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Relationship between fluorescence characteristics and molecular weight distribution of natural dissolved organic matter in Lake Hongfeng and Lake Baihua, China

YUE Lanxiu[1,2], WU Fengchang[1], LIU Congqiang[1], LI Wen, FU Pingqing[1], BAI Yingchen[1], WANG Liying[1], YIN Zuoying[1] & LÜ Zhicheng[1]

1. State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China;
2. Shougang Institute of Technology, Beijing 100041, China

Correspondence should be addressed to Wu Fengchang (email: wufengchang@vip.skleq.cn)

Abstract Dissolved organic matter (DOM) is one of the most interesting and difficult problems in recent years due to its important functions in the ecological and environmental system and the complexity of its chemical composition and structure. It is well accepted that fluorescence characteristics and molecular weight distribution are two important parameters in the DOM characterization. However, the relationship between them is still unknown. In this study, fluorescence and molecular weight distribution of DOM in Lake Hongfeng, Lake Baihua and their rivers, and their relationship were investigated using the combination of fluorescence spectroscopy and high-performance size exclusion chromatography (HPSEC) with on-line UV absorbance and fluorescence detectors. The results show that there were two obvious humic-like fluorescence peaks (Peaks A and B) in DOM from lake water. But there was another obvious protein-like fluorescence peak (Peak C) in DOM from river water. The humic-like fluorescence material consisted of DOM fraction with smaller molecular weight, ranging from 1.0 to 3.0 kDa, while the protein-like fluorescence material mainly consisted of DOM fraction with MW larger than 2.0 kDa. The calculation of MW using HPSEC was related to the UV absorbance wavelength chosen.

Keywords: high-performance size-exclusion chromatography, dissolved organic matter, molecular weight, UV absorbance, fluorescence spectra.

Dissolved organic matter (DOM) in natural waters is a mixture of organic compounds with complicated chemical composition and structure, originating from animal and plant debris in soils and aquatic environments. It is well known that DOM usually consists of aromatic compounds and aliphatic chain carbohydrates with a number of functional groups, e. g. amido, carboxyl, hydroxyl, sulfur alcohol and its molecular weight (MW) ranges from several hundred to several ten thousand Dalton (Da)[1]. DOM plays an important role in aquatic eco-environmental system. DOM can bind metal ions, thus reducing their bioavailability and toxicity to aquatic organisms[2,3]. DOM can also bind organic and inorganic contaminants and result in the increase of their solubility and transport ability in aquatic environments[4]. In the drinking water treatment processes, DOM can participate in the formation of by-product disinfection, for example, the trihalomethane formation potential is mainly related to the low - intermediate MW DOM[5,6].

DOM is one of the most interesting and difficult problems in aquatic environments in recent years. Fluorescence characteristics and MW distribution are the important parameters in the DOM characterization[7−10]. Methods often used for the determination of MW include ultrafiltration, flow field flow fractionation, vapour-pressure osmometry, ultracentrifugation and gel permeation chromatography with each having its advantages and disadvantages. High-performance size-exclusion chromatography (HPSEC) has been extensively used in the studies of MW distribution of DOM. HPSEC has many advantages[11−17], for example, water samples do not have to be pre-concentrated or purified, number-averaged molecular weight (M_n) and weight-averaged molecular weight (M_w) can be calculated simultaneously, equipment stability, simple operation and the use of small sample volume. Detectors used in HPSEC usually include on-line dissolved organic carbon (DOC) detector, UV variable wavelength detector and fluorescence detector. Molecular fluorescence spectroscopy can be used for DOM investigation since DOM has many organic compounds of aromatic structure or conjugate chromophores and unsaturated aliphatic chains having various energy ranges of $\pi\rightarrow\pi^*$ transitions. DOM usually consists of a mixture of complicated organic compounds, e.g. humic and fulvic substances,

hydrophilic organic acids, carboxyl acid, amino acid and carbohydrates, and their fluorescence characteristics contain a lot of information, e.g. structure, functional groups, conformation, homogeneousity and MW. Fluorescence spectroscopy has been extensively used in characterizing DOM with different origins, e. g. ocean, river, lake and soil[18−23], for example, synchronous fluorescence spectra and three-dimensional excitation/emission matrix fluorescence spectroscopy (3DEEM), in which fluorescence information can be obtained when excitation and emission wavelengths are simultaneously changed. Compared to other methods, e.g. nuclear magnetic resonance (NMR) spectroscopy and GC-MS, 3DEEM has many advantages: high sensitivity, less sample volume used, without destroying samples, and direct measurement of natural water samples.

