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RESEARCH PAPER

Separation and characterization of sulfonates in dissolved organic matter from industrial wastewater by solid phase extraction and high-resolution mass spectrometry

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Abstract

Electrospray ionization (ESI) source combined with high-resolution mass spectrometry (HRMS) has been successfully used to characterize the molecular composition of dissolved organic matter (DOM) in industrial wastewaters. However, sulfonates are commonly presenting in these wastewaters and showing strong ionization suppression for the ESI analysis, which seriously affects the characterization of other DOM components. In this paper, a novel method based on polymer anion exchange (PAX) solid phase extraction (SPE) was proposed to separate DOMs from petroleum refining and coal coking wastewaters into hydrophobic neutral, hydrophobic acids, and hydrophobic strong acids; the fractions were analyzed by negative-ion ESI Fourier transform ion cyclotron resonance mass spectrometry. The results show that PAX-SPE separated sulfonates from wastewaters and enabled the molecular characterization of oxygen-containing compounds, which are the major component of DOM in the wastewaters. Semi-quantitative analysis of sulfonates in the wastewaters was carried out and the results show that these compounds account for 11% and 2% in total organic carbon for the refinery and coal coking wastewaters, respectively. PAX-SPE with stepwise elution and followed by high-resolution mass spectrometry analysis enables a comprehensive characterization for the molecular composition of industrial wastewater DOM. The method has potential for extensive application and the results are instructive for the understanding of the molecular composition of DOM in industrial wastewaters.

Keywords Industrial wastewater · Dissolved organic matter · Sulfonates · High-resolution mass spectrometry · Molecular characterization

Introduction

With the discharge standards becoming stricter, the cost of industrial wastewater treatment is increasing. Even so, the quality of discharged water is still hard to meet the discharge standard. Dissolved organic matter (DOM) is an important organic pollutant in industrial wastewater, which varies greatly in concentration and composition among different samples [1, 2]. DOM has super complex molecular composition; a better understanding on chemical composition of DOM in wastewaters is instructive for the design and optimization of water treatment technologies.

Generally, bulk properties such as COD (chemical oxygen demand) were used to evaluate the quality of industrial wastewater, but the changes of pollutants in the treatment process cannot be well characterized without molecular level information. In the past decade, negative-ion electrospray ionization (ESI) combined with high-resolution mass spectrometry (HRMS) has been successfully applied to analyze the molecular composition of wastewater DOMs in many researches relevant to environmental science and engineering [3–6]. Most acidic oxygen-containing compounds (including phenols, carboxylic acids, and sulfonic acids) and neutral nitrogen-containing compounds in DOM have been characterized by the approach [7–10]. However, the ionization efficiency of these compounds is largely different in ESI, among which sulfonates have very strong ionization suppression effect on carboxylic acids and phenols [11, 12]. This will lead to a loss of

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compositional information of other compounds in DOMs [13, 14]. Sulfonates are widely used in various fields including chemical industry, pesticide, paper making, and so on, accounting for about 70% of the total production of surfactant [15]. For instance, sulfonate surfactants are often added as dispersants to reduce the viscosity of coal slurry and make it have better fluidity and manifold in coal processing [16, 17]. In addition, a large amount of sulfonate oil-displacing agent are injected into the stratums during crude oil exploitation. These synthetic sulfonates will eventually migrate into wastewaters, affecting the molecular characterization of DOM by ESI MS analysis. Therefore, separation of sulfonates from other organic compounds is required to achieve a comprehensive characterization of the DOMs in wastewaters.

At present, liquid-liquid extraction (LLE) and reversed solid phase extraction (R-SPE, such PPL, C_{18}, and HLB) are commonly used to separate DOM from water [18–20]. However, LLE is only applicable to wastewaters containing high concentrations (g/L magnitudes) of sulfonates and usually used to recover raw materials or products from wastewaters [21–23]. R-SPE by single-step elution cannot separate acidic oxygen-containing compounds from sulfonates [8, 24, 25]. The polymer anion exchange (PAX) SPE filled with a hydrophilic styrene-divinylbenzene copolymer modified by quaternary ammonium salt has a good retention ability for ionic compounds carrying acidic groups in water, and the different polar compounds have a different retention ability. PAX has good adsorption effect in the range of pH 0–14 [7]. Therefore, PAX has a potential advantage on separating sulfonates from acidic oxygen-containing compounds in wastewater DOMs.

In this paper, a novel method based on PAX-SPE was proposed to separate DOMs from electric desalination wastewater from a petroleum refinery and coal coking wastewater from a coal chemical plant. DOMs from the wastewaters were separated into three fractions according to the acidity of compounds, namely hydrophobic neutral (HON), hydrophobic acids (HOA), and sulfonic acids (HOSA). The subfractions were analyzed by negative-ion ESI coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in order to track the trend of various compounds in the separation process, and to reveal the comprehensive composition of DOM in wastewaters from the molecular level. Sulfonic acids in the PPL (a reversed styrene-divinylbenzene copolymer sorbent SPE) and PAX-HOSA extracts were semi-quantitively characterized by negative-ion ESI Orbitrap MS. The separation efficiency of the PAX method on sulfonates was evaluated according to the total organic carbon (TOC) and semi-quantitative results by ESI Orbitrap MS.

Sample and method

Reagents and samples

Methanol and ultrapure water (all LC-MS grade) were purchased from Fisher Scientific (USA). Analytical grade reagents of formic acids, ammonium, sodium hydroxide, and hydrochloric acid (HCl, 1 mol/L) were obtained from Sinopharm (China). Sodium 4-n-octylbenzenesulfonate ($C_{14}H_{21}NaO_3S$, 98%) was obtained from Alfa Aesar (China).

