Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 67 (2005) 103–111
Talanta
www.elsevier.com/locate/talanta

Comparative study for differentiation of aquatic humic-type organic constituents by capillary zone electrophoresis using polyvinyl alcohol-coated capillary

Juhani Peuravuori [a,*], Tero Lehtonen [a], Viia Lepane [b], Kalevi Pihlaja [a]

[a] *Department of Chemistry, Physical Chemistry, University of Turku, FIN-20014 Turku, Finland*
[b] *Department of Analytical Chemistry, Tallinn Technical University, 19086 Tallinn, Estonia*

Received 7 October 2004; received in revised form 24 January 2005; accepted 15 February 2005
Available online 17 March 2005

Abstract

Capillary zone electrophoresis (CZE) with UV detection (254 nm) was applied to characterize aquatic dissolved humic matter (DHM) from different environmental sources (lake, river and sea waters, in all 15 different samples). A series of separation examples of DHMs using a polyvinyl alcohol (PVA)-coated silica open tubular capillary were carried out in a phosphate buffer (40 mM) as a background electrolyte at neutral acidity (pH 6.8). The separative power of electropherograms was reasonable and the reproducibility was above the mark. Each electropherogram was characteristic of the corresponding humic sample. Special functional fulvic and humic acids or their overall mixtures separated with XAD, DAX and DEAE sorbing solids as well as the original dissolved organic matter (DOM) were nicely differentiated according to their environmental sources. The PVA coating of open tubular silica capillaries seems to be very potential in electrophoretic characterization and separation of different humic solutes at neutral acidities with low sample concentrations thus permitting a workable technique, in a growing series of CZE studies, for better compared results from different studies.

Keywords: Capillary zone electrophoresis; Polyvinyl alcohol-coated capillaries; Humic substances; Differentiation

1. Introduction

Hundreds of CZE analyses have been carried out at short notice in the studies of macromolecular natural organic matter (NOM) using numerous electrolytes at different acidities (e.g., see Refs. [1–4]). It is essential to bear in mind that several operational definitions between so-called humic substances (or more accurate humic matter, HM) and the abbreviations of NOM and DOM are in reality like a line on the water. The electrophoretic separations have been performed almost invariably by uncoated fused-silica capillaries using basic (pH 8–9) background electrolytes and different complexing agents (e.g., borate buffers) or other powerful modifiers. As a general rule, these studies have one relation in common: their contribution to the advancement of our understanding of so-called humic substances, excepting some special cases (e.g., see Ref. [5]), is mostly limited because it is quite impossible to compare results from different studies, and it is not known whether variations are caused by sample properties or experimental conditions.

It has been demonstrated [6–8] that miscellaneous manipulations of acidity, buffer composition and other variables will generate informative electropherograms for humic solutes by using simple uncoated fused-silica capillaries. However, according to the general literature, many problems, e.g., the electro-osmotic flow (EOF), sorption effect and the influence of powerful complexing agents, exist in studies of humic solutes by uncoated fused-silica capillaries. For that reason, it is meaningful to try reducing the variation of several variables by utilizing coated capillaries mainly due to two reasons [9]: (1) to prevent the wall interaction with the

* Corresponding author. Fax: +358 2 3336700.
E-mail address: juhpeur@utu.fi (J. Peuravuori).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.02.011

sample and (2) to reduce the EOF effect. Neutral coatings are usually bi-layers, consisting first a bound phase to shield the silanol groups followed by a hydrophilic polymer to prevent hydrophobic interaction. This property makes them ideal for the separation of complex samples, containing different sorts of ionic functional groups and hydrophobic structural components, enhancing the resolution of the electropherograms by suppressing the EOF effect and reducing the band broadening induced by analyte–wall interactions [10–12].

Unaccountably, the utilization of coated open tubular capillaries for suppression of EOF in CZE studies of so-called humic substances is relatively uncommon and only a few comments can be found (e.g., see Ref. [13]). On the contrary, the molecular sieving effect, especially the utilization of different non-cross-linked polymer solutions, has been more popular in the humus chemistry [14–18]. This modification (so-called physical gels or entangled polymer solutions), generally applied by uncoated silica capillaries, increases buffer viscosity, mostly generates better separative power through the sieving effect and also serves dynamic deactivation against the inner capillary wall.

On the other hand, it has been recently demonstrated [19] by a thorough study using different CZE applications that the simple PVA coating (a non-charged polymer consisting of maximum hydrophilicity) of silica capillaries offers a practical technique for the CZE characterization of aquatic humic solutes with moderate resolution of electropherograms even without extra inorganic or organic modifiers. This previous work [19] also spoke, irrespective of the fact that humic constituents in urea solutions undergo slow changes resulting from denaturation process or some formation of humic complexes with buffer solutions may also be potential, for that the overall effect of the urea modifier on the resolution of electropherograms was practically rather slight compared with the pure water as the sample solvent, i.e. structural changes of DHM–DOMs were nonexistent, and no formation of humic complexes with the applied buffer solution was observable, especially when using PVA-coated open tubular capillary. On the contrary, the acidity and concentration of the applied phosphate buffer solution were the most critical parameters.

