Complexation of trace organic contaminants with fractionated dissolved organic matter: Implications for mass spectrometric quantification

Selene Hernandez Ruiz, Samanthi Wickramasekara, Leif Abrell, Xiaodong Gao, Benny Chefetz, Jon Chorover

Department of Soil, Water and Environmental Science, University of Arizona, 1177 E 4th St., Tucson AZ 85721, USA
Arizona Laboratory for Emerging Contaminants, University of Arizona, 1040 East 4th St., Tucson AZ 85721, USA
Department of Soil and Water Sciences, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Highlights
- Suwannee River natural organic matter is composed dominantly of hydrophobic acids.
- Fluorescence of protein and fulvic constituents was quenched by reaction with PPCPs.
- PPCP reaction with hydrophilic acids diminished LC–MS/MS recovery.

Abstract
Interaction with aqueous phase dissolved organic matter (DOM) can alter the fate of trace organic contaminants of emerging concern once they enter the water cycle. In order to probe possible DOM binding mechanisms and their consequences for contaminant detection and quantification in natural waters, a set of laboratory experiments was conducted with aqueous solutions containing various operationally-defined “hydrophilic” and “hydrophobic” freshwater DOM fractions isolated by resin adsorption techniques from reference Suwannee River natural organic matter (SRM). Per unit mass of SRM carbon, hydrophobic acids (HoA) comprised the largest C fraction (0.63 ± 0.029), followed by hydrophilic-neutrals (HiN, 0.11 ± 0.01) and acids (HiA, 0.09 ± 0.017). Aqueous solutions comprising 8 mg L⁻¹ DOC of each SROM fraction were spiked with a concentration range (10–1000 lgl⁻¹) of bisphenol A (BPA), carbamazepine (CBZ), or ibuprofen (IBU) as model target compounds in 24 mM NH₄HCO₃ background electrolyte at pH 7.4. Contaminant interaction with the SROM fractions was probed using fluorescence spectroscopy, and effects on quantitative analysis of the target compounds were measured using direct aqueous-injection liquid chromatography tandem mass spectrometry (LC–MS/MS). Total quenching was greater for the hydrophilic fractions of SROM and associations were principally with protein-like and fulvic acid-like constituents. Whereas LC–MS/MS recoveries indicated relatively weak interactions with most SROM factions, an important exception was the HiA fraction, which diminished recovery of CBZ and IBU by ca. 30% and 70%, respectively, indicating relatively strong molecular interactions.

1. Introduction
The continuous disposal of pharmaceuticals and personal care products (PPCPs) into wastewater, followed by their incomplete removal during treatment, results in their eventual release into freshwater sources including rivers, lakes and/or groundwater (Kolpin et al., 2002; Cahill et al., 2004; Sharma et al., 2009). For decades, it has been suggested that dissolved organic matter (DOM) plays a mediating role in the fate of these organic contaminants (Chin et al., 1997; Leenheer and Croue, 2003; Yamamoto et al., 2004). An established conceptual framework for predicting the impacts of organic matter on hydrophobic organic pollutant fate has led to attempts to predict the affinity of many PPCPs for organic matter based on contaminant hydrophobicity, e.g., through the use of octanol–water partition coefficients (K_{ow}) (Tolls, 2001; Pan et al., 2009). Indeed, complexation of more polar PPCPs with DOM often exceeds that predicted from log K_{ow} values alone, likely because bond formation involves not only hydrophobic
interactions, but also polar interactions mediated through O, N, and S containing functional groups of PPCPs and DOM (Tolls, 2001; Pan et al., 2009). Thus, the diverse physico-chemical properties of both PPCPs and DOM undoubtedly influence the types and strengths of bonds formed.

