

Reviewer 3:

Review of “Characterization of water-soluble brown carbon chromophores from wildfire plumes in the western US using size exclusion chromatography” by Azzarello et al.

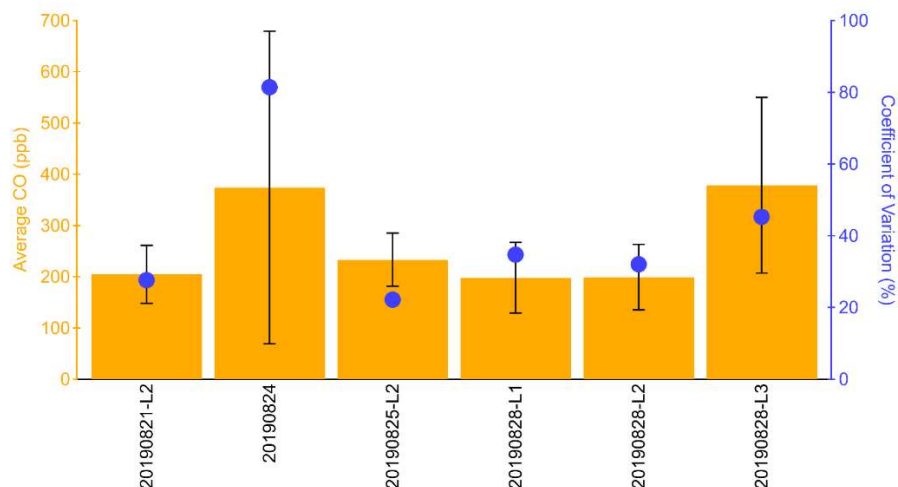
General comments: This manuscript presents results on a comparison of offline and online brown carbon (BrC) measurements for wildfire smoke collected from the Twin Otter aircraft during FIREX-AQ. The authors found that the BrC was dominated by smaller chromophores (<500 Da) and that there was not a consistent decrease in BrC with plume age (0-5 hours). They also found differences in the spectra between online and offline measurements and attribute this to solvent effects. Overall this is a very well written paper with a clear summary of the results. I have a few minor comments that should be addressed before this paper is accepted.

We appreciate the detailed comments of the Reviewer. We have addressed each in detail below, where our responses are in highlighted blue and changes to the manuscript are indicated in **bold blue**. We have undertaken additional experiments and added new text and figures to the manuscript and SI. The changes are detailed below.

Specific comments:

1. It is noted that there isn't a consistent trend in BrC with plume age, as has been reported in other papers looking at these fires. Here the BrC is normalized to the average CO concentration. How much variation was observed in this CO mixing ratio during the sample collection period and how much variation was observed in general in the fire? How does this variation compare to previous studies using this normalization method?

The CO mixing ratio that corresponds to the sampling duration of each aqueous sample was determined. The average CO mixing ratio and standard deviation for each flight is displayed in new Figure S9. To represent the variation observed in CO for each flight, the figure includes the coefficient of variation which represents the standard deviation as a percent of the mean; a greater coefficient of variation indicates more variability. Intercomparison of total measured absorption to CO was our focus since this is a standard way to assess the evolution of BrC while considering plume dilution.



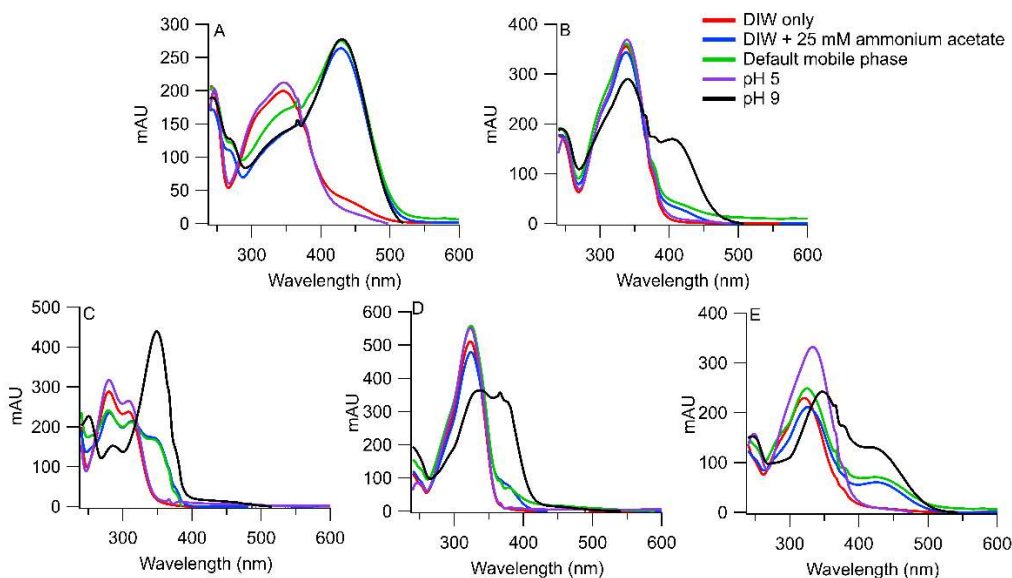
“Figure S9. Average CO measured and standard deviation for each flight and the blue markers denote the coefficient of variation, which represents the standard deviation as a percent of the mean.”