The main objective of this work was to demonstrate the practical adaptation of PVA-coated silica open tubular capillary for CZE analyses of different DHM–DOMs. Phosphate buffer (40 mM) with pH value (6.8) characteristic for natural fresh waters was applied to CZE characterization and differentiation of 15 aquatic DHM–DOM samples originating from different environmental sources. The solid DHM–DOM samples were dissolved in 5 M urea–water for slightly improving the resolution of electropherograms. Also, some electrophoretic parameters were calculated for different DHM–DOM samples. The multidimensional data set so obtained was examined with principal component analysis (PCA) for confirming the fingerprinting ability of the different electropherograms.

2. Experimental

2.1. Reagents

All solutions and buffers were prepared from analytical (p.a. or HPLC) chemicals and ultra-pure water (Elgastat UHQ-PS). Forty millimolars of phosphate buffer at pH 6.8 was prepared from sodium dihydrogen phosphate and disodium hydrogenphosphate. All the solutions were degassed in an ultrasonic bath and filtered through 0.45 μM PTFE membrane filters (Titan) before the experiments.

2.2. Capillary

The dimensions of PVA-coated silica capillary (Agilent G1600-61419) were: 100 μm internal diameter, 64.5 cm total length and 56.0 cm effective length. The capillary was pre-washed daily before sample injections for 15 min with the electrolyte solution at 940×10^2 Pa and conditioned for 2 min without pressure with −10 kV voltage. At the end of daily experiments the capillary was rinsed with the electrolyte solution (5 min), water (15 min) and finally dried with airflow (10 min). For more thorough cleaning the capillary was rinsed with 10 mM phosphoric acid.

2.3. Instrumentation

CZE experiments were performed on an HP^{3D}CE capillary electrophoresis system (Hewlett-Packard) equipped with a diode array detector at 254 nm. Operation of the instrument, data collection and analyses were controlled by ChemStation software. The polarity was negative, voltage of −10 kV, temperature 25 °C, total run time 35 min. Samples were injected hydrodynamically using pressure of 5000 Pa for 10 s. Dissolved organic carbon (OC) measurements were performed on Shimadzu TOC-5050 Carbon Analyser utilizing catalytic oxidation at 680 °C.

Relative $UV_{254\,nm}$-absorbances {(UV absorbance at 254 nm/OC of the original sample, mg l^{-1}) × 100} were calculated for the electropherograms irrespective of the fact that the actual OC concentration of the migrated sample is not exactly known but the influence of the viscosity on injection volumes between different samples was assumed to be slight and quite constant. The standardized relative $UV_{254\,nm}$-absorbances make the results more comparable with each other. The common meaning of the $UV_{254\,nm}$–OC ratio in the water chemistry is to indicate the aromaticity of the sample.

2.4. Samples

Natural fresh water humic samples were isolated from the Lake Savojärvi (S: February 1988, SS: September 1994) and from the Lake Mustajärvi (Mu: October 1999) situated in southwestern part of Finland. The International Humic Substances Society reference samples of Nordic aquatic humic

acid (No.HA, code IR105H) and fulvic acid (No.FA, code IR105F) were isolated from the runoff water of a Norwegian mire. Other humic samples were isolated from the territory of Estonia: one river water sample from the River Pirita (Pirita, January 2001), two sea water samples from the estuary of the River Pirita (PiritaM, February 2001) and the seaside of the island Saaremaa in the Baltic Sea (MaasiM, February 2001), two lake water samples from the Lake Koigi (Koigi, August 2000) and from the Lake Maardu (Maardu, November 2000).

The DHM–DOMs from the S and SS samples were isolated with the well-known conventional sorbing solid technique at pH 2 using the Amberlite® XAD-8 resin and further divided at pH 1 into functional humic (HA)- and fulvic (FA)-acid fractions using the method applied to the Nordic reference humic samples. Furthermore, in the case of the SS water sample the DEAE cellulose was applied for isolating the integrated whole of practically all humic solutes at the natural acidity and this DHM–DOM sample is sub-labelled as SS.DEAE. In the case of the water sample Mu, the DHM–DOM was isolated with the XAD technique using both the non-ionic acryl ester-based Amberlite® XAD-8 resin (isolated humic extracts are sub-labelled as Mu.XAD) and the Supelite™ DAX-8 resin (isolated humic extracts are sub-labelled as Mu.DAX). The isolation procedure with the DAX-8 resin was analogous to that of XAD-8 resin. Likewise, the DEAE isolation procedure was applied to the Mu water sample and the macromolecular organic acids are sub-labelled as Mu.DEAE. The humic solute isolates SS.DEAE, Mu.XAD, Mu.DAX and Mu.DEAE were not, however, further divided at pH 1 into functional HA- and FA-type acid subfractions. The isolation procedures and the chemical characteristics of the freeze-dried No.FA, No.HA, S.FA, S.HA, SS.FA, SS.DEAE, Mu.XAD, Mu.DAX and Mu.DEAE humic fractions have been reported [20,21] in detail previously.

The DHM–DOMs of the Estonian fresh and sea water samples were isolated also with the XAD column technique using exceptionally the non-ionic Sigma–Aldrich XAD-16 resin. The obtained humic concentrates (Pirita, PiritaM, Koigi, Maardu and MaasiM) were not divided at pH 1 into functional HA- and FA-type subfractions but they represented the integrated whole of the DHM–DOM in question. The XAD-16 isolation procedure as well as some basic chromatographic properties of the different Estonian aquatic DHM–DOMs have been reported previously [22,23].

