containing 1 mL of anhydrous ACN such that the amount of PAA in the sample is equivalent to that of the gas-phase experiments. Specifically, 6, 12, 25, 50, 100, 199, 249, 373, and 498 μg of PAA were chosen, which are equivalent to 25, 50, 100, 200, 400, 800, 1000, 1500, and 2000 ppb gas phase concentrations when dispersed into 80 L of air, respectively. An excess of cyclohexene (100 μL, 0.99 mmol) was added to the vial, which was then capped and vortexed for 1 min. The mixture reacted for approximately 24 h at 297 ± 3 K (defined as room temperature in this study (RT)) without further agitation. Following this, 1.5 mL of 0.178 mM 1-bromohexane (internal standard, IS) in *n*-hexane was added. The solution was once again vortexed for 1 min to promote even mixing. The layers were allowed to settle for 5 min, and then 100 μL of the hexane layer was removed to a 2 mL amber vial with a 250 μL glass insert (allowing for maximum sample retrieval), which was subsequently analyzed via GC–MS (see below).

Control experiments were performed in the absence of added PAA solution as well as with the addition of HP or AA stock solutions prepared in DI H_2O at various concentrations and ratios as described in the results. Additional control experiments were performed with CHO solutions prepared by combining neat CHO purchased from Sigma-Aldrich with ACN in concentrations of 0.041–3.27 M (matching concentrations of PAA solutions as described above). For each experiment, 2 μL of CHO solution was added to 5 mL of ACN, extracted with hexane as described above, and analyzed by GC–MS. For comparison to the CHO samples, PAA samples were also prepared in 5 mL of ACN. Five milliliters of ACN was used in initial experiments, but after testing indicated that the use of 1 mL of ACN improved recovery, all remaining experiments were performed as such. Experiments pertaining to the use of 5 mL are presented in the manuscript strictly when relative comparisons were made between treatments using 5 mL.

PAA Chamber Creation and Sorbent Tube Sampling.

PAA solutions with concentrations ranging from 0.041 to 3.27 M were prepared as described above. A 2 μL aliquot of the prepared PAA solution was dispersed into 80 L of air, resulting in concentrations of 25–2000 ppb. Chamber experiments were performed at RT in a collapsible Teflon 100 L reaction chamber, the construction of which is described elsewhere.[39] The chamber was equipped a 6.4 mm Swagelok (Solon, OH) Teflon tee fitting, into which 2 μL of prepared PAA solution was delivered. The aliquot of PAA was delivered to the chamber to create the PAA atmosphere as follows: compressed air from the NIOSH facility passed through anhydrous calcium sulfate ($CaSO_4$) (Drierite, Xenia, OH) and 4 Å molecular sieves (Sigma Aldrich, St. Louis, MO) to remove moisture and flowed into a humidifying chamber, where it was mixed to the predetermined relative humidity (RH) of 50 ± 3%. A mass flow controller (MKS, Andover, MA) regulated the air flow rate to 5 L/min entering the chamber, and each chamber was filled to 80 L.

Following the creation of the PAA atmosphere, 60 L of the total 80 L of PAA-containing air was pulled through a 350 mg XAD-7 sorbent tube (SKC Inc., PA) via a personal sampling pump (Pocket Pump Touch, SKC Inc., PA) for 4 h at 250 mL/min. The 4 h sampling period was determined based on the maximum capacity of the reaction chamber and was supported by several examples in literature wherein personal sampling was conducted for time intervals shorter than the standard 8 h shift and were subsequently used to determine a full-shift TWA.[40–42] For data shown in Table 4, PAA-containing air was collected at 1 L/min for 15 min (URG-3000–02Q, Chapel Hill, NC). After collection, the sorbent tube was broken, and the entire amount of XAD-7 sorbent in the tube (350 mg total) was transferred to a clean 7 mL clear glass vial equipped with a Teflon septum cap. ACN (1 mL) and an excess of cyclohexene (100 μL, 0.99 mmol) were added, and the sample was vortexed for 1 min. The mixture reacted for 24 h at room temperature with no further agitation, after which 1.5 mL of 0.178 mM 1-bromohexane (IS) in *n*-hexane solution was added. The sample was vortexed for 1 min and allowed to settle for approximately 5 min. A 100 μL aliquot of the hexane layer was then transferred to a 2 mL amber vial with a 250 μL glass insert for GC–MS analysis as described below.

Calibration Curve Experiments.

Calibration Curve A (Liquid Phase).—

Calibration curve A was constructed using the data obtained from the liquid-phase experiments discussed above (0.041–3.27 M). Aliquots containing 6, 12, 25, 50, 100, 199, 249, 373, and 498 μg of PAA were used for the experiments, and three replicates were performed for each μg quantity.

