Title

Quantification of Malondialdehyde in Atmospheric Aerosols: Application of the Thiobarbituric Acid Method

Journal

Aerosol and Air Quality Research, 22(7)

ISSN

1680-8584

Authors

Gonzalez, David H
Paulson, Suzanne E

Publication Date

2022

DOI

10.4209/aaqr.220037

Peer reviewed

Quantification of Malondialdehyde in Atmospheric Aerosols: *Application of the Thiobarbituric Acid Method*

David H. Gonzalez, and Suzanne E. Paulson*

[1]*University of California at Los Angeles, Department of Atmospheric and Oceanic Sciences, 405 Hilgard Ave., Los Angeles, CA 90405*

*Corresponding Author. Tel: 310-206-4442; Fax: 310-206-5219

E-mail address: paulson@atmos.ucla.edu

Abstract

Based on available toxicity data, malondialdehyde (MDA; O=CHCH$_2$CH=O) has been designated as a potential human carcinogen. A handful of studies suggest that MDA forms in the gas and aerosol phase in the troposphere, potentially contributing to inhalation toxicity, yet it has never been quantified in ambient air. The thiobarbituric acid (TBA) acid assay for (MDA) has been used as a marker for reactive oxygen species (ROS), oxidative stress, and lipid peroxidation in biological samples for decades. Here we apply the TBA assay to estimate the amount of MDA in ambient fine particulate matter ($PM_{2.5}$) for the first time, in samples containing biomass burning/urban aerosol from Fresno, CA, and urban aerosol from Los Angeles. We found 0.31– 0.75 ng m^{-3} MDA in the particle phase, similar to the low end, but about to three orders of magnitude lower than the upper end of reported concentrations of the common C_3 oxygenates methylglyoxal and malonic acid. Additionally, we investigated the response in the assay to seven common small oxygenates, and found interference only from acrolein, and that only when the acrolein was at millimolar concentrations, well above expected levels in aerosol extracts. In sum, this work suggests that MDA is present at moderate levels in biomass burning and urban aerosols; more may be in the gas phase.

Keywords: Carbonyl Quantification, Biomass Burning, Urban Aerosol, Aerosol Toxicity, Assay Interference.

1. INTRODUCTION

Malondialdehyde (MDA) has been widely used as an indicator of aqueous reactive oxygen species (ROS), lipid peroxidation, oxidative stress, and rancidity in food products (Buege and Aust 1978, Halliwell and Gutteridge 1981, Zeb and Ullah 2016, Agarwal and Majzoub 2017). MDA is a product of lipid peroxidation (Buege and Aust 1978, Del Rio et al. 2005). and OH-mediated oxidation of 2-deoxyribose, and it has been widely used to assess oxidation in human, animal, and ecotoxicity applications (Halliwell and Gutteridge 1981, Gutteridge and Halliwell 1988, Genaro-Mattos et al. 2009). MDA has also been shown to be mutagenic and carcinogenic, resulting in its classification as a possible human carcinogen (Millar 1991), and to contribute to atherosclerosis, suggesting a role more significant than simply an indicator for oxidation in biological systems (Basu and Marnett 1983, Niedernhofer et al. 2003, Del Rio et al. 2005, Papac-Milicevic et al. 2016).

Two studies have reported formation of gas phase MDA formation in laboratory organic photooxidation experiments. Liu et al. (1999) observed MDA formation from photooxidation of several aromatic oxidation products including 2-butenedial, 4-oxo-pentenal, and 1,3-butadiene in the gas phase in an environmental chamber. Zhou et al. (2014) found that ozonolysis of polyunsaturated fatty acids at the surface of an aqueous layer produces gaseous MDA. Furthermore, Beeby et al. (1987) found that photolysis of glycolaldehyde in aqueous solutions produced MDA, suggesting MDA can also form in bulk aerosol or cloud water.

Destaillats et al. (2002) reported identification of MDA in ambient air in San Francisco, CA, using a derivatization method coupled with High-Resolution Gas Chromatography/Ion Trap Mass Spectrometry. The authors stated that MDA co-eluted with an internal standard that was distinguishable by interpreting a combination of electron ionization, methane chemical

ionization and derivative chemical ionization spectra. Their experimental design was not able to quantify its concentration and did not distinguish between gas or particle phase MDA.

Okochi and Brimblecombe (2002) used a bond contribution method to estimate a Henry's Law Constant for gas-particle partitioning for MDA, estimating a value of 1.4×10^4 M atm^{-1}, below the value ($\sim10^5$ M atm^{-1}) needed for the majority of MDA to partition into cloud and fog droplets. Their model predicted that 8-9% of gas phase MDA would partition into a pH 6 fog droplet when [MDA] = 10-10^{-4} ppb, but that by pH 2, partitioning would be negligible. This pH dependence is understood by recognizing that aqueous malondialdehyde prefers the enol form (Fig. 1a), pK_a = 4.7, which is more soluble than the dicarbonyl; at low pH the dialdehyde is favored, reducing its solubility substantially. Their model further predicts that MDA complexation of Cu(II) and Ni(II) at the droplet surface would enhance MDA partitioning (Okochi and Brimblecombe, 2002).

MDA has most commonly been measured in biological and other systems via derivatization with thiobarbituric acid (TBA) (Halliwell and Gutteridge 1981, Gutteridge and Halliwell 1988, Genaro-Mattos et al. 2009), the approach used here. The method reacts two TBA molecules with MDA, in the presence of acid and heat to form a TBA_2-MDA adduct that can be measured with absorption or fluorescence spectroscopy (Fig. 1, reaction 2). Interferences have been reported from MDA precursors such as carbohydrates that can form small amounts of $(TBA)_2$-MDA upon heating, and from other carbonyls that form non-MDA adducts with TBA and absorb or fluoresce at similar wavelengths (Waravdekar and Saslaw 1959, Morales and Munné-Bosch 2019). Such interferences are greatly reduced by using high performance liquid chromatography (HPLC) or mass spectrometry to detect TBA_2-MDA (Moselhy et al. 2013, Domijan et al. 2015).

Here, for the first time, we apply the TBA method to estimate MDA concentrations in urban and biomass burning aerosols (BBA) particles smaller than 2.5 microns in diameter ($PM_{2.5}$). We also characterize the fluorescence of the extracts with detailed excitation-emission (EEM) scans and investigate the potential of seven small oxygenated species common in the atmosphere for their potential to interfere with the MDA signal in the TBA assay.

2. METHODS

2.1 Materials

Malondialdehyde tetrabutyl ammonium salt (≥ 96%), 2-thiobarbituric acid (≥ 96%), acrolein (analytical standard), formaldehyde (36.5%-37.5% in water), sodium formate (99.9%), and oxalic acid (99.9%) and sodium malonate dibasic monohydrate (Bioextra) were purchased from Sigma-Aldrich. 0.1 N sulfuric acid was purchased from Titripur®. HPLC-grade acetonitrile was purchased from Omnisolve. HPLC-grade methanol was purchased from Fischer Scientific, and 15mL Falcon tubes (Corning Brand) were obtained from Thermo Scientific. Ultra-high purity Argon and Nitrogen were purchased from AirGas. Glyoxal (40% in water) and methylglyoxal (40% in water) were purchased from Tokyo Chemical Industry.