The weighted DHM–DOM samples were first dissolved in known volumes of water for verifying their capacity for OC. The weighted DHM–DOM samples were further dissolved in 5 M urea and filtered (0.45 μm) before the CZE analyses. The calculated OC contents of different DHM–DOM concentrates in their 5 M urea solutions were between 13 and 141 mg l^{-1}. The applied urea concentration in the original SS water sample was also 5 M. All DHM–DOM solutions were analysed after 5 days of preparation, according to a previous study [19], for obtaining practically enough reproducible results. The aromaticity for Estonian DHM–DOM samples (Koigi, Pirita, PiritaM, Maardu and MaasiM) was estimated using the known literature values measured by 13C $NMR_{solid\ state}$ [20,21,24,25] for the aromaticities of No.-, S.-, SS.- and Mu.DHM samples (10 examples). Syringic acid served as an extraordinary reference compound for proportioning absorptive capacities (integrated areas of obtained electropherograms) of different DHM–DOMs at 254 nm to the known aromaticities obtained by 13C NMR. The fit ($aromaticity_{254\ nm}$ versus $aromaticity_{13C\ NMR}$) thus generated was quite reliable ($r^2 = 0.98$) permitting the estimation of the aromaticities for the five unknown DHM–DOM samples.

3. Results and discussion

3.1. CZE of aquatic DHM–DOM in phosphate buffer

The electropherograms of 15 different aquatic humic samples are shown in Figs. 1–3 (also blanks were analysed to confirm that absolutely no extra peaks, caused by the buffer, were detected with the PVA-coated capillary). Table 1 shows some essential characteristics and electrophoretic parameters connected with Figs. 1–3. The reproducibility of the electropherograms for all DHM–DOM samples as well as for the syringic acid was good. The precision was estimated as an average standard deviation of the mean electropherogram within the whole migration time range using duplicate determinations (differences between replicated electropherograms were moderate constant confirming the validity of the technique). Small differences between estimated (254 nm) aromaticities and corresponding literature values (13C NMR) speak for the validity of the choice of syringic acid as a reference compound in computation the unknown aromaticities of Estonian DHM–DOMs. The inclusion of syringic acid also contributed to the interpretation of the electropherograms on the ground that aromatic structural subunits of DHM–DOM constituents resemble it.

The average electrophoretic mobilities in Table 1 (AEM, $m^2\ s\ V^{-1}$) were calculated for the DHM–DOM peaks taking into account the electrophoretic velocity (ν_e, m s^{-1}) and the electric field strength (E, V m^{-1}) according to the equation: $AEM = \nu_e/E = (L_d/t_m)/(V/L_t)$,where L_d is the length of the capillary to the detector, L_t the total length of the capillary, V the applied voltage and t_m is the migration time. The AEM as a normalized parameter permits, in principle, the comparisons with similar literature samples. However, the utilization of many literature AEM values, especially generated by uncoated bare fused-silica capillaries, is quite insignificant resulting from so-called ‘humic humps’. Accordingly, the different AEM values given in the literature reference [1] are quite close to each other, independent of the DHM–DOM sample, thus producing a weak discriminating factor. On the other hand, the AEM values of the present study in Table 1 vary in the case of all DHM–DOM isolates from -2.72×10^{-8} to $-4.12 \times 10^{-8}\ m^2\ s\ V^{-1}$. This range of variation is significantly greater than that given in the literature reference [1] for several fresh water DHM–DOM samples