Many previous studies have showed that MW distribution and fluorescence characteristics of DOM are significant in understanding its structure, origin and eco-environmental effects[7−10,19−23], for example, DOM removal efficiency of flocculation and active carbon adsorption was closely related to MW[7,8]. Competitive adsorption of low MW DOM on organic contaminants resulted in the decrease of the removal efficiency[9]. And larger MW DOM had a stronger ability to bind trace metals while the adsorption of low MW DOM on particulates was faster than that of large MW DOM. Senesi and Miano[24,25] reported that various humic and fulvic acids of the same origin can be distinguished using fluorescence spectroscopy. Fluorescence spectroscopy can also characterize the origin, chemical and structural properties of DOM with different types of fluorescence[19 − 23,26]. However, the relationship between fluorescence characteristics and MW distribution is still not clear.

In the present work, Lake Hongfeng and Lake Baihua and their rivers in Qingzang Plateau, southwestern China were chosen as a study case. 3DEEM and HPSEC with on-line UV variable wavelength and fluorescence detectors were combined to characterize MW distribution and fluorescence of DOM, and their relationship. The chemical composition and structure of DOM were also discussed.

1 Samples and experiments

1.1 Sampling

Water samples were collected from Lake Hongfeng (HF) and Lake Baihua (BH), situated in the suburb of Guiyang City, southwestern China, and Nanming River These two lakes are the main source for drinking, industrial and agricultural irrigation of Guiyang City. HF-S-0 m, HF-N-0 m, BH-1-0 m and BH-2-0 m were collected from the surface water of lakes. River water samples were collected from Nanming River, which flows through Guiyang City. The river has been polluted by human and industrial wastes. XHPQ, XYCQ and SHKS were collected from the river at inflow, city area and outflow city. The physical and chemical parameters of all samples are shown in Table 1. Humic acid (HA) was purchased from Fluka Company, and was used for references. Water samples were filtered through precombusted (450℃ for 5 hours) glass-fiber filters (Waterman, GF/F) shortly after sampling, and samples were stored in the refrigerator (4℃) before further analyses.

1.2 Experiments

3DEEM spectra of DOM were recorded with a fluorescence spectrophotometer (Hitachi, Model F-4500) equipped with a 150-W Xenon arc lamp as the excitation source. A PMT voltage of 700V and an automatic response time were used. The ratio of signal and noise was higher than 110. Spectra were recorded using 5-nm excitation and emission slits, and were collected at a scan speed of 1200 nm/min. The spectra were corrected automatically for instrumental bias. Wavelengths were

Table 1 The physical and chemical parameters of samples in lake and river waters[a)]

Samples	pH	DOC/mg·L^{-1}	DO/mg·L^{-1}	Cl^-/mg·L^{-1}	Si/mg·L^{-1}	Chla/μg·L^{-1}	NO_2^-/mg·L^{-1}	NO_3^-/mg·L^{-1}	$H_2PO_4^-$/mg·L^{-1}	SO_4^{2-}/mg·L^{-1}
XHPQ	8.36	3.94	6.10	35.6	6.75	22.6	0.168	0.55	0.26	127.0
XYCQ	8.21	3.26	3.68	23.3	5.23	16.4	0.145	2.75	—	116.0
SHKS	8.14	4.83	5.64	39.3	2.68	36.7	0.112	—	0.72	154.0
HF-S-0m	8.60	2.78	7.60	5.04	0.42	41.6	0.062	1.57	—	66.01
HF-N-0m	8.67	2.89	5.99	5.36	—	31.4	0.054	1.51	—	68.37
BH-1-0m	8.38	2.25	7.15	5.40	0.73	36.8	0.089	1.81	—	95.86
BH-2-0m	8.44	2.35	6.90	5.46	1.23	65.5	0.076	2.01	—	94.88

a) –, not measured.

set from 220 to 400 nm for excitation, and from 250 to 500 nm for emission. Samples were kept at a constant temperature (20 ± 1° in water bath) before placing into quartz cells. Raman scattering of Milli-Q water was used for monitoring the instrumental condition, without evident errors. Sigma Plot software was used to obtain 3DEEM spectrum.