2. Looking at Figure 1, the black markers have a line fit to them that appears to extend beyond the data shown in the figure. Is there data not shown in the figure?

Figure 1 displays absorption at 300 nm measured by the SEC-UV for analysis normalized to CO as a function of plume age. The purpose of the linear fit is to represent whether BrC absorption was increasing or depleting as a function of plume age and to compare these findings to the literature. The linear fit for the flight denoted by the black markers does not extend past the data shown; the data shown in Figure 1 is not cut-off in any way.

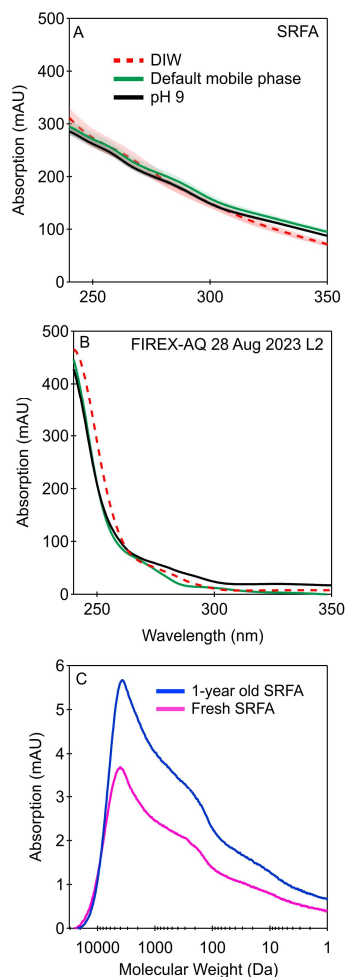
3. For the solvent effects, pH has also been demonstrated to play an important role in shifts in the absorption of BrC. What was the pH (estimated) of the water for the PILS analysis. Do you expect a difference compared to the pH that is found in the water collected for offline analysis? The idea of pH for organics in organic solvents is complicated, but the pH of the water in the online measurements may play a role in the differences observed.

We agree that pH can play a role in absorption shifting. To investigate the impact the pH of the mobile phase has on wavelength dependent absorption, the ammonium acetate solution was adjusted to pH 5 and pH 9 prior to the addition of the acetonitrile. Several compounds that contribute to brown carbon absorption (4-nitrocatechol, vanillin, 7-hydroxycoumarin, 4-hydroxy-3-methoxy cinnamaldehyde, and mixture of the four compounds) and SRFA were analyzed in the following mobile phases: DIW, DIW with 25 mM ammonium acetate, the default mobile phase (not pH controlled), pH 5, and pH 9. There is red shifting when the pH was greater than the pK_a of the compound. We acknowledge that numerous studies have investigated the impact directly acidifying and alkalizing a SRFA sample have on absorption – there is a slight increase in absorption with increasing pH (Phillips et al., 2017). These results are shown in new Figure S6.