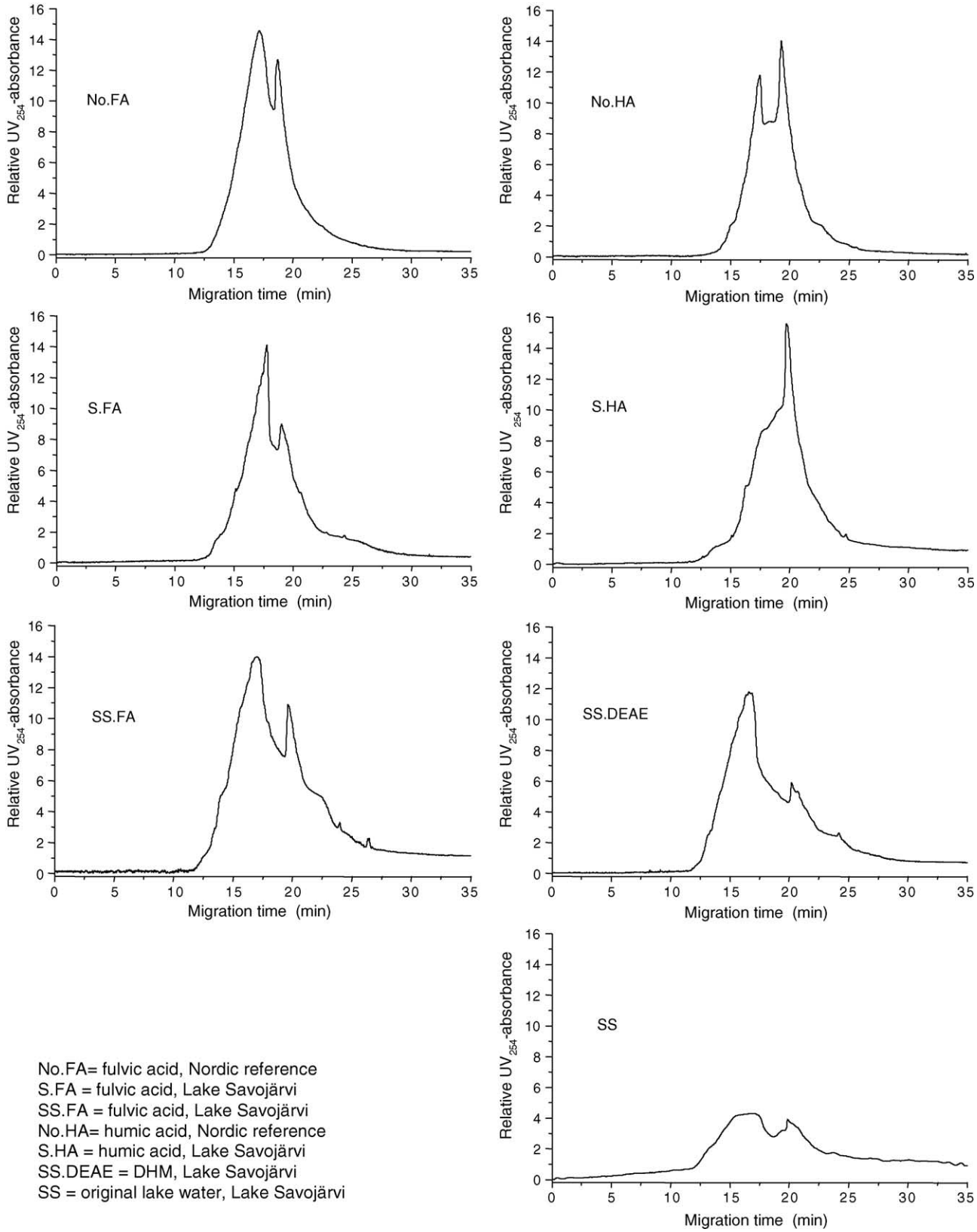

Fig. 1. Electropherograms of different lake water DHM-DOM fractions and original lake water DOM in 40 mM phosphate buffer (pH 6.8). CZE conditions: voltage −10 kV; PVA coated capillary, 64.5 cm × 100 μm i.d. (effective length 56.0 cm); injection hydrodynamic 5000 Pa for 10 s; samples 0.15 mg ml^{-1} in 5 M urea; detection 254 nm; applied sorbing solids were XAD-8 resin and DEAE cellulose. In the case of SS.DEAE the DHM-DOM isolate is not divided into functional HA- and FA-type subfractions.

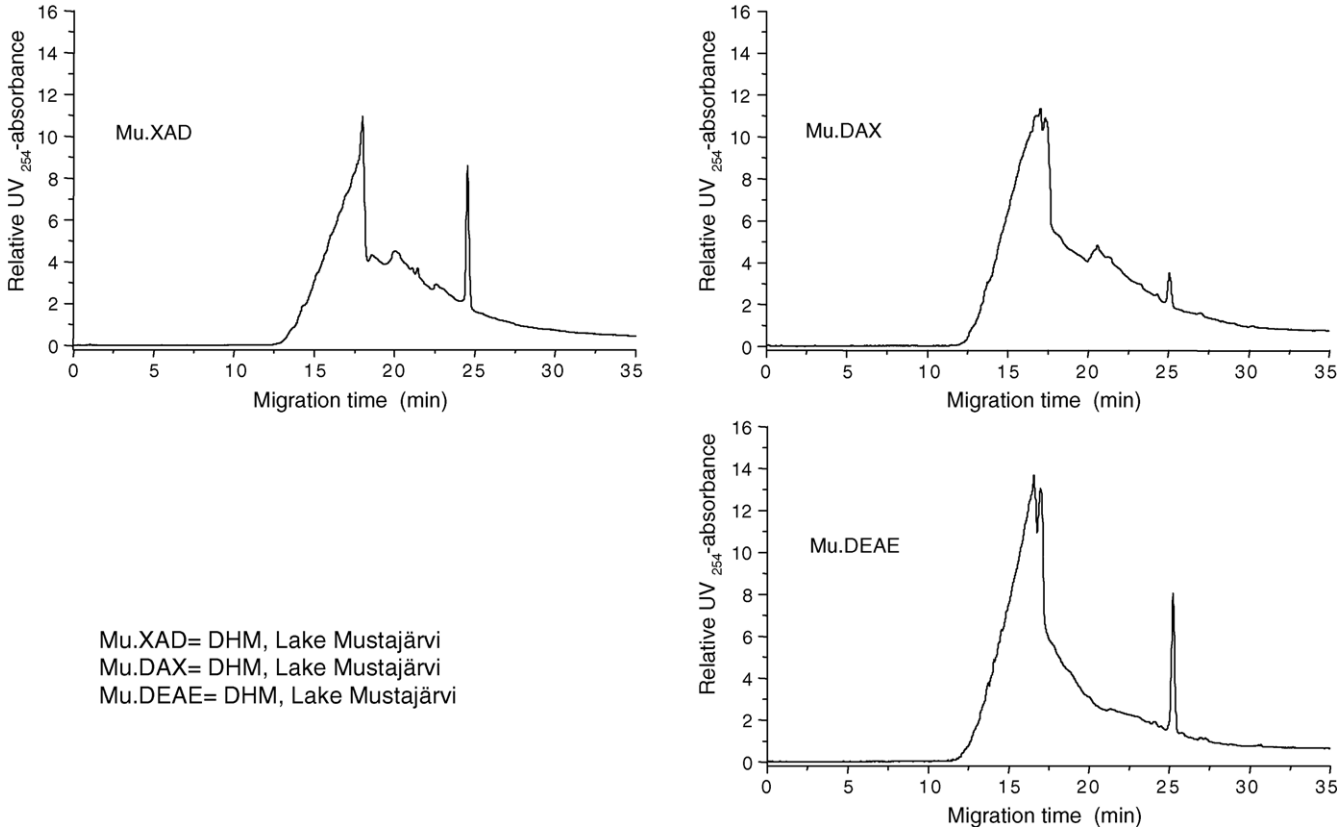

Fig. 2. Electropherograms of three different lake water DHM–DOM fractions. CZE conditions the same as in Fig. 1. Applied sorbing solids were XAD-8 and DAX-8 resins and DEAE cellulose. DHM–DOM isolates were not divided into functional HA- and FA-type subfractions.