The electric desalting wastewater (EDW) was the backwash efflux of the crude oil electric desalting unit in a petroleum refinery. The coal coking wastewater (CCW) was a concentrate effluent from the biochemical unit of a wastewater treatment plant in a coal chemical plant. The wastewaters were filtered by a 0.45-μm filter membrane and sealed in a dark place at 4 °C before analysis.

Solid phase extraction and fractionation of wastewaters

PPL cartridgeextraction

Activation and elution PPL cartridge methods refer to Dittmar et al. [26], and the process diagram is shown in Fig. 1 (left). The PPL cartridge (Bond Elut-500 mg 6 mL, Agilent, USA) was activated by 20 mL methanol and 20 mL ultrapure water with pH = 2. One milliliter EDW and 5 mL CCW were diluted to 20 mL. The diluted wastewater samples were acidified to pH = 2 with HCl and then passed through the PPL cartridge at a rate of 5 mL min/L, respectively. After loading sample, the cartridge was washed with 20 mL acidic water (pH = 2, HCl) to remove salts and polar compounds that cannot be retained on the adsorbent, and then dried with nitrogen purge at room temperature. After that, the cartridge was washed with 10 mL methanol to obtain the DOM, which was labeled as PPL fraction.

PAX cartridge extraction

Activation and elution PAX cartridge methods refer to Fang et al. [7], and the process diagram is shown in Fig. 1 (right). A PAX cartridge (Bond Elut Plexa PAX 500 mg, 6 mL, Agilent, USA) was activated by 20 mL methanol and 20 mL water (pH = 12, NaOH). One milliliter EDW and 5 mL CCW were diluted to 20 mL, respectively. The diluted wastewaters were alkalified to pH = 12 with NaOH and then passed through the PAX cartridge at a rate of 5 mL/min. After loading sample, the cartridge was washed with 20 mL water (pH = 12, NaOH) to remove salts. The PAX cartridge was eluted with 10 mL methanol, 10 mL methanol with 2 v% HCOOH, and 10 mL methanol with 1 v% HCl to obtain

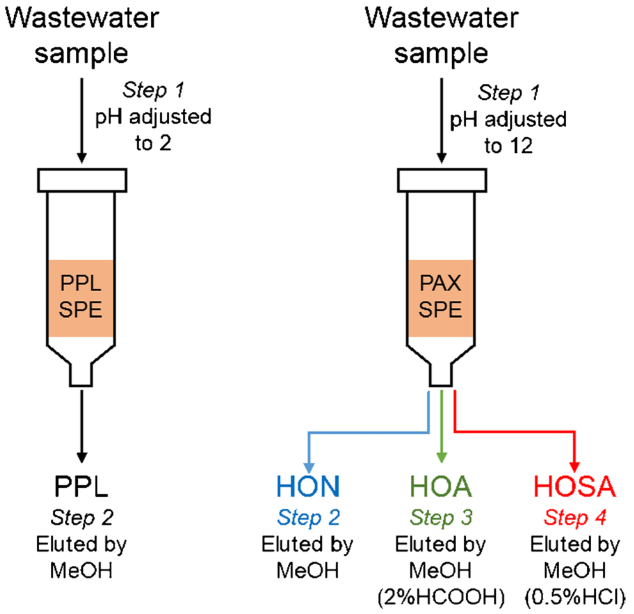

Fig. 1 Scheme of the solid phase extraction of DOM in industrial wastewaters with PPL cartridge (left) and PAX cartridge (right)

hydrophobic neutral (HON), hydrophobic acids (HOA), and hydrophobic strong acids (HOSA), respectively. The fraction of HON, HOA, and HOSA fractions were dried with nitrogen purge and redissolved with 10 mL methanol under 70 °C.

Total organic carbon

A total organic carbon (TOC) analyzer (TOC-5000A, Shimadzu, Japan) was used to determine the total organic carbon in the raw wastewaters and their subfractions. For DOM fractions, a known volume of methanol solution of the DOM was blow-dried by nitrogen at 70 °C and then dissolved in 20 mL of ultrapure water for TOC analysis (TOC results are converted to the concentration under raw water volume).

Negative-ion FT-ICR MS analysis

The HON, HOA, HOSA, and PPL fractions were analyzed using an Apex-ultra FT-ICR mass spectrometer (Bruker, Germany) equipped with a 9.4-T superconducting magnet and an Apollo II electrospray ion source. The samples were diluted with methanol to a concentration about 100 mg/L and injected into the electrospray source at 250 μL/h with a syringe pump. The operating conditions for negative-ion ESI were as follows: spray shield voltage, 4.0 kV; capillary column introduce voltage, 4.5 kV; capillary column end voltage, −320 V. The mass range was set at *m/z* 150–800. A 4 M word size was selected for the time domain signal acquisition. The signal-to-noise ratio (S/N) was enhanced by adding 128 domain transients.

Mass spectrometry data analysis

Molecular formula assignment was carried out using an in-house software during data analysis [27, 28]. Briefly, the FT-ICR MS was calibrated using a known homologous series of the SRFA sample (standard matter obtained from IHSS, USA), which contains high relative abundances of oxygen-containing compounds. For FT-ICR MS, mass peaks with S/N greater than 4 were exported to a data sheet. For Orbitrap MS, mass lists of Orbitrap MS were internally calibrated with a high-abundance homologous series and extracted using Xcalibur Qual Browser (Thermo Fisher). After averaging all scans in the collected 2-min spectrum, noise peaks with relative intensity below 0.1% were discarded.

