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Quantification of Quinones in Environmental Media by Chemical Tagging with Cysteine-Containing Peptides Coupled to Size Exclusionary Separation

Anil Timilsina, Srinidhi Lokesh, Abrar Shahriar, Travis Numan, and Yu Yang*

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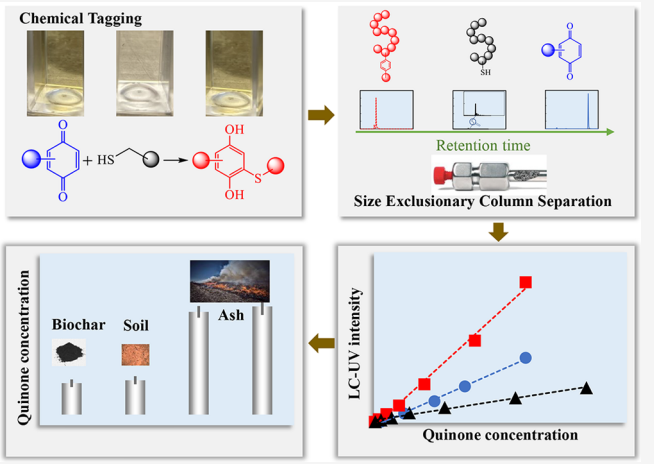
ABSTRACT: Quinones are one of the most important redox-reactive organic compounds in natural environments, such as soil, water, and sediment, playing an important role in regulating the environmental processes and biogeochemical cycles of critical elements under climate change, including the influences of extreme events such as wildfires. However, to date, no existing methods can quantify quinones in complex environmental media. To overcome this challenge, a quantification method was developed by coupling chemical tagging of quinones by cysteine-containing nonaromatic peptides (Cpep) through a Michael addition reaction with size exclusionary chromatography (SEC) separation and ultraviolet (UV) analysis—leveraging on the characteristic absorbance of aromatic rings at 254 nm and molecular size of peptide. The method was demonstrated using model quinones, including 1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), and 1,4-anthraquinone (AQ), with a detection limit of 3.3, 0.7, and 0.2 μM, respectively. Concentrations of quinones in water extractions of biochars, soils, and wildfire-derived ashes were determined to range from 0.8 to 14 μM and were positively correlated with their redox reactivity determined by a chemical assay. This method provides a novel rapid quantification of quinones in complex environmental media as well as a quick assessment for redox reactivity and opens up new avenues for studying environmental transformation and remediation of contaminants.



INTRODUCTION

Quinones are one of the most important classes of organic carbon in natural soil and water environments for their redox reactivity, with significant implications on the biogeochemical cycles of numerous elements (e.g., carbon (C), nitrogen (N), phosphorus (P), iron (Fe) and others), as well as on the degradation of pollutants, natural attenuation, and water treatment.[1−5] Natural organic matters in soils and water were determined to contain reversible redox sites,[6] for which quinones are critical constituents.[7] Quinones can be used by microbes as electron donors/acceptors and accelerate the reduction of Fe-bearing minerals, such as goethite,[8] hematite,[9] and ferrihydrite,[10] and promote the release of mineral-bound organic matter and associated nutritional elements, such as C and N.[11−13] Understanding the fate of quinones is also important for predicting the impact of climate change on the biogeochemical cycles of critical elements (C, N, P, and otehrs), including the influences of wildfires.[14−16] In the redox reactions of arsenic (As), semiquinones (one-electron reduction form of quinones) can act as electron acceptors and regulate the oxidation state and mobility of As.[17] Degradation of organohalogen compounds, important for natural attenuation and engineering treatments of contaminated water bodies, can also be promoted by the presence of quinones.[5−7,18]

Despite the significance of quinones in environmental and geochemical reactions as well as engineering applications for pollutant removal, such as the use of biochars, no available techniques can quantify quinones in complex natural environmental samples. Electron paramagnetic resonance (EPR) analysis has been widely used to demonstrate the presence of semiquinone radicals based on the detection of unpaired electrons,[19,20] but quantification of quinones by EPR is hampered by weak signals, the presence of other types of environmentally persistent free radicals (EPFR), the stability of