(from -3.8×10^{-8} to -4.0×10^{-8} $m^2\,s\,V^{-1}$), thus speaking for the significance of the coating of the silica capillaries. It was also possible in the present study, because of the moderately good resolutions of the electropherograms, to determine the accurate electrophoretic mobilities (EM) for the separated distinct sub-constituents (peaks), as shown in Table 1.

Fig. 1 demonstrates that electropherograms of functional FA-type subfractions of DHM–DOM resemble enough each other. The same phenomenon also occurs but weaker in the case of functional HA-type subfractions. Despite the fact that exactly the same XAD isolation technique was applied for these natural fresh waters originating from different environmental conditions, these humic solute fractions must play a role as certain definite entities and they cannot be merely accidental ones of the isolation procedure, as reported previously [20]. The electropherogram of the SS.DEAE differed relatively much from those of S.FA. SS.FA or S.HA. This is quite natural because the SS.DEAE fraction was isolated at the natural acidity by the DEAE cellulose technique, and this isolate was not acid-precipitated into functional FA- and HA-type subfractions. The close similarity between the electropherograms of SS.DEAE and the corresponding original water sample SS is readily understandable because the SS.DEAE isolate accounts for about 80% of the OC in the original lake water [26]. In Fig. 1 the distinct and well-resolved electropherogram of the original water sample SS is remarkable taking into account its very low organic carbon content.

Fig. 2 shows the electropherograms for the three DHM–DOM isolates obtained by three different sorbing solids from the lake water sample Mu. The electropherograms of the Mu.DHM isolates obtained by XAD-8 and DAX-8 resins and DEAE cellulose resemble closely each other. This similarity is, in all probability, result of the fact that these isolates were not fractionated at pH 1 into functional FA and HA subfractions but they represented the integrated whole of the applied isolation technique in question. The characteristic feature in Fig. 2 is the existence of a sharp peak at the migration time of about 25 min. Because this sharp peak is present in all electropherograms it evidently represents a true electrophoretic fraction being representative of the special nature of the lake water Mu.

Fig. 3 summarizes the electropherograms for two lake waters, two sea waters and one river water DHM–DOMs collected from Estonia. All these five samples were isolated by a non-ionic styrene–divinylbenzene XAD-16 resin following the common so-called XAD column-technique based on certain hydrophobic–hydrophilic interactions between organic solutes and the sorbing solid under the preadjusted, very acidic conditions. These DHM–DOM isolates were not further divided into functional FA and HA subfractions. The most striking outcome is, in addition of the very