Elucidating the nature of PPCP-DOM interactions is complicated by the multi-disperse and heterogeneous nature of DOM, which is comprised of a diverse array of biomolecules, molecular fragments, and partially humified degradation products that derive from both plant and microbial sources (Leenheer and Croué, 2003; Leenheer, 2004). Natural DOM mixtures are polydisperse, and contain constituents with varying levels of hydrophilicity, and a wide range of functional group chemistry (Leenheer, 1981; Leenheer et al., 2003). As a result, DOM is observed to undergo sorptive fractionation in natural, aqueous heterogeneous systems where trends in aqueous-solid phase partitioning depend on DOM and particle surface chemistries (Chorover and Amstadi, 2001; Zhou et al., 2001; Guo and Chorover, 2003). Sorptive fractionation on ion exchange or hydrophobic resins is likewise employed in analytical organic geochemistry to isolate operationally-defined pools of DOM for further interrogation (Leenheer, 1981; Chefetz et al., 1998). For instance, through LC-MS/MS infusion experiments of fractionated DOM (Wickramasekara et al., 2012) found that the hydrophilic acid fraction produced the greatest matrix suppression of several PPCPs.

In the present work designed to study DOM-PPCP interactions, Suwannee River natural organic matter (SROM, an International Humic Substances Society standard reference material) was fractionated (Leenheer, 1981; Chefetz et al., 1998) into operationally-defined hydrophobic (Ho) and hydrophilic (Hi) acids (A), bases (B) and neutrals (N). Interactions of the resulting DOM fractions with model PPCPs were then probed at environmentally relevant pH and ionic strength. Two analytical techniques were used for complementary purposes: fluorescence spectroscopy was used to elucidate the molecular components of DOM fractions whose fluorescence is quenched by interaction with PPCPs, and direct aqueous injection tandem mass spectrometry was used to assess the relative strength of potential bond formation, particularly with respect to its effect on analyte chromatographic separation and quantification of contaminant analytes. In a prior study of unfracionated DOM solutions, we found that despite reproducible quenching of DOM fluorescence principally in protein-like and fulvic acid-like regions, PPCP recoveries by LC-MS/MS were essentially unimpaired (Hernandez-Ruiz et al., 2012). Hence, the same PPCPs were selected for use in the current study to probe interactions with DOM constituents in more detail for direct comparison to that prior work: bisphenol-A (BPA), carbamazepine (CBZ), and ibuprofen (IBU) (Table S1). All of these contaminants have been observed to survive secondary wastewater treatment, and to accompany treated wastewater discharges into the environment (Snyder et al., 2003; Dickenson et al., 2011).

2. Materials and methods

Suwannee River NOM standard (1R101N, reverse osmosis isolation) was purchased from the International Humic Substances Society (IHSS, http://www.humicsubstances.org/sources.html). All other materials were ACS reagent grade or better and water used for experiments was ultrapure treated (18 MΩ and UV).

2.1. SROM fractionation

SROM was fractionated into hydrophilic (Hi) and hydrophobic (Ho) acids (A), neutrals (N) and bases (B) with resin-based approach. The XAD-8, Amberlyst 15 and Amberlyst 21 resins were purchased from Sigma–Aldrich (St. Louis, MO), cleaned and preconditioned according to Allard et al. (1991). The fractionation procedure was a slight modification of published methods (Leenheer, 1981; Chefetz et al., 1998). See Supplementary materials for details.

2.2. Infrared spectroscopy

Subsamples of freeze-dried SROM fractions were dissolved in ultrapure water for collection of Fourier transform infrared (FTIR) spectra. The GRAMS/AI software Thermo Electron Corp. (Milford, MA) was used for peak deconvolution to determine the position and area of bond vibrations. See Supplementary materials for details.

2.3. Incubation of PPCPs with SROM fractions

Stock solutions for BPA, CBZ, and IBU were prepared at 10.0 mg L⁻¹ in nanopure water with 0.01% (v/v) MeOH co-solvent for 48 h. Substock solutions were then prepared at 20, 40, 400, 2000 μg L⁻¹ in an LC-MS/MS compatible 24 mM volatile NH₄HCO₃ buffer titrated with 0.06 M HCl to pH 7.4 for CBZ and IBU experiments. The limits of detection (signal to noise ratio > 3) were 50 μg L⁻¹ for BPA, 0.15 μg L⁻¹ for CBZ and 0.16 μg L⁻¹ for IBU. Therefore, for BPA, only the 400 and 2000 μg L⁻¹ solutions were used in the LC-MS/MS measurements. SROM fractions were dissolved in the same buffer at 16 mg L⁻¹ DOC stock concentration. PPCP-SROM fraction incubations were performed in triplicate by adding 2 mL of each DOC stock (16 mg L⁻¹) to 2 mL of substock PPCP solution to give a final volume of 4 mL. Negative controls comprising SROM fractions alone (no PPCPs), and positive controls with PPCPs at their respective concentrations but with no SROM, were included. The 4 mL bottles with no headspace were then incubated for 24 h in an end-over-end shaker. A 3 mL aliquot was then transferred to a quartz cuvette for fluorescence spectroscopy and the 1 mL was transferred to autosampler vial for LC–MS/MS analysis.