2.2 Aerosol Sample Collection

Four ambient $PM_{2.5}$ aerosol samples are tested here, one from Fresno (36.82 ^{o}N, 119.74 ^{o}W) and three from Los Angeles, CA (34.07° N, 118.44° W). The Fresno sample contained mixed urban aerosol mixed with biomass burning aerosol from residential wood burning in the surrounding areas, and was collected on an a 406 cm^2 Teflon-coated glass fiber filter from Sept 10 – 16, 2015 (Gonzalez et al. 2017). Urban $PM_{2.5}$ from Los Angeles, CA (Urban LA) was collected on the roof of the Math Sciences Building at UCLA. Urban LA samples were

collected on acid washed and pre-weighed PTFE filters (PALL, 47 mm 2 µm pore size) using an URG cyclone at 92.5 L Min^{-1}, corresponding to a cut size of 2.5 microns. Three samples and three blanks were collected for approximately 24 hours each during March 27^{th}-30^{th} 2019. The mass of collected particles was determined immediately after collection using a microbalance (1 µg precision, ME 5, Sartorius). To remove charge on the PTFE filters, a charge neutralizer was passed over the filter for 30 seconds before weighing. The Fresno BBA sample mass 467 µg in^{-2} , corresponding to average PM concentrations of 3.0 µg m^{-3}, of which about 270 µg in^{-2} was BBA. The content of BBA was characterized with optical absorption using an aethalometer (Paulson et al. 2019). The fraction of the sample comprised of BBA is at the higher end of observed BBA fraction compared to earlier measurements in Fresno (Paulson et al. 2019). The three urban LA samples had PM masses of 201 µg, 551 µg, and 835 µg, corresponding to average PM concentrations of 1.5 µg m^{-3}, 4.1 µg m^{-3}, and 6.3 µg m^{-3} respectively (Table 1). These values are on the low end for the West Los Angeles site, but such low values are common in the spring.

2.3 Application of the 2-Thiobarbituric Acid Method to Measure MDA in Ambient $PM_{2.5}$

$PM_{2.5}$ filter samples and blanks were placed in 15 mL Falcon tubes and extracted in 7.5 mL HPLC-grade methanol for 1 hour at room temperature in the dark. The extraction volume and time were chosen to allow all soluble organic constituents to dissolve. Extracting samples in the dark minimizes the possibility of photochemical reactions that may change the composition of the aerosol extract. Methanol was selected because it is a good solvent for small oxygenates and evaporates easily. The filters were then removed, and the methanol extracts were evaporated to dryness using a gentle stream of N_2 at room temperature and reconstituted in 720 µL of milliQ water (adjusted to pH 3 with H_2SO_4), followed by addition of 4 mM TBA (30 µL of 100 mM TBA), and incubation at 100ºC for 1.25 hours. 2.4 Estimation of Malondialdehyde using 2-Thiobarbituric Acid

A wide variety of protocols have been reported for HPLC-fluorescence detection of TBA_2-MDA in biological samples, but no protocols for the TBA assay applied to PM extracts were available. We performed quantification of TBA_2-MDA using High-Performance Liquid Chromatography (HPLC) with a fluorescence detector (Shimadzu RF-10AXL). A reversed phase C-18 chromatography column (GL Sciences Inc., Intersil ODS-2, 5 µm, 4.6 x 250 mm) and guard column (Thermo Scientific, ODS Hypersil JAVELIN Filter, 5 µm, 4 x 10 mm) separated analytes, and peaks were analyzed with Chromperfect Software (Justice Laboratory Software). Because the TBA_2-MDA adduct is most stable under acidic conditions (pH 2-3) (Guillén-Sans et al. 1997) and an eluent of 7:3 acetontirile:milli-Q water (18MΩ) acidified to pH 3 (with 0.1 N sulfuric acid) was suggested by Fukunaga et al. (1995), we performed the assay at pH 3. The eluent was continuously degassed with a gentle stream of argon and delivered at a rate of 1.0 mL min^{-1}. The TBA_2-MDA adduct eluted at 6 minutes, and fluorescence was measured at E_x/E_m = 530 nm/550nm.

The HPLC was calibrated daily with four MDA standards ranging from 0.25 to 2.5 µM. The method detection limit was about 0.1 µM. A typical TBA_2-MDA calibration curve is shown in Figure 2a; calibration slopes were within ± 12% of one another Calibration standards were prepared from pH 3 stock solutions of 100 mM TBA and 20 mM malondialdehyde tetrabutylammonium salt serially diluted to 20 µM MDA. TBA stock solution was prepared in a Teflon bottle with stirring and heating (90°C) for approximately 15 minutes until all TBA was dissolved. The TBA was used immediately after preparation because precipitants form approximately 20 minutes after removal from the hot plate. 30 µL TBA was added to 626 - 711 µL pH3 MilliQ water, then 9.4 – 94 µL aliquots of the 20 µM MDA stock solution were added for a total volume of 750 µL. The resulting solutions were capped and incubated in a boiling water bath (100 °C for 1.25 hours, after which the calibration solutions turned a pink-purple

color; blanks did not change color. Solutions were cooled in a refrigerator at 4 ºC for 15 minutes and analyzed with the HPLC immediately.

2.3 Excitation-Emission Matrix Spectra and Interfering Compounds (3D Fluorescence)

The Excitation-Emission Matrix (EEM) scan mode (Lumina Fluorometer, Thermo Scientific) was used to determine fluorescence features of MDA calibrations, BBA extracts, PM samples and potential interfering compounds. Scans were performed every 5 nm in both excitation and emission space, using 10 nm excitation and emission slit widths and 20 ms integration time for each step. The instrument scanned at 60 nm per second. Figure 3 shows an EEM for a 1 μM MDA standard after reaction with 4 mM TBA. Fluorescence contours indicate a fluorophore with peak fluorescence centered at E_x/E_m = 530 nm/550nm, corresponding to the TBA_2-MDA adduct (Del Rio et al. 2005, Moselhy et al. 2013, Domijan et al. 2015).

To characterize potential interfering compounds, we made 10 mM solutions of formaldehyde, formate, oxalic acid, malonate, glyoxal, methylglyoxal, and acrolein and incubated them in the presence of 4 mM of TBA adjusted to pH 3 (with H_2SO_4) heated at 100ºC for 1.25 hrs.

