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Analytica Chimica Acta 407 (2000) 193–202

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Size-exclusion chromatographic descriptors of humic substances

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Received 14 July 1999; received in revised form 13 October 1999; accepted 18 October 1999

Abstract

An approach to the generation of size exclusion chromatographic (SEC) descriptors of humic substances (HS) is proposed. It implies a calculation of initial, central and normalised statistical moments and some of their ratios from the raw SEC-data. The proposed descriptors extend the pool of common molecular weight (MW) characteristics (average MW's and their ratios) up to 20 parameters, which is important for the purposes of classification and prognostic modelling. Original software was developed to facilitate the computation of molecular weight distribution (MWD) and SEC-descriptors of HS from raw chromatographic data. The developed approach was applied to two model compounds and three humic materials. The discriminatory power of the calculated descriptors was roughly estimated on the example of three humic materials of different origin with the use of a multivariate variance analysis. Weight average MW and a ratio of the *z*- to weight average distribution coefficients were found to be the best discriminators, whereas the significance level of discrimination provided by the second moment and M_z/M_w-ratio was lower than 95%. ©2000 Elsevier Science B.V. All rights reserved.

Keywords: Humic substances; Size-exclusion chromatography; Molecular weight; Descriptors; Discrimination

1. Introduction

Molecular weight (MW) is a fundamental characteristic of each chemical substance. That is why it is considered as an important molecular descriptor when deriving prognostic structure-activity models that use constitutional descriptors [1]. In the case of an individual compound, MW can be uniquely defined by a single value, whereas MW of the natural random macromolecular compounds such as humic substances (HS) is always a distribution. HS are ubiquitous in the environment and comprise from 50 to 80% of the organic matter in soil and water ecosystems [2]. Due to their polyfunctional character, HS are capable of binding both to heavy metals and to organic compounds [3]. As a result, they influence greatly speciation of the chemicals released into the environment. To predict the reactivity of HS using quantitative structure-activity relationships (QSAR) technique, a generation of different constitutional molecular descriptors including those of molecular weight distribution (MWD) is of a great importance.

Size exclusion chromatography (SEC) is one of the most widely used techniques for the investigation of MWD of HS [4]. Up to today, the quantitative information on MW characteristics of HS is mostly limited to an operationally derived number of fractions which depends on the conditions of SEC-fractionation of HS and a range of MW of each fraction [5,6]. However, as it was shown by our previous results [7,8], the multi-

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PII: S0003-2670(99)00814-4

modal character of SEC-elution curves of HS is a consequence of the non-size exclusion effects caused by electrostatic interactions between gel and HS, rather than a reflection of their true MWD. Under conditions of suppression of charge interactions provided by the use of buffer as an eluent, the SEC-chromatograms of HS are characterised by a unimodal distribution [9–11]. The latter can be rather precisely characterised with the use of the number- and weight-average MW's and of their ratio — polydispersity. This approach is widely adopted in polymer chemistry [12]. The results of its application to HS are reported in [10].

However, the three above-mentioned parameters do not contain all the information on MWD of the polymer which can be of importance both for prognostic modelling and classification of HS. To obtain additional information on the MWD, more excessive treatment of the raw SEC-elution curve of HS is needed. In this work, we concentrated our efforts on deriving MW descriptors of HS by calculating statistical moments of different orders and their ratios from the raw SEC-chromatograms. As far as we are aware, this approach has not been used yet for deriving the molecular descriptors of HS. An applicability of the proposed descriptors for the purposes of discrimination of HS of different origin has been demonstrated.

The objective of this work was to develop an approach for deriving molecular descriptors of HS from the SEC-data.

2. Theoretical background

2.1. Calculation of average MW's and polydispersity from the raw SEC-elution curve

The unique feature of the SEC-elution curve is that it contains information about MW and polydispersity of the fractionated polymer. To reveal this information, a calibration curve of the chromatographic system, expressed as

$$V_{\mathrm{R}} = f(M) \tag{1}$$

should be determined. Eq. (1) relates the values of retention volume V_{R} of the analyte to its MW *M*. To determine an exact analytical expression of the calibration curve, polymers of known MWD (or of the known peak-, number- or weight-average MW's) — MW standards or individual substances are analysed with the chosen chromatographic system. On the basis of the known V_{R} values of the standards, the calibration equation can be calculated as follows [13]:

$$\log(M) = \sum_{i=0}^{n} B_i V^i \tag{2}$$

where B_i stands for the calibration coefficients.