2.4. Fluorescence measurements

To elucidate the DOM-PPCP interactions, excitation-emission matrices (EEMs) of each SROM fraction were collected in the absence and presence of PPCP analytes. EEMs were obtained with a Jobin Yvon Horiba/Spek Fluoromax-4 fluorometer (Edison, NJ) equipped with a xenon lamp as excitation source. The 3.0 mL subsample for each treatment was placed in a square quartz cuvette cell (light path 10 mm × 10 mm) for EEM collection. The signal to reference detector ratio was collected and EEMs were produced using the FluorEssence software with excitation and emission wavelength ranges of 200–450 nm (5 nm slit) and 250–650 nm (2 nm slit), respectively, both at 5 nm increments. Quenching of DOM fluorescence by PPCP interaction was calculated by subtracting the EEM for DOM fraction-PPCP treatment from that of the corresponding DOM fraction alone. The contribution of the PPCP molecules themselves to total fluorescence in DOM-PPCP systems was negligible relative to that of bulk DOM, such that quenching EEMs represent accurately the diminished fluorescence of a given DOM fraction in the presence of PPCP. Moreover, no inner filtering effects were evident during trial experiments with DOM (Hernandez-Ruiz et al., 2012).

The fluorescence intensity was integrated beneath all five EEM regions previously characterized as (I) “tyrosine-like”, (II) “tryptophan-like”, (III) “fulvic acid-like”, (IV) “microbial byproduct-like”, and (V) “humic acid-like” based on a large reference sample set (Chen et al., 2003). The fluorescence volume (Φ) beneath all
regions of the sample fluorescence (bulk SROM or fractions) EEM surface was calculated as:

\[ \phi = \sum_{k} \frac{I_{\text{Em}k} - I_{\text{Em}k}^{\text{D}}}{I_{\text{Em}k}} \Delta \lambda_{\text{Ex}} \Delta \lambda_{\text{Em}} \]  

(1)

where \( \Delta \lambda_{\text{Ex}} \) is the excitation wavelength interval, \( \Delta \lambda_{\text{Em}} \) is the emission wavelength interval and \( I \) (\( \Delta \lambda_{\text{Ex}} \) and \( \Delta \lambda_{\text{Em}} \)) is the fluorescence intensity >10^4 at each excitation–emission wavelength pair. The intensity cut-off of 10^4 was applied to remove background noise in the SROM samples (e.g., apparent fluorescence at \( \lambda_{\text{Em}} < \lambda_{\text{Ex}} \) (Lakowicz, 1999). For the resulting quenching EEMs [SROM–(SROM + PPCP)] the quenching volume is:

\[ \phi_Q = \sum_{k} \frac{I_{\text{Em}k} - I_{\text{Em}k}^{\text{D}}}{I_{\text{Em}k}} \Delta \lambda_{\text{Ex}} \Delta \lambda_{\text{Em}} \]  

(2)