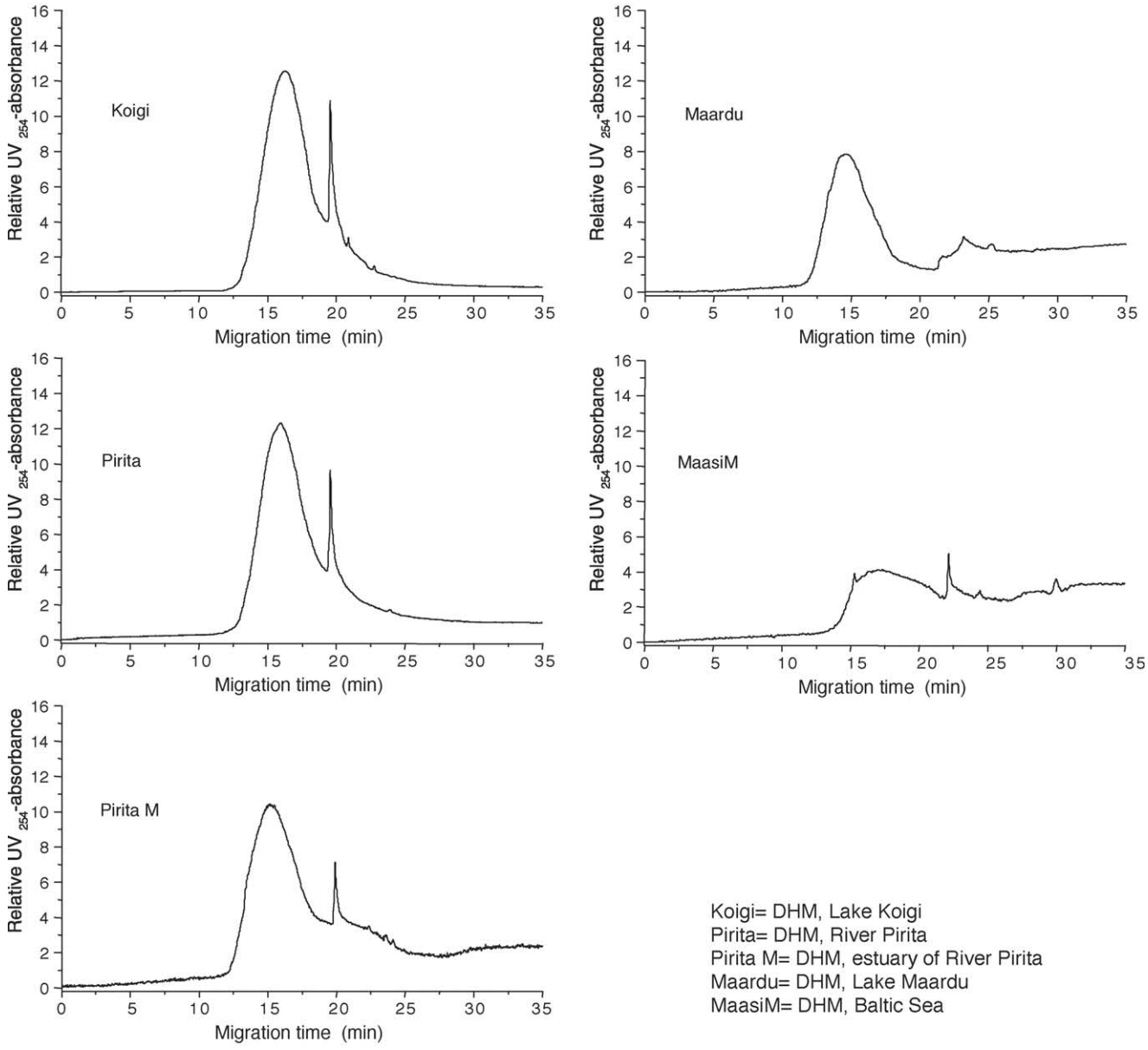

Fig. 3. Electropherograms of isolated water DHM–DOM fractions originated from different aquatic environments. CZE conditions the same as in Fig. 1. Applied sorbing was XAD-16 resin. DHM–DOM isolates were not divided into functional HA- and FA-type subfractions.

close resemblance between the electropherograms of the DHM–DOMs obtained from the River Pirita and its estuary, also their uniformity with that obtained from the Lake Koigi. The electropherograms of Koigi, Pirita and PiritaM were characterized by sharp 'shoulder' peaks at the migration time of about 20 min. The shapes of these three electropherograms resemble roughly those presented in Fig. 1 for functional FA-type subfractions. This, together with the fact that functional FA subfractions of DHM–DOMs retained on non-ionic sorbing solids at acidic conditions account for about 85% of the total DHM–DOMs in organically coloured waters common to Nordic countries, Northern Russia and Canada, supports the view that a general resemblance dominates between hydrophobic humic solutes originating from similar environmental conditions and that the products obtained are at least to a certain degree real and not just accidental outcomes of the applied non-ionic sorbing solid matrix [27]. The close resemblance prevailing between the DHM–DOMs obtained from the River Pirita and its estuary speaks for the important and permanent effect of the surface runoff on the sea water DHM–DOM. The electropherogram of the Lake Maardu is totally different from all the others obtained from fresh waters in the present study. One reason for this irregularity may be the special water composition of this lake (a polluted lake in the district of Tallinn [23]). The PVA-coated capillary was also able to generate a reasonably informative electropherogram for a sea water DHM–DOM (Baltic Sea, MaasiM) despite its low organic carbon content.

Table 1
Characteristics of DHM–DOM samples together with electrophoretic data

Sample	Peak maximum (min)	EM[a] ($\times10^{-8}$) ($m^2\,s\,V^{-1}$)	Average peak maximum (min)	AEM[b] ($\times10^{-8}$) ($m^2\,s\,V^{-1}$)	OC[c] ($mg\,l^{-1}$)	Aromaticity		Precision[f] (±%)
						254 nm (%)[d]	13C NMR (%)[e]	
No.FA	17.15 18.72	−3.51 −3.22	17.18	−3.50	75.1	36.5	37.1	17.7
S.FA	17.78 19.01	−3.39 −3.17	17.79	−3.38	76.3	30.4	27.8	16.3
SS.FA	16.93 19.63	−3.56 −3.07	16.92	−3.56	76.6	29.9	25.9	15.8
No.HA	17.47 19.30	−3.45 −3.12	19.3	−3.12	77.3	39.6	45.2	16.7
S.HA	19.73	−3.05	19.73	−3.05	74.7	38.0	39.2	15.1
SS.DEAE	16.62 20.20 24.18	−3.62 −2.98 −2.49	16.63	−3.62	75.4	32.9	32.0	14.8
SS (original water)	16.91 19.87	−3.56 −3.03	16.89	−3.56	17.7	16.5	18.0	13.5
Mu.XAD	17.99 18.59 20.06 22.59 24.55	−3.35 −3.24 −3.00 −2.66 −2.45	18.01	−3.34	71.0	31.0	34.7	18.2
Mu.DAX	17.36 20.59 25.07	−3.47 −2.92 −2.40	17.05	−3.53	72.1	31.5	34.8	14.4
Mu.DEAE	16.57 16.98 25.22	−3.63 −3.55 −2.39	16.58	−3.63	60.5	33.8	33.9	15.7
Koigi	16.25 19.57	−3.70 −3.08	16.22	−3.71	141.2	37.0		18.5
Pirita	15.95 19.55	−3.77 −3.08	15.94	−3.78	97.5	35.0		16.3
PiritaM	15.11 19.92	−3.98 −3.02	15.12	−3.98	22.8	17.5		23.5
Maardu	14.60 23.17 25.18	−4.12 −2.60 −2.39	14.60	−4.12	19.2	14.0		15.1
MaasiM	15.30 16.81 22.16 24.44 29.98	−3.93 −3.58 −2.72 −2.46 −2.01	22.15	−2.72	13.1	5.8		22.8
Syringic acid[g]	29.04	−2.07	29.04	−2.07	163.5			2.8

[a] Electrophoretic mobility at the peak maximum.
[b] Average electrophoretic mobility.
[c] Carbon content in the 5 M urea solution.
[d] Estimated at 254 nm.
[e] Literature value [20,21,24,25].
[f] Relative reproducibility of the mean electropherogram ($P_{0.95}$).
[g] 4-Hydroxy-3,5-dimethoxybenzoic acid [CAS 530-57-4].