The MW of DOM was determined using HPSEC. The HPSEC system consisted of a high pressure Hewlett Packard 1100 pump and two on-line detectors in series: a UV variable wavelength detector and a 1046A fluorescence detector. A 7725i manual sampler with a 20 μL loop injector was used. The fluorescence detector was one dimension with given excitation/emission wavelengths. YMC 60 column (6 mm i.d×300 mm L) was used for separation, and it was packed with silica diol gel material (particulate size: 5 μm, pore-size: 60Å). HPSEC was carried out at a constant temperature (25℃). The mobile phase (pH=6.8) was phosphate buffer (0.03 mol·L^{-1} NaCl, 0.001 mol·L^{-1} NaH_2PO_4 and 0.001 mol·L^{-1} Na_2HPO_4), and the flow rate was 0.5 mL·min^{-1}.

The common standard sodium polystyrene sulfonates (PSS) were used for MW calibration[14−17]. PSS with 6.8 kDa, 4.3 kDa, 1.4 kDa, 210 Da and acetone were used as standards. These standards were diluted by mobile phase and their concentrations were 100 mg·L^{-1}. The elution volume of standards was linear with logarithm of MW. Under the same experiment condition, MW of DOM can be calculated by the linearity. The averaged MW (M_n and M_w) and polydispersity (ρ) were determined using eqs. (1)—(3)[13,14,17]:

$$M_n = \sum_{i=1}^{n} h_i \Big/ \sum_{i=1}^{n} (h_i / M_i), \quad (1)$$

$$M_w = \sum_{i=1}^{n} (h_i * M_i) \Big/ \sum_{i=1}^{n} h_i, \quad (2)$$

$$\rho = \frac{M_w}{M_n}, \quad (3)$$

where n is the detector's response number, h_i is the response of the HPSEC chromatogram at the elution volume "i", M_i is the MW of the fraction at the elution volume "i", M_w is the weight averaged molecular weight, M_n is the number averaged molecular weight.

2 Results and discussion

2.1 3DEEM fluorescence characteristics of DOM

The 3DEEM spectra were obtained at the simultaneous scan of emission and excitation spectra. Fig. 1 shows 3DEEM characteristics for DOM in water samples. As shown in Fig. 1, two distinct fluorescence peaks (Peaks A and B) can be observed for lake and river water samples, the emission wavelengths of both peaks occurred in the visible region, ranging from 412 to 434 nm, while the excitation wavelengths of both peaks occurred in the UV region, they were 305—335 nm and 239—241 nm for Peaks A and B, respectively. Peaks A and B are similar to those reported in previous studies for humic substances in aquatic environments[3,18], and are usually referred to as humic-like fluorescence. Excitation/emission (Ex/Em) wavelengths of Peak A in lake water samples were similar, approximately at 310—314/416—417 nm. But they were 320—335/420—425 nm for river water samples. Compared to lake water, river water had a third unique Peak C with Ex/Em 278—281 nm/334—353 nm wavelengths (Fig. 1). The observed Peak C is similar to the fluorescence of protein material reported in previous studies[14,19,21,22], and is often referred to as protein-like fluorescence.

2.2 Effects of various UV absorbance detection wavelengths chosen on the MW calculation of DOM

Fig. 2 shows the HPSEC chromatograms of XHPQ and HA at variable wavelengths of 230, 254, 280, 300 and 350 nm. There was a single peak with some sub-peaks in HPSEC chromatogram of HA, and there were several peaks, which were not completely separated, in the HPSEC chromatogram of XHPQ. These HPSEC chromatograms are similar to those of DOM with different origins[11,13]. Fig. 1 also shows that the shape of the chromatograms at different UV absorbance wavelengths was quite similar. But both the peak heights and peak elution volume decreased with increasing UV wavelength. This indicates that the measurement of MW of DOM increased as increasing UV wavelength was chosen.