2.5. PPCPs spike and LC–MS/MS recovery

The recovery of spiked PPCPs following incubation was accomplished using a tandem mass spectrometer (LC–MS/MS) equipped with an Acquity Ultra Performance Liquid Chromatograph and triple quadrupole Quattro Premier XE mass spectrometer, with a sample organizer from Waters Corporation (Milford, MA). All treatments conducted in 24 mM NH₄HCO₃, pH 7.4, in a 24 mM NH₄HCO₃ buffer and pH of 7.4 in the presence and absence of DOM. Analytes were separated following 5 min direct injection onto a reverse phase column maintained at 40°C with a gradient mobile phase from 80:20% (v/v) water:acetonitrile at flow rate 0.3 ml min⁻¹. Recovery was quantified using multiple reaction monitoring (MRM). Fragmentation transitions, collision energies (CEs), capillary voltages (CVs), cone voltages (CNVs), and desolvation temperatures (DTs) are provided in Table S1. Mass lynx software from Waters Corp. (Milford, MA) was used for identification and quantification of analytes. An ANOVA/Tukey’s statistical test (95% CI) was used in Origin 8.5 (Northampton, MA) to assess variance in recoveries from all treatments.

3. Results

3.1. Characterization of SROM hydrophilic and hydrophobic fractions

Quantitative mass balance indicates that, per unit SROM carbon, HoA is the most prevalent fraction (0.63 ± 0.029), followed by HiN (0.11 ± 0.006), HiA (0.09 ± 0.0017), HoN (0.06 ± 0.012), HoB (0.02 ± 0.002) and HiB (0.02 ± 0.007) with a cumulative standard deviation of 0.073 and a total DOC recovery of 93%; (Wickramasekara et al., 2012) observed similar fractionation percentages. FTIR spectra and fluorescence EEMs are unique for each of the obtained fractions (Fig. S1), and consistent with distinct mechanisms of sorptive fractionation (see Supplementary materials for details).

3.2. Fluorescence quenching

Quenching EEMs for each SROM fraction – PPCP pair followed a similar trend irrespective of the contaminant concentration and thus a representative EEM per SROM fraction-PPCPs set is presented (Figs. 1–3).

The addition of BPA to all SROM fractions resulted in reproducible quenching in the low excitation region (200–250 nm) of the matrix throughout the entire emission range corresponding to regions I, II, and III (Fig. 1A–F). However, additional quenching of region V in HoA suggests that aromatic moieties are also involved in molecular interactions. For both basic fractions (HiB and HoB) additional quenching is observed in region IV, attributed to carbohydrates (Her et al., 2003) and aromatic amino acids such as tryptophan and tyrosine (Chen et al., 2003; Her et al., 2003; Hudson et al., 2007; Henderson et al., 2009). Fluorescence quenching by CBZ was apparent in regions I, II and III for all SROM fractions (Fig. 2A–F). However, HoA again displayed prominent quenching in region V (Fig. 2D) and HoB in region IV and V (Fig. 2E). IBU quenched DOM fluorescence in regions I, II and III (Fig. 3A–F) for

Fig. 1. Difference (quenching) EEM of DOM fractions by BPA [(EEM of DOM fraction) – (EEM of DOM fraction + PPCP)]. All treatments conducted in 24 mM NH₄HCO₃, pH 7.4, and 8 mg L⁻¹ of DOC and BPA at 200 and 1000 μg L⁻¹.
all fractions, except for HiN, for which minimal quenching was observed. However, as with CBZ and BPA, addition of IBU also quenched the HoA fraction in region V.

The total normalized quenching plot (normalized to its corresponding bulk SROM or fraction) for bisphenol-A shows that the HiN, HiA and HiB fractions were quenched to a greater degree across treatment concentrations (Fig. S3A). Similarly CBZ quenched the HiB, HiN and HoB fraction to a greater extent the other fractions (Fig. S3B). IBU quenched the HiA, followed by the bulk SROM and the HiB fraction (Fig. S3C).
3.3. LC–MS/MS recovery of PPCPs post SROM fractions exposure

Negative controls, (no PPCP added) produced zero percent recoveries for the three contaminants tested, thus target analytes were undetected in the SROM fractions themselves. All BPA-SROM fractions (200–1000 µg L⁻¹) yielded no statistically significant difference in LC–MS/MS recovery relative to respective positive controls (Fig. 4A). For CBZ, the HiA fraction yielded a significant reduction in recovery (ca. 30%) at all spiked CBZ concentrations. The HoN produced an apparent increase in CBZ recovery (>100%) relative to positive controls at the 10, 20, 100 and 1000 µg L⁻¹ (Fig. 4B). The recovery of IBU in presence of most SROM fractions did not differ significantly from the corresponding positive controls, with the exception again being the HiA fraction, which produced recoveries of about 28% at all spiked concentrations (Fig. 4C).