3. RESULTS AND DISCUSSION

3.1 Malondialdehyde in Fresno BBA and Los Angeles $PM_{2.5}$

3.1.1 Concentrations

All HPLC analyses of the derivatized PM extracts exhibited a signal that matched that of the MDA standards, with a retention time of 6 minutes and fluorescence at E_x/E_m = 530/550 nm, indicating the presence of TBA_2-MDA. We used varying amounts of the Fresno PM

sample to test dependence of the signal on aerosol mass and found a linear relationship (Fig. 2b). The estimated concentration of MDA in the Fresno sample was 0.31 ± 0.02 ng m^{-3} or $(10.2 \pm 0.6) \times 10^{-3}$ ng MDA (μg PM)$^{-1}$ (Figure 4). Urban LA samples contained 51 to 97ng MDA corresponding to approximately 0.41, 0.75, and 0.55 ng m^{-3} or 0.25, 0.18, and 0.087 ng μg^{-1} respectively (Tab. 1 and Fig. 4).

While we were unable to find reports of MDA concentrations in urban aerosols, we can compare concentration measurements to the concentrations of similar C_3 oxygenated organic compounds in ambient urban $PM_{2.5}$ (Destaillats et al. 2002, Ho et al. 2010, Kawamura et al. 2013, He et al. 2014, Ho et al. 2015, Shen et al. 2018). Reported concentrations for methylglyoxal, the 1, 2-carbonyl isomer of MDA (Fig. 1c), range from 0.8 – 242 ng m^{-3} in urban $PM_{2.5}$ (Destaillats et al. 2002, Ho et al. 2010, Kawamura et al. 2013, He et al. 2014, Ho et al. 2015, Shen et al. 2018). Malonic acid, a structurally similar molecule containing two carboxylic acids instead of two aldehyde groups (Fig. 1c; we note that there is no known pathway for oxidation of MDA to form malonic acid in the atmosphere), has been reported at concentrations in the range 17.6 - 233 ng m^{-3} in urban $PM_{2.5}$ (Ho et al. 2010, He et al. 2014, Ho et al. 2015). Thus, our reported range of 0.31 – 0.75 ng m^{-3} MDA are similar to the low end of methylglyoxal and up to three orders of magnitude lower than the upper limits of the concentrations of both methylglyoxal and malonic acid.

3.1.2 EEM Scans of Fresno BBA and Los Angeles $PM_{2.5}$

Figure 5a shows an EEM of the extract of 467 μg of Fresno $PM_{2.5}$, without addition of TBA. While urban samples typically do not exhibit fluorescence, the biomass burning HUmic-Like Substances (HULIS) in the Fresno sample are strongly fluorescent. The sample's two peaks centered at E_x/E_m = 350/460 nm and E_x/E_m = 330/410 nm are characteristic of HULIS, and are similar to Fulvic Acids (Graber and Rudich 2006, Kuang 2017). Figure 5b shows an

EEM scan for the same Fresno sample reacted with 4 mM TBA. After processing, the sample retains some of the fluorescence features of HULIS and gains a fluorescent feature matching the TBA_2-MDA fluorophore centered at E_x/E_m = 530/550 nm. Interestingly, there is another fluorophore centered at E_x/E_m = 455/470 nm with a similar trapezoidal shape as the TBA_2-MDA fluorophore. This fluorophore could arise from a TBA-aldehyde adduct of a different dicarbonyl species. Possible identities for this species are discussed below.

Figures 6a-c show EEM scans of the three concentrated Urban LA $PM_{2.5}$ extracts after reaction with 4 mM TBA. Concentrated extracts of Urban LA $PM_{2.5}$ without addition of TBA had no observable fluorescence. EEMs for all three samples show the characteristic fluorescence of the TBA_2-MDA adduct centered at E_x/E_m = 530/550 nm. Additional trapezoidal-shaped peaks are also observed in these samples, including the same E_x/E_m = 455/470 nm peak observed in the Fresno sample, and a second satellite peak at E_x/E_m = 640/665 nm.

While TBA may react with many aldehydes, the formation of a fluorophore requires addition of two TBA molecules, forming a conjugated system connecting the two aromatic rings, a system that is only possible for molecules with odd numbers of carbon atoms in the backbone and two aldehyde groups. However, despite running the TBA assay on multiple C_1 – C_3 oxygenated compounds (Fig. 1c) we were not able to produce any fluorescence features other than the one matching MDA, and that was only observed in trace quantities for acrolein (below). Generally, any substitution in the fluorophore will red-shift the peak, suggesting that the peak at 640/655 nm could be from methyl malondialdehyde (methylpropanedial) or another malondialdehyde with a substitution at the center carbon. The explanation for the peak at 455/470 nm is less clear, although its wavelengths might suggest one carbon bridging the two aromatic rings rather than three.

229 **3.2 Potential Interferences with the TBA Assay**

230 **3.2.1 Other Small Oxygenates**

231 We tested the common small oxygenates expected in ambient samples for their ability
232 to react with TBA and produce a product with the same or similar fluorescence characteristics
233 as the MDA-TBA_2 adduct. Formaldehyde, formic acid, oxalic acid, malonate, glyoxal and
234 methylglyoxal (Fig. 1c) produced no measurable fluorescence anywhere in the E_x/E_m spectrum.
235 Of all compounds tested, only acrolein produced any measurable fluorescence. The signal for
236 acrolein appears at the same retention time in the HPLC and has the same fluorescence features
237 as the TBA_2-MDA adduct. Triplicate samples of 1 mM and 10 mM acrolein were reacted with
238 4 mM TBA under oxygenated conditions produced 0.45 ± 0.07 μM and 0.87 ± 0.2 μM MDA
239 respectively, corresponding to 0.004% -0.008% conversion of acrolein to MDA. As it is
240 unlikely that acrolein would make up more than a few % of aerosol mass, acrolein is unlikely
241 to contribute measurably to the TBA_2-MDA signals for the aerosol extracts.

242 The MDA associated with acrolein may have been present in the bottle from the
243 manufacturer, or it may have been produced via acid hydration of the acrolein followed by
244 oxidation, as proposed in Figure 7. Under this mechanism, protonation of the alkene group
245 produces a primary and secondary carbocation, followed hydration that produces 2-
246 hydroxypropanal and 3-hydroxypropanal. Two possible oxidation products of these hydration
247 products are glyoxal and MDA. The hydration of acrolein to 3-hydroxypropanal has been
248 identified under acidic conditions (Pressman and Lucas 1942, Melicherčík and Treindl 1981,
249 Campadelli et al. 1983), but we could find no studies identifying MDA as a product of acrolein
250 hydration and oxidation. Furthermore, primary carbocations are known to be less stable than
251 secondary carbocations. Thus, formation of MDA from acrolein should be a minor pathway,
252 consistent with the very low observed yield.