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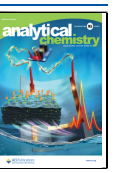

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semiquinones, and the variation in quinone structures.[21−23] Other approaches, such as semiquantification of trimethyl phosphite-tagged quinones by ^{31}P nuclear magnetic resonance (^{31}P NMR), were used for measuring quinones, and it is challenging to measure low-concentration quinones in complex environmental samples.[24] So far, none of the existing methods can quantify total quinones in environmental samples, which is critical for understanding their redox reactivity.

Coupling a specific chemical tagging reaction with quantitative analyses can provide opportunities to measure quinones in environmental samples where relevant efforts are still limited.[24,25] This study aimed to develop a novel method for quantifying low-molecular-weight quinones in environmental samples through chemical tagging with cysteine-containing peptides (Cpep) coupled with size exclusion chromatography (SEC) and ultraviolet (UV) absorption at 254 nm (Figure 1). Previous research has shown that the

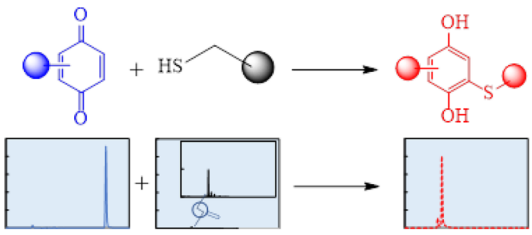

Figure 1. Scheme of the reaction used to quantify quinones in this study. Balls (blue, black, and red) represent substituted groups in quinones, cysteine-containing nonaromatic peptides, and adducts. Size-exclusionary chromatography-ultraviolet (UV) spectra show the representative results for quinones (long retention time and high absorption), cysteine-containing nonaromatic peptides (short retention time and low absorption with inset showing enlarged result), and adducts (short retention time and high absorption).

Michael addition reaction between quinones and (thiol groups in) cysteine or Cpep can tag peptides and proteins in biological samples,[26] but to the best of our knowledge, such a reaction has never been used for tagging quinones in environmental samples. In this study, a high-molecular-weight nonaromatic Cpep was used to tag low-molecular-weight quinones in environmental samples specifically, and the high-molecular-weight tagged products were chromatographically resolved using SEC and quantified by UV absorbance at 254 nm. A nonaromatic Cpep was specifically used for this study to minimize background absorbance at 254 nm. This entire procedure took around 60 min (30 min for tagging and 30 min) for SEC-UV analysis. Model quinones, including 1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), and 1,4-anthraquinone (AQ), were utilized to demonstrate this technique and develop calibration curves for the quantification of quinones. The selectivity of this tagging method was investigated by analyzing the influences of possible co-occurring organic compounds with common functional groups such as phenolic, carboxylic, and catechol groups. Finally, quinones in environmental samples were analyzed using the method developed, and correlations between the quinone concentrations and the redox reactivity of samples were analyzed.

RESULTS AND DISCUSSION

Analysis of Model Compounds. Adducts formed by the Michael addition reaction between Cpep and model quinones (BQ, NQ, AQ) were captured based on the absorption of aromatic rings at 254 nm with a short retention time (9.0−10.9 min) after SEC separation (Figure 2). Cpep (nominal MW = 1123) has the highest absorbance at 214 nm and was eluted at the retention time of 8.6 min, consistent with the elution pattern of standards through the SEC column when its absorbance at 254 nm was low (intensity of 4.7 for 100 μM Cpep) (SI, Figures S2, S3). Differently, the model compounds were eluted with longer retention times of 12.6 and 24.4 min for BQ and NQ, respectively, when AQ was not eluted out within 30 min. The Michael addition reaction products were eluted with relatively short retention times of 9.0, 9.0, and 10.9 min for the adducts of BQ-Cpep (MW = 1231), NQ-Cpep (MW = 1279), and AQ-Cpep (MW = 1329), respectively, consistent with the short retention time of standard polymer (MW = 1000) at 10 min. Cpep adducts exhibit significantly higher absorbance (intensity of 23−200) at 254 nm than Cpep.