The UV absorbance of DOM was mainly related to the unsaturated conjugated double bond in organic molecular structure. Previous studies have shown that high MW DOM had a larger proportion of aromatic and unsaturated conjugated double bonds than small MW DOM[11,13]. Therefore, the higher MW DOM fraction had a higher molar unit absorptivities than the smaller MW DOM fraction. The molar absorptivities of DOM decreased with increasing UV absorbance wavelength chosen[17]. Thus, the larger MW fraction had higher

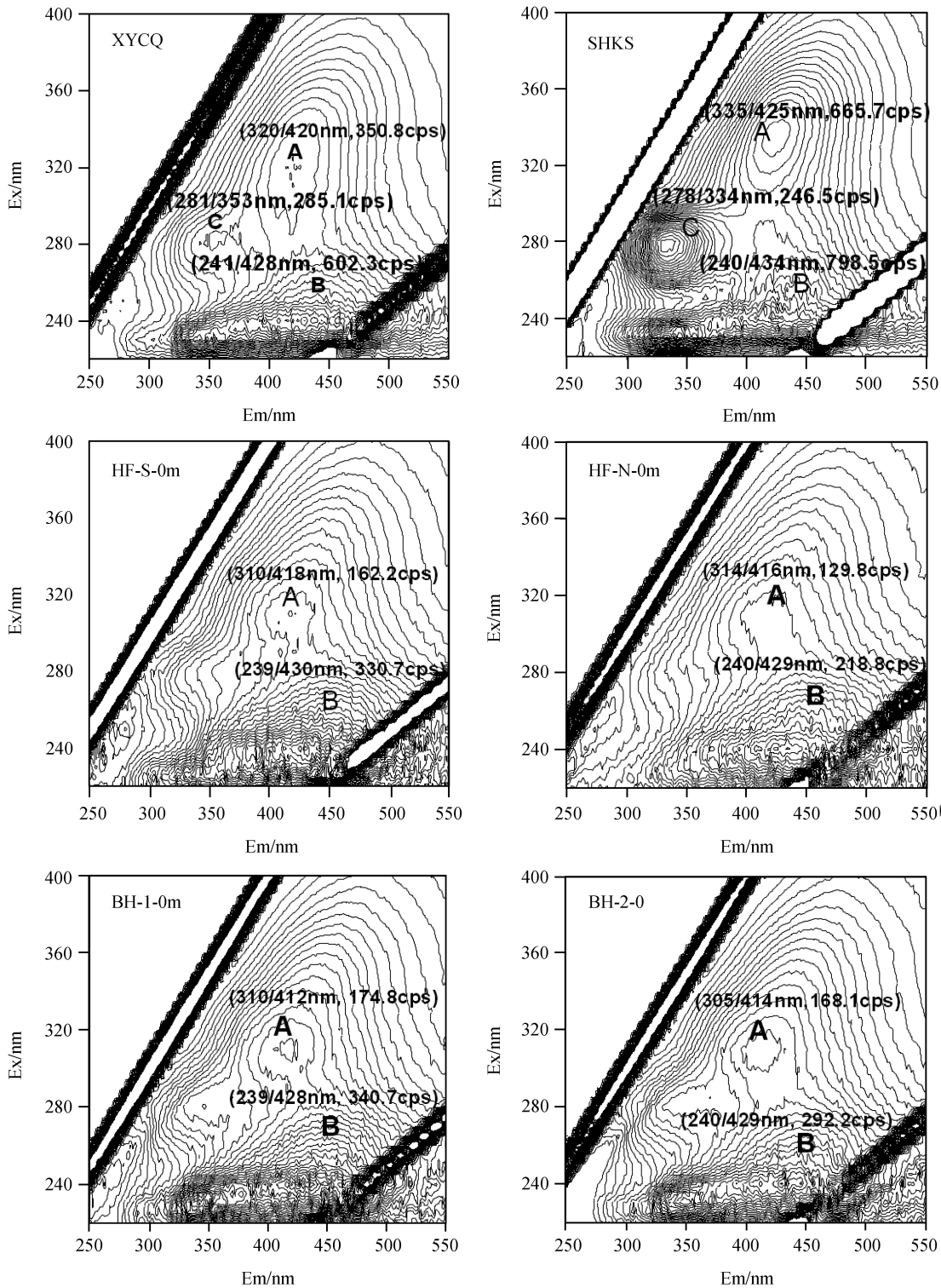

Fig. 1. 3DEEM plots of lake and river samples.

responses at the same wavelength than smaller MW fraction. As the detector wavelength increased this difference became more obvious. The larger MW fraction had higher response, and smaller MW fraction had lower response due to the lower molar absorptivities. As the wavelength increased, smaller MW fraction

