Light absorption and scattering properties of indole secondary organic aerosol prepared under various oxidant and relative humidity conditions

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ABSTRACT
Secondary organic aerosol (SOA) from indole was produced in a smog chamber under different relative humidity (RH) and with different oxidants (O3, OH, \(\text{OH}^+\text{NOx}\), and \(\text{NO}_3\)). The mass absorption coefficients were measured by extracting SOA in methanol and taking absorption spectra in the 200-700 nm range. Chromophoric compounds were tentatively identified by liquid chromatography – spectrophotometry – mass spectrometry. One of the strongest chromophores was nitroindole, produced in high abundance by both \(\text{NO}_3\) and \(\text{OH}^+\text{NOx}\) oxidation. Indole SOA was also prepared in an oxidation flow reactor (OFR) using the same set of oxidants under variable RH conditions. Densities of the OFR-generated SOA particles ranged from 1.05 g/cm\(^3\) for indole + O\(_3\) SOA to 1.33 g/cm\(^3\) for indole + OH SOA. The real (\(\rho\)) and imaginary (\(\kappa\)) refractive indices were quantified using a broadband cavity-enhanced spectrometer (BBCES) over the 360–425 nm spectral range. The values of \(\kappa\) ranged from 10\(^{-2}\) to 10\(^{-1}\) at 405 nm, which is comparable to those for humic-like substances and at least an order of magnitude larger than \(\kappa\) values of SOA produced from other biogenic and anthropogenic volatile organic compounds. The \(\kappa\) values for the indole + \(\text{NO}_3\) SOA had a strong and non-monotonic dependence on RH, suggesting two competing reaction mechanisms for the chromophores, highlighting the complex effect of water on the absorption coefficient of SOA from heterocyclic aromatic precursors. Strong absorption of indole SOA may contribute to the degradation of visibility near its emission sources, which include animal husbandry facilities and stressed plants.

1. Introduction
Atmospheric particulate matter absorbs and scatters incoming solar radiation, which has important implications for air quality, visibility and climate change (Chen 2021). Secondary organic aerosol (SOA) accounts for a considerable fraction of airborne particulate matter (Hodzic et al. 2016) and is largely formed through the oxidation of biogenic and anthropogenic volatile organic compounds (VOCs), followed by gas-particle partitioning of the low volatility oxidation products (Hallquist et al. 2009). Aqueous phase processing of organic compounds can also add to the SOA burden in the atmosphere, where watersoluble volatiles are taken up into the particle phase and are then oxidized to form low-volatility products (Herrmann et al. 2015).

Brown carbon (BrC) is defined as the aerosol organic matter that strongly absorbs solar radiation across the near-UV (300-400 nm) and visible ranges (Andreae and Gelencser 2006; Laskin, Laskin, and Nizkorodov 2015; Moise, Flores, and Rudich 2015; Saleh 2020). While BrC is not as light-absorbing as black carbon, there is enough BrC in the atmosphere to measurably reduce the single scattering albedo and contribute to the radiative forcing of climate by aerosols. Examples of BrC include primary organic aerosol (POA) produced by biomass burning (Hettiyadura et al. 2021; Lack et al. 2013; Li et al. 2020a; Li et al. 2020b; Samburova et al. 2016) and SOA generated by photooxidation of polycyclic aromatic compounds (He et al. 2022; Lin et al. 2015; Liu et al. 2015; Siemens et al. 2022). It is important to identify major BrC sources, both primary and secondary, to more accurately predict the effect of BrC on visibility and climate.
Indole is a heterocyclic nitrogen-containing VOC emitted by certain plants during stress and flowering events when its emissions become substantial. For example, in the San Joaquin Valley, an agricultural region in California, indole was measured at levels as high as 4.7–18 μg m⁻³, which was comparable to or greater than concentrations of myrcene, a dominant monoterpene (Gentner et al. 2014). Once emitted, indole plays an important role in plant ecology, for example, by attracting pollinators (Zito, Dotterl, and Sajeva 2015). Another important source of indole is the microbial processing of L-tryptophan in the intestines or feces of livestock animals (Le et al. 2005). The microbial processing of L-tryptophan has been incorporated into a model to simulate its effects on the air quality in the South Coast Air Basin (Aguilera et al. 2017). The indole-derived BrC is not likely to contribute to visibility reduction in remote areas with high-NOx conditions, 200 ppb NO is expected to convert to NO₂ but still contribute to BC extinction could not be modeled because the light absorption properties of this SOA were unknown at the time. The first objective of this work is to study indole oxidation by the most important atmospheric oxidants (OH in the absence and presence of NOₓ, O₃ and NO₃), thus providing valuable data for modeling the effects of indole on climate forcing and visibility.