Knowing the calibration curve *V*(*M*), differential function *W*(*M*) of the MWD can be obtained from the SEC-chromatogram *F*(*V*) by replacing retention volumes *V* with *M* according to Eq. (2). This transformation can be described as follows [13]:

$$F(V)\,\mathrm{d}V = W(M)\,\mathrm{d}M \tag{3}$$

$$W(M) = F[V(M)]\left|\sum_{i=0}^{n}(iB_i(\ln 10)[V(M)]^{i-1})M\right|^{-1} \tag{4}$$

On the basis of the known MWD, the number-, weight- and *z*-average MW's (M_n, M_w and M_z, respectively) of the analysed polymer can be calculated according to the following equation:

$$\bar{M} = \frac{\sum N_i M_i^{r+1}}{\sum N_i M_i^{r}} = \frac{\sum W_i M_i^{r}}{\sum W_i M_i^{r-1}} \tag{5}$$

where W_i is the total weight of and N_i is the number of the molecules of a MW M_i; *i* is incrementing over all the MW's presented in the sample. A value of $\bar{M}$ calculated at $r=0$ corresponds to M_n, at $r=1$ to M_w and at $r=2$ to M_z.

Proceeding from the summation to integration and fulfilling all the necessary transformations, an expression for the calculation of M_n, M_w and M_z values directly from the SEC-chromatogram can be obtained [13]:

$$\bar{M} = \frac{\int_{V_0}^{V_k} M^{r}(V)F(V)\,\mathrm{d}V}{\int_{V_0}^{V_k} M^{r-1}(V)F(V)\,\mathrm{d}V} \tag{6}$$

where *F*(*V*) is an ordinate of a SEC-chromatogram, *M*(*V*) is the calibration curve, and V_0 and V_k are retention volumes of the starting and endpoints of the chromatogram, respectively. A value of $\bar{M}$ calculated at $r = 0$ corresponds to M_n, at $r = 1$ to M_w, at $r = 2$ to M_z.

The above average MW's and their ratios serve as the most important characteristics of the MWD of the polymer. The M_w/M_n ratio characterises polydispersity, or a breadth of the MWD of the sample. M_z/M_w ratio is more sensitive to high MW fractions of the sample.

2.2. Calculation of statistical moments of a SEC-elution curve and their ratios

To increase the significance and the range of applicability of any parameters calculated from the SEC-elution curve, it should be converted into a scale of distribution coefficients (K_d). The latter is defined as [14]

$$K_\mathrm{d} = \frac{(V_\mathrm{R} - V_0)}{(V_\mathrm{t} - V_0)} \tag{7}$$

where V_0 and V_t are void and total volume of the SEC-column, respectively.

K_d is a more fundamental characteristic of the SEC-properties of an analyte than V_R [14]. It does not depend on the geometrical size and quality of packing of the gel in the SEC-column and could be compared for different chromatographic systems. The corresponding SEC-elution curve $F(K_\mathrm{d})$ as for any other distribution can be described numerically by use of the statistical moments. This approach is widely used for studying the regularities of the chromatographic processes [13].

The initial statistical moments can be calculated according to the standard procedure [15]. The corresponding expressions for a SEC-chromatogram are as follows:

$$m_n = \frac{\int_{K_{\mathrm{d}_0}}^{K_{\mathrm{d}_k}} F(K_\mathrm{d}) K_\mathrm{d}^n \,\mathrm{d}K_\mathrm{d}}{\int_{K_{\mathrm{d}_0}}^{K_{\mathrm{d}_k}} F(K_\mathrm{d}) \,\mathrm{d}K_\mathrm{d}} \tag{8}$$

where m_n stands for the moments of the nth order, $n = 1, 2, 3, 4\ldots$; F_i is a height of the SEC curve at K_{d_i}; K_{d_0} and K_{d_k} are distribution coefficients at the starting and end points of the chromatogram.