4. Discussion

4.1. PPCP quenching of DOM fluorescence

Quenching is the result of aqueous phase PPCP-DOM complexation that might be mediated by one or more of several mechanisms of intermolecular association, including hydrophobic, H-bonding, electrostatic, and van der Waals interactions. Mechanisms of interaction leading to the quenching behavior observed (Figs. 1–3) are, therefore, likely dependent on both PPCP and SROM fraction chemistry and structure. At pH 7.4, IBU is anionic and, based on logDOW values, it exhibits the lowest hydrophobicity of the three compounds because of its charge. Conversely, neutral BPA is the most hydrophobic and CBZ is intermediate in hydrophobicity. Hence, based on charge and logDOW characteristics, we hypothesized that BPA and CBZ would exhibit strongest interaction with Ho DOM fractions via van der Waals and/or hydrophobic interactions, whereas IBU was expected to interact with charged Hi DOM fractions either via anion exchange (e.g., on protonated amide or amino groups) or via cation bridging interaction (e.g., with dissociated DOM carboxyl groups).

Quenching of DOM fluorescence was observed to occur in nearly all PPCP-SROM fraction pairs (Figs. 1–3). The greatest total quenching was measured (Fig. S3) for the BPA-HiN, CBZ-HiB, and the IBU-HiA and the lowest quenching was observed for BPA-HoN, CBZ-HoN, and IBU-HiN which is qualitatively inconsistent with the above hypotheses. Indeed, the trends in the extent of quenching indicate the importance of the hydrophilic SROM fractions with basic and acidic functional groups for interactions with neutral and acidic organic contaminants.

A secondary hypothesis was that the hydrophobic DOM fractions would be preferentially quenched in the “humic-acid like” regions of the EEMs, which represent the more aromatic moieties,
particularly by neutral PPCPs (BPA and CBZ). Results revealed that, for HoA, quenching in region V was indeed consistently observed (Figs. 1–3). In fact, there were significant differences in quenching patterns between HoA and all other fractions that showed predominant quenching in the fulvic-acid-like and protein-like regions of the EEMs, and even anionic IBU gave rise to significant quenching of the “humic-acid-like” region of HoA (Fig. 3).

Peptides and proteins (e.g., including amino acids tryptophan and tyrosine) may be zwitterionic at pH 7.4, whereas polyphenols and polysaccharides bear net negative charge due to carboxyl group dissociation. Aliphatic molecules exhibiting a lower degree of carboxylation should exhibit lower net negative charge, as they comprise a greater prevalence of neutral aliphatic and aromatic moieties that are suggested to drive the associations with neutral compounds. Aliphatic molecules exhibiting a lower degree of carboxylation should exhibit lower net negative charge, as they comprise a greater prevalence of neutral aliphatic and aromatic moieties that are suggested to drive the associations with neutral compounds.

The interactions between the SROM fractions and BPA that gave rise to quenching are likely due to van der Waals forces (Sun et al., 2007), π–π interactions between aromatic moieties and/or hydrogen-bonding. Similarly, CBZ could participate in π–π interactions between the stilbene CBZ moiety (Bai et al., 2008) and the aromatics in fulvic acid-like and protein-like fluorophores, in addition to hydrogen bonding. In contrast, IBU produced significant quenching of all fractions except HIN in the protein-like and the fulvic acid regions. Since the carboxylic group of IBU is deprotonated at the experimental pH, anion exchange and/or cation bridging could be mediating the interactions among the carboxylic groups of the SROM fractions and IBU in addition to the hydrogen bonding, π–π and hydrophobic interactions and/or van der Waals associations between the aromatic regions of the protein-like and fulvic acid-like groups.

Despite most fractions being predominantly quenched in the protein-like and fulvic acid-like regions of the EEMs, the additional quenching of HoA in the humic acid region is of particular importance being that this fraction accounts for about 63% of the total DOC contribution. The humic-acid region quenching by CBZ is consistent with prior observations (Bai et al., 2008). Unlike that study, however, the present experiment probed the low excitation (200–250 nm) range as well, that showed significant response.