A molecular formula calculator generated matching formulae according to elemental combinations of $^{12}C_{1-60}$, $^{1}H_{1-120}$, $^{14}N_{0-5}$, $^{16}O_{0-30}$, and $^{32}S_{0-3}$. The mass accuracy window was set to 1.0 ppm in the formula assignment section. All elemental formulae should meet basic chemical criteria: (1) the number of H atoms should be at least 1/3 that of C atoms and cannot be greater than that of 2C + N + 2; (2) the sum number of N and H atoms should be even; and (3) the H/C and O/C value should be restricted to be less than 3 and 1.5, respectively.

Semi-quantitative analysis of sulfonates by negative-ion Orbitrap MS

Sulfonates in the wastewaters and their fractions were semi-quantified by an Orbitrap Fusion MS (Thermo Fisher Scientific, USA). The HOSA and PPL fractions of these two wastewaters samples were diluted 10 times with methanol and were divided into 10 equal parts. The $C_{14}H_{21}NaO_3S$ was added into the diluted samples as the internal standards for semi-quantitative analyses by negative-ion ESI Orbitrap MS. The concentrations of internal standards were 0, 0.5, 1, 3, and 5 μg•L^{-1} in EDW and were 0, 5, 10, 30, and 50 μg•L^{-1} in CCW, respectively. The samples were injected into the electrospray source at 300 μL/h with a syringe pump. The main parameters were set as follows: sheath gas flow rate, 5.0 Arb; auxiliary gas flow, 2.0 Arb; ion transfer tube temperature, 300 °C; evaporation temperature, 20 °C. The MS resolution was up to 500,000 (@ *m/z* 200). Negative-ion spray voltage is from A1 (2600 V) to A6 (3600 V). Each mass spectrum was detected in 2 min with a mass range of *m/z* 150–800. Tandem mass spectrometry (MS/MS) analysis was carried out by high-energy collision dissociation (HCD) with collision energy of 0−45 eV.

The MS detected sulfonate ions instead of sulfonate compounds, so the quantitative results are based on the sulfonic acids. The highest peak in the sample was selected as the base peak; the intensity of internal standard was divided by the base peak intensity to obtain the normalized relative intensity. The response coefficient of sulfonic acids was obtained according to the fitting curve of the signal intensity of the normalized internal standard peak and the molar concentration of internal standard. Three spectra with different concentrations of internal standard were selected for analysis of sulfonic acid content.

According to the internal standard of different concentrations, the linear response curve and equation can be obtained: $y = Kx$+A. x is the relative intensity, and y is the internal standard molality; the slope K is obtained by fitting internal standard molality concentration with the relative intensity of the internal standard. The mass concentration of sulfonic acids is defined as ρ, and the organic carbon concentration contributed by sulfonic acids is defined as ρ_{TOC}.

Formula for calculating the mass concentration of sulfonic acids:

$$\rho = \sum \text{(Peak intensity of sulfonic acids/Base peak intensity)} \times \text{Molecular weight} \times K \tag{1}$$

Formula for calculating the organic carbon concentration contributed by sulfonic acids:

$$\rho_{\text{TOC}} = \sum(\text{Peak intensity } \textit{of} \text{ sulfonic acids/Base peak intensity} \times K \times \text{carbon number} \times 12) \tag{2}$$

Results and discussion

TOC recoveries of the SPE separation

DOMs in the wastewaters of EDW and CCW were fractionated by the PAX and PPL extraction into HON, HOA, HOSA, and PPL fractions. The yields in TOC and their actual TOC in raw water are listed in Table 1. For EDW, a total of 64.38% of TOC was extracted from the water by PAX, including HON (24.0%), HOA (28.0%), and HOSA (12.5%), while the yield by PPL is 29.6%. For CCW, a total of 30.48% of TOC was extracted from the water by PAX, including HON (10. 8%), HOA (12.9%), and HOSA (6.7%), while the yield by PPL is 22.8%. The TOC results were the average values of triple measurements.

TOC results showed that, compared with PPL, PAX had a higher TOC recovery for the wastewaters. Previous studies have shown that the TOC recovery for surface water by the PPL method was about 30–70% [26]. In this study, the TOC recoveries of PPL in the two kinds of industrial wastewaters were both below 30%. The one reason was that volatile organic compounds in industrial wastewaters accounted for a higher proportion in TOC, and these volatile components in the eluent would lose in the process of nitrogen blowing. On the other hand, the industrial wastewaters contain more weak polar DOMs than natural waters. These weak polar compounds tend to be permanently adsorbed on the column.

HON and HOA fractions have similar yields in these industrial wastewaters, and are the main component of the DOMs extracted by PAX. The HOSA fraction was eluted by methanol with HCl, which enriched sulfonic acids. Comparing with HON and HOA fractions, TOC concentration of HOSA fraction is the lowest, which indicated that sulfonates had a low contribution to TOC and only accounted for a little few parts of wastewaters DOMs. This conclusion will be further discussed in the "Semi-quantitative analysis of sulfonates by negative-ion ESI Orbitrap MS" section.

The PAX SPE has obvious advantages over reverse phase retention SPE in TOC yield. Selecting an appropriate DOM extraction method can help us obtain more DOM from wastewaters.