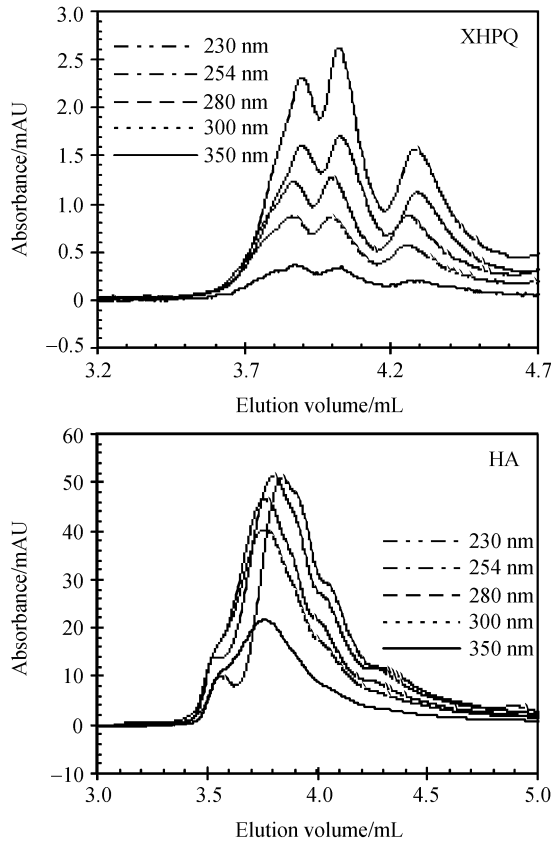

Fig. 2. HPSEC chromatograms of XHPQ and HA with different UV absorbance wavelengths.

appeared "invisible", resulting in the peak shifted to the larger MW. The calculated M_w and M_n increased with increasing wavelength chosen for XHPQ and HA (Table 2). The relative increase in M_w and M_n for XHPQ was 49.1% and 52.5%, respectively. The increase for HA was 12.5% and 19.4%, respectively. This suggests that the same UV detection wavelength should be chosen when HPSEC was used to characterize MW distribution of different origin DOM.

2.3 MW distribution of different fluorescence materials

To investigate MW distribution characteristics of different fluorescence materials, HPSEC was used with the combination of on-line UV absorbance and fluorescence detectors. Fig. 3 shows the HPSEC chromatograms with different Ex/Em wavelengths chosen. The A, B and C curves were the HPSEC chromatograms detected at Ex/Em wavelengths of Peaks A, B and C, respectively.

Humic-like fluorescence substances (Peaks A and B) had similar MW distribution (Fig. 3). The HPSEC chromatogram at Peak A Ex/Em wavelengths had 3 to 4 major peaks, which were similar to those at Peak B wavelengths. This indicates that they had similar MW distribution. However, the smallest MW and elution volume were not the same, suggesting their subtle differences in MW distribution and averaged MW. The

Table 2 M_w, M_n and polydispersity (ρ) of DOM obtained by HPSEC at different UV absorbance wavelengths

	XHPQ			HA		
Wavelength /nm	M_w /Da	M_n/Da	ρ	M_w/Da	M_n/Da	ρ
230	2318	2206	1.05	2354	1732	1.36
254	2085	1803	1.16	2513	1843	1.36
280	2091	1806	1.16	2501	1897	1.32
300	2179	1875	1.16	2545	1930	1.32
350	2174	1856	1.17	2662	2113	1.26

Table 3 The averaged molecular weight of different fluorescence materials and contribution of each fraction to the total(%)

Sample	Peak	M_w/Da	M_n/Da	ρ	>3.0 kDa	3.0−2.0 kDa	2.0−1.0 kDa	< 1.0 kDa
SHKS	A	1763	1499	1.2	2.2	35.1	46.2	16.5
	B	1685	1351	1.2	2.8	33.0	40.5	23.7
	C	2916	1835	1.6	12.4	54.7	16.8	16.1
XYCQ	A	1820	1527	1.2	2.3	39.4	42.3	16.0
	B	1845	1515	1.2	3.7	41.0	37.5	17.8
	C	2975	1893	1.6	18.6	44.3	21.5	15.6
HF-S-0 m	A	1848	1591	1.2	2.3	46.9	40.3	10.5
	B	1709	1460	1.2	7.7	46.6	33.3	12.5
HF-N-0 m	A	1846	1558	1.2	1.7	42.2	40.8	15.3
	B	1788	1467	1.2	2.4	39.8	39.1	18.8
BH-1-0 m	A	1824	1571	1.2	1.9	41.1	38.7	18.3
	B	1793	1473	1.2	1.5	39.5	44.5	14.6
BH-2-0 m	A	1850	1572	1.2	1.3	43.0	41.1	14.6
	B	1834	1531	1.2	3.0	40.5	39.5	17.1