The formation of Cpep-quinone adducts was confirmed by time-of-flight mass spectrometry (TOF-MS) analysis of captured features (Figure 2; SI, Figure S4). In ESI+ mode, Cpep [M+H$^+$] with m/z = 1123.429 and Cpep [M+2H^{2+}] with m/z = 562.218 were captured; however, quinones were not captured due to their low ionization in ESI, as reported previously.[27] New features with m/z = 1231.450, 1279.453, and 1329.466 were captured for the [M+H$^+$] adduct products of BQ-Cpep, NQ-Cpep, and AQ-Cpep, respectively, for the reaction products. It was noted that the adduct product of BQ-Cpep was the hydroquinone form,[26] but the adduct products of NQ-Cpep and AQ-Cpep were quinones, possibly due to the reoxidation in the presence of oxygen or quinones, consistent with published reports.[28,29]

Additional analysis for the reaction of BQ with Cpep in the presence of other model compounds for possible co-occurring functional groups demonstrated the selectivity of Cpep toward quinone functional groups. The SEC-UV peaks for cinnamic acid (CnA) (with a carboxylic group), coumaric acid (CoA) (with a carboxylic group and phenolic group), and caffeic acid (CaA) (with a carboxylic group and catechol group) were eluted at 18, 20, and 21 min, respectively (SI, Figure S5). After mixing with Cpep for 30 min, no adducts were captured for all of these compounds. BQ spiked to a mixture of these possible co-occurring compounds reacted with Cpep as efficiently as between only BQ and Cpep.

Calibration Curves and Detection Limit. Good linearity was obtained between the signal area and concentrations of quinones (R^2 > 0.98) from the SEC UV analysis (SI, Table S1). For environmental samples, the range of quinone concentrations can be calculated using model quinone-based calibration curves (Figure 3). The detection limits for BQ, NQ, and AQ were 3.3, 0.7, and 0.2 μM, respectively. To validate the method for complex environmental samples, an aqueous extract of Swiss biochar was used as representative complex matrix. The method recovery determined for Swiss biochar extract (SBE) was 98.47 ± 4.59, 95.09 ± 3.40, and 95.51 ± 5.20% for BQ, NQ, and AQ spiked to SBE. When no existing methods can quantify total quinones in environmental samples, as a comparison, the reported EPFR in biochar samples ranged from 0.06 × 10^{18} to 521 × 10^{18} spins/g,[30] equal to 0.1−865.44 μmol/g (based on only standard of 2,2-diphenyl-1-picrylhydrazyl radical) comparable to the detection limit of quinones demonstrated for this method. The method detection limit can be further improved using a larger volume of samples for

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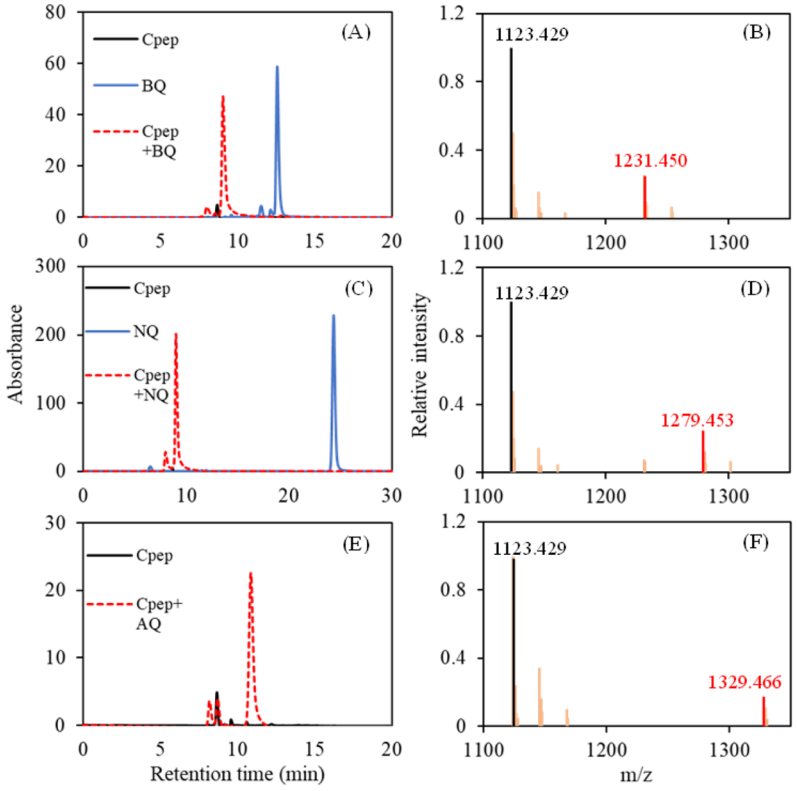