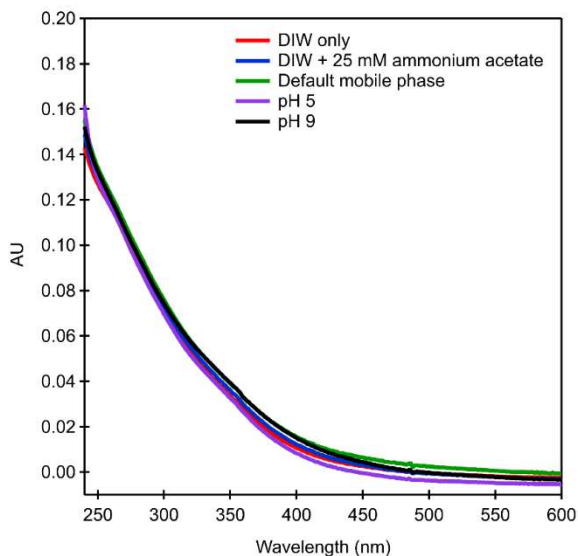
“Figure S6. Absorption as a function of wavelength measured in various mobile phases of (a) 4-nitrocatechol (b) 4-hydroxy-3-methoxy cinnamaldehyde (c) vanillin (d) 7-hydroxycoumarin and (e) a mixture of the four compounds by a diode array detector. The default mobile phase consists of 1:1 mixture of acetonitrile and DIW with 25 mM ammonium acetate. The purple and black traces represent pH controlled mobile phases, where the pH of the ammonium acetate solution was adjusted to pH 5 and pH 9.”

The pH of the PILS water was approximately 5. Since the aqueous samples flowed through the liquid waveguide capillary cell prior to its collection in the polypropylene tubes, we do not expect a difference in pH of between the online analysis and aqueous samples. To assess the impact of pH and mobile phase on a complex mixture, we also measured the absorption of a SRFA aqueous solution and a FIREX-AQ aqueous sample with the abovementioned mobile phases (Figure 4 and Figure S8). In contrast to the individual BrC compounds, no major changes in the spectral shape were observed under different mobile phase conditions. To confirm these results, we measured the absorption of SRFA in each solvent using an Agilent spectrophotometer (Figure S7).

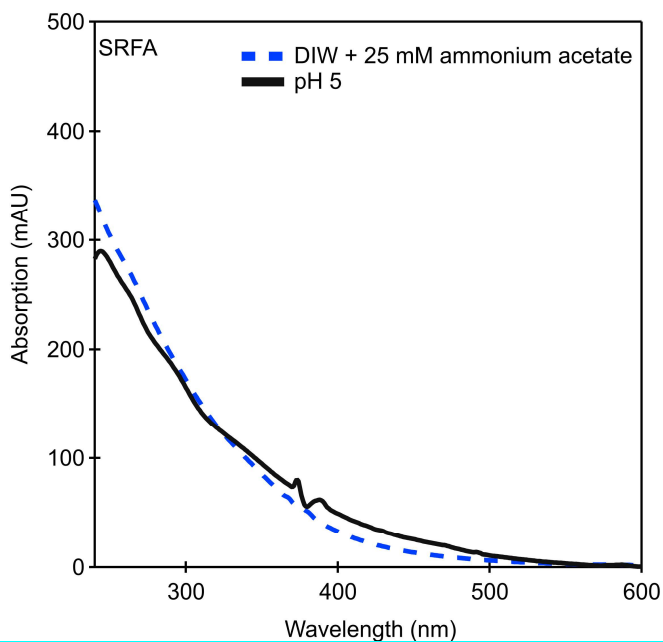


“Figure 4. Absorption as a function of wavelength of (a) SRFA and (b) a FIREX-AQ aqueous sample collected on 28 Aug 2019 L3. The default mobile phase consists of 1:1 mixture of acetonitrile and DIW with 25 mM ammonium acetate. The purple and orange traces represent

pH controlled mobile phases, where the pH of the ammonium acetate solution was adjusted to pH 5 and pH 9 prior to the addition of the acetonitrile.”



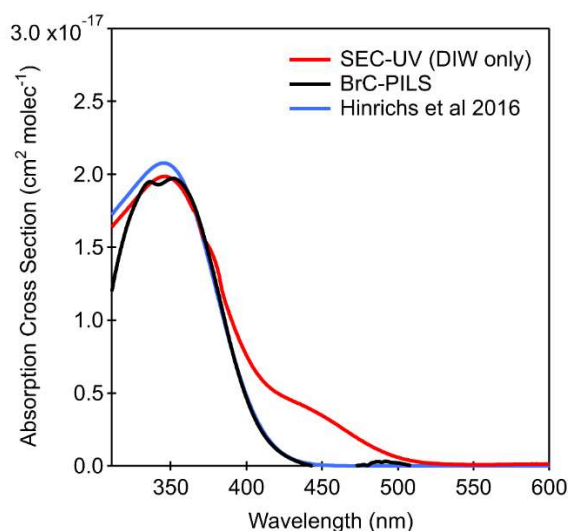
“Figure S7. Absorption as a function of wavelength of SRFA measured using a Agilent 8453 UV-visible Spectroscopy System. A solution of 15 $\mu\text{g/mL}$ SRFA was diluted by 50 % by the mobile phase and then transferred to the cuvette prior to measurement. The default mobile phase consists of 1:1 mixture of acetonitrile and DIW with 25 mM ammonium acetate. The purple and orange traces represent pH controlled mobile phases, where the pH of the ammonium acetate solution was adjusted to pH 5 and pH 9 prior to the addition of the acetonitrile.”