Calibration Curve B (Liquid + Sorbent).—Samples for generation of calibration curve B were prepared in triplicate for each *μ*g quantity as follows. Sorbent from a blank 350 mg XAD-7 sorbent tube, 1 mL of ACN, and 2 *μ*L of PAA solution with a known concentration were added to a clean 7 mL glass vial equipped with a septum cap. This mixture was vortexed for 1 min to promote an even distribution of solvent and analyte throughout the sorbent. Following this, 100 *μ*L of cyclohexene was added, and the sample was vortexed for 1 min. This mixture reacted for 24 h at RT without further agitation and then was extracted by adding 1.5 mL of 0.178 mM 1-bromohexane in *n*-hexane and vortexed for 1 min. After the layers settled, 100 *μ*L of the hexane layer was removed to a 2 mL amber vial with a 250 *μ*L glass insert and was analyzed via GC–MS. Aliquots containing 6, 12, 25, 50, 100, 199, 249, 373, and 498 *μ*g of PAA were used for the experiments.

Fortified Sampler Experiments.

A 350 mg XAD-7 sorbent tube was loaded with 2 *μ*L of a PAA solution with a known concentration (prepared as described above), and clean air at 50% RH from a Teflon chamber was pulled across the spiked sorbent tube for 4 h at a rate of 250 mL/min. Following sampling, the sorbent tube was processed as described above and analyzed via GC–MS. Aliquots containing 6, 12, 25, 50, 100, 199, 249, 373, and 498 *μ*g of PAA were used for the experiments, and three replicates were performed for each *μ*g quantity.

Sorbent Capacity Experiments.

A solution of PAA (2 *μ*L, 3.27 M, 498 *μ*g) was injected into a Teflon tee and was subsequently combined with 80 L of 50% RH air to create an atmosphere containing 2000 ppb of PAA within the chamber, as described above. An additional XAD-7 sorbent tube (containing 175 mg of sorbent) was inserted into the sampling train behind the initial 350 mg XAD-7 sorbent tube. A personal sampling pump was then connected to the opposite end of the second sorbent tube, and 250 mL/min of PAA-spiked air from the chamber was pulled onto sorbent tubes for 4 h. After sampling, the tubes were processed separately as described above and analyzed by GC–MS.

Sorbent Tube Storage Experiments.

Chambers containing 50, 100, and 199 *μ*g of PAA were prepared, corresponding to 200, 400, and 800 ppb, respectively, in 80 L of 50% RH air as described above in PAA Chamber Creation and Sorbent Tube Sampling. The PAA-containing air was sampled through a 350 mg XAD-7 sorbent tube via a personal sampling pump for 4 h at 250 mL/min (total of 60 L). The sorbent tube was then tightly capped, sealed with parafilm, and stored at RT or −20 °C in the absence of light for 72 h. Following storage, the sample was processed as described above, with the exception that 5 mL of ACN was used, and the corresponding data were normalized using data from experiments without storage that used the 5 mL extraction protocol described in PAA Liquid-Phase Experiments.

Stability and Variable Sampling Time Experiments.

The stability of PAA and the flexibility of sampling time were evaluated using 800 ppb chambers containing 199 *μ*g of PAA and 80 L of 50% RH air (preparation described above).

Two different treatments were conducted: (1) collection of a 15 L sample over the course of 1 h at a rate of 250 mL/min ($n = 2$ chambers) and (2) collection of a 15 L sample over the course of 15 min at a rate of 1 L/min ($n = 3$ chambers). Four samples were collected from each chamber prepared. Following the allotted time periods, each of the samples was processed as described above.

GC–MS Analysis.

All samples were analyzed using an Agilent (Santa Clara, CA) 7890B GC coupled to an Agilent 240 Internal EI/CI ion trap MS. Compound separation was achieved by an Agilent HP-5MS (0.25 mm I.D., 30 m long, 0.25 μm film thickness) column and the following GC oven parameters: 40 °C for 2 min, then 5 °C min^{-1} to 200 °C, then 25 °C min^{-1} to 280 °C, and held for 5 min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. For each sample, 1 μL was injected in splitless mode with the injector temperature at 230 °C. The ion trap MS was tuned using perfluorotributylamine (FC-43). Full-scan ionization spectra were collected from 40 to 1000 (m/z). Extracted-ion chromatogram (XIC) peak areas were ratio-corrected by peak areas of the IS. JMP 15.1.0 (SAS Institute, Cary, NC) was used for statistical comparisons using ANOVA followed by Tukey–Kramer multiple comparisons method using a $P < 0.01$ threshold or Student's t test using a significance threshold of $P < 0.05$.

RESULTS AND DISCUSSION

Evaluation of PAA Oxidation in the Liquid Phase.