The second objective of this work is to explore the effects of relative humidity (RH) on the BrC properties of indole SOA. The absorption coefficient of organic aerosols appears to be quite sensitive to their chemical composition because it is controlled by a handful of chromophores (Laskin, Laskin, and Nizkorodov 2015). Recent studies have demonstrated that the absorption coefficient of SOA produced from aromatic (Cui et al. 2021) and heterocyclic aromatic compounds is RH-dependent (Kasthuriaacheti et al. 2020). A strong effect of RH was recently reported for optical properties of toluene SOA produced under high-NOₓ conditions (Mitra et al. 2022). In this study, we report a complex effect of RH on the absorption coefficient of indole SOA, suggesting at least two distinct competing mechanisms for BrC production from indole.

2. Methods and materials

2.1. Smog chamber SOA generation

Indole SOA was generated in a 5 m³ Teflon chamber at different levels of RH (<2%, 25%, 50%), which was measured by a HMT333 probe (Vaisala Inc., Finland). No inorganic seed aerosol was used to avoid interference with the off-line mass-spectrometric analysis of SOA. Indole (99% purity, Sigma-Aldrich) was dissolved in methanol (LC-MS grade, 99.9% purity, Honeywell) and evaporated into the chamber to obtain an initial mixing ratio of 200 ppb, which is equivalent to 960 µg m⁻³. The injector and inlet lines were heated to 70 ℃ to minimize losses of indole on the injector surfaces. While 200 ppb of indole was used in most experiments to collect sufficient mass for analysis, several experiments were done at lower mixing ratios (50 ppb and 100 ppb) to test the dependence of indole SOA optical properties on the starting indole mixing ratios (see Figure S1). In the nitrate radical experiments, NO₃ was generated in the chamber by introducing O₃ and NO in a small excess of the 2:1 ratio (500 ppb O₃ and 200 ppb NO). Under these conditions, 200 ppb NO is expected to convert to ~200 ppb NO₃ (a fast conversion of NO to NO₂ followed by slower conversion of NO₂ to NO₃ but still only taking a few seconds), with ~100 ppb of residual ozone. Because NO₃ is much more reactive toward indole than O₃, with rate constants of 1.3 × 10⁻¹⁰ cm³ molec⁻¹ s⁻¹ for indole+NO₃ compared to 5 × 10⁻¹⁷ cm³ molec⁻¹ s⁻¹ for indole + O₃ (Atkinson et al. 1992).
et al. 1995), indole primarily reacts with NO₃ under these conditions. The ozonolysis experiments were conducted in the presence of 1 ppm O₃. In the OH photooxidation experiments, hydrogen peroxide was introduced into the chamber by evaporation of a 30 wt% solution of H₂O₂ in water (Fisher Scientific) into a clear air flow to achieve an initial mixing ratio of 2 ppm. UV-B lamps (FS40T12/UVB, Solarc Systems, Inc.) were turned on to initiate the photooxidation, which led to [OH] concentration of \( \sim 1.4 \times 10^6 \) molecules cm\(^{-3}\). Table 1 summarizes SOA preparation conditions for the chamber experiments.

Throughout the experiment, the particle size distribution was monitored with a scanning mobility particle sizer (SMPS; TSI 3936) equipped with a condensation particle counter (CPC; TSI 3775). A particle sizer (SMPS; TSI 3936) equipped with a condensation particle counter (CPC; TSI 3775). A proton-transfer-reaction time-of-flight mass spectrometer (PTR-ToF-MS; Ionicon model 8000) monitored the decay of indole, as well as the formation of volatile products. The PTR-ToF-MS had a resolving power \( m/Δm = 5 \times 10^6 \) and was operated with the following settings: drift tube temperature of 60°C, drift tube voltage of 600 V, normalized electric field strength of \( E/N \sim 135 \) Td \( (1.35 \times 10^{-15} \text{ V cm}^{-2}) \), and inlet flow of 0.2 SLM. When SOA reached a peak concentration in the chamber, UV irradiation was stopped, and the chamber was allowed to cool for 2 h. One filter was collected per chamber run. Replicate samples were collected from separate experiments to confirm the results. The amount of the collected SOA material on each filter was determined gravimetrically. The SOA compounds, permitting the calculation of the solution mass concentration, \( C_{\text{mass}} \) (g cm\(^{-3}\)) from gravimetric measurements. The SOA methanol extract was analyzed by ultraviolet-visible (UV-Vis) spectrophotometry using a dual beam Shimadzu UV-2450 instrument, with pure methanol used as reference.