The values of the above-defined initial statistical moments depend on the position of the chromatogram relative to the beginning of the coordinates and on its scale. To diminish a dependence of the derived descriptors on the particular chromatographic system, the central statistical moments (relative to the mean) [15] are to be used. The latter are determined only by the shape of the chromatogram. Central statistical moments can be calculated by use of the following equation:

$$mc_n = \frac{\int_{K_{\mathrm{d}_0}}^{K_{\mathrm{d}_k}} F(K_\mathrm{d})(K_\mathrm{d} - m_1)^n \,\mathrm{d}K_\mathrm{d}}{\int_{K_{\mathrm{d}_0}}^{K_{\mathrm{d}_k}} F(K_\mathrm{d}) \,\mathrm{d}K_\mathrm{d}} \tag{9}$$

where mc_n stands for the central moments of the nth order, and m_1 is the first moment. Under SEC conditions, response of the detector is proportional not to a molar, but to a weight concentration of an analyte; that is why, m_1 is equivalent not to a number-, but to a weight-average K_d — $(K_\mathrm{d})_w$.

mc_1 is identical to 0. mc_2 is a measure of how widely values are dispersed from the mean. mc_3 characterises asymmetry of a chromatogram: $mc_3 > 0$ indicates that the chromatogram is skewed to the front boundary of the chromatographic zone, $mc_3 < 0$ to the rear boundary of the zone. mc_4 characterises a degree of the curvature of a distribution in the region of its maximum. The moments of higher order are difficult to interpret.

To characterise the shape of the distribution, two normalised statistical moments — *skewness* and *kurtosis* — are usually calculated. Skewness is defined as [16]

$$\mathrm{skew} = \frac{mc_3}{mc_2^{3/2}} \tag{10}$$

Positive skewness indicates a distribution with an asymmetric tail extending towards more positive values and vice versa. If the chromatogram is symmetric, skew = 0. Kurtosis is defined as [16]

$$\mathrm{kurt} = \left(\frac{mc_4}{mc_2^2}\right) - 3 \tag{11}$$

Positive kurtosis indicates a peaked distribution relative to the normal one. Negative kurtosis indicates a relatively flat distribution. Kurtosis of the normal distribution equals 0.

Calculation of the ratios of initial statistical moments of the low orders returns values of average distribution coefficients (K_d) according to Eq. (12):

$$\bar{K}_\mathrm{d} = \frac{\int_{K_{\mathrm{d}_0}}^{K_{\mathrm{d}_k}} K_\mathrm{d}^r \, F(K_\mathrm{d}) \, \mathrm{d}K_\mathrm{d}}{\int_{K_{\mathrm{d}_0}}^{K_{\mathrm{d}_k}} K_\mathrm{d}^{r-1} \, F(K_\mathrm{d}) \, \mathrm{d}K_\mathrm{d}} \tag{12}$$

where a value of $\bar{K}_\mathrm{d}$ corresponds to number $(K_\mathrm{d})_n$-, weight $(K_\mathrm{d})_w$- and z $(K_\mathrm{d})_z$-average distribution coefficients at $r=0$, 1 and 2, respectively.

An advantage of the use of average K_d values in comparison with the similar average MWs is their lower sensitivity to chromatographic noise. The latter is tremendously magnified upon transforming chromatograms into MWD's. In addition, average K_d values are calculated without use of a calibration curve. This means that the errors of determination of the calibration curve do not influence the calculated parameters. This suggests higher stability of the values of average K_d's in comparison with those of MWs. The average K_d's can be very useful for prognostic modelling. The latter usually implies a linear relationship between molecular descriptors and the property. If the relationship of interest is non-linear, the ratios of statistical moments might have much higher predictive power than their linear combination.

The ratios of the initial statistical moments of higher than the fourth-order can be very unstable due to high sensitivity to experimental errors. This hinders their use as descriptors for predictive modelling and they were not considered in this study.

3. Materials and methods

Humic materials used were soil fulvic acid (FST), soil humic acid (HST) and sum of peat humic and fulvic acids (T4) isolated according to techniques that have been described elsewhere [17,18]. Concentrated stock solutions of humic materials (100–500 mg organic carbon (C) l^{-1}) were prepared by dissolving the dried material in 0.1 M NaOH. All the stock HS solutions prior to the SEC analysis were diluted (100–200 times) with the 0.028 M phosphate buffer used as the mobile phase to obtain the working concentration of HS in the range of 1–4 mg C l^{-1}. An injection volume was 2 ml.

Model compounds used were glucose (purissimo, Merck) and polydextran 10 kDa (Merck). The latter has been designated as PDX in Tables 1–3 and Figs. 2 and 3.