The study was initiated by determining appropriate conditions for the model Prilezhaev reaction. Cyclohexene was chosen as the alkene precursor since the corresponding epoxide, CHO, is commercially available and because it provided a distinctive signal in the gas chromatogram for subsequent quantification. As outlined in the Methods and Materials section, PAA (249 μg, 3270 nmol) was combined with ACN and cyclohexene (81 mg, 0.99 mmol). Following an organic extraction, the Prilezhaev epoxide product was analyzed via GC–MS, and the results are shown in Figure 1. The chromatogram on the left depicts the product from the reaction in the presence of 3270 nmol of PAA (Figure 1A, blue trace) as well as those of analogous reactions performed with an equivalent amount of HP (Figure 1A, red trace) and AA (Figure 1A, green trace), both of which exist in equilibrium with PAA in solution. A prominent feature was observed at 8.7 min in the chromatogram trace shown in blue, and analysis of the associated mass spectrum revealed a feature at 98 m/z, which is consistent with the molecular ion of CHO, as well as a base peak of 83 m/z that is related to the loss of a neutral methyl radical (Figure 1B). To confirm this assignment, the mass spectrum was compared to that of an analytical CHO standard purchased from Sigma Aldrich (Figure 1C) and was found to be consistent. The identity of CHO was further confirmed by a NIST library search of the experimentally collected spectra.

The chromatograms associated with the HP and AA treatments in Figure 1A also depicted minor CHO peaks at 8.7 min. As such, extensive quantitative experiments were designed to determine if the observed minor CHO peaks were due to HP and AA or were due to background levels derived from the cyclohexene stock solution. ACN solutions were prepared containing 10 equiv (3270 nmol, 111 μg, 1000 ppb in 80 L of air) and 100 equiv (32,700 nmol, 1112 μg, 10,000 ppb in 80 L of air) of HP relative to the quantity of PAA in solution (327 nmol, 25 μg, 100 ppb in 80 L of air). High quantities of HP and AA were chosen to challenge the assay to assure specificity. The different combinations of PAA and HP are listed in Table 1, and all CHO peak area response data are normalized to the negative control (sample A). A minor peak corresponding to CHO was detected from the negative control, indicating that the cyclohexene standard contains a small amount of CHO. The addition of 3270 nmol of HP to cyclohexene had no effect on the amount of CHO observed versus the negative control (sample B vs A). The 32,700 nmol HP treatment, conversely, resulted in a 1.9-fold increase in CHO formation above the baseline amount detected in the negative control, which was statistically significant ($P < 0.01$). Despite this, the response is minor relative to the increase observed in the presence of PAA. Notably, 327 nmol of PAA alone induced a nearly 600-fold response above the baseline amount found in the negative control. More importantly, there was no effect of the 10-fold or 100-fold excess of HP on the ability to accurately quantify PAA, indicated by almost identical responses despite the quantity of HP added (Table 1D–F).

The same experiment was performed using AA, another component present in PAA solutions. As shown in Table 2, 3270 and 32,700 nmol of AA (196 and 1964 μg, respectively) had no effect on the oxidation of cyclohexene in the presence or in the absence of PAA. These data demonstrate that employing the Prilezhaev reaction provides specificity for PAA without the need for additional reagents and could negate the use of a separate sampling cassette, both of which are hallmarks of many other techniques that could introduce complications, such as cross-reactivity and flow rate limitations, into the sampling process.[27,31,43]

In the process of developing the sorbent technique for PAA measurement, it was necessary to determine whether cyclohexene was quantitatively being oxidized to CHO by PAA. Experiments were conducted to compare peak area responses of a CHO standard versus CHO created via oxidation of cyclohexene by PAA. The CHO standard was subjected to the same conditions as the PAA/cyclohexene mixture, which in this case involved the addition of CHO solution to ACN followed by extraction in *n*-hexane containing IS. As shown in Figure 2, the peak area response was similar between the CHO standard and CHO derived from PAA across all concentrations. Percent differences between pairs were 38% (80 nmol), 5% (160 nmol), 10% (330 nmol), 6% (650 nmol), 2% (1310 nmol), 13% (2620 nmol), and 13% (3270 nmol); however, these differences did not reach statistical significance (criterion: $P < 0.05$). These data support the conclusion that the oxidation reaction is complete after the 24 h reaction time and that there is molar equivalence between the amount of PAA added to the reaction and the resultant amount of CHO product.

Assessment of Recovery Using Sorbent.

Two calibration curves were constructed to understand the effect of sorbent on analyte recovery (Figure 3). Calibration curve A (shown in blue) was constructed using the data from the 1 mL liquid-phase experiments containing 6 to 498 μg of added PAA, which is equivalent to 25–2000 ppb of PAA in the gas phase (80 L air). Calibration curve B, shown in red in Figure 3, was constructed through liquid-phase experiments using the same amounts of PAA with sorbent added to better reflect conditions during air sampling. Both calibration curves exhibit a large linear range for calibration, ranging from 6 to 498 μg of PAA and exhibiting R^2 values exceeding 0.99. However, the addition of the sorbent led to a decrease of approximately 27% in the slope compared to the liquid-phase data. Similar experiments were conducted using a standard CHO solution, and in instances where sorbent was added, loss of recovery was also observed compared to CHO samples without sorbent (data not shown). These data indicate that the presence of sorbent material led to reduced recovery of the CHO product.