### 2.2. SOA MAC and k values

The weighted filters were extracted in 5 mL of methanol (LC-MS grade, 99.9% purity, Honeywell) and shaken on a shaker for 5 min. The filter color changed from colored to white suggesting most of the light-absorbing compounds were extracted. Furthermore, the UV absorbance of the extract after the first extraction was at least a factor of 20 larger than that after the second extraction (Figure S3). Since we can reasonably expect that all indole oxidation products that retain one or both aromatic rings must have strong \( \pi \rightarrow \pi^* \) absorption bands in the UV range, we can conclude that the initial extraction dissolved >95% of the SOA compounds, permitting the calculation of the solution mass concentration, \( C_{\text{mass}} \) (g cm\(^{-3}\)) from gravimetric measurements. The SOA methanol extract was analyzed by ultraviolet-visible (UV-Vis) spectrophotometry using a dual beam Shimadzu UV-2450 instrument, with pure methanol used as reference.

Mass absorption coefficients (MAC) of the SOA material were calculated as a function of wavelength \( (\lambda) \) from the measured base-10 absorbance, \( A_{10}^{\text{solution}}(\lambda) \), the path length, \( b = 1\text{ cm} \), and \( C_{\text{mass}} \):

\[
MAC(\lambda) = \frac{A_{10}^{\text{solution}}(\lambda) \times \ln(10)}{b \times C_{\text{mass}}} \quad (1)
\]

The imaginary part of the refractive index, \( k \), which describes the absorptive properties of the particle material was calculated from MAC values using Equation (2).

### Table 1. SOA production conditions in both the smog chamber and the oxidation flow reactor (OFR). The starting mixing ratios and estimated OH concentrations are listed.

<table>
<thead>
<tr>
<th>SOA Production Method</th>
<th>Oxidant</th>
<th>Indole (ppb)</th>
<th>NO (ppb)</th>
<th>NO₃ (ppb)</th>
<th>O₃ (ppb)</th>
<th>OH (molec cm(^{-3}))</th>
<th>( N_2O_5 ) (ppb)</th>
<th>RH values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber</td>
<td>OH</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>( 1.4 \times 10^6 )</td>
<td>–</td>
<td>&lt;2%, 25%, 50%</td>
</tr>
<tr>
<td>Chamber</td>
<td>NO₃</td>
<td>200</td>
<td>200</td>
<td>–</td>
<td>500</td>
<td>–</td>
<td>–</td>
<td>&lt;2%, 25%, 50%</td>
</tr>
<tr>
<td>Chamber</td>
<td>O₃</td>
<td>200</td>
<td>–</td>
<td>1000</td>
<td>–</td>
<td>( 1.4 \times 10^6 )</td>
<td>–</td>
<td>&lt;2%, 25%, 50%</td>
</tr>
<tr>
<td>Chamber</td>
<td>OH + NO₃</td>
<td>200</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>( 2.3 \times 10^9 )</td>
<td>–</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>OFR</td>
<td>OH</td>
<td>200</td>
<td>–</td>
<td>130</td>
<td>–</td>
<td>( 2.3 \times 10^9 )</td>
<td>–</td>
<td>38%</td>
</tr>
<tr>
<td>OFR</td>
<td>OH + NO₃</td>
<td>200</td>
<td>–</td>
<td>670</td>
<td>–</td>
<td>( 2.3 \times 10^9 )</td>
<td>–</td>
<td>38%</td>
</tr>
<tr>
<td>OFR</td>
<td>OH + NO₃</td>
<td>200</td>
<td>–</td>
<td>2400</td>
<td>–</td>
<td>( 1.7 \times 10^9 )</td>
<td>–</td>
<td>38%</td>
</tr>
<tr>
<td>OFR</td>
<td>O₃</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>20000</td>
<td>–</td>
<td>–</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>OFR</td>
<td>NO₃</td>
<td>200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1000</td>
<td>–</td>
<td>&lt;2%, 25%, 51%, 76%</td>
</tr>
</tbody>
</table>
We used the average measured effective density of $\rho = 1.21 \text{ g cm}^{-3}$, as discussed below. The estimated uncertainty in $\text{MAC}$ is limited by the uncertainty of gravimetric measurements and completeness of extraction, and is likely to be below 20%. The uncertainty in $k$ is somewhat higher because of the use of average density instead of the actual density (which was not measured for the smog-chamber generated SOA), but it is likely to be below 25%.