3.2.2 Reactive Oxygen Species and Potential Formation of MDA in the Assay

There is some potential for formation of MDA in the assay itself. This would most likely happen via an oxidation reaction, mediated by hydroxyl radicals or other reactive oxygen species. Any hydroxyl radicals or other reactive oxygen species that might form during the heating phase of the assay should be scavenged by the large excess (4 mM) of TBA in the solution. Nonetheless, for some calibration samples, a gentle stream of argon was bubbled through the solution for approximately 1 minute prior to TBA addition and incubation to remove oxygen and reduce ROS generation during the assay. No differences were observed (data not shown), indicating that oxygen does not impact the condensation reaction between TBA and MDA, and ROS did not affect the calibration.

3.3 Potential Sources of Atmospheric MDA

MDA in particles could arise via reactions on the particles themselves, or partitioning from the gas phase, either directly into the particles or into cloud or fog droplets followed by incorporation into the particles once the droplet re-evaporates. Liu et al. (1999) reported formation of malondialdehyde from gas phase photo-oxidation of butadiene and unsaturated dicarbonyls. The Henry's law coefficient of methylglyoxal (and several other aldehydes) estimated from recent field measurements indicate that the Henry's Law partitioning coefficient for methylglyoxal could be ~ 10^8 M atm^{-1}, much higher than values reported earlier (~10^4 M atm^{-1}) (Betterton and Hoffmann 1988, Lee and Zhou 1993, Shen et al. 2018). Since MDA and methylglyoxal have similar theoretical Henry's Law constants (~10^4 - 10^5 M atm^{-1}) (Okochi and Brimblecombe 2002, Shen et al. 2018) and similar molecular structures, it is possible that they share similar gas-particle partitioning behavior. MDA has a higher boiling point (108 °C) than methylglyoxal (72 °C), and thus may partition into the aqueous phase even more readily than methylglyoxal. Further potentially enhancing partitioning is the formation of

277 the enol form (above the pK_a of 4.7) although this is expected to be more likely to play a role
278 in clouds and fog, as aerosols are believed to often be too acidic (pH 0 – 2) for this to be relevant
279 (Weber et al. 2016). MDA complexation to Cu(II) and Ni(II) at the droplet surface may also
280 enhance MDA uptake (Okochi and Brimblecombe 2002).

281 It is also possible that reactions photochemical (Beeby et al. 1987) or dark oxidation
282 reactions within aerosol waters result in MDA production. MDA is expected as an oxidation
283 product from 1,4 dienes, and 1, 3 unsaturated aldehydes; and it has been observed as an
284 oxidation product of butadiene oxidation (Liu et al. 1999, Liu et al. 1999). Further,
285 polyunsaturated fatty acids are MDA precursors in biological systems, and it is well
286 documented that aqueous oxidation of 2-deoxyribose sugar produces MDA (Halliwell and
287 Gutteridge 1981, Gutteridge and Halliwell 1988). Atmospheric aerosols contain some
288 biological material, including whole or fragmented bacteria and viruses, and fragments of plant
289 material, material that is composed of a variety of polyunsaturated fatty acids and
290 polysaccharides such as cellulose, hemicellulose, lignin, and free sugars including deoxyribose.

291 **3.4 MDA Toxicity**

292 MDA has been classified as a potential occupational carcinogen, although no reference
293 exposure limits (RELs) have been established (NIOSH 1990). Many small aldehydes exhibit
294 toxicity, although their reference exposure limits vary widely; the reference limits for chronic
295 exposure range from 0.35 μg m^{-3} for acrolein, 9 for formaldehyde and 140 μg m^{-3} for
296 acetaldehyde (OEHHA 2021). While the REL for acrolein is in the same range as our
297 measurements of MDA in the particle phase, it has yet to be established that MDA is as toxic
298 as acrolein. The MDA concentrations in the particle phase are significantly lower than the
299 RELs for formaldehyde and acetaldehyde. Future studies should aim to measure both gas and
300 particle phase MDA concentrations for toxicology assessment.

3.5 Conclusions

The thiobarbituric acid assay has been successfully applied to measure MDA in ambient aerosol particles. Other compounds found in aerosols do not appear to present significant interferences for the assay. Levels of MDA in urban samples, including one containing a significant contribution from biomass burning, were moderate at ~0.5 ng m^{-3}. This concentration is at the low end of observed concentrations of similar small carbonyl compounds in ambient aerosols, but it may contribute to toxicity of ambient air in combination with gas-phase MDA and other toxic species.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Prof. Alam Hasson of the California State University, Fresno for the BBA sample used in this work. We thank J. Adlin Scott for measurement of BBA in the Fresno sample. We thank J. Puna Bauman for assistance in some of the preliminary experiments leading to this work.

DISCLAIMER

The authors have no disclaimers to disclose.

REFERENCES

Agarwal, A. and A. Majzoub (2017). "Laboratory tests for oxidative stress." Indian journal of urology: IJU: journal of the Urological Society of India **33**(3): 199.

Basu, A. K. and L. J. Marnett (1983). "Unequivocal demonstration that malondialdehyde is a mutagen." Carcinogenesis **4**(3): 331-333.

Beeby, A., D. b. H. Mohammed and J. R. Sodeau (1987). "Photochemistry and photophysics of glycolaldehyde in solution." Journal of the American Chemical Society **109**(3): 857-861.

Betterton, E. A. and M. R. Hoffmann (1988). "Henry's law constants of some environmentally important aldehydes." Environmental science & technology **22**(12): 1415-1418.

Buege, J. A. and S. D. Aust (1978). [30] Microsomal lipid peroxidation. Methods in enzymology, Elsevier. **52:** 302-310.

Campadelli, F., C. Tosi, E. Santoro and L. Roberti (1983). "Spectroscopic investigation on the acrolein hydration." Spectroscopy letters **16**(8): 601-611.

Del Rio, D., A. J. Stewart and N. Pellegrini (2005). "A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress." Nutrition, metabolism and cardiovascular diseases **15**(4): 316-328.

Destaillats, H., R. S. Spaulding and M. J. Charles (2002). "Ambient air measurement of acrolein and other carbonyls at the Oakland-San Francisco Bay Bridge toll plaza." Environmental science & technology **36**(10): 2227-2235.

Domijan, A. M., J. Ralić, S. Radić Brkanac, L. Rumora and T. Žanić-Grubišić (2015). "Quantification of malondialdehyde by HPLC-FL–application to various biological samples." Biomedical Chromatography **29**(1): 41-46.

Fukunaga, K., K. Takama and T. Suzuki (1995). "High-performance liquid chromatographic determination of plasma malondialdehyde level without a solvent extraction procedure." Analytical biochemistry **230**(1): 20-23.

Genaro-Mattos, T. C., L. T. Dalvi, R. G. Oliveira, J. S. Ginani and M. Hermes-Lima (2009). "Reevaluation of the 2-deoxyribose assay for determination of free radical formation." Biochimica et Biophysica Acta (BBA)-General Subjects **1790**(12): 1636-1642.