The loss of recovery due to the sorbent and the extraction process precluded the use of neat standards for calibration in determining recovery. As such, all subsequent calibrations in this work were performed using calibration curve B, wherein PAA standards were added to sorbent, reacted with cyclohexene, and subjected to the extraction procedure, which would be considered a process-matched approach. Using this approach, the recovery of PAA after sampling was determined from fortified samplers prepared as described in the methods. Calibration curve B was utilized to determine the quantity of PAA recovered in the form of CHO product for each of the fortified samplers, and the % recovery was calculated using eq 2 across different concentrations. Recovery values were 94, 70, 84, 93, 101, 98, and 100%, corresponding to 25, 49, 99, 199, 249, 373, and 498 μg of PAA fortified onto the samplers, respectively. Overall, the calculated recovery values using the process-matched approach averaged 91%. Consequently, when combined with loss incurred due to sorbent adsorption and extraction, the total recovery was estimated to be less than 73%, which is below the recommended recovery threshold of 75% for fully validated air sampling methods.[44] Further optimization experiments, such as sequential extractions of PAA from the sorbent, are expected to improve extraction efficiency and sensitivity as well as allow calibration using neat standards. However, the data support that the current approach using process-matched calibration standards appropriately controlled for loss incurred by sorbent adsorption.

$$\%\text{recovery (PAA)} = \frac{\text{measured } \mu\text{g collected from fortified sampler}}{\text{theoretical } \mu\text{g}} \times 100 \tag{2}$$

Using calibration curve B, the limit of detection (LOD) of the current technique was estimated utilizing the following definition: the minimal amount of analyte that provided a response that was three standard deviations greater than the response of a blank sample.[44] Using the average response of three blank fortified sorbent samples (XIC/IS = 1.63×10^{-4}) and the standard deviation of the blank samples (SD = 2.7×10^{-5}), the LOD was estimated to be 14.9 μg of PAA, which corresponded to 60 ppb PAA in 80 L of air. Multiplication of the LOD by 3.3 yielded the estimation of the LOQ at 49 μg or 198 ppb PAA in 80 L of air.

To ensure that the sorbent tubes were able to capture PAA at higher concentrations, the sampling train was modified to include a second sorbent tube (see Methods and Materials). In a series of experiments sampling air containing 2000 ppb of PAA, it was determined that the amount of PAA recovered on the second tube was not significantly different from the blank (Table S1). This indicated that one 350 mg XAD-7 sorbent tube in the sampling train was sufficient to capture gas-phase PAA up to 2000 ppb and that loss to the sampling pump as a result of saturation of the sorbent was negligible.

Chamber Sampling.

A series of experiments were conducted to determine the feasibility of the technique to accurately quantify PAA from the gas phase. PAA atmospheres across six concentrations were prepared, and the PAA was collected onto sorbent tubes using personal sampling pumps over 4 h. Following the Prilezhaev reaction, the product was analyzed by GC–MS. The peak area ratios were applied to calibration curve B, and figures of merit were determined for the PAA atmosphere quantification via the CHO product (Table 3). The bias of each sorbent tube measurement was determined using eq 3.

$$\%\text{bias} = \frac{\text{measured } \mu\text{g} - \text{theoretical } \mu\text{g}}{\text{theoretical } \mu\text{g}} \times 100 \tag{3}$$

By averaging the absolute values of the bias measurements, an overall bias estimate of 10.7% was determined. This value exceeded recommended guidelines of less than 10% for fully validated methods.[44] The precision of the method for 19, 23, 37, 75, and 186 μg was measured as <10% relative standard deviation (RSD) across independent chamber experiments. The 149 μg sample showed slightly higher variability at 16% RSD across independent experiments. This discrepancy is likely related to chamber preparation rather than the sorbent sampling method itself. The observation that the other five test atmospheres exhibited RSD values of less than 10% supports this reasoning.

Storage Effects on Adsorbed PAA.