### 2.3. Chromophore identification

Additional SOA sample filters were collected for high performance liquid chromatography – photo diode array spectrophotometry – high resolution mass spectrometry (HPLC-PDA-HRMS) measurements as described previously (Montoya-Aguilera et al. 2017), for which one-quarter of the filter was extracted using 350 $\mu$L acetonitrile (CH$_3$CN, gradient grade, $\geq$99.9% purity). The completeness of extraction of light-absorbing compounds was confirmed by visual inspection of the filter, which returned to the white color of a clean filter after the extraction. The first set of experiments used a Scherzo SM-C$_{18}$ column (Intakt USA) for HPLC separation (referred to as Column A). Solvents A and B used during liquid chromatography were HPLC grade acetonitrile and water, respectively. The gradient elution protocol included a 3 min hold at 10% solvent A, a 45 min linear gradient to 80% solvent A, a 16 min hold at this level, a 1 min return to 10% solvent A, and another hold until the total scan time of 90 min. The column was maintained at 25°C. The UV–Vis spectra of the eluted fractions of SOA were measured with a PDA detector from 250 to 700 nm. The electrospray ionization (ESI) settings in the positive ionization mode were $+4.5 \text{kV}$ spray potential, 35 units of sheath gas flow, 8 units of auxiliary gas flow, and 5 units of sweep gas flow. The Thermo LTQ Orbitrap high resolution mass spectrometer was operated at a mass resolving power of $\approx 10^5$ at $m/z$ 200. The HPLC-PDA-HRMS analysis was done as described in (Lin et al. 2016; Montoya-Aguilera et al. 2017).

An additional set of HPLC-PDA-HRMS experiments was performed two years later (the delay was due to the COVID-19 disruptions of 2020–21) using a different instrument configuration. These experiments

\[
k(\lambda) = \frac{\text{MAC}(\lambda) \times \rho \times \lambda}{4\pi}
\]
utilized ultra-performance liquid chromatography coupled with a Thermo Q-Exactive Plus Orbitrap mass spectrometer equipped with an ESI source. Filters were extracted using a solvent consisting of 1:1 (v/v) mixture of acetonitrile (Sigma Aldrich, purity ≥ 99.9%) and water (Sigma-Aldrich, HPLC grade). A Luna 1.6 μm Omega Polar C18 (150 × 2.1 mm) column was used for separation (referred to as Column B). Solvents A and B, which were used for mobile phase, were HPLC-grade acetonitrile (with 0.1% formic acid) and water (with 0.1% formic acid), respectively. The gradient elution protocol included a 3 min hold at 5% solvent A, an 11 min linear gradient to 95% solvent A, a 2 min hold at this level, and a linear gradient back to 5% solvent A in preparation for the next run. The column was maintained at 30°C. The UV–vis spectra were measured with a PDA detector from 250 to 700 nm. The ESI settings in the positive ionization mode were +4.0 kV spray potential, 35 units of sheath gas flow, 10 units of auxiliary gas flow, and 8 units of sweep gas flow. The instrument was operated in the positive ion mode with a mass resolving power of 105 at m/z 200. Data analysis was conducted using MZmine 2.53 software package (Pluskal et al. 2010). Molecular formulas were assigned to detected signals ([M + H]+) satisfying the following constraints: C1-40H0-80O0-35N0-5 with a mass tolerance of 0.001 Da; element count heuristics 0.3 H/C 3; N/C 4, and O/C 3.

**2.4. Oxidation flow reactor experiments**

Oxidation flow reactor (OFR) experiments were conducted for direct measurements of the optical properties of airborne indole SOA. SOA was generated at four different OFRs settings to simulate indole oxidation initiated by OH radical (with and without NOx), O3, and NO3 radical. No seed aerosol was used in the OFR experiments. The experimental system is described in detail in previous publications (He et al. 2018; He et al. 2021). The OFR conditions used are summarized in Table 1.

Briefly, a potential aerosol mass (PAM) OFR was used for OH-initiated oxidation. Pure indole (99% purity, Sigma-Aldrich) was placed in a bubbler and was introduced into the PAM by a gentle N2 flow to obtain an estimated initial mixing ratio of 200 ppb. For the SOA generation through OH oxidation, a total flow of 4.2 L min⁻¹ of N2 and 0.3 L min⁻¹ O2 at RH of 36–38% was used in the PAM with a corresponding residence time of 184 s. The OH concentration was controlled by changing the UV light intensity. To
simulate SOA formation in the presence of NOx, 1%, 2%, and 4% N2O were added to the PAM chamber as a NOx source (Lambe et al. 2017), with corresponding NOx concentrations of 0.13, 0.67, and 2.4 ppm measured at the exit of the OFR (Model T200, Teledyne, USA). Most of the measured NOx is expected to be present in the OFR in the form of NO2 but some NO3 can be produced under OFR conditions, complicating the interpretation of our data. Using a simplified box model including the rate constants for the indole + OH, NO3, and O3 reactions, we estimated the average NO/NO2 ratios of 0.0077, 0.011, and 0.0096, and the average OH/NO3 ratios of 0.10, 0.078, 0.059 at the 1%, 2%, and 4% N2O cases, respectively. However, the model predicts that most of the indole is oxidized within seconds of being added to the reactor, when the chemistry is still dominated by OH + NO/NO2, and not by NO3 oxidation. The OH exposures were regulated to \( \sim 4.2 \times 10^{11} \text{ molecules cm}^{-3} \text{ s} \), which were determined by measuring the decay of the SO2 (Model 49i, Thermo Fisher Scientific, USA). Due to the limitation of the lamp power, the OH exposure was limited to \( \sim 3.1 \times 10^{11} \text{ molecules cm}^{-3} \text{ s} \) for the experiments conducted with 4% N2O.