Gonzalez, D. H., C. K. Cala, Q. Peng and S. E. Paulson (2017). "HULIS Enhancement of Hydroxyl Radical formation from Fe (II): Kinetics of Fulvic Acid-Fe (II) Complexes in the Presence of Lung Anti-Oxidants." Environmental Science & Technology.

Graber, E. and Y. Rudich (2006). "Atmospheric HULIS: How humic-like are they? A comprehensive and critical review." Atmospheric Chemistry and Physics **6**(3): 729-753.

Guillén-Sans, R., I. Vicario and M. Guzmán-Chozas (1997). "Further studies and observations on 2-thiobarbituric acid assay (fat autoxidtion) and 2-thiobarbituric acid-aldehyde reactions." Food/Nahrung **41**(3): 162-166.

Gutteridge, J. and B. Halliwell (1988). "The deoxyribose assay: an assay both for'free'hydroxyl radical and for site-specific hydroxyl radical production." Biochemical Journal **253**(3): 932.

Halliwell, B. and J. Gutteridge (1981). "Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts." FEBS letters **128**(2): 347-352.

He, N., K. Kawamura, K. Okuzawa, P. Pochanart, Y. Liu, Y. Kanaya and Z. Wang (2014). "Diurnal and temporal variations of water-soluble dicarboxylic acids and related compounds in aerosols from the northern vicinity of Beijing: Implication for photochemical aging during atmospheric transport." Science of the Total Environment **499**: 154-165.

Ho, K., R.-J. Huang, K. Kawamura, E. Tachibana, S. Lee, S. Ho, T. Zhu and L. Tian (2015). "Dicarboxylic acids, ketocarboxylic acids, α-dicarbonyls, fatty acids and benzoic acid in PM 2.5 aerosol collected during CAREBeijing-2007: an effect of traffic restriction on air quality." Atmospheric Chemistry and Physics **15**(6): 3111-3123.

Ho, K., S. Lee, S. S. H. Ho, K. Kawamura, E. Tachibana, Y. Cheng and T. Zhu (2010). "Dicarboxylic acids, ketocarboxylic acids, α-dicarbonyls, fatty acids, and benzoic acid in urban aerosols collected during the 2006 Campaign of Air Quality Research in Beijing (CAREBeijing-2006)." Journal of geophysical research: atmospheres **115**(D19).

Kawamura, K., K. Okuzawa, S. Aggarwal, H. Irie, Y. Kanaya and Z. Wang (2013). "Determination of gaseous and particulate carbonyls (glycolaldehyde, hydroxyacetone, glyoxal, methylglyoxal, nonanal and decanal) in the atmosphere at Mt. Tai." Atmospheric Chemistry and Physics **13**(10): 5369-5380.

Kuang, X. M. (2017). Formation of Reactive Oxygen Species by Ambient Particulate Matter: Probing causative agents and the underlying mechanism, UCLA.

Lee, Y. N. and X. Zhou (1993). "Method for the determination of some soluble atmospheric carbonyl compounds." Environmental science & technology **27**(4): 749-756.
Liu, X., H. E. Jeffries and K. G. Sexton (1999). "Atmospheric photochemical degradation of 1, 4-unsaturated dicarbonyls." Environmental science & technology **33**(23): 4212-4220.
Liu, X., H. E. Jeffries and K. G. Sexton (1999). "Hydroxyl radical and ozone initiated photochemical reactions of 1, 3-butadiene." Atmospheric Environment **33**(18): 3005-3022.
Melicherčík, M. and Ľ. Treindl (1981). "Kinetics and mechanism of the oxidation of acrolein, crotonaldehyde, and methacrolein with cerium (IV) sulfate." Chemical Papers **35**(2): 153-163.
Millar, J. D. (1991). "Carcinogenicity of acetaldehyde and malonaldehyde, and mutagenicity of related low-molecular-weight aldehydes.".
Morales, M. and S. Munné-Bosch (2019). "Malondialdehyde: facts and artifacts." Plant physiology **180**(3): 1246-1250.
Moselhy, H. F., R. G. Reid, S. Yousef and S. P. Boyle (2013). "A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC." Journal of lipid research **54**(3): 852-858.
Niedernhofer, L. J., J. S. Daniels, C. A. Rouzer, R. E. Greene and L. J. Marnett (2003). "Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells." Journal of Biological Chemistry **278**(33): 31426-31433.
NIOSH (1990). Title 29 Code of Federal Regulations Part 1990 DHHS (NIOSH) Publication Number 91-112.
OEHHA (2021). OEHHA Acute, 8-hour and Chronic Reference Exposure Level (REL) Summary. Sacramento, CA, USA.
Okochi, H. and P. Brimblecombe (2002). "Potential trace metal–organic complexation in the atmosphere." The Scientific World Journal **2**: 767-786.
Papac-Milicevic, N., C.-L. Busch and C. J. Binder (2016). "Malondialdehyde epitopes as targets of immunity and the implications for atherosclerosis." Advances in immunology **131**: 1-59.
Paulson, S. E., P. J. Gallimore, X. M. Kuang, J. R. Chen, M. Kalberer and D. H. Gonzalez (2019). "A light-driven burst of hydroxyl radicals dominates oxidation chemistry in newly activated cloud droplets." Science Advances **5**(5): eaav7689.DOI: 10.1126/sciadv.aav7689.
Pressman, D. and H. Lucas (1942). "Hydration of Unsaturated Compounds. XI. Acrolein and Acrylic Acid1." Journal of the American Chemical Society **64**(8): 1953-1957.
Shen, H., Z. Chen, H. Li, X. Qian, X. Qin and W. Shi (2018). "Gas-Particle Partitioning of Carbonyl Compounds in the Ambient Atmosphere." Environmental Science & Technology **52**(19): 10997-11006.DOI: 10.1021/acs.est.8b01882.
Waravdekar, V. and L. Saslaw (1959). "A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehydethiobarbituric acid reaction." Journal of Biological Chemistry **234**(8): 1945-1950.
Weber, R. J., H. Guo, A. G. Russell and A. Nenes (2016). "High aerosol acidity despite declining atmospheric sulfate concentrations over the past 15 years." Nature Geoscience **9**(4): 282-285.
Zeb, A. and F. Ullah (2016). "A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods." Journal of analytical methods in chemistry **2016**.
Zhou, S., L. Gonzalez, A. Leithead, Z. Finewax, R. Thalman, A. Vlasenko, S. Vagle, L. Miller, S.-M. Li, S. Bureekul, H. Furutani, M. Uematsu, R. Volkamer and J. Abbatt (2014). "Formation of gas-phase carbonyls from heterogeneous oxidation of polyunsaturated fatty acids at the air–water interface and of the sea surface microlayer." Atmospheric Chemistry and Physics **14**(3): 1371-1384.