3.1. SEC-analysis of HS

The experimental setup was as described elsewhere [5,9]. All the analyses were conducted at the facilities of the Division of Water Chemistry of the Engler–Bunte Institute of the University of Karlsruhe. An on-line DOC detector (Gräntzel, Germany) was used to register an elution curve. Data acquisition was by Rhothron (Germany) on an Atari-compatible computer. A SEC column was 25 mm × 200 mm with a column packing of Toyopearl HW-50S (Toyo Soda, Japan) that is a rigid, hydrophilic matrix prepared from a copolymerisation of ethylene glycol and methacrylate polymers. Phosphate buffer (concentration of 0.028 M, pH 6.2, I (ionic strength) ~0.1 M) was used as an eluent at a flow rate of 1 ml min^{-1}. The SEC column was calibrated by a set of polydextrans of the peak MWs given in the brackets (0.83, 4.4, 9.9, 21.4, 43.5, 2000 kDa), mono- and oligosaccharides (180, 342, 504 Da), glycerol (92 Da) and methanol (37 Da). Blue dextran (2000 kDa) served as a void volume (V_0) probe, and methanol as a total volume (V_t) probe. V_0 and V_t accounted for 26.0 and 66.8 ml, respectively.

3.2. Data treatment

The numerated signals from the detector were recorded every 0.1 min into a data file. The obtained file was read by a self-designed 'GelTreat'-program for further treatment. The program runs on the PC under Windows 95. It allows to perform a pre-treatment of the raw elution curve, and calculation of the calibration curve, of the MWD and of the statistical moments. The pre-treatment of a raw elution curve implies base line correction and smoothing. As a rule, linear base line correction was applied to the region of 22–60 ml, which cut off the noise in the low MW range (<120 Da) of the MWD-curve. Smoothing was conducted manifold by beta-splines. It was necessary because of an extremely high sensitivity of the calculated MWD to the chromatographic noise, in particular, in the region of low MWs. The program also allows the cutting off of a single peak out of the