SOA generation from ozonolysis of indole under dry (<2% RH) conditions was performed in a glass bulb OFR (20 L in volume) with an initial O3 concentration of 20 ppm. A high O3 concentration was used due to the low reactivity of indole toward ozone and a short residence time in the glass reactor (~20 min at the total flow of 1.0 L min\(^{-1}\)). To simulate NO3 oxidation of indole, N2O5, synthesized ahead of time from the NO2 reaction with ozone, was used as the source of the NO3 radical in the same glass bulb OFR (Model 49i, Thermo Fisher Scientific, USA). The RH in the OFR was set to 60% to examine the effect of RH on aerosol optical properties.

The chemical composition of the OFR-generated SOA particles was probed online with a Time-of-Flight Aerosol Mass Spectrometer (ToF-AMS), and the data was analyzed using SQUIRREL 1.62 A software with PIKA 1.22 A. For all the experiments, the particle size distribution was monitored by the SMPS.

### 3. Results and discussion

#### 3.1. Particle densities and elemental ratios

Particle densities are necessary for calculating the imaginary refractive index of the SOA material from solution absorbance. Assuming that the SOA particles are spherical and non-porous, the particle effective densities can be calculated by comparing the aerodynamic diameter \( (d_{\text{aero}}, \text{obtained from the AAC}) \) and the mobility diameter (measured by SMPS):

\[
\rho_{\text{eff}} = \frac{d_{\text{aero}}}{d_{m}} \times \rho_0 \tag{3}
\]

The resulting densities of SOA particles produced in the OFR varied from 1.05 g/cm\(^3\) for O3 oxidation to 1.33 for low-NOx OH oxidation (Table S1). This is comparable to densities of known indole oxidation products: isatin (1.47 g/cm\(^3\)), anthranilic acid (1.40 g/cm\(^3\)), indigo dye (1.20 g/cm\(^3\)), isatoic anhydride (1.52 g/cm\(^3\)), and 3-oxindole (1.20 g/cm\(^3\)). Our previous study of indole + OH SOA (Montoya-Aguilera et al. 2017) assumed a higher density of 1.4 g/cm\(^3\), which means we slightly underestimated the yield of indole SOA and overestimated the MAC values reported in that work. In view of the relatively narrow spread of the densities measured for OFR SOA (Table S1), we elected to use the average of the measured effective densities (1.21 g/cm\(^3\)) to calculate \( k \) from
MAC of chamber-generated SOA using Equation (2). The uncertainty in MAC and k introduced by using this assumption is below 15%.

The elemental ratios of SOA particles produced in the OFR are listed in Table S1. For the indole + O₃ SOA, the N/C ratio of 0.112 is comparable to the N/C ratio in the indole itself (C₈H₇N, N/C = 0.125) suggesting that the products are likely dominated by the functionalized indole rather than its decomposition products. The indole + NO₃ SOA have slightly higher ratios ranging from 0.114 to 0.119, which is consistent with the expected nitration of the indole ring in some of the products. The O/C ratio of ~0.5 for the indole + NO₃ SOA, is higher than that expected for nitroindole (C₈H₆N₂O₂, O/C = 0.25), suggesting that the products must contain additional oxygen-containing functional groups, such as hydroxyl or carbonyl. The indole + OH + NOₓ SOA has the strongest dependence of the elemental ratios on the reaction conditions: the O/C ratios decrease from ~1 to ~0.7, and N/C ratios increase from 0.109 to 0.130 as the fraction of N₂O in the OFR increases from 1% to 4%. This points to a rather significant change in oxidation regime with the increased NOₓ level. In the absence of the detailed mechanism of indole oxidation, we have not been able to model this behavior, but it is likely that high concentrations of NO₂ leads to direct reactions between OH-indole adducts (commonly formed in the OH oxidation of aromatic compounds) with NO₂ instead of oxygen.