The effects of storage of the sorbent tubes on the stability of PAA were preliminarily investigated. Atmospheres were created containing 200, 400, and 800 ppb PAA in 80 L of air, which corresponded to 50, 96, and 199 μg of PAA, and chambers were sampled using sorbent tubes as previously described. The sorbent tubes were then subjected to one of three treatments: (1) processed for the Prilezhaev reaction as normal, which consists of a 24 h derivatization of cyclohexene via PAA followed by extraction and analysis by GC–MS, (2) capped and stored for 72 h at room temperature followed by derivatization and processing, or (3) capped and stored for 72 h at −20 °C followed by derivatization and processing. The corrected product peak areas from stored samples were normalized by the corrected product peak areas from corresponding sorbent tubes processed normally (i.e., treatment 1), and data are shown in Figure 4. Compared to samples that were immediately processed following collection, the data from sorbent stored at room temperature for 72 h indicated a loss of PAA. The worst instance was observed for the chamber containing 50 μg of PAA, in which only 12% of PAA was recovered from the sorbent following the 72 h period compared to the control. Storage at −20 °C, in contrast, largely preserved PAA on the sorbent tubes, with an average of 110% of signal maintained across the three concentrations. Additional experiments to determine the maximum threshold of storage time are warranted and will be explored in future studies.

Stability of PAA in Chambers and Variable Sampling Periods.

Repeated sampling experiments were performed to determine the stability of PAA within the chambers. However, size limitations precluded a series of 4 h, 60 L sampling events on the same chamber. To circumvent this issue, stability of chamber sampling was assessed through four 1 h, 15 L repeated measurements, wherein a chamber containing 80 L of PAA-spiked air (800 ppb, 199 μg) was sampled onto four separate 350 mg XAD-7 sorbent tubes in sequence rather than collection on a single tube. Each sorbent tube was processed individually, and the variability between consecutive samples within a given chamber was calculated to be 4.4% RSD, indicating that the PAA atmospheres remained stable across 4 h. The microgram quantity of recovered PAA from each is presented in Figure S1. For both sets of repeated measurements, 1 h sample tubes yielded an average of 39 μg of PAA, which constituted 105% of the theoretical amount expected within the 15 L samples (37 μg). Further, it was shown that the summation of the 1 h sampling series collected similar quantities of PAA compared to the average 4 h sampling experiment ($n = 6$) (Figure S1).

Scalability of the sampling time was also preliminarily assessed through a series of experiments wherein sampling occurred for 15 min at a rate of 1 L/min using chambers containing 80 L of PAA-spiked air (800 ppb, 199 μg), which is reflective of current PAA testing methods.[25–27] The average of four 15 min replicates from three separate chambers is shown in Table 4. Across the three chambers, the amount of PAA recovered differed minimally from the 37 μg anticipated from a 15 L sample at 800 ppb, as the average absolute bias estimate was 7.2%. Further, the low average relative standard deviation of 6.7% indicates that there was little variation in the PAA atmosphere within the chamber during sampling.

The initial assessment of the LOD of 14.9 μg corresponds to 0.32 ppm in 15 L of air, which falls below the current ACGIH STEL of 0.4 ppm. However, it is recognized that additional optimization of sensitivity is necessary to reach the threshold requirement for LOD in validated methods, which is 10% of the exposure limit[45] or 0.04 ppm for the current STEL. Further, analogous experiments with 15 L collection at varying concentrations are necessary in future studies.

SUMMARY AND CONCLUSIONS

Recent full-shift measurements of PAA within hospital environments indicate that workers experienced exposure symptoms in response to low concentrations of PAA in the air during full work shifts. Such findings support the need for additional full-shift TWA measurements to document the impact of longer exposures on workers.[15,16] Current published PAA methods have been developed based on a 15 min sampling period, collecting 15 L of air; however, the performance of these methods with longer sampling periods has not been established.[25–27]

The new technique presented here used sorbent tube sampling to collect PAA from air samples and applied the Prilezhaev epoxidation to indirectly quantify PAA via its reaction with cyclohexene to form CHO. It takes advantage of the specificity of the Prilezhaev epoxidation toward the use of a peroxyacid as the oxidant, is resistant to interferences from

100-fold excesses of HP and AA without additional capture strategies, and is sensitive to PAA to an LOD of 60 ppb in 80 L of air. Further, the technique developed has a large dynamic sampling range, with the highest tested concentration of 2000 ppb posing no issues to the capacity of the sorbent tube used. Preliminary storage experiments indicated that at −20 °C, PAA is stable on a sealed XAD-7 sorbent tube for up to 72 h post collection, which could prove useful in scenarios in which delays in processing may occur. The assessment of various sampling times was also integral to understanding the potential flexibility of the technique with further optimization.

Overall, these results indicate initial feasibility of the current technique in scenarios where 8 h work shift sampling is needed, requiring the use of only two sorbent tubes and, by extension, less interruption of worker activity. In addition to the insights obtained from the current study, further optimization to improve the recovery as well as assessment of field sampling capabilities will allow for further understanding of the health effects of longer periods of exposure to PAA in the workplace.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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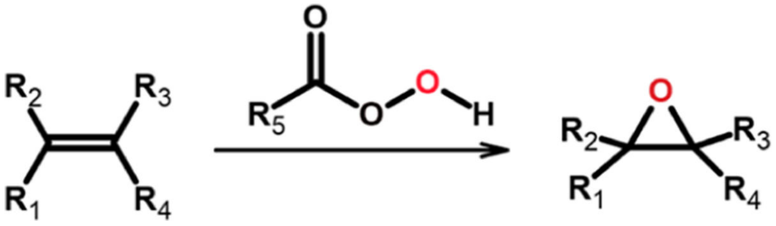

Scheme 1.