Molecular composition of extracted DOMs

All PAX and PPL extracts from EDW and CCW were analyzed by negative-ion ESI FT-ICR MS and the broadband mass spectra can be found in Supporting Information. Mass peaks were unambiguously assigned to specific molecular composition based on accurate mass values obtained by the MS. Molecular composition of each fraction is different from others, which can be observed visually from Fig. 2. Compounds detected in HON and HOA fraction are mainly O3-O5 and O3-O7 class species, respectively, while O3S1

Table 1 TOC of the electric desalting wastewater (EDW) and coal coking wastewater (CCW) and their fractions extracted by PAX and PPL (mg/L)

Sample	Raw water	PAX				PPL
		HON	HOA	HOSA	Total	
EDW	227±7	54.5±1.4 (24.0%±0.6%)	63.4±1.6 (28.0%±0.9%)	28.3±2.2 (12.5%±1.0%)	146.2±5.3 (64.4%±2.3%)	67.3±1.2 (29.6%±0.5%)
CCW	1220±12	131.5±6.2 (10.8%±0.5%)	158.0±10.1 (12.9%±0.8%)	82.3±3.0 (6.7%±0.2%)	371.8±19.3 (30.5%±1.6%)	277.6±12.1 (22.8%±1.0%)

and O4S1 class species are dominant in HOSA and PPL fractions. The mass scale expanded mass spectra indicate that the oxygen-containing compounds with low oxygen atom number were mainly enriched in HON fraction. More oxygen-containing compounds with high oxygen atom number were enriched in HOA fraction. HOSA and PPL fractions were highly similar, and only OxS class species peaks could be detected.

The relative abundance plots of class species assigned from negative-ion ESI FT-ICR MS of EDW are shown in Fig. 3, in which compounds with different double bond equivalent (DBE) of each class species are shown in different colors. A total of 18, 30, and 10 class species were assigned in HON, HOA, and HOSA fractions, respectively. Only seven class species were found in PPL fraction. Class species detected in the three fractions of PAX included O1-O10, O2S-O6S, NO1S-NO4S, NO1-NO8, and N2O4-N2O7. The HON and HOA fractions were dominated by oxygen-containing compounds, accounting for 62% and 94% in relative abundance of all assigned compounds, respectively. The sulfur compounds (including OxS and NOxS) were dominant in HOSA fraction, accounting for 98%. The class species assigned in PPL fraction include O2-O4 and O3S-O6S, among which sulfur compounds are dominant (98%) (see Table S1 in Supporting Information).

The MS results for PPL and HOSA fractions were similar (as shown in Fig. 3). The Ox classes with low oxygen number were detected but in very low relative abundance. O3S1 class species were predominant; O4S1-O6S1 class species were also detected in low relative abundance. The HON and HOA fractions contained a variety of oxygen-containing and nitrogen-containing compounds, which could not be detected in PPL fraction due to ionization suppression by extremely high response of sulfonic acids in the ESI analysis.

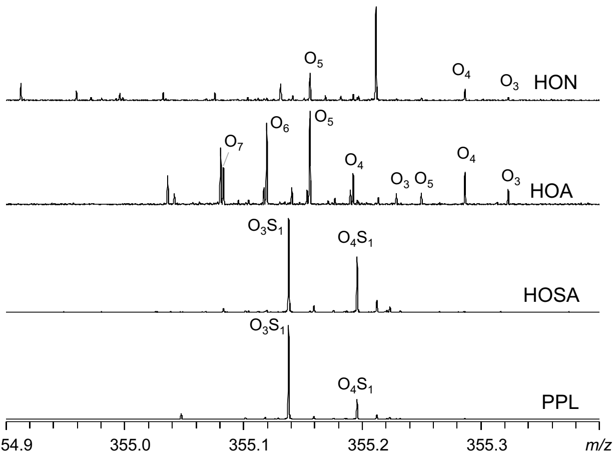

Fig. 2 Mass scale expanded FT-ICR mass spectra at *m/z* 355 of the HON, HOA, HOSA, and PPL fractions from the electric desalting wastewater

Figure 4 shows the relative abundance plots of class species assigned from negative-ion ESI FT-ICR MS of CCW. A total of 44, 41, and 48 heteroatom class species were assigned in HON, HOA, and HOSA fractions, respectively. There were 27 sulfur-containing class species in HOSA fraction. Only 39 class species were found in PPL fraction, among which 28 class species contained sulfur atom. Class species identified in three fractions of PAX were O1-O14, O3S-O13S, NO2S-NO12S, N2O3S-N2O9S, NO1-NO10, N2O1-N2O7, and N3O1-N3O6. The HON and HOA fractions were dominated by oxygen-containing compounds, accounting for 80% and 68% in relative intensity of all assigned compounds, respectively. And the sulfur compounds (including OxS, NOxS, N2OxS) in HOSA fraction accounted for an absolute majority, accounting for 93% (Table S1). In addition, the class species assigned in PPL fraction were O1-O6, O3S-O9S, NO2S-NO9S, N2O2S-N2O8S, NO1-NO6, and N2O1-N2O5, among which sulfur compounds accounted for an absolute majority, reaching 92%, which was similar to HOSA fraction (Table S1). Compared with PPL fraction, more information about DOM molecular compositions was detected in subfractions of CCW after PAX separation.