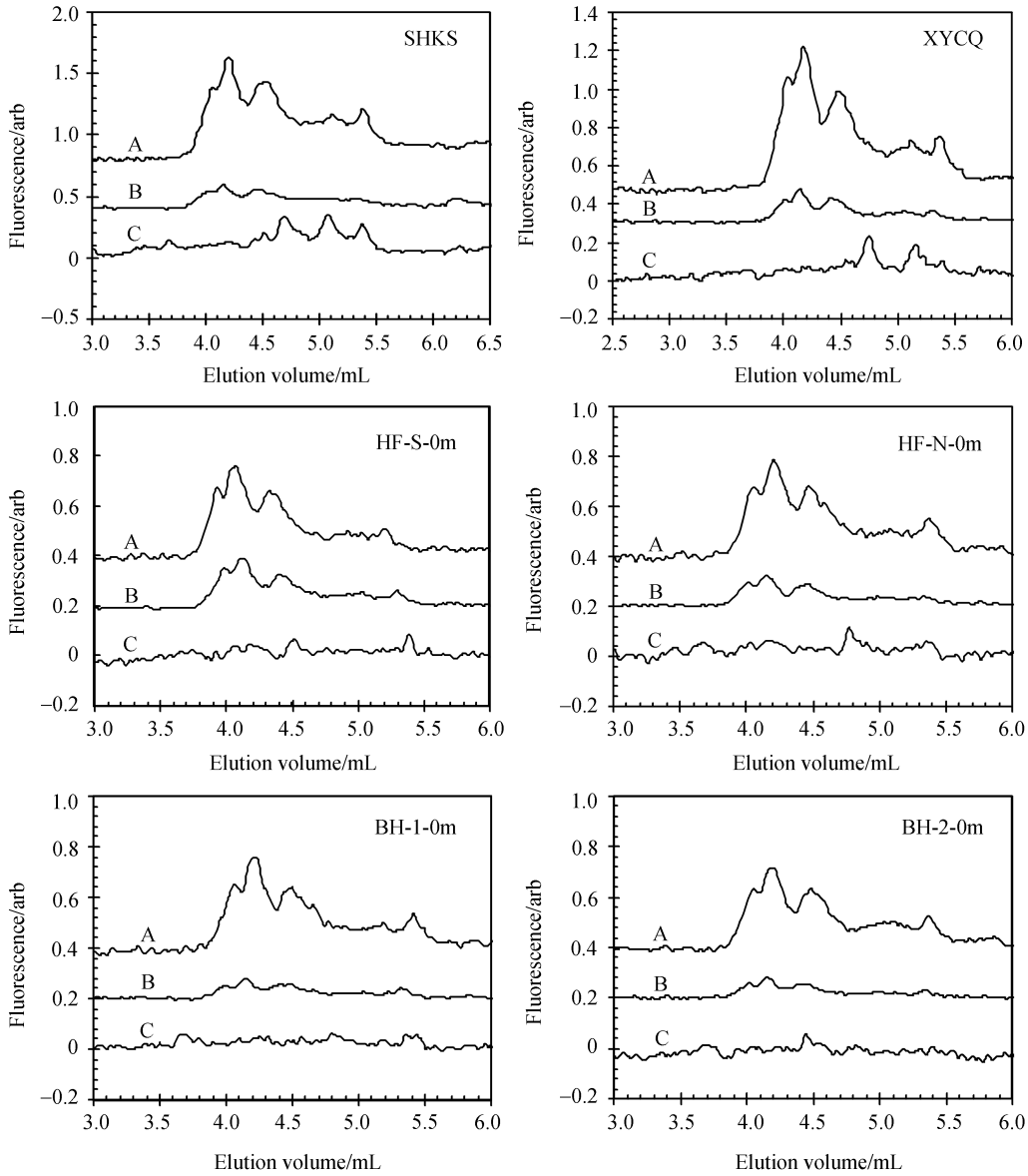

Fig. 3. HPSEC chromatograms of different fluorescence materials.