“Figure S8. Absorption as a function of wavelength of SRFA measured in a 25 mM ammonium acetate solution and in a mobile phases controlled to pH 5.”

4. Solvent effects can play a role in the position of the absorption, but I've seen less discussion around changing the overall intensity of the absorption. Do you think that the increase for the offline measurements can be attributed to this, or are there other possible reasons for that increase?

The offline SEC-UV analysis and the online absorption measurements by the BrC-PILS were converted to Mm^{-1} , a common unit used to express the magnitude of BrC absorption. The total absorption by the SEC-UV measurements is approximately an order of magnitude greater than the BrC-PILS measurements at 300 nm. We are confident that the optical system and the diode array detector can measure absorption that are within agreement. For example, we compared absorption measurements of 4-nitrocatechol by the LWCC of the BrC-PILS and the diode array detector converted to absorption cross section, showing good agreement (Figure S13 below).



“Figure S13. Absorption cross section of 4-nitrocatechol measured by injection onto the LWCC of the BrC-PILS and onto the diode array detector of the SEC-UV set-up compared to Hinrichs et al. 2016.”

The investigation of pH impact on absorption prompted the investigation of other plausible explanations to describe the discrepancies between the online BrC absorption measurements and offline SEC-UV measurements. Major differences between the online and offline analyses are the use of acetonitrile for the chromatographic separation and that the aqueous samples were stored prior to the SEC-UV analysis.

Therefore, in considering possible pH, solvent, and storage effects, section 3.4 was retitled and rewritten:

Section 3.4 was titled: 3.4 Solvents affect the measured absorption spectra

This was changed to:

“3.4 Investigating the impact of solvent effects, pH, and storage effects on absorption spectra”

Section 3.4 first addresses solvation effects (Lines 337 to 376):

“First, we assess solvation effects due to changes in the mobile phase composition. The PILS solubilizes BrC in pure water for the online measurements to facilitate absorption measurements (Weber et al., 2001). In contrast, the mobile phase used for the offline SEC-UV analysis was a mixture of acetonitrile and DIW with 25 mM ammonium acetate. Chromatographic packing materials are often incompatible with pure water and require a mixture with an organic solvent to elute compounds from the stationary phase or, in SEC separations, to prevent sorption to the stationary phase. For this reason, chromatographic partitioning-based separations occur in aqueous-organic mixtures, where the composition can be deliberately modified to optimize interactions of the target molecules between the stationary phase and mobile phase. In SEC, non-size exclusion interactions between the analyte and stationary phase are dominated by electrostatic and hydrophobic interactions (Hong et al., 2012). If the analyte and stationary phase are identically charged, ion exclusion effects can occur, resulting in an earlier elution time as the analyte is prevented from entering the pores. If the analyte and stationary phase are oppositely charged, adsorption can result, leading to a later elution time. Hydrophobic effects can occur if the analyte interacts with hydrophobic sites of the column matrix (Hong et al., 2012). The purpose of adding ammonium acetate to the mobile phase is to increase the ionic strength of the mobile phase and facilitate ion-pairing, which suppresses electrostatic interactions between the stationary phase and the polar and charged functional groups. The organic solvent used in our mobile phase was acetonitrile, which has been shown to be unreactive towards typical BrC components and has been recommended as an inert solvent for BrC extraction and analysis (Walser et al., 2008; Bateman et al., 2008; Chen et al., 2022). Therefore, we do not expect the mobile phase to chemically alter BrC compounds while effective at mitigating column stationary phase-analyte interactions.