According to the molecular composition, the O3S1 classes are likely organic sulfonic acids, while O4S1 classes could be organic sulfuric or sulfonic acids. In order to investigate the structure of these sulfur-containing compounds, the peaks of *m/z* 201.0227 ($C_8H_9O_4S$), *m/z* 234.0230 ($C_{11}H_8NO_3S$), and *m/z* 249.0227 ($C_9H_{12}O_4S_1$) in CCW were characterized by MS/MS analysis using the Orbitrap MS (results are shown in Figure S3 which can be found in Supporting Information). The fragment ion peaks at *m/z* 80.9650 and *m/z* 79.9570, which show high relative intensities in the MS/MS spectra, were identified as HO_3S^- and O_3S^{2-}. The results imply that the CHOS and CHNOS classes contain sulfonic acid groups. The base peak fragment of *m/z* 249.0227 ($C_{12}H_{10}O_4S_1$) in CCW-PPL fraction indicates that the sulfonic acid group was attached to the naphthalene ring directly. It was known that benzene and naphthalene sulfonates are important organic chemicals, and are usually added as dispersants in coal processing. For instance, sodium β-naphthalene sulfonate is a commonly used additive in coal chemical production [29]. The HOSA and PPL fractions were similar in class species, and the main compounds with strong relative abundances were OS or NOS classes. However, the relative abundances of NOS classes were higher than OS classes in HOSA fractions, while that in PPL fraction was opposite. The base peak in PPL fraction, which corresponding to $C_{12}H_{10}O_4S_1$, decreased significantly in HOSA; this may be due to the loss in the separation.

HON and HOA fractions of the two industrial wastewater samples have similar composition in compound classes, and were dominated by compounds containing multiple oxygen

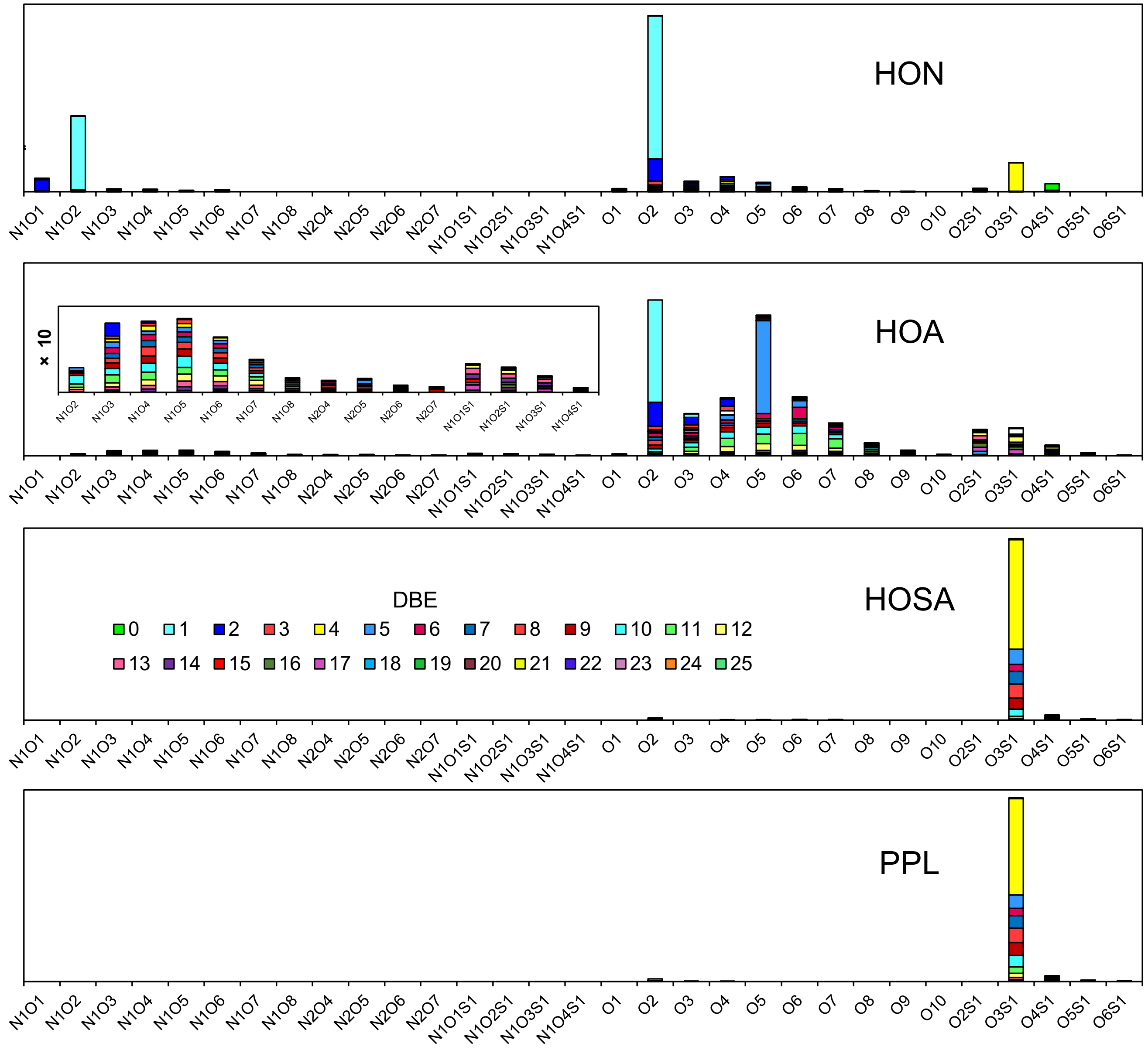

Fig. 3 Relative abundance plots of compound class species in HON, HOA, HOSA, and PPL fractions of the electric desalting wastewater (EDW) detected by negative-ion ESI FT-ICR MS

atoms, which could be polyphenol or naphthenic acids. The sulfonic acids would be separated and enriched in HOSA fraction by PAX (Figure S4). Generally, PAX separation enables to detect the oxygen-containing acidic compounds in the wastewaters.