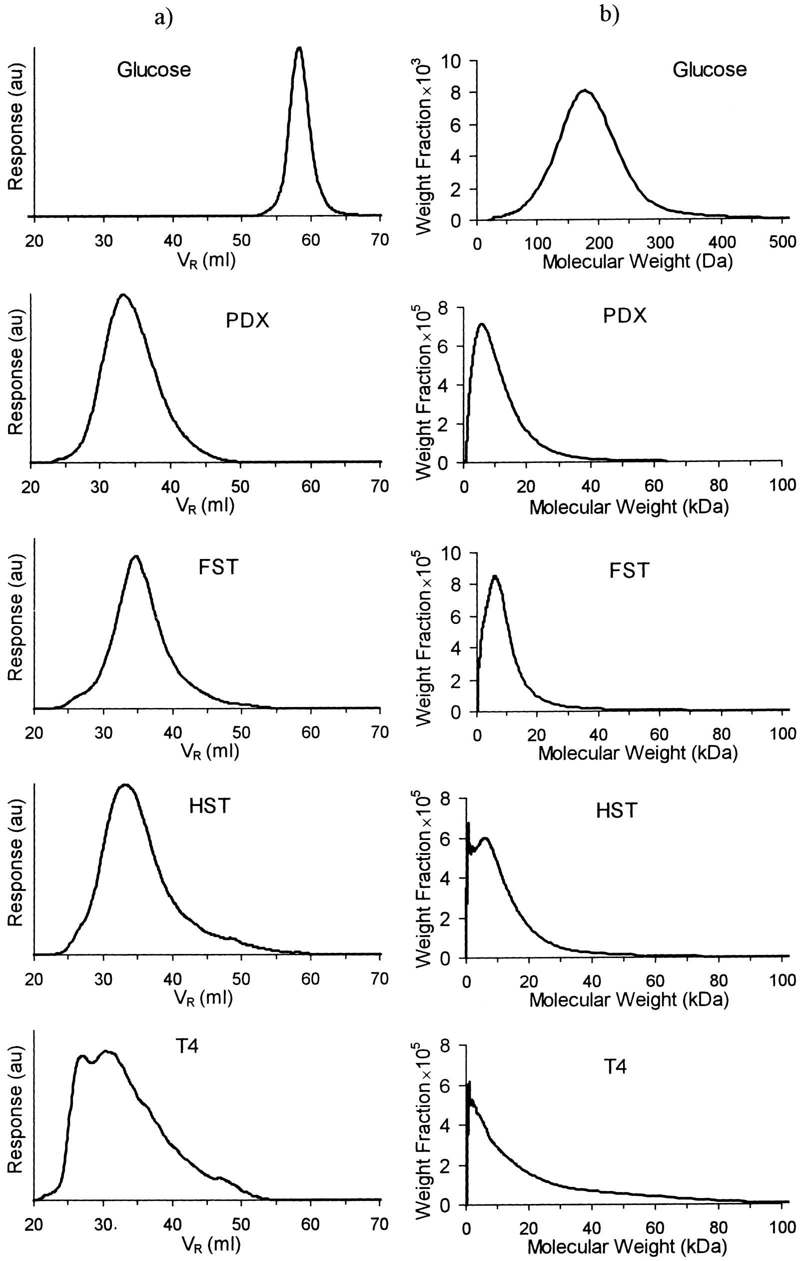

Fig. 1. (a) SEC-curves of two model compounds and three humic materials. Column-packing Toyopearl HW-50S, eluent 0.028 M phosphate buffer, concentration of HS: 1–4 mg C l^{-1}, $V_0 = 26.0$ ml, $V_t = 66.8$ ml. (b) Differential MWD's calculated from the SEC-curves of two model compounds and three humic materials given in Fig. 1a.

multimodal chromatogram for further treatment as described above.

For the above set of the standards, the best description of the calibration curve was reached with the following polynomial ($r^2 = 0.998$):

$$\log M = 10.22 - 0.33 \times V_R + 0.0055 \times V_R^2 - 3.92 \times 10^{-5} \times V_R^3 \qquad (16)$$

Table 1
Average MW's of two model and three humic materials used in this study

Sample	M_{peak} (kDa)	M_n (kDa)	M_w (kDa)	M_z (kDa)
Glucose	0.183 ± 0.004[a]	0.173 ± 0.005	0.193 ± 0.005	0.217 ± 0.007
PDX	10.9 ± 0.3	7.8 ± 0.7	13.0 ± 0.5	25 ± 2
FST	8.7 ± 0.1	5.2 ± 0.4	11.0 ± 0.5	25 ± 4
HST	11.2 ± 0.5	5.4 ± 0.4	13.3 ± 0.6	26 ± 3
T4	30 ± 10	4.38 ± 0.25	22.9 ± 0.6	53.6 ± 1.5

[a] ± corresponds to standard deviations at $n=4$ (glucose, HST, T4), $n=3$ (PDX) and $n=6$ (FST).

On the basis of this calibration curve, the pre-treated chromatograms were transformed into the corresponding MWD's. Statistical moments and their ratios were calculated in accordance with the equations given in Section 2.

4. Results and discussion

4.1. SEC-chromatograms and MWD's of HS

To demonstrate the approach described for the quantitation of SEC-chromatograms, the raw SEC data on three humic materials were used. Two of them were chosen from among 40 others as possessing the most symmetric (FST) and the most asymmetric (HST) chromatographic distribution, whereas T4 was chosen as an intermediate case. Such a choice was made to elucidate more clearly the specific features of the raw SEC-elution curves and the corresponding MWD's by use of the SEC-descriptors defined in Section 2.

The SEC-analyses on HS were conducted under conditions which yield an elution curve with unimodal distribution. In this study, this was obtained using 0.028 M phosphate buffer as an eluent together with an equilibration of an HS sample with this mobile phase prior to the analysis.

The SEC-chromatograms of two model compounds (glucose and polydextran 10 kDa) and of three humic materials in the scale of a retention volume are given in Fig. 1a. The data on glucose and polydextran are included to enable a comparison of the corresponding parameters of HS with those of the individual substance or the relatively monodisperse polymer. As can be seen, under the given conditions, all the SEC-chromatograms of HS are characterised with a unimodal distribution, but contain small subpeaks and shoulders. The corresponding differential weight fraction distributions are given in Fig. 1b. The MWD-curves of HS are characterised by rather strong noise in the region of low MW's.

The corresponding average MW's of the model compounds and target humic materials are given in Table 1. The M_n values of the humic materials varied from 4.38 to 5.4 kDa (T4 and HST, respectively), whereas the M_w of the same materials were 22.9 and 13.3 kDa, respectively. The values of M_{peak} of all three HS samples were close to M_w. This looks reasonable because response of the DOC-detector is proportional to an eluted weight fraction of the analyte.