While chemical changes caused by our mobile phase are unlikely, it is possible that other solvent effects on absorption could be occurring. Effects of solvent on molecular absorption are well established in the photochemistry literature (Lignell et al., 2014; Mo et al., 2017; Zheng et al., 2018; Lyu et al., 2021; Chen et al., 2022; Dalton et al., 2023). The polarity of the solvent affects the absorption wavelength by changing stabilization of the ground and/or excited states. With a decrease in solvent polarity, (acetonitrile-water is less polar relative to pure water), this can lead to a decrease in stabilization of the ground state of BrC compounds (such as 4-nitrocathol), but this effect is molecule dependent. The impact of solvation on red and blue spectral shifting will likely be several nanometers, which could contribute to the observed differences in the offline and online absorption measurements. Previous work has shown that acetonitrile could disrupt π - π interactions between BrC molecules, which could cause the liberation of adsorbed low MW BrC chromophores from larger chromophores or disrupt BrC aggregates (Lyu et al., 2021). Smaller, less conjugated systems typically absorb in the ultraviolet-blue wavelength region, and their $\pi \rightarrow \pi^*$ transition red shifts when more conjugated systems are fused together (Gorkowski et al., 2022). Thus, we would expect absorption measurements in the presence of acetonitrile to be blue-shifted relative to those in pure water. This represents a possible explanation for greater absorption intensity at lower wavelengths measured in the offline SEC-UV analysis compared to the online analysis.”

The influence of solvent pH in section 3.4 now reads (Line 377 to 407):

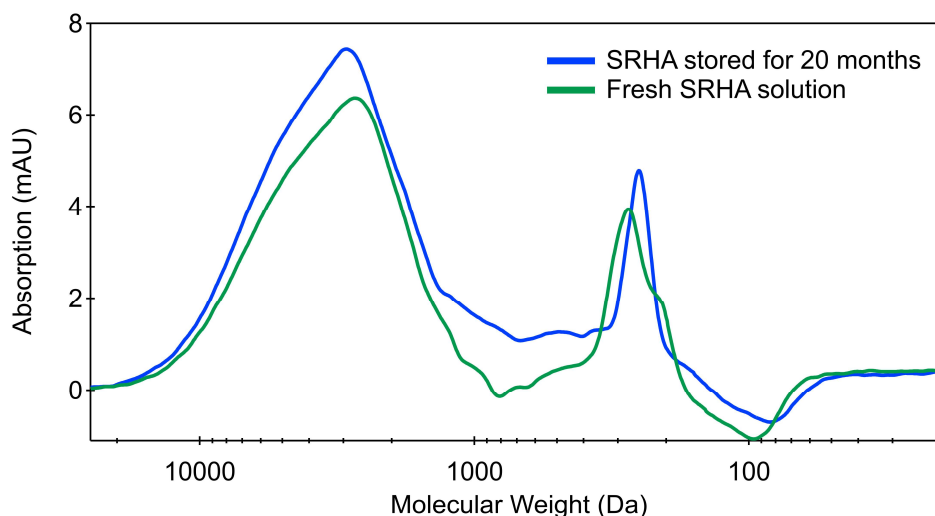
“Second, we assess the pH of the sample matrix, which is known to affect the absorption profile of BrC compounds. Multiple studies have investigated the impact of pH on wavelength-dependent absorption. For example, Phillips et al. 2017 directly adjusted the pH of SRFA and biomass-burning derived aqueous extracts (with sodium hydroxide or hydrochloric acid) and observed no measurable shift in the spectra to shorter or longer wavelengths; however they did observe that as the pH increased, there was an increase in the magnitude of absorption, which was more pronounced at higher wavelengths. The pH of the default mobile phase solution was 7.2, while the pH of the deionized water solutions in the PILS was approximately 5 (due to carbon dioxide dissolution). To investigate the impact pH has on BrC absorption, we measured several compounds that have been shown to contribute to BrC absorption (4-nitrocatechol, vanillin, 7-hydroxycoumarin, 4-hydroxy-3-methoxy cinnamaldehyde, and mixture of the four compounds) under different solvent and pH conditions: DIW, DIW with 25 mM ammonium acetate, as well as the mobile phase at pH 5, 7.2, and 9. When the matrix conditions have a pH greater than the pK_a of the compound in question, the species will deprotonate, resulting in a shift to longer wavelengths (Hinrichs et al., 2016). For compounds with a pK_a between 5 and 9 (i.e., 4-nitrocatechol, 7-hydroxycoumarin, vanillin), we observed this phenomenon (Figure S6). To assess the impact of pH and mobile phase on a complex mixture, we also measured the absorption of a SRFA aqueous solution and a FIREX-AQ aqueous sample with the abovementioned mobile phases (Figure 4 and Figure S8). In contrast to the individual BrC compounds, no major changes in the spectral shape were observed under different mobile phase conditions. To confirm these results, we measured the absorption of SRFA in each solvent using a separate spectrophotometer (Figure S7). This suggests that the pK_a of the majority of functional groups in the absorbing compounds present were less than 5 or above 9. Nitroaromatic compounds typically have pK_a values between 5 and 8; suggesting low levels of this class of compounds present in the aqueous samples. This observation is comparable to the online BrC-PILS analysis; for aqueous absorption, Washenfelder et al. 2022 observed the average absorption contribution at 365 nm of 4-nitrocatechol was less than 1.1 % and the summed contribution to absorption by 2-nitrophenol, 4-nitrophenol, 4-nitrocatechol, 4-nitroguaiacol, and 2,4-dinitrophenolate was less than 3.6 %. Since the absorption profile of SRFA and the FIREX-AQ sample appear similar in all mobile phase conditions, we have no evidence that pH of the mobile phase in the SEC separation conditions impacts the wavelength dependent absorption of the FIREX-AQ aqueous samples.”