The PAX ion-exchange solid phase extraction cartridge was filled with a hydrophilic styrene-divinylbenzene filler modified by quaternary ammonium salt, which had a polar stationary phase. The quaternary amine radical group (CH_2-N^+-R_3) carries positive ions after activation and is a strong anion exchanger. The pH plays an important role in the presence of compounds in water. When pH > 10, acidic compounds in wastewaters DOMs (including phenols, carboxylic acids, and sulfonic acids) are in ionic form and more easily adsorbed by the quaternary ammonium type modified adsorbent [7, 19]. When the water sample adjusts to strong alkaline (high pH), the acidic oxygen-containing compound would be strongly attracted on the cartridge by filler with positive ion modified quaternary amine group. The other part of neutral dissolved organic matter will be adsorbed by a hydrophilic styrene-divinylbenzene polymer. When using methanol as solvent (pH = 7), the HON fraction, which contains fewer oxygen atoms, is preferentially eluted. The ionization constant (KPa) of formic acid (HCOOH) is 3.75 and

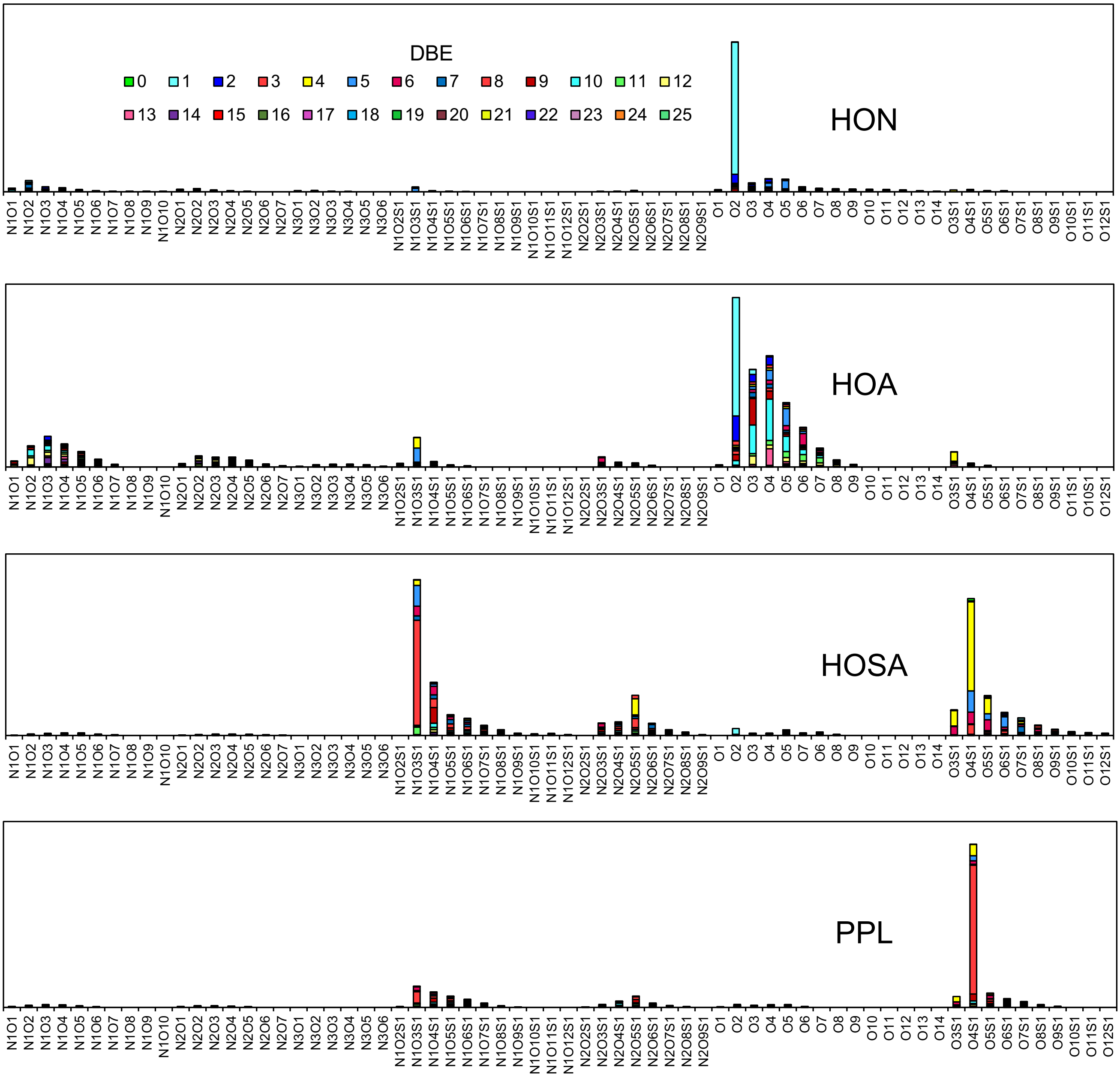

Fig. 4 Relative abundance plots of compound class species in HON, HOA, HOSA, and PPL fractions of the coal coking wastewater (CCW) detected by negative-ion ESI FT-ICR MS

of benzenesulfonic acid is 0.7. Methanol/HCOOH preferentially elutes acidic compounds carrying phenolic hydroxyl and carboxyl groups. Sulfonic acids have the strongest association with quaternary amine radical group. HCl (KPa = −8.0) is more acidic than benzene sulfonic acids. Therefore, methanol mixed with HCl can elute sulfonic acids.