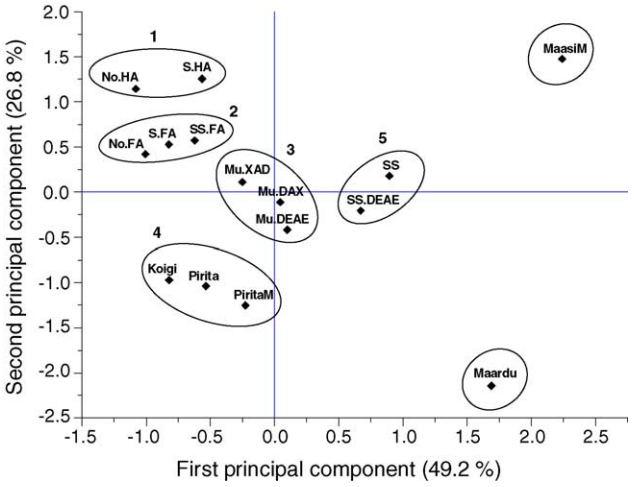

Fig. 4. Graphical 2D perspective of projections of the electrophoretic data set on the first two principal components for the distribution of the different DHM–DOM isolates.

The electropherogram of the sea water sample MaasiM and its aromaticity in Table 1 verify the special nature of the sea water DHM–DOM in comparison with the organic composition of the fresh water DHM–DOMs.

3.2. *Differentiation of electrophoretically characterized DHM–DOMs according to their origin*

For better extraction of the small differences obtained by CZE analyses and to find a stronger similarity–dissimilarity between samples together with their environmental effects, the electropherograms of Figs. 1–3 were slightly manipulated. The multidimensional data set of the electropherograms was examined closer with a statistical–graphical principal components analysis (PCA) [28]. The original data set (3500 data points, interval 0.01 min) was reduced, owing to the computational capacity, to smaller size (229 data points, migration time between 12.04 and 28.00 min, interval 0.07 min) yielding enough resolving power. For the PCA, from the 15×229 matrix (15 objects, DHM–DOM isolates, and 229 variables, relative $UV_{254\,nm}$-absorbances) variances and covariances were computed. From these, Eigenvectors and Eigenvalues for the original data set have been extracted. The fundamental idea of the PCA is to reduce the numerous variables and to seek for linear combinations of those variables explaining most of the variability. Accordingly, PCA (Eigenanalysis) and subsequent inspection of the Eigenvector plots is one of the first and foremost procedures that can be done when tackling a multidimensional data set.

Fig. 4 shows the 2D scatter-plot of projections (referred to also as scores) of the original data set on the first two principal components (the overall level of statement is 76% of the total variance for the first two PCs). PC1 serves as a coarse discriminating factor and the unresolved variation contributes to more specific discrimination between the samples on PC2. The most powerful discriminating effects on PC1 had those electrophoretically separated DHM–DOM's sub-constituents whose migration times were about 13–15 and 21–26 min. Similarly, on PC2 the interrelation between the different DHM–DOM samples was predominantly based on those electrophoretically separated DHM–DOM's sub-constituents whose migration times were about 13–15 and 17–21 min.

The different DHM–DOM samples in Fig. 4 were divided into five main groups: (1) (No.HA, S.HA); (2) (S.FA, SS.FA, No.FA); (3) (Mu.XAD, Mu.DAX, Mu.DEAE); (4) (Koigi, Pirita, PiritaM); (5) (SS, SS.DEAE). The sea water sample MaasiM and the lake water sample Maardu formed their own positions as outliers. The same similarity–dissimilarity between the different DHM–DOM samples is also partly foreseeable directly from the original electropherograms of Figs. 1–3 but the multidimensional projection permits general features of the data set in compressed form.

Fig. 4 shows that the groups 1, 2, 4 and partly 3 are closely located at the negative values of PC1. On PC2 the groups 1, 2 and 4 are clearly separated from each other but the interrelation of the groups 3 and 5 on PC2 is equal. The DHM–DOM samples of Koigi, Pirita and PiritaM isolated by the XAD-16 resin formed a separate group of their own (4). The most likely reason for this is the utilization of the XAD-16 sorbing solid instead of XAD-8 or DAX-8 resins but the local environmental conditions can also have their special influence on the composition of the DHM–DOM. The same effect of the applied sorbing solid on the interrelation of the different DHM–DOM samples is also slightly seen in the case of the DEAE cellulose isolates (samples Mu.DEAE and SS.DEAE, express a coarse structural analogy). It is notable that the XAD-16 isolates were not freeze-dried after isolation as was the case with the XAD, DAX and DEAE isolates. However, in a previous study it has been shown [29] that the freeze-drying process had no significant effect on the spectroscopic properties of the bulk of humic solutes speaking for the stability of the structural composition, i.e. fluorescence properties of the studied DHM–DOM solutes were practically equal before freeze-drying and after re-dissolving the dried solid matter.