MW distribution of Peak C material was different from humic-like fluorescence material, DOM in river water had smaller absorbance HPSEC and there was nearly no absorbance chromatogram in lake water. As seen in Fig. 3, the elution volume of Peak C fluorescence material was larger than that of Humic-like fluorescence material, indicating its lower MW characteristics. Because of the relative MW of DOM obtained by HPSEC when the detector wavelength was at Ex/Em 280/340 nm (Peak C), the standard material had a longer elution time than that at the other two Ex/Em wavelengths (Peaks A and B). Thus, Peak C fluorescence material actually had a larger MW than Peaks A and B.

The averaged MW and contribution of each MW fraction of DOM in different fluorescence materials are shown in Table 3. The estimated MW distribution was consistent with the discussion above. Peak A material was composed of larger MW fraction (3.0─1.0 kDa), accounting for 79.8% ─ 87.2 % of total DOM. The second largest fraction was the MW fraction smaller than 1.0 kDa, accounting for 17% ─ 24%. And the smallest fraction was the MW fraction larger than 3.0 kDa, only accounting for 1.9% ─ 3.7%. The contribution of each fraction of Peak B was similar to that of

Peak A, mainly consisting of fractions with MW smaller than 3.0 kDa. This indicates that humic-like substances had similar chemical composition and structure, mainly containing smaller MW organic compounds. The M_w of protein-like substances was larger than 2.0 kDa, obviously larger than that of humusic-like substances (1685－1845 Da). This suggests that protein-like substances had larger MW. The dispersibility of both humic-like and protein-like fluorescence substances was lower than 2.0 kDa, showing a narrow MW distribution.

Because of the complexity of chemical composition and limit of investigation method, it is difficult to characterize DOM. Due to the low concentration of DOM, the separation and characterization methods were different from the traditional organic analyses. Molecular fluorescence spectroscopy and HPSEC have been the effective methods to characterize DOM. Our observations further confirm that the DOM in lake and river waters had two kinds of fluorescence materials (humic-like and protein-like), which are similar to DOM of other origins. The humic-like fluorescence material can be divided into two fractions: Peaks A and B. Our observations also confirm that HPSEC was effective to characterize MW distribution of DOM and the estimated MW was related to the UV absorbance wavelength chosen. However, the relationship between structure and composition of different fluorescence materials is poorly understood. This study conducted by using HPSEC with on-line UV absorbance and fluorescence detectors offers novel methods to solve the scientific problem as described above. Our results also show that the MW distribution of different fluorescence materials was obviously different. This was supported by many previous studies[3,11,19,22,27]. Humic-like fluorescence mainly came from organism debris with long-term degradation, which was supposed to have a smaller MW distribution, whereas, protein-like fluorescence was usually related to recent biological activities, and was newly produced and was without much degradation, thus was supposed to have larger MW distribution.

Peak A humic-like fluorescence material extensively occurred in DOM with different origins. But Peak B did not occur in all DOM, which was reported to be possibly related to humic substances[27]. But the chemical composition and structure of Peaks A and B are still not known. Our results suggest that Peaks A and B fluorescence substances had similar MW distribution and may have similar origin and structure.

MW distribution was closely related to the chemical composition, structure and other physical characteristics[3,6,10,27,28]. The larger MW DOM had a higher proportion of phenyl ring structure and unsatuated double bonds, and smaller MW DOM had a higher proportion of chemical functional groups. Thus, our results are significant for the further investigation of DOM characteristics and eco-environmental effects.

3 Conclusions

This study shows that the combination of 3DEEM and HPSEC with on-line UV absorbance and fluorescence detectors had superiority on the DOM characterization in aquatic environments. Different fluorescence substances had obviously different MW distributions. Humic-like fluorescence substances occurred in DOM fractions with MW smaller than 3.0 kDa, while protein-like fluorescence substances mainly occurred in DOM fractions with larger MW. Those techniques and results are of significance for further studies of DOM sources, chemical composition, structure and its geochemical behavior.

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