The ratios of the average MW's of the target humic materials are given in Fig. 2. The highest polydispersity (M_w/M_n ratio of 5.2 ± 0.3) was observed for T4, whereas the much lower corresponding values for FST and HST were 2.1 ± 0.2 and 2.5 ± 0.3 kDa, respectively. These values indicate rather large polydispersity of the target humic materials and substantially exceed those of 1.5–1.9 reported in [10] for different aquatic fulvic acids and dissolved organic matter. Such a disagreement can be related to the

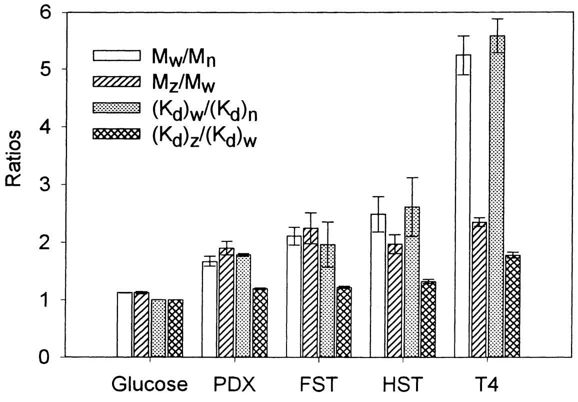

Fig. 2. Average MW's and K_d's for two model compounds and three humic materials. Error bars correspond to standard deviations at $n=4$ (glucose, HST, T4), $n=3$ (PDX) and $n=6$ (FST).

Table 2
Average K_d's calculated from the corresponding SEC-curves given in Fig. 1a

Sample	$(K_d)_{peak}$	$(K_d)_n$	$(K_d)_w$	$(K_d)_z$
Glucose	0.794 ± 0.003[a]	0.790 ± 0.003	0.792 ± 0.003	0.795 ± 0.003
PDX	0.183 ± 0.003	0.113 ± 0.005	0.201 ± 0.008	0.24 ± 0.01
FST	0.211 ± 0.002	0.13 ± 0.04	0.235 ± 0.004	0.287 ± 0.008
HST	0.181 ± 0.006	0.09 ± 0.01	0.218 ± 0.004	0.29 ± 0.01
T4	0.09 ± 0.03	0.034 ± 0.002	0.192 ± 0.003	0.340 ± 0.008

[a] ± corresponds to standard deviations at $n = 4$ (glucose, HST, T4), $n = 3$ (PDX) and $n = 6$ (FST).

different sources and isolation procedures of the target humic materials used in both studies. Of particular importance can be the use of different detectors for quantification of the SEC-elution curve. In this study, a DOC-detector was used, which is very sensitive to low MW, non-UV active impurities. This causes enhanced tailing of the chromatogram, and as a result, leads to higher values of polydispersity. In the work of Chin et al. [10], UV-SEC-profiles were used, which are not sensitive to low MW impurities and the rear front of the chromatogram has much smaller tailing. However, UV-detection has another disadvantage connected with the possible distortions of the recorded chromatographic distribution due to differences in absorptivities of HS fractions of different MW's.

It should be noted that, in contrast to M_w/M_n, the M_z/M_w ratios for the same HS samples had a much tighter range of variation of 1.95–2.34. The largest difference in M_w/M_n and M_z/M_w values was observed for the most polydisperse T4 sample.

4.2. Numerical description of the SEC-chromatograms of HS

The average K_d's of the model compounds and target humic materials are given in Table 2, and their ratios are given in Fig. 2. For all the materials under study, except for the individual compound — glucose, the much larger difference between the values of $(K_d)_w/(K_d)_n$ and $(K_d)_z/(K_d)_w$ ratios is observed compared to that of the corresponding MW ratios. This could be indicative of the higher discriminatory power of these parameters in comparison with the MW-analogues. In addition, all the determined $(K_d)_w/(K_d)_n$ ratios (except for that of the glucose) were bigger than $(K_d)_z/(K_d)_w$ ones. This was not the case for the MW: for polydextran and FST, M_w/M_n ratio was smaller than the M_z/M_w. Hence, calculation of the K_d-ratios provides additional information on the peculiarities of the SEC-elution curve of humic materials.

Consideration of the statistical moments of the corresponding SEC-chromatograms (Table 3) shows rather high variability of central statistical moments among the HS samples studied. It is of importance that mc_2 changes similar to the M_w/M_n ratio. Given that both parameters characterise a breadth of the chromatographic distribution, this can indicate the validity of the values obtained. For all three HS samples, only positive values of mc_3 are obtained. This is also reasonable because visual examination of the chromatograms under consideration (Fig. 1a) shows that they are skewed to the front boundary of the chromatographic zone. Among the HS samples, the

Table 3
Statistical moments calculated from the corresponding SEC-curves given in Fig. 1a