421

422

423

424

425

426

427

428

429

430

431

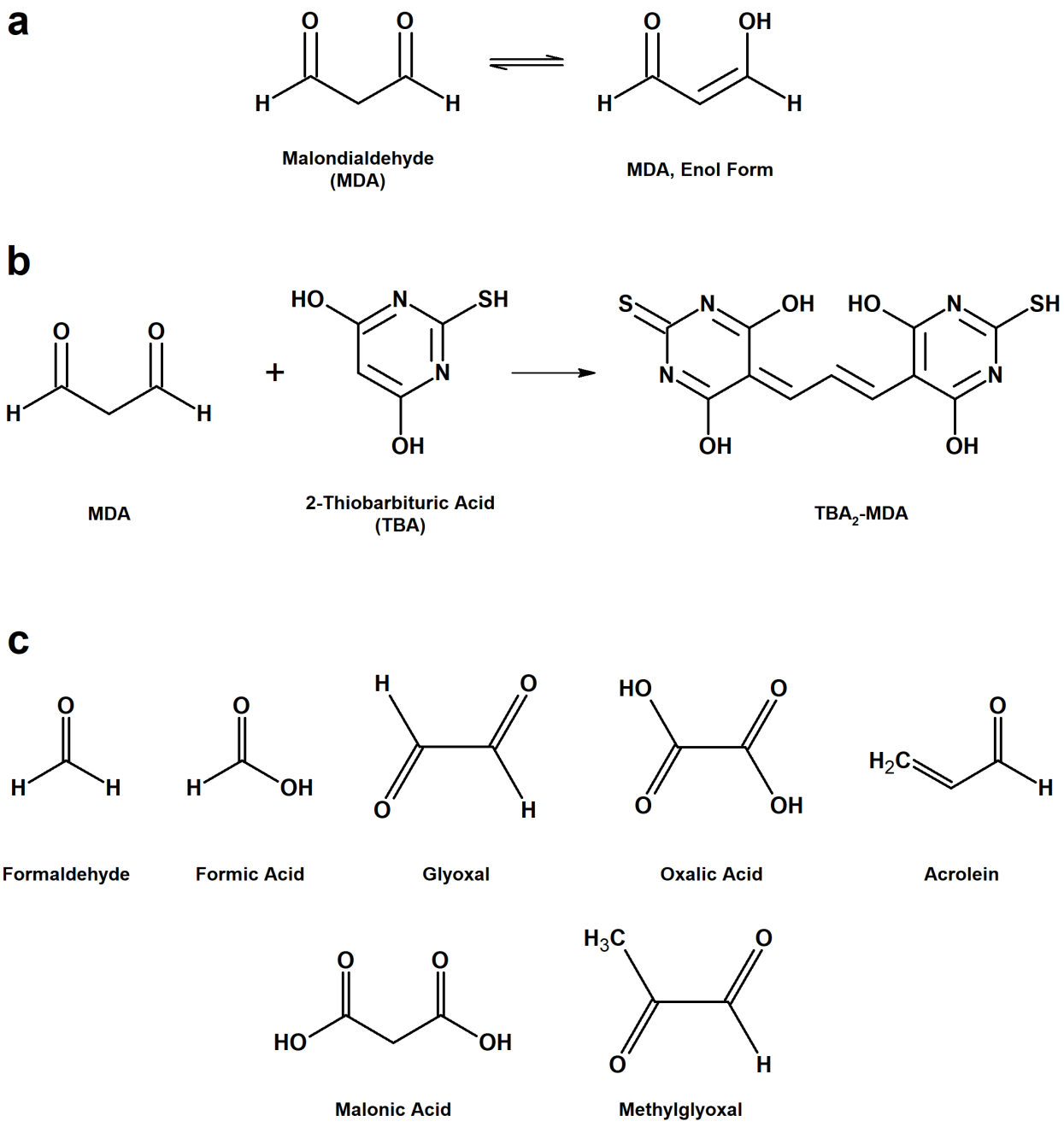
432 **TABLE 1. AMBIENT SAMPLE RESULTS**

Sample	Aerosol Mass on filter (μg)[a]	Aerosol Mass Conc. (μg/m^3)	MDA on filter (ng)	MDA Conc. (ng/m^3)	MDA per aerosol mass (ng/μg)	Fresno BBA content
Fresno (Sept 10-16, 2015)	467	3	51	0.33 ± .02	0.11 ± .01	266
Los Angeles 1 (Mar 27, 2019)	201	1.5	51	0.41 ± .02	0.25 ± .01	–
Los Angeles 2 (Mar 28, 2019)	551	4.1	97	0.75 ± .01	0.18 ± .01	–
Los Angeles 3 (Mar 29, 2019)	835	6.3	72	0.55 ± .06	0.09 ± .01	–

433 [a]Mass on whole 47 mm filter for LA samples; mass on 1" square punch from Fresno filter.

434

435



436

437 **Figure 1. (a)** Malondialdehyde aqueous equilibrium reaction. **(b)** Condensation Reaction of

438 MDA and TBA to form TBA_2-MDA. **(c)** C_1 – C_3 compounds tested for interference in the

439 TBA assay.

440

441

442

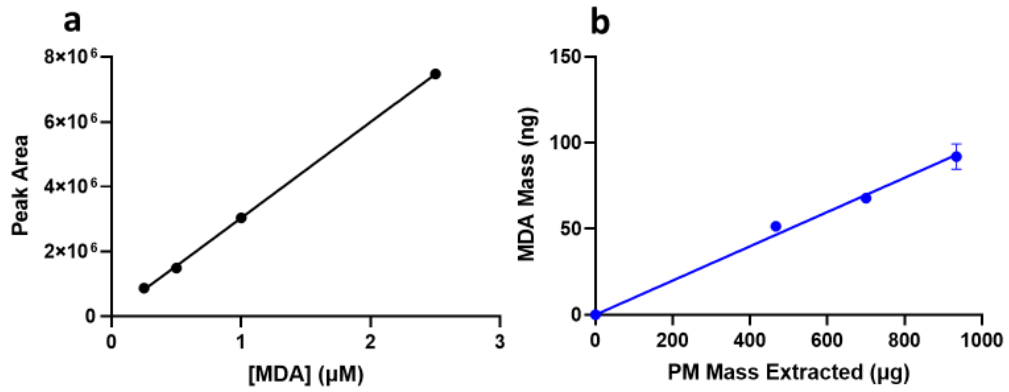


Figure 2. (a) Calibration curve for TBA_2-MDA adduct measured with HPLC-Fluorescence. Peaks eluted at a retention time of 6 minutes. (b) Mass of MDA measured from TBA assay for different quantities of Fresno urban/biomass burning aerosol. Error bars indicate ±1σ of three values measured on the HPLC from the same sample extract.