Figure 5 shows the relative abundance plots of DBE versus carbon number of O3S1 classes assigned from the negative-ion ESI FT-ICR mass spectra of HOSA and PPL fractions from EDW and CCW. In EDW-HOSA fraction, O3S1 class species were mainly distributed in the range of C12-C38, and DBE was in the range of 0–14. O3S1 class species in EDW-PPL fraction mainly distributed in the range of C8-C38 and DBE was in the range of 0–18. O3S1 compounds of C17-C19 with DBE = 4 should be benzene sulfonates with long alkyl chain, which are commonly used as raw materials of surfactants. This kind of sulfonate is widely distributed in various industrial wastewaters. In CCW-HOSA fraction, O3S1 class species mainly distributed in the range of C6-C24, and DBE was in the range of 0–9. O3S1 class species in CCW-PPL fraction mainly distributed in the range of C9-C21 and DBE

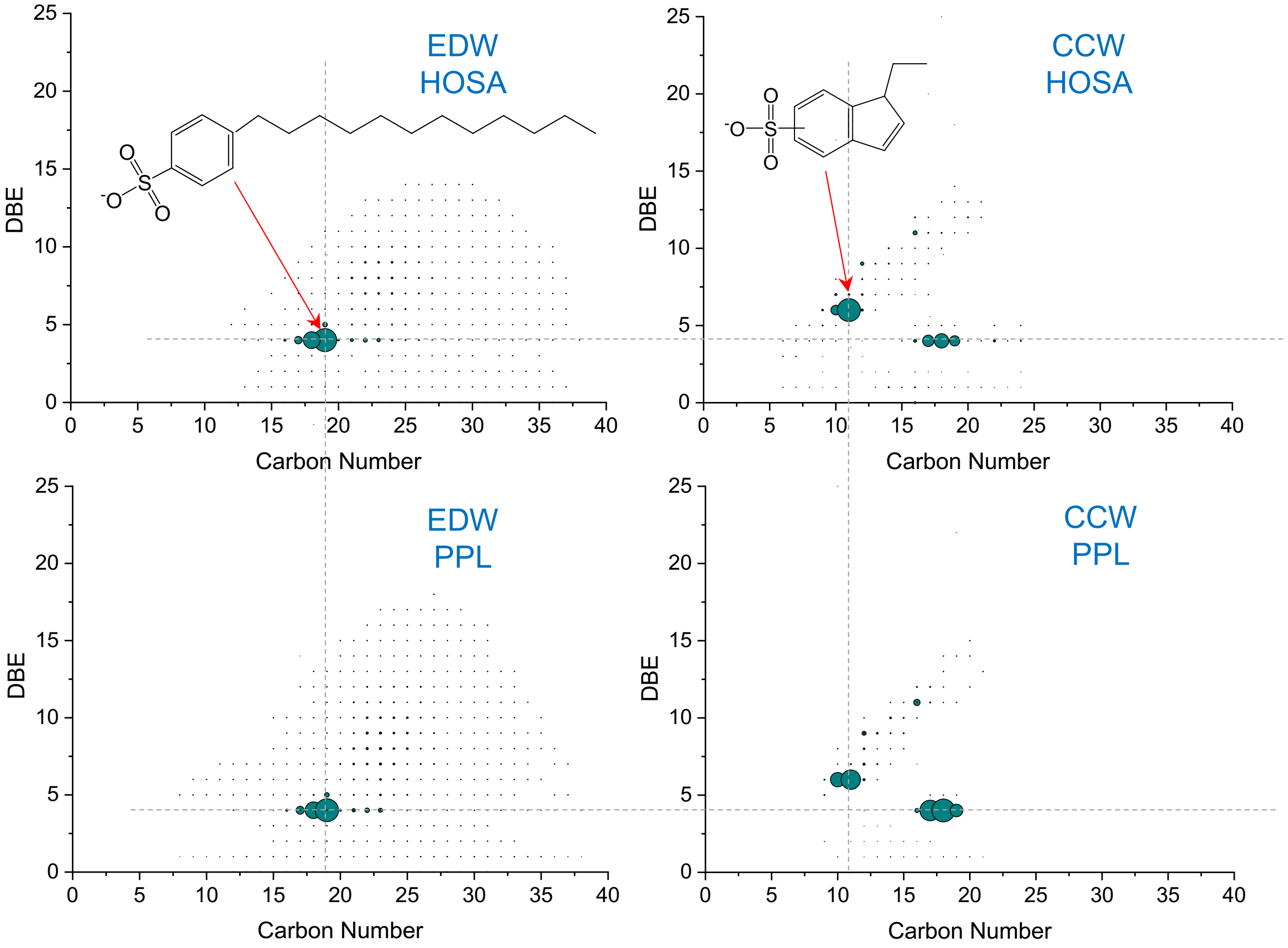

Fig. 5 Ion relative abundance plots of DBE versus carbon numbers for O3S1 classes in electric desalting wastewater (EDW) and coal coking wastewater (CCW) detected by negative-ion ESI FT-ICR MS. Bubble size indicative of its relative abundance. (note: The supposed structures were proposed based on the molecular composition, and was just possible but not detected.)

was in the range of 4–15. The FT-ICR MS results indicate that the sulfonate compositions in the two water samples are different, which proves that the sulfonate with different sources and structures can be separated and enriched in industrial wastewaters by PAX solid phase extraction sequential elution.