The Prilezhaev Reaction for Synthesis of Epoxides from Peracids and Alkenes

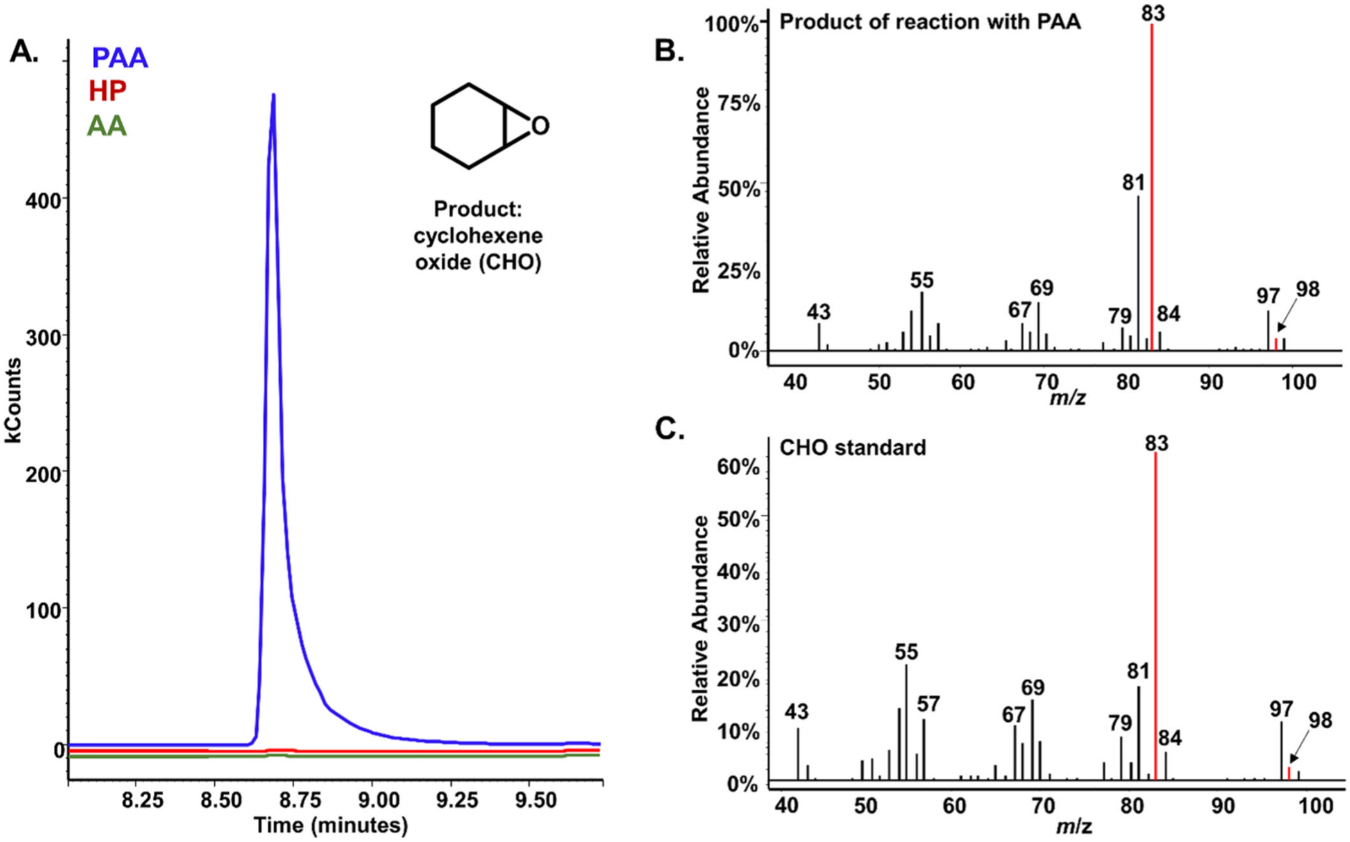

Figure 1.

(A) Product’s 83-ion-extracted chromatograms from the reaction of cyclohexene with PAA (blue trace), HP (red trace), or AA (green trace). [Reactant] = 3270 nmol; equivalent to 1000 ppb in gas phase (in 80 L of air). (B) Mass spectrum at 8.7 min of CHO product. (C) Mass spectrum at 8.7 min of CHO standard solution.