### 3.2. Dependence of the mass-absorption coefficient on the oxidant and relative humidity

Figure 1 shows the MAC(λ) values of indole SOA samples prepared under different oxidation and RH conditions. Indole itself has several absorption bands between 200–290 nm, but its absorption coefficient is negligible in the near-UV and visible spectra range (Friedel and Orchin 1951). In contrast, the MAC(λ) spectra for indole SOA prepared under all smog chamber conditions extend into the visible range, similar to absorption spectra of common brown carbon aerosols (Laskin, Laskin, and Nizkorodov 2015). However, there are striking differences between the spectra. For example, the peak at 350 nm (Figure 1d) is a prominent feature in the indole + NO₃ SOA spectrum. Based on chemical analysis (see below), this peak is attributed to nitroindole (C₈H₆N₂O₂), which is a significant finding because only a single nitration step is required to produce this strong near-UV absorber from indole. The 350 nm peak is also observable in the indole + OH + NOₓ SOA (Figure 1b) but absent for the indole + O₃ (Figure 1c) and indole + OH SOA (Figure 1a) systems, which is fully consistent with its assignment to nitroindole. Another striking difference is the significant abundance of indigo dye distinguished by its characteristic absorption band at 600 nm (Weinstein and Wyman 1956) in the indole + NO₃ SOA prepared under <2% or 25% RH. The indigo dye peak is the strongest under dry conditions but becomes much weaker at 50% RH. It is also present in indole SOA prepared by OH and O₃ oxidation, but it is smaller in abundance than in the indole + NO₃ SOA.

The MAC values from Figure 1 were used to calculate the imaginary refractive index values using Equation (2) for chamber-generated SOA (Figures 2–4a) and compared to the k values obtained using BBCES for OFR-generated SOA (Figures 2–4b). We do not expect the k values between the two measurement methods to be the same because of different SOA formation conditions. For example, the indole + OH particles probed in the BBCES setup are more oxidized (i.e., aged) than in the smog chamber,
with \(3.1 \times 10^{11} - 4.2 \times 10^{11}\) molecules cm\(^{-3}\) s and \(1.5 \times 10^{10}\) molecules cm\(^{-3}\) s, respectively. This difference in oxidation likely corresponds to differences in chemical composition and chromophores present. The differences in the measurement approach (BBCES vs. UV-Vis) may also contribute to the differences in \(k\) values. BBCES measures optical properties of the actual SOA material in aerosolized particles, whereas the UV-Vis method measures a spectrum from dissolved SOA material. For example, strong solvatochromic effects were observed in the indigo dye spectra with large peak shifts between the spectra recorded in solution and in the solid phase (Weinstein and Wyman 1956). Nonetheless, the \(k\) values obtained from the two independent methods are of the same order of magnitude, suggesting the effects of oxidation and SOA preparation methods on the \(k\) values and the solvatochromic shifts are relatively small in this case.

The MAC values can also be used to calculate the Absorption Ångström Exponent (AAE), an empirical parameter used to describe the wavelength dependence of the absorption coefficient. The resulting values of AAE ranged from 4.9 to 6.5 (Figure S4). (Saleh 2020) classified the strength of absorption by BrC based on the values of \(w = AAE-1\) and \(k\) at 550 nm (at the peak of the solar spectrum). Based on the magnitude of the \(k\) values, indole SOA falls in between “very weakly absorptive BrC” (VW-BrC) and “weakly absorptive BrC” (W-BrC). However, the AAE values of indole SOA are lower than those typically observed for W-BrC (5-8) (Saleh 2020) (Figure S5), making its absorption spectrum overlap better with the solar spectrum.

Indole + OH SOA \(k\) values increase with increasing RH (Figure 2a) and with increasing amounts of NO\(_x\) (Figure 2b and c). The RH dependence of \(k\) for indole + O\(_3\) SOA (Figure 3) has the same trend as the photooxidation samples (Figure 2): the \(k\) values generally increase with increasing RH.

Unlike SOA prepared by photooxidation or ozonolysis, the \(k\) values for indole + NO\(_3\) SOA do not have a monotonic dependence on RH (Figure 4), both in smog chamber and OFR data. Instead, the \(k\) values for indole + NO\(_3\) SOA are highest for SOA prepared under dry conditions (<2% RH), then decrease at 25% RH, and subsequently increase at 50% RH, a trend captured by both experimental methods (Figures 4a–c). This non-monotonic RH dependence can also be seen when considering the RH dependence of individual chromophores that form in SOA under different conditions, as discussed below. We note that a similar non-monotonic dependence of absorption coefficient on RH was recently reported for toluene + OH/NO\(_x\) SOA (Mitra et al. 2022).