Semi-quantitative analysis of sulfonates by negative-ion ESI Orbitrap MS

Sulfonates often have extremely high relative abundances in negative-ion ESI MS spectra due to their high ionization efficiency [11]. Therefore, sulfonic acid peaks with high relative abundance may not indicate a high concentration of sulfonates in the wastewaters. To investigate the effect of sulfonates on the MS characterization, the sulfonates in PPL and HOSA fractions were semi-quantitatively analyzed by negative-ion ESI Orbitrap MS with the using of sodium 4-n-octylbenzenesulfonate as an internal standard.

According to the intensity of base peak (*m/z* 339.1999 for EDW; *m/z* 201.0227 for CCW-HOSA, *m/z* 249.0227 for CCW-PPL), the intensity of internal standard (*m/z* 269.1217) was normalized. Correlation coefficient of the semi-quantitative fitting curve was $R > 0.99$ for between internal standard concentration and peak intensity of the four fractions (Figure S5).

The mass spectrograms with internal standard concentrations of 0, 1, and 5 μg•L^{-1} were selected to calculate the concentration of sulfonic acids in EDW, and internal standard concentrations of 0, 10, and 30μg•L^{-1} were selected in CCW. The results were averaged three times for semi-quantitative calculations, respectively, and SRD was less than 7 of each group, which proves that the semi-quantification of HRMS is feasible (Table S2).

The semi-quantitative results of sulfonates (based on sulfonic acids) in each fraction were compared with TOC results in Tables 1 and 2. TOC of the EDW-PPL fraction was 67.3 mg/L, and TOC of sulfonic acids was 25.6 mg/L. The organic carbon content provided by sulfonic acids accounted for 38.0% of the total organic carbon in the PPL fraction. TOC of the CCW-PPL fraction was 277.6 mg/L, and TOC of sulfonic acids 22.8 mg/L. The organic carbon content contributed by sulfonic acids accounted for 8.2% of the total organic carbon in the PPL fraction. There was a great difference between the organic carbon concentration of sulfonic acids by semi-quantitative mass spectrometry and the total organic carbon concentration of PPL fraction, indicating that sulfonic acids were only a small part of DOMs extracted by PPL. However, FT-ICR MS only gave the molecular composition of sulfonic acids due to ionization suppression, and the composition information of other compounds (such as naphthenic acids and phenols) was missing.

TOC of the EDW-HOSA fraction was 28.3 mg/L, and TOC of sulfonic acids was 25.4 mg/L. The organic carbon content contributed by sulfonic acids accounted for 89.5% of total organic carbon in the HOSA fraction. TOC of the CCW-HOSA fraction reached 82.3 mg/L, and TOC of sulfonic acids was 18.7 mg/L. The content of organic carbon provided by sulfonic acids accounted for 22.7% of total organic carbon in the HOSA fraction (Tables 1 and 2). Compared with PPL fraction, TOC concentration of sulfonic acids obtained by the semi-quantitative analysis was closer to that of HOSA fraction.

The concentration of sulfonic acids in HOSA fraction in CCW was slightly lower than that of PPL fraction, which may be due to the decrease in the relative abundance of the aromatic sulfonic acids with high molecular condensation degree (e.g., $C_{12}H_{10}O_4S_1$) in the HOSA fraction. This may be caused by irreversible adsorption of aromatic sulfonic acids on the PAX cartridge.

The semi-quantitative results reveal that sulfonates in the EDW and CCW contribute about 11% and 2% TOC, respectively. However, these compounds have absolute dominance in the ESI mass spectra and heavily suppress the response of oxygen compounds which are major components of the DOM. A separation based on the PAX extraction is effective for the molecular characterization of DOM in wastewaters which contain sulfonates, even if they are in very low concentration.

Table 2 Sulfonic acids and carbon of sulfonic acid concentration of HOSA and PPL fractions detected by negative-ion Orbitrap MS (mg/L)

Fraction	Sulfonic acids	TOC concentration of sulfonic acids
EDW-HOSA	37.8	25.4
EDW-PPL	37.4	25.6
CCW-HOSA	37.1	18.7
CCW-PPL	41.3	22.8

Conclusions

An SPE separation approach based on anion exchange cartridge was proposed for the comprehensive molecular characterization of DOM from different industrial wastewaters. The DOMs in the wastewaters were separated into hydrophobic neutral (HON), hydrophobic acid (HOA), and hydrophobic strong acid (HOSA) fractions by PAX anion exchange SPE. PAX separated sulfonates from wastewaters and enables the molecular characterization by ESI MS of oxygen-containing compounds, which are the major component of DOM in the wastewaters. Semi-quantitative analysis results show that sulfonates account for 11% and 2% in total organic carbon for the refinery and coal coking wastewaters, respectively. However, these compounds have absolute dominance in the ESI mass spectra and heavily suppress the response of oxygen compounds which are major components of the DOM. PAX cartridge extraction with stepwise elution and followed by high-resolution MS analysis enables a comprehensive characterization for molecular composition of industrial wastewater DOM. The method has potential for an extensive application and the results are instructive for the understanding on the molecular composition of DOM in industry wastewaters.

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Author contribution W. Deng, C. He, and H. Huang designed and conceived the experiments. W. Deng and C. He carried out the experiments and data processing. All authors co-wrote the manuscript.

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Declarations

Consent for publication All authors have approved the version to be published.

Conflict of interest The authors declare no competing interests.

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