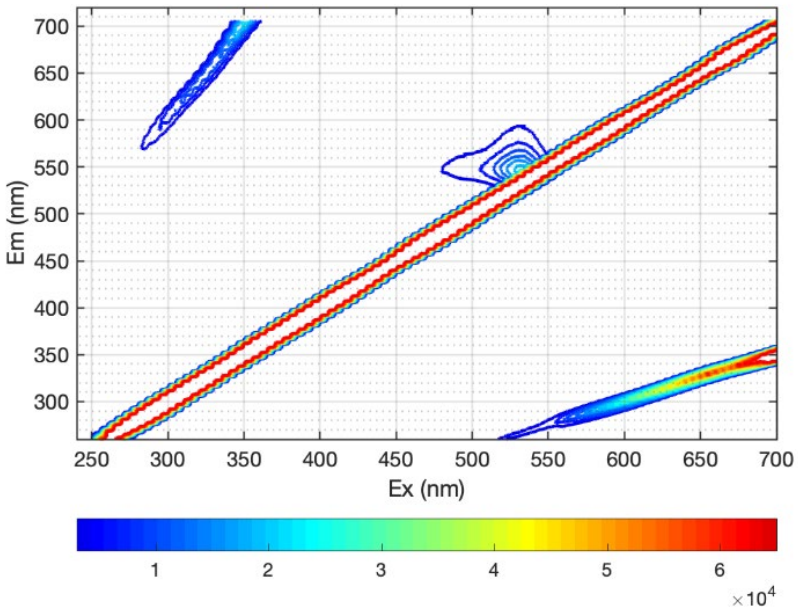


Figure 3. Excitation-emission matrix scan of 1 µM MDA assayed with 4 mM TBA in pH 3.

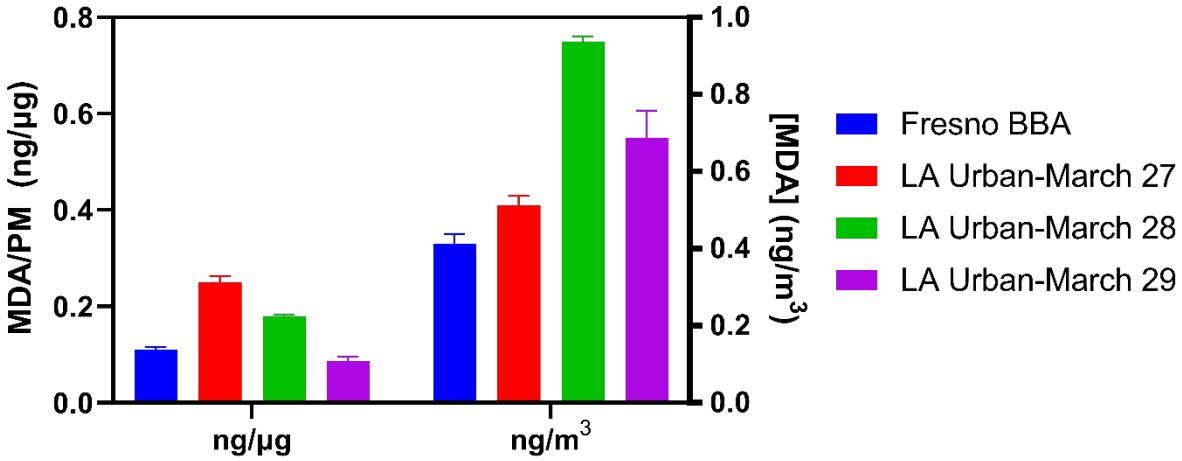


Figure 4. MDA measured with the TBA assay for the Fresno biomass burning aerosol (BBA, blue bar) and urban Los Angeles $PM_{2.5}$ (Urban LA; red, green, and purple bars) extracts. Error bars indicate ±1σ of three values measured on the HPLC from the same sample extract.

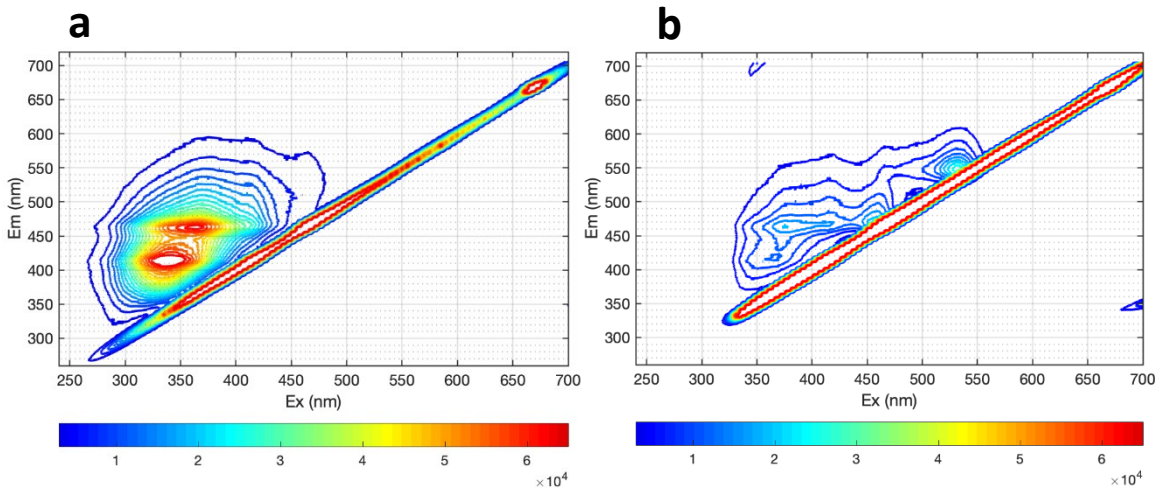


Figure 5. Excitation-emission matrix of (a) 467 µg Fresno BBA extracted in methanol and reconstituted in aqueous pH 3 solution and (b) the same extract after reaction with 4 mM TBA.