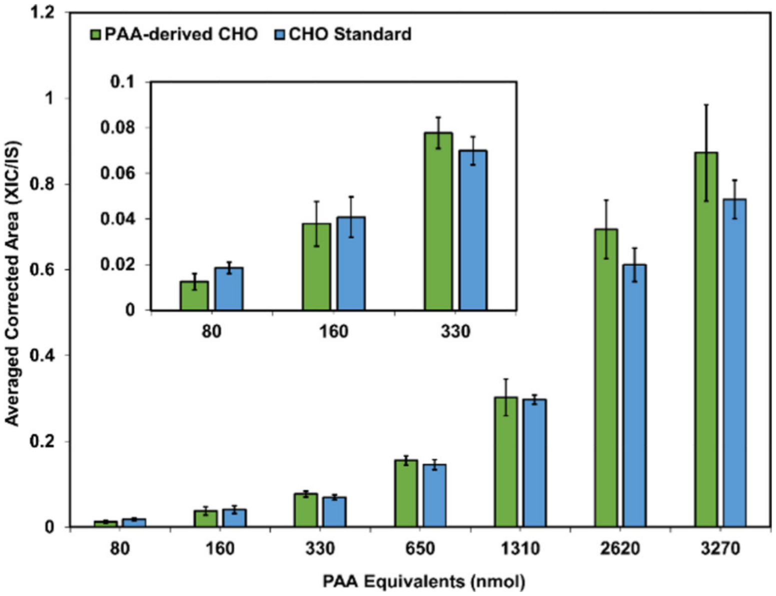

Figure 2.

Comparison of average corrected peak areas of CHO standard and CHO created via oxidation of cyclohexene by PAA. The *y* axis represents the average ratio of the XIC peak area divided by the peak area of the IS across at least three replicates for each treatment. The *x* axis represents the amount of CHO standard (blue bars) or the amount of PAA added to the oxidation reaction (green bars) in nanomoles. Inlay provides magnification of the lower values. Error bars indicate standard deviation. Percent differences between pairs were 38% (80 nmol), 5% (160 nmol), 10% (330 nmol), 6% (650 nmol), 2% (1310 nmol), 13% (2620 nmol), and 13% (3270 nmol).

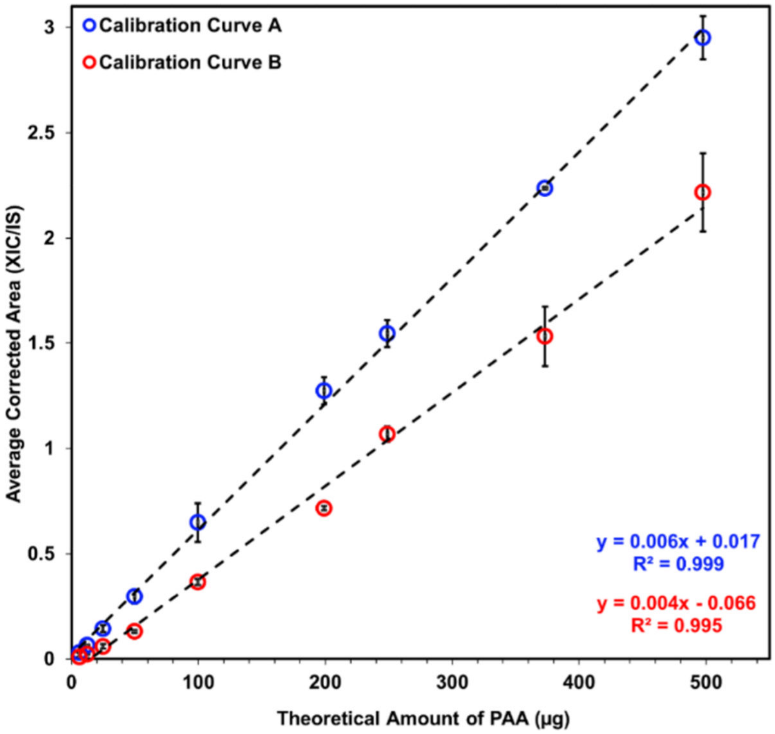

Figure 3.

Calibration curves constructed via (A) liquid-phase experiments (1 mL ACN, 2 *µ*L PAA solution, and 100 *µ*L cyclohexene) and (B) liquid + sorbent experiments (1 mL ACN, 2 *µ*L PAA solution, 100 *µ*L cyclohexene, and 350 mg XAD-7 sorbent). The *y* axis represents the average of XIC area of the product peak divided by the IS peak area across three replicates for each concentration. The *x* axis represents the theoretical amount of PAA (*µ*g) dispersed in 80 L of air. Error bars reflect standard deviation.