Overall, the \(k\) values reported here are remarkably high, even at visible wavelengths. For example, at 405 nm, which is a common wavelength in aerosol optical instruments, indole + OH SOA \(k\) values range between 0.03 and 0.05 for low-NO\(_x\) and higher NO\(_x\) conditions, with the measured largest value of 0.12 in the OFR measurements. Indole + O\(_3\) SOA \(k\) values at 405 nm range from 0.02 – 0.05, while indole + NO\(_3\) SOA \(k\) values range from 0.04 – 0.08. This is comparable to \(k\) values observed for products of reaction of NO\(_3\) with non-soluble tar balls (Li et al. 2020b). For reference, at 405 nm, x-pinene + OH (biogenic SOA), naphthalene + OH + NO\(_x\) (anthropogenic SOA), and guaiacol + OH (biomass burning SOA) \(k\) values are orders of magnitude smaller, reported to be between 0.0002 – 0.001, 0.001 – 0.018, and 0.0005-0.001, respectively (He et al. 2022; Lambe et al. 2013; Moise, Flores, and Rudich 2015). On the other hand, \(k\) values measured in this work are comparable with strongly absorbing biomass burning OA reported in our earlier study (Li et al. 2020b). This makes indole SOA a uniquely efficient type of BrC compared to SOA produced from other precursors. In fact, indole SOA \(k\) values are most similar to those from highly absorbing Humic-like substances (HULIS) such as Suwannee River Fulvic Acid (SRFA) with values between 0.036 – 0.056 (Flores et al. 2014). Furthermore, other biogenic SOA formed from nitrate radical oxidation (isoprene, x-humulene, \(\beta\)-caryophyllene, etc.) have been found to have a negligible \(k\) values at 405 nm (He et al. 2021), highlighting the uniquely efficient light absorption of indole + NO\(_3\) SOA.

### 3.3. Chromophoric compounds in SOA

HPLC-PDA-HRMS analysis was used to identify individual chromophores in the indole + O\(_3\) and indole + NO\(_3\) SOA samples, extending our previous study of the indole + OH SOA system (Montoya-Aguilera et al. 2017). These experiments were not done for the indole + OH + NO\(_x\) SOA. In the case of indole + NO\(_3\) SOA, which had a non-monotonic dependence of MAC on RH, this analysis was done for SOA produced under dry (<2% RH), 25%, and 50% RH conditions. A representative HPLC-PDA chromatogram is shown in Figure 5. Table S2 lists the individual chromophores and their proposed structures based on elemental formulas obtained from the HRMS analysis and reference UV-Vis absorption
Altogether, 28 individual compounds can be tentatively identified, including 6 monomers, 20 dimers, and 2 trimers. Monomeric compounds are especially relevant to this discussion, as the dimers and trimers are much less likely to form under lower, atmospherically relevant concentrations of indole in the atmosphere. A noteworthy finding is that the mass absorption coefficients of indole $+$ NO$_3$ SOA are relatively insensitive to the starting indole mixing ratio (Figure S1), which may suggest that the dimers are produced by particle-phase chemistry as opposed to gas-phase chemistry.

Three chromophores (tryptanthrin, indigo dye, and indirubin) are observed in all three SOA types, and seven additional chromophores are shared by two SOA types. Indigo dye, while not unique to the indole $+$ NO$_3$ SOA, is the chromophore contributing to the prominent peak at 600 nm in the indole $+$ NO$_3$ SOA absorbance spectrum (Figure 1d).

As already mentioned, the $k$ values for indole $+$ NO$_3$ SOA exhibit a non-monotonic dependence on RH. To examine this further, the most abundant compounds absorbing between 350 $-$ 450 nm were identified by examining the correlation between chromatographic peaks detected by both PDA and mass spectroscopy and the normalized intensities of that peak (Figure 6). It is important to note that this set of experiments was carried out later in the project using a shorter chromatography column (Column B), and, thus, the retention times of the identified compounds do not overlap with...
those listed in Table S2. However, the order in which they elute is the same.

While the abundances of some of the detected chromophores, such as tryptanthrin (C_{15}H_{10}N_{2}O_{2}), exhibit a monotonic dependence on RH, some of them follow a non-monotonic trend similar to the $k$ values. For example, nitroindole (C_{8}H_{6}N_{2}O_{2}) abundance in indole$^{+}$NO_{3} SOA decreases when RH changes from <2% to 25% but increases again when at 50% RH. Nitroindole is most likely responsible for the non-monotonic $k$ value dependence, as it is the most abundant compound in indole$^{+}$NO_{3} SOA under all RH conditions, accounting for 20% of the total ion abundance. A chromophore that is unique to indole$^{+}$NO_{3} SOA (see Table S2), with an assigned chemical formula of C_{15}H_{10}NO, also follows this RH-dependence trend. Other chromophores follow the opposite trend with abundance increasing from <2% to 25% RH, then decreasing from 25% RH to 50% RH, e.g., for indoxyl red (C_{16}H_{10}N_{2}O).