Sample	m_2 $(\times 10^3)$	m_3 $(\times 10^3)$	m_4 $(\times 10^3)$	mc_2 $(\times 10^3)$	mc_3 $(\times 10^3)$	mc_4 $(\times 10^3)$
Glucose	630 ± 5[a]	502 ± 6	402 ± 6	1.99 ± 0.09	0 ± 0.03	0.018 ± 0.002
PDX	48 ± 4	13 ± 2	3.9 ± 0.9	8 ± 1	0.2 ± 0.1	0.20 ± 0.04
FST	67 ± 3	23 ± 2	9 ± 1	12 ± 1	1.0 ± 0.3	0.7 ± 0.1
HST	63 ± 4	22 ± 2	9 ± 1	15 ± 2	1.6 ± 0.2	0.9 ± 0.2
T4	65 ± 2	28 ± 2	14 ± 1	28 ± 1	4.8 ± 0.8	2.9 ± 0.4

[a] ± corresponds to standard deviations at $n = 4$ (glucose, HST, T4), $n = 3$ (PDX) and $n = 6$ (FST).

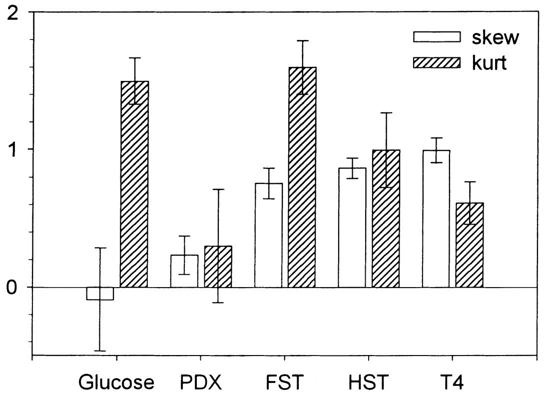

Fig. 3. *Skewness* and *kurtosis* calculated from the SEC-curves of two model compounds and three humic materials given in Fig. 1a. Error bars correspond to standard deviations at $n=4$ (glucose, HST, T4), $n=3$ (PDX) and $n=6$ (FST).

maximum value of mc_3 (the highest asymmetry) was found for T4, and the lowest one for FST.

Two more parameters characteristic of the shape of the chromatographic distribution — *skewness* and *kurtosis* — are given in Fig. 3. All the *skew* values (except for glucose) are positive, reflecting a skewness of the raw-chromatograms towards a front edge of the chromatographic zone. The maximum skewness (0.99) is characteristic of T4, the minimum one (−0.1) being characteristic of glucose. The *kurtosis* values for the SEC-chromatograms of all the materials studied are positive, reflecting more peaked distribution relative to the normal one. The biggest *kurtosis* values are found for glucose (1.5) and FST (1.6). In general, it should be noted that both *skewness* and *kurtosis* values are characterised with much bigger standard deviations in comparison with those of the K_d's or MW ratios.

4.3. Variance analysis of the SEC-descriptors of HS

To rank roughly the validity of the 20 SEC- descriptors under consideration for the purposes of HS classification, the multivariate variance analysis was conducted under the assumption that each of the three target humic materials represents a separate class of HS. For this purpose, a variance of the parameter within the class or, in our case, within the results of the parallel SEC-determinations of the same humic material (within SD), was calculated and compared to a variance between the classes, in our case, between the results of the SEC-determinations of the three

Table 4
Statistical significance of discrimination between three humic materials under study provided by use of the proposed SEC-descriptors

SEC-descriptor	Between SD[a]	Within SD[a]	Calculated F[b]	p
M_{peak}	4.39×10^{8}	2.60×10^{7}	17	0.00045
M_n	1.24×10^{6}	1.20×10^{5}	10.3	0.0030
M_w	1.78×10^{8}	3.38×10^{5}	527	1.2×10^{-11}
M_z	1.14×10^{9}	1.12×10^{7}	103	7.7×10^{-8}
$(K_d)_{\mathrm{peak}}$	1.93×10^{-2}	3.23×10^{-4}	60	1.2×10^{-6}
$(K_d)_n$	1.00×10^{-2}	6.50×10^{-4}	15	0.00065
$(K_d)_w$	2.24×10^{-3}	1.65×10^{-5}	136	1.8×10^{-8}
$(K_d)_z$	3.89×10^{-3}	8.40×10^{-5}	46	4.4×10^{-6}
M_w/M_n	1.29×10	6.78×10^{-2}	190	3.0×10^{-9}
M_z/M_w	1.50×10^{-1}	4.06×10^{-2}	3.7	0.059
$(K_d)_w/(K_d)_n$	1.66×10	1.65×10^{-1}	101	8.4×10^{-8}
$(K_d)_z/(K_d)_w$	3.87×10^{-1}	1.16×10^{-3}	334	1.4×10^{-10}
Skew	6.89×10^{-2}	9.25×10^{-3}	7.4	0.0090
Kurt	1.23	4.39×10^{-2}	28	4.8×10^{-5}
m_2	2.26×10^{-5}	8.89×10^{-6}	2.5	0.12
m_3	4.64×10^{-5}	3.66×10^{-6}	13	0.0014
m_4	3.99×10^{-5}	1.44×10^{-6}	28	5.2×10^{-5}
mc_2	3.28×10^{-4}	2.47×10^{-6}	133	2.0×10^{-8}
mc_3	1.79×10^{-5}	2.07×10^{-7}	86	1.9×10^{-7}
mc_4	6.52×10^{-6}	6.45×10^{-8}	101	8.3×10^{-8}