The influence of storage effects in section 3.4 reads (Line 408 to 444):

“Third, we assess the potential effect of storage on the aqueous samples measured by SEC-UV. A recent study by Resch et al. 2023 observed that biomass burning-derived filter extracts stored at temperatures above freezing may undergo compositional changes that can increase in signal for various compounds. Hydrolysis reactions include converting alkenes to alcohols and esters to carboxylic acids, and the breakdown of oligomers. The hydrolysis of oligomers such as dimer esters stored in an aqueous solution can result in an increase in precursor monomers as decomposition products leading to an increase in signal (Zhao et al., 2018; Resch et al., 2023). Further, ammonium and alkylamines have been observed in high levels in biomass burning aerosols (Di Lorenzo et al., 2018); aqueous reactions between dicarbonyls (e.g., glyoxal, methylglyoxal) with ammonium and amines may also contribute to an increase in absorption intensity at pH 4 to 7 (Powelson et al., 2014; Yang et al., 2023). The FIREX-AQ aqueous samples had a pH of 5 and were stored at 4 °C for two years prior to analysis. Assuming they contained dicarbonyl compounds and reduced nitrogenous species, it is possible reactions leading to products that can contribute to greater absorption during storage occurred. To further

investigate the impacts of storage on a complex aqueous mixture, we measured the absorption spectra of two SRFA solutions: one freshly made and one stored for one year at 4 °C. We observed an increase in absorption in the aged SRFA solution, in which integrated absorption was 39 % higher than the freshly-made solution. This same effect was also observed with SRHA solutions (Figure S14). Thus, it is possible that processes during storage could have led to increased absorption measured in the offline SEC samples.

Among the three processes discussed here, we conclude that the storage of aqueous extracts is the most plausible explanation for the higher absorption observed in the offline samples from FIREX-AQ. If hydrolysis reactions are occurring, we might expect this to impact the MW profile (i.e., SEC elution times). We examined the MW profile of freshly-made and one year-aged SRFA solutions (Figure 4C). The increase in absorption with storage does not measurably affect the molecular size-resolved absorption of the mixtures. The same effect was observed for SRHA (Figure S14). This demonstrates that any storage-induced changes in these complex mixtures of organic molecules have a minimal impact on the molecular weight relative to the wide MW range of the SEC column. The MW of the BrC species would have to change by ~ 100 Da to be noticeable on the MW scale of our separation (250 Da to 75 kDa). Such a drastic change in MW is unlikely the case in most hydrolysis reactions. Thus, our results above in which we broadly categorize MW species to be less than or greater than 500 Da are likely robust. The SEC separation of the aqueous samples signify that low MW (<500 Da) chromophores contribute more to total absorption than higher MW (>500 Da), this finding is supported by previous SEC-UV analyses of BrC aged less than 10 hrs (Di Lorenzo et al., 2017; Lyu et al. 2021). The consistent MW profiles between the freshly-made and stored solutions of SRFA and SRHA reasonably suggest that storage did not have a major impact on the MW of BrC.”



“Figure S14. Size separation of a fresh SRHA solution which was then re-run 20 months later.”

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