The ToF-AMS results from the OFR experiments for the indole$^{+}$NO_{3} SOA also exhibited non-monotonic trends of various molecular fragments as a function of RH (Figure S6). The AMS chemical families that showed a similar RH dependence to the measured $k$ values were the C_{6}H_{10}O^{+} and C_{7}H_{12}O_{2}^{+} (Figure S6). In contrast, the NO$^{+}$ and C_{x}H_{y}O$^{+}$ families followed the opposite RH dependence to the $k$ values. Based on AMS data, the NO$^{+}$ to NO_{2}^{+} ratios for indole$^{+}$NO_{3} SOA generated at <2%, 25%, 51%, and 76% RH were 4.3, 5.9, 5.6, and 6.9 respectively. While the NO$^{+}$ to NO_{2}^{+} ratios have not been systematically measured for heterocyclic compounds, they are similar to the values reported for organonitrates (~3–5), and higher than values reported for ammonium nitrate (~1.5–2.4) (Bruns et al. 2010; Farmer et al. 2010). Therefore, we do not think these results are affected by the presence of inorganic nitrate in the particles. While we do not have an explanation for the complex RH dependence of AMS peak abundances, the data suggest that at least two competing mechanisms operate, with one favoring dry conditions and the other favoring humidified conditions.

4. Conclusions and implications

This study has examined the light-absorbing properties of indole SOA formed through ozonolysis, photochemical OH oxidation in the presence and absence of NOx, and dark nitrate radical oxidation. In all cases, the SOA contains strong chromophores, resulting in high imaginary refractive index ($k$ ranging from $10^{-2}$ to $10^{-1}$ at 405 nm) values comparable to those for humic-like substances and at least an order of magnitude larger than $k$ values of SOA produced from other biogenic and anthropogenic sources. This means that in areas with elevated indole emissions, such as downwind of animal husbandry facilities, this
type of SOA can contribute to visibility degradation. It will be of interest to test this assumption with modeling studies of strong agricultural emissions using the data obtained in this work. It would also be of interest to directly observe indole oxidation products under these conditions. To aid in detection, Figure S7 provides full ToF-AMS spectra of OFR-generated indole + NO₃ SOA.

This is one of very few studies that examine the effect of RH on the optical properties of SOA. Both gas-phase and particle-phase processes leading to SOA formation, and aging can be affected by the presence of water vapor. Gaseous water molecules are known to react with Criegee intermediates formed in ozonolysis (Chao et al. 2015; Neeb et al. 1997), with a strong effect on particle nucleation and chemical composition (Bonn, Schuster, and Moortgat 2002; Zhao et al. 2015). Water molecules partitioned into aerosol particles can slowly hydrolyze organonitrates formed during NO₃ and OH/NO₃ oxidation (Bean and Hildebrandt Ruiz 2016; Liu et al. 2012; Takeuchi and Ng 2019) and ester hydroperoxides produced from reactions of Criegee intermediates with organic acids (Zhao et al. 2018). RH can also have an indirect effect on SOA formation chemistry through its effect on particle viscosity, which strongly affects the size distribution and composition of SOA particles (Reid et al. 2018; Shiraiwa et al. 2013). Finally, the particle acidity is strongly RH-dependent, which could affect the chemistry of products derived from indole due to the presence of a basic -N(H)-group in these molecules. It is not clear whether the complex RH dependence for the absorption coefficient of indole + NO₃ SOA is driven by gas-phase or particle-phase processes. This represents an important gap in our understanding of these complex RH effects as the wall losses of gases in smog chambers strongly depend on RH (Huang et al. 2018). We hope that this work will prompt further studies into the RH-dependent mechanism of nitration of atmospherically-relevant aromatic and heterocyclic aromatic compounds over a wider range of oxidation conditions (e.g., at lower VOC concentrations and under conditions that minimize wall loss effects).

Disclosure statement

No potential conflict of interest was reported by the authors.

Author contributions

YR and SAN designed the experiments; JM, NNA, LTF, and VJB did the smog chamber experiments; QF did the OFR experiments; PL, JL, AL, and VJB recorded off-line mass-spectrometric data; JM, QF and VJB analyzed the data and wrote the manuscript; all authors edited the manuscript.

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