Figure 2. High-performance liquid chromatography (HPLC-UV) (A, C, E) and time-of-flight mass spectrometry (ESI-TOF-MS) profiles (B, D, F) of quinones and cysteine contained peptide (Cpep) reactions: (A) and (B) for 50 μM 1,4-benzoquinone (BQ) + 100 μM Cpep reaction, (C) and (D) 50 μM 1,4-naphthoquinones (NQ) + 100 μM Cpep reaction, and (E) and (F) for 50 μM 1,4-anthraquinone (AQ) + 100 μM Cpep reaction, respectively.

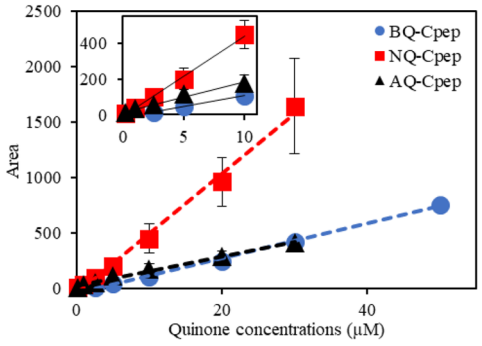

Figure 3. Calibration curves for model quinone compounds: BQ, 1,4-benzoquinone; NQ, 1,4-naphthoquinone; AQ, 1,4-anthraquinone. Note: Error bars of standard deviation can be smaller than the symbol size.

concentration and analysis. When our method is applied directly to the extractable components of environmental media, such as biochars, absolute quantification requires optimal extraction; such extraction methods are under our current investigation.

Quantification of Quinones in Environmental Samples and Link with Redox Reactivity. Cpep-quinone adducts were captured for all environmental samples by coupling the chemical tagging with Cpep, SEC separation, and UV (254 nm) measurement (SI, Figures S6, S7). Quinone concentrations in environmental samples ranged from 4 to 14 μM, from 0.8 to 4 μM, and from 0.9 to 9.5 μM, when using the calibration curves based on BQ, NQ, and AQ, respectively (Figure 4). The calculated concentrations of quinones varied by a factor of 2−4 folds upon the calibration curves used, for

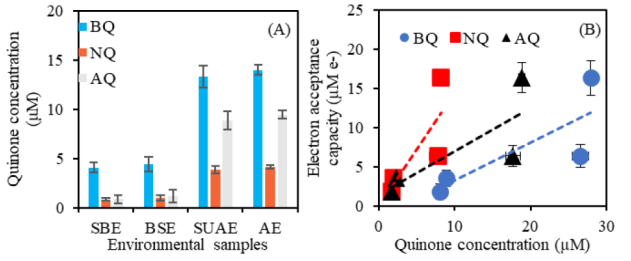

Figure 4. Quinone concentrations (A) and their correlation with redox reactivity measured by a chemical assay (B) of environmental samples. Environmental samples include Swiss biochar extract (SBE), background soil extract (BSE), soil-under-ash extract (SUAE), and ash extract (AE). Calibration curves based on 1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), and 1,4-anthraquinone (AQ) were used to quantify quinone concentrations. Error bars represent standard deviations from duplicate samples.