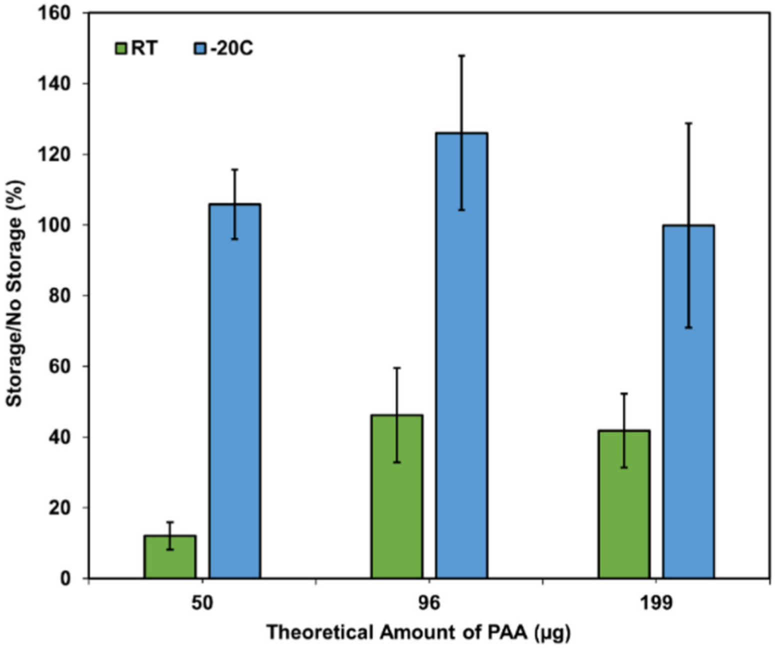

Figure 4.

The effect of post-collection sorbent tube storage at RT or −20 °C for 72 h on PAA atmosphere measurements. The *y* axis is depicted as a percentage of normalized data from stored samples with respect to non-stored samples. The *x* axis represents the amount of PAA in micrograms (*μ*g) introduced into 80 L of air in each storage experiment. Error bars indicate standard deviation from an average of at least three replicates per treatment.

Table 1.

Effect of PAA and HP Combinations on the CHO Peak Area Relative to the Negative Control

sample ID	PAA (nmol)	HP (nmol)	corrected peak area[a]	ratio[b]	SD[c]
A	0	0	2.41×10^{-4}	1.0	
B	0	3270	2.70×10^{-4}	1.1	0.2
C	0	32,700	4.65×10^{-4}	1.9[d]	0.2
D	327	0	0.143	592.6	66.4
E	327	3270	0.144	597.8	22.8
F	327	32,700	0.137	565.8	3.1

[a] Three replicates for each sample treatment were performed.

[b] Ratio of corrected peak area relative to sample A. Statistical comparisons were made within the group containing A, B, C and the group containing D, E, F.

[c] Standard deviation.

[d] Significantly different from samples A and B, $P < 0.01$.

Table 2.

Effect of PAA and AA Combinations on the CHO Peak Area Relative to the Negative Control

sample ID	PAA (nmol)	AA (nmol)	corrected peak area[a]	ratio[b]	SD[c]
A	0	0	2.41×10^{-4}	1.0	
B	0	3270	3.12×10^{-4}	1.3	0.3
C	0	32,700	2.16×10^{-4}	0.9	0.05
D	327	0	0.143	592.6	66.4
E	327	3270	0.144	595.3	27.3
F	327	32,700	0.142	589.2	6.4

[a] Three replicates for each sample treatment were performed.

[b] Ratio of corrected peak area relative to sample A. Statistical comparisons were made within the group containing A, B, C and the group containing D, E, F.

[c] Standard deviation.

Table 3.

Figures of Merit for PAA Atmosphere Collection via the Prilezhaev Sorbent Tube Technique across Various Concentrations

target PAA concentration (ppb)	**target PAA amount (μg)**[a]	**bias (%)**[b]	**precision (% RSD)**[c]	***n***[d]
100	19	19.6	2.8	4
125	23	9.1	1.8	3
200	37	−5.3	9.8	5
400	75	−13.5	6.1	4
800	149	−11.2	16.1	6
1000	186	5.3	9.4	4
	overall estimate[e]	**10.7**	**7.7**	

[a] Theoretical amount of PAA in 60 L of air sampled.

[b] Defined by eq 3.

[c] Relative standard deviation.

[d] Number of replicate experiments.

[e] Determined by averaging the absolute values of all bias values and RSD values, respectively.

Table 4.

The Recovery and Bias of PAA when Sampling for 15 min at 1 L/min

sample ID	**PAA (ppb)**[a]	**corrected peak area**[b]	**% RSD**	**recovered PAA (μg)**[c]	**% bias**
A	800	0.109	6.3	40	7.3
B	800	0.103	6.6	38	3.5
C	800	0.079	7.1	33	−10.9

[a] Concentration in ppb is related to a chamber containing 80 L of air.

[b] Represents an average of peak areas. $n = 4$ for each sample ID.

[c] Represents the number of micrograms of PAA recovered from a 15 L sample calculated using calibration curve B.