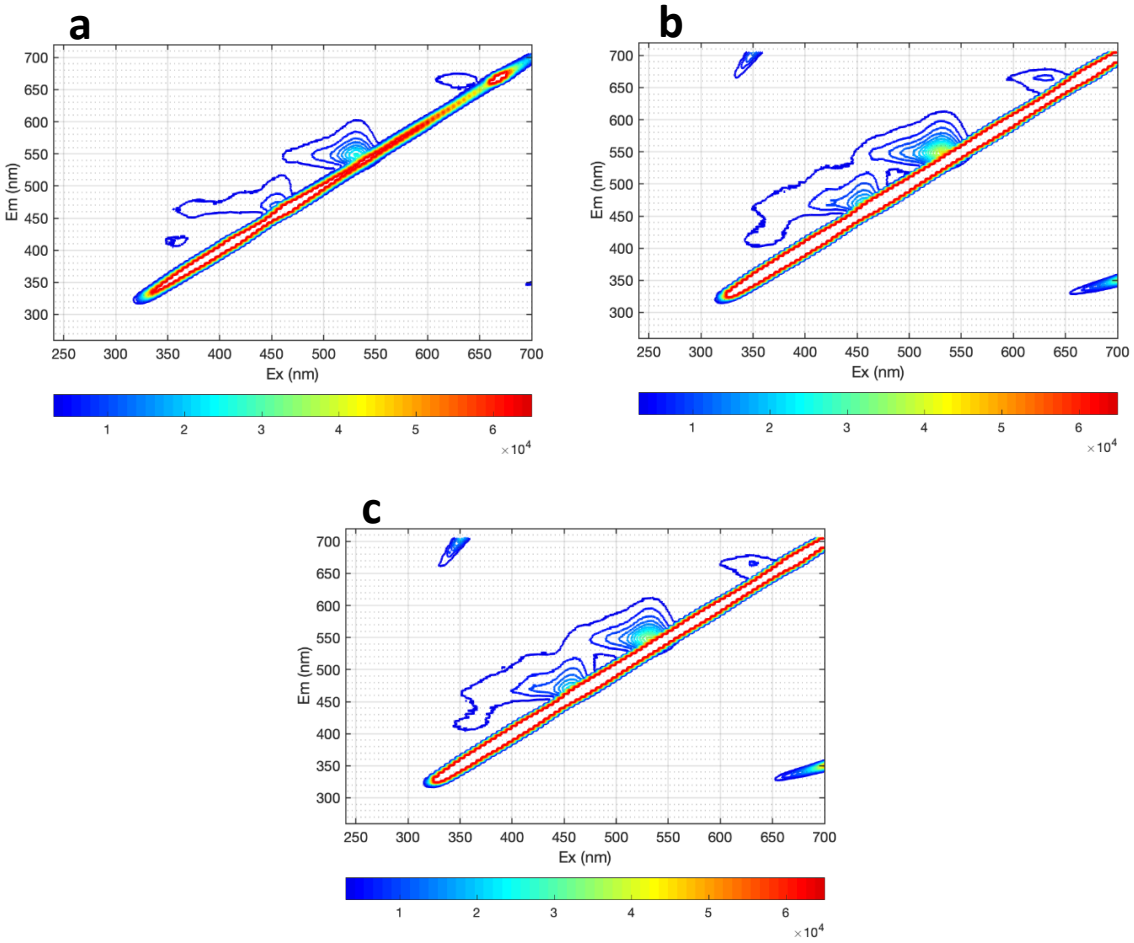
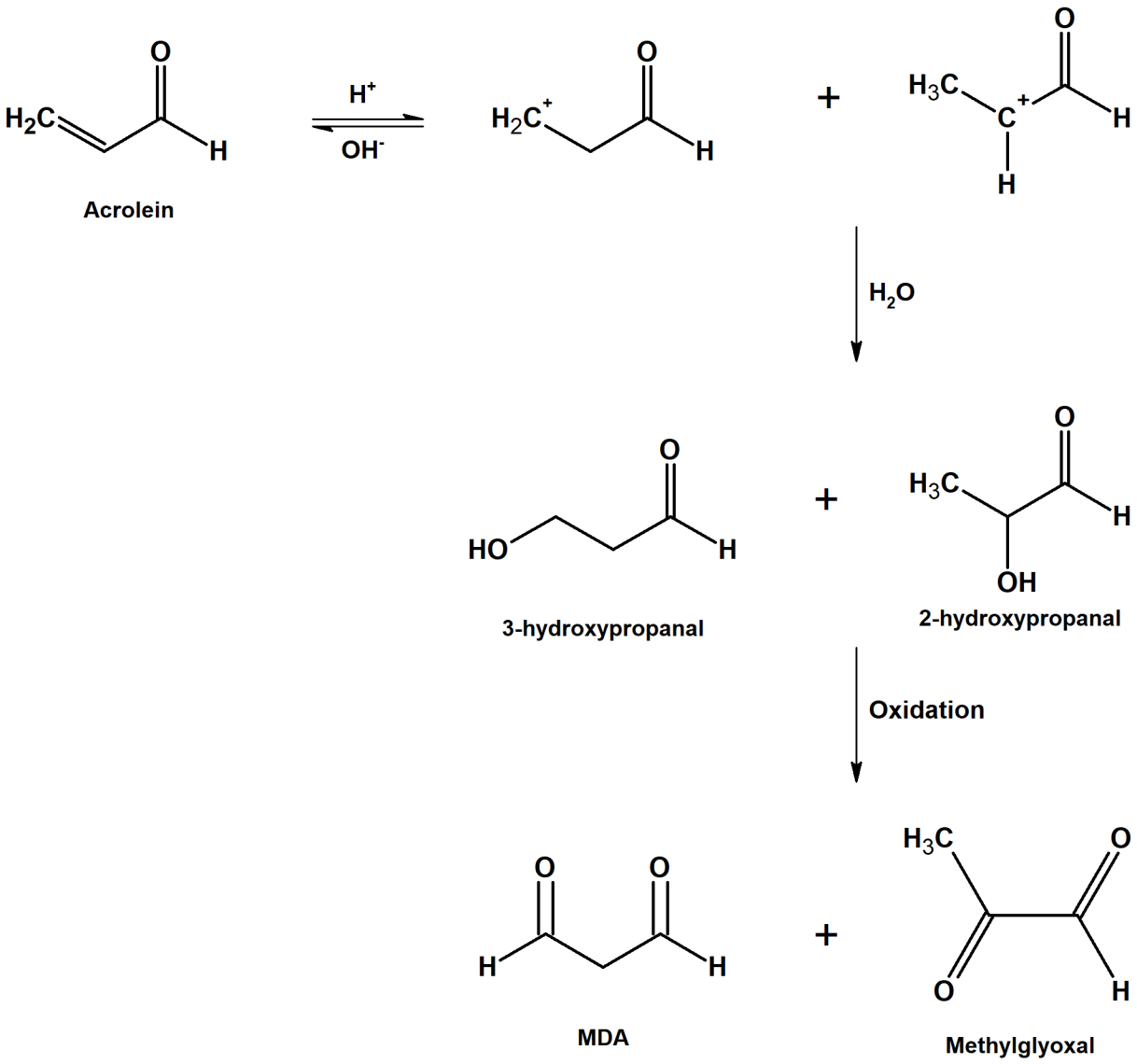


Figure 6. Excitation-emission matrix spectra for three urban Los Angeles $PM_{2.5}$ (samples collected on different days) assayed with 4 mM TBA in aqueous pH 3 solution. The samples had masses of (a) 201 µg (c) 551 µg (c) 835 µg (Tab. 1). The diagonal features in the center and at $E_x/E_m \sim 680/320$ nm and 320/650 nm respectively are scattering artifacts inherent to the spectrometer.



557

558 **Figure 7.** Proposed mechanism for conversion of acrolein to MDA under acidic, oxygenated
559 conditions.

560