which improvement is needed in the next-step investigation to identify the dominant structures of low molecular weight quinones in environmental samples. Among the tested environmental samples, the quinone concentrations were found highest in ash extract (AE) (4.1−14.0 μM), followed by soil under ash extract (SUAE) (3.9−13.3 μM). The background soil extract (BSE) and SBE had similar quinone concentrations. When normalized to the dissolved organic carbon (DOC) concentration, quinones represented a

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Anal. Chem. 2023, 95, 12575−12579

significant fraction of DOC for SBE (11.34% of DOC) (SI, Table S2). The Swiss biochar used in this experiment was produced by pyrolysis at 700 °C, which was demonstrated to promote the formation of quinone structures.[31] A recently developed colorimetric assay determined the EPFR in biochars ranged 1.57−38.66 × 10^{18} spins/g (using suspension of 10 mg biochar/1 mL) equivalent to 26−642 μM spins in biochar extracts, correspondence to carbon-centered radicals according to a g-factor of 2.0024−2.0032 of EPR analysis.[32] The accurate contributions of semiquinone to EPFR require further investigation, but comparisons of our measurement of total quinones to EPFR concentrations indicate the semiquinones might be relatively a small fraction of EPFR.

The chemical assay of redox reactivity revealed the electron-accepting capacity of environmental samples ranging from 1.86 μM in SBE to 16.37 μM in AE (SI, Figure S8). There was a significant positive correlation (Pearsons correlation coefficient $r = 0.81$, $p < 0.01$) between concentrations of quinones in environmental samples and their electron-accepting capacity (Figure 4). Assuming all quinones were fully reduced to hydroquinones during the microbial reduction for the electron-accepting capacity measurement, the electron-accepting capacity of environmental samples was calculated to be 1.7−8.1, 1.9−8.9, 7.8−26.6, and 8.2−27.0 μM electrons for SBE, BSE, SUAE, and AE, respectively, comparable with those measured by the chemical assay. All of these results supported the contribution of quinones to the redox reactivity of these environmental samples. Similar studies on biochemical assay of redox reactivity measurement for Swiss biochar aqueous extracts (1.2 mg-C/L) measured electron accepting capacity of 0.05 mol e^-/mol-C (equal to 5 μM electron acceptance capacity),[33] which is close to our chemical assay measurement and lies within the range of redox reactivity speculation via quinone quantification.

In summary, this study developed a novel rapid method for quantifying quinones in complex environmental samples, which has been needed for a long time. Measurement of quinones in representative samples supported their important roles in redox reactivity when further analysis can more accurately determine the speciation and properties of quinones, with implications for the reduction/oxidation of Fe-bearing minerals, mobilization of carbon and other nutritional elements under climate change including the influences of wildfires, degradation of redox-sensitive contaminants, and application of biochars in engineering treatments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c01224.

Text S1 for experimental details. Figures S1−S8 including chemical structures of model compounds, SEC-UV spectra of peptide standards and quinone-peptide adducts, TOF-MS isotope spectra of reaction products for model quinones, SEC-UV spectra for the influences of co-occurring compounds, SEC-UV spectra of environmental samples, and redox reactivity assessment results. Table S1 showing linear regression modeling data of calibration curves. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Yu Yang − *Department of Civil and Environmental Engineering, University of Nevada, Reno, Reno, Nevada 89557, United States;* orcid.org/0000-0002-7568-0202; Email: yuy@unr.edu

Authors

Anil Timilsina − *Department of Civil and Environmental Engineering, University of Nevada, Reno, Reno, Nevada 89557, United States*

Srinidhi Lokesh − *Department of Civil and Environmental Engineering, University of Nevada, Reno, Reno, Nevada 89557, United States*

Abrar Shahriar − *Department of Civil and Environmental Engineering, University of Nevada, Reno, Reno, Nevada 89557, United States*

Travis Numan − *Department of Civil and Environmental Engineering, University of Nevada, Reno, Reno, Nevada 89557, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.3c01224

Author Contributions

Anil Timilsina and Srinidhi Lokesh contributed to the work equally.

Notes

The authors declare no competing financial interest.

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