[a] The corresponding mathematical expressions are given in text by Eqs. (17) and (18).

[b] To be compared with a tabular value of F-criterion at $f_1=2$, $f_2=11$, $p=0.05$: $F=3.98$ [16]. See text for more explanations.

target humic materials (between SD). The corresponding mathematical expressions are given below [16]:

$$\text{Between SD} = \frac{\sum_{j=1}^{m} n_j (\bar{x}_j - \bar{\bar{x}})^2}{m-1} \qquad (17)$$

$$\text{Within SD} = \frac{\sum_{j=1}^{m} \sum_{i=1}^{n_j} (x_{ji} - \bar{x}_j)^2}{n-m} \qquad (18)$$

where

m	number of the samples;
n_j	number of chromatograms of the sample *j*;
$n = \Sigma n_j$	total number of chromatograms;
$\bar{\bar{x}}$	total average value of parameter (averaged by all chromatorgams);
$\bar{x}_j$	average value of the parameter for chromatograms of the sample *j*;
x_{ji}	value of the parameter for the chromatogram *i* of the sample *j*;

A ratio of a variance between the classes to a variance within the classes (designated as *F* in Table 4) estimates a significance of the discrimination provided by the parameter. If the calculated *F*-value is higher than the corresponding tabulated *F*-criterion, the parameter allows a discrimination between the classes at the given significance level, and consequently, can be used for the purposes of discrimination or classification. The higher the *F*-value is, the better the discriminatory power of the parameter that is to be expected.

In our case, the number of the samples (*m*) was three, and the total number of chromatograms (*n*) was 14. This yields the degrees of freedom: $f_1 = 2$ and $f_2 = 11$. The corresponding tabulated value of *F*-criterion at $p = 0.05$ is 3.98 [16]. The calculated *F*-values and significance levels *p* are given in Table 4. As can be seen, the calculated *F*-values below the tabulated one at $p = 0.05$ (2.5 and 3.7) were obtained only for two parameters (m_2 and M_z/M_w, respectively). The highest *F*-values of 527 and 334 were obtained for M_w and $(K_d)_z/(K_d)_w$, respectively. This allows a preliminary conclusion that the SEC-descriptors under consideration can be very useful for the purpose of discrimination between the humic materials of different origin. To estimate a discriminatory power of each of the proposed descriptors more generally, a much bigger set of the humic materials is to be used. Application of the SEC-descriptors for the purposes of discrimination and classification of humic materials of different origin and fractional composition is our immediate goal.

5. Conclusions

A new approach to numerical description of SEC-chromatograms of HS is proposed. It is based on the calculation of initial, central and normalised statistical moments and some of their ratios from the raw SEC-elution curves. Introduction of these parameters into a set of usually determined MW characteristics (average MW's and their ratios) allowed us to extend a set of SEC-descriptors up to 20 parameters. Their applicability to the discrimination of humic materials was demonstrated on examples of three HS samples of different origin and fractional composition.

Acknowledgements

We extend our deepest appreciation to Prof. F. Frimmel and Dr. G. Abbt-Braun from the Division of Water Chemistry, University of Karlsruhe (Germany) for putting LC-DOC facilities at the disposal of Dr. Perminova during her work as a visiting scientist and S. Hesse from the same institution for an invaluable technical assistance. Our thanks to Dr. A. Garmash (Department of Chemistry, Lomonosov Moscow State University, Russia) for very useful comments and discussions on the manuscript. Two anonymous referees are deeply appreciated for the constructive comments which allowed us to improve the quality of the manuscript. R. Havemann Scholarship is acknowledged for providing a stipend to A. Kudryavtsev during his Ph.D. studies.

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