4.2. Effects of PPCPs-DOM interaction on analyte quantification

Liquid chromatography tandem mass spectrometry has been used previously to probe complex formation among biological molecules. The bonding strength and stability of proteins and ligands with other organic molecules has been assessed by monitoring their MS signal intensity relative to a control (Loo, 2000; Daniel et al., 2002; Bolbach, 2005). For instance, proteins and other biomolecules can interact weakly via hydrophobic interactions and/or van der Waals associations in addition to hydrogen bonding, but then be separated chromatographically to become labile in the gas phase during MS analysis, whereas stronger interactions, such as electrostatic bridging between an opposite-charged organic species or stable cation bridging between carboxylic acids can produce a signal reduction in comparison to a control (Loo, 2000; Schug and Lindner, 2005).

The near 100% LC–MS/MS recoveries of BPA, CBZ, and IBU in the presence of most fractions (Fig. 4) suggests that the majority of interactions giving rise to fluorescence quenching are relatively weak. An important exception was discovered, however, by reaction of HIA with CBZ and IBU, where recoveries decreased to ca. 70% and 30% of the values obtained in controls, respectively (Fig. 4). The chemistry of HIA, which differs significantly from that other fractions, is evidently responsible for this effect. The HIA EEM had the strongest peak in the “fulvic acid-like region” (Fig. S2), which is consistent with intense vibrations deriving from carboxylic and sugar-ring vibrations as observed by FTIR. These DOM constituents interact strongly with more polar target analytes (Navon et al., 2011) and, according to the present study, prevent their quantitative recovery via mass spectrometry. Based on the fluorescence spectroscopy data, plausible mechanisms of IBU–HIA interaction include cation bridging, whereas CBZ–HIA interaction could also include hydrogen bonding at carboxyl or amino functionalities and/or π–π interactions with the ring groups of the CBZ. Likewise, (Maoz and Chefetz, 2010) reported that HIA produced the highest complexity coefficient for CBZ at pH 7, indicating that this fraction may play an important role in environmental fate of CBZ and IBU. It is noteworthy that HIA comprises a relatively low (ca. 9%) contribution to the total SROM carbon mass. Thus, although it has clear potential to strongly interact with ionized PPCPs, its impact on PPCP binding to SROM in surface waters will depend on its prevalence relative to other fractions. Moreover, the near 100% recovery of all three contaminants in presence of bulk SROM (Hernandez-Ruiz et al., 2012) suggests that when the HIA fraction is in its native bulk SROM mixture it has a diminished impact on recovery from the bulk, either because of its lower fractional abundance in that case, or because of its aggregate formation (and hence blocking of reactive sites) in the presence of other SROM components. A comparable effect was observed by Wickrnesakeara et al. (2012), who showed via MS/MS infusion experiments that the HIA fraction produced the most significant decrease in signal MS signal intensity, quantified as a negative matrix effect.

5. Conclusions

The HIA fraction of SROM gave rise to reduced mass spectrometric recovery of spiked CBZ and IBU irrespective of contaminant concentration, suggesting the importance of carboxylic acid functional groups in DOM–PPCP molecular interactions. Thus, chemical interactions of polar contaminants with DOM components of acidic hydrophilic character might preclude the accurate quantitative assessment of similar organic contaminants. Fluorescence spectroscopy proved to be a complementary, efficient and sensitive probe of PPCP interaction with freshwater SROM fractions. PPCPs interacted mostly with protein-like and fulvic acid-like moieties. Probable SROM interactions for all three model contaminants include hydrophobic interactions in addition to hydrogen bonding, π–π interactions and/or cation bridging.

Acknowledgments

Research support was provided by the Binational Agricultural Research and Development (BARD) fund, Grant # IS-3822-06, the Water Research Foundation (Award #4269) and the University of Arizona Water Sustainability Program. The comments and views detailed herein may be necessarily reflecting the views of the Water Research Foundation, its officers, directors, affiliates, or agents. Analyses in the ALEC were supported by NSF Grant CBET-0722579.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.11.059.

References