4. Conclusions

- The results emphasize the applicability of the PVA-coated capillary for the characterization and differentiation of aquatic DHM–DOM by CZE. The separative power of electropherograms was moderately good using a phosphate buffer as a background electrolyte at neutral pH and relative low concentration of urea–water as a modifier of the sample itself.
- The PCA analysis of the electrophoretic data revealed the existence of five different sub-constituents among the analysed DHM–DOM samples. However, it is rather difficult to draw thorough conclusions as to the PCA analysis because the CZE is an extremely sensitive method containing certain internal distractions in relation to the

very heterogeneous nature of aquatic DHM–DOM. This being the case, the PCA analysis readily overstress the influence of the applied isolation technique on the minor structural differences between different DHM–DOM's compositions, even if a certain general resemblance of humic constituents originated from similar environmental conditions is assuredly seen.

- Irrespective of the fact that it is in all probability impossible to separate the original very complex and heterogenous DHM–DOM polyelectrolytes to distinct sharp electrophoretic patterns by electrophoretic methods, the PVA-coated capillaries offer a tool to work with very low sample concentrations and enable rapid fingerprint characterizations of different DHM–DOM samples.

Acknowledgements

The authors thank the International Office of Turku University and Turku University Foundation for financial support to Dr. V. Lepane. The authors are thankful to Prof. Pekka Mäntsälä (Department of Biochemistry and Food Chemistry, University of Turku) for the possibility of the CZE analyses.

References

[1] P.K. Egeberg, S.O. Bergli, J. Chromatogr. A 950 (2002) 221.
[2] E. Parlanti, B. Morin, L. Vacher, Org. Geochem. 33 (2002) 221.
[3] P. Schmitt-Kopplin, J. Junkers, J. Chromatogr. A 998 (2003) 1.
[4] M. Übner, V. Lepane, M. Lopp, M. Kaljurand, J. Chromatogr. A 1045 (2004) 253.
[5] M.L. Pacheco, E.M. Peña-Méndez, J. Havel, Chemosphere 51 (2003) 95.
[6] A.W. Garrison, P. Schmitt, A. Kettrup, Water Res. 29 (1995) 2149.
[7] P. Schmitt-Kopplin, A.W. Garrison, E.M. Perdue, D. Freitag, A. Kettrup, J. Chromatogr. A 807 (1998) 101.
[8] P. Schmitt-Kopplin, N. Hertkorn, A.W. Garrison, D. Freitag, A. Kettrup, Anal. Chem. 70 (1998) 3798.
[9] K.D. Altria, J. Chromatogr. A 856 (1999) 443.
[10] D. Belder, D. Stöckigt, J. Chromatogr. A 752 (1996) 271.
[11] D. Belder, K. Elke, H. Husmann, J. Chromatogr. A 868 (2000) 63.
[12] D. Belder, A. Deege, H. Husmann, F. Kohler, M. Ludwig, Electrophoresis 22 (2001) 3813.
[13] M. De Nobili, F. Fornasier, G. Bragato, in: J. Drozd, S.S. Gonet, N. Senesi, J. Weber (Eds.), The Role of Humic Substances in the Ecosystems and in Environmental Protection, Polish Society of Humic Substances, Wroclaw, 1997, pp. 97–101.
[14] A.E. Barron, D.S. Soane, H.W. Blanch, J. Chromatogr. A 652 (1993) 3.
[15] R. Dunkel, H.-H. Rüttinger, K. Peisker, J. Chromatogr. A 777 (1997) 355.
[16] M. De Nobili, G. Bragato, A. Mori, Acta Hydroch. Hydrobiol. 26 (1998) 186.
[17] M. De Nobili, G. Bragato, M. Mori, J. Chromatogr. A 863 (1999) 195.
[18] L. Pokorná, M.L. Pacheco, J. Havel, J. Chromatogr. A 895 (2000) 345.
[19] J. Peuravuori, V. Lepane, T. Lehtonen, K. Pihlaja, J. Chromatogr. A 1023 (2004) 129.
[20] J. Peuravuori, K. Pihlaja, Anal. Chim. Acta 363 (1998) 235.
[21] J. Peuravuori, P. Ingman, K. Pihlaja, R. Koivikko, Talanta 55 (2001) 733.
[22] V. Lepane, J. Chromatogr. A 845 (1999) 329.
[23] V. Lepane, Characterization of Aquatic Humic Substances by Size Exclusion Chromatography and Capillary Electrophoresis. Ph.D. Dissertation, Tallinn Technical University, 2001 Estonia, pp. 51.
[24] J. Peuravuori, Anal. Chim. Acta 429 (2001) 75.
[25] J. Peuravuori, K. Pihlaja, Anal. Chim. Acta 337 (1997) 133.
[26] J. Peuravuori, K. Pihlaja, N. Välimäki, Environ. Int. 23 (1997) 453.
[27] J. Peuravuori, K. Pihlaja, in: L.M.L. Nollet (Ed.), Handbook of Water Analysis, Marcel Dekker Inc., New York, 2000, pp. 387–408.
[28] Statistica for Windows, Statistica® 6.0, StatSoft Inc., Tulsa, 1996.
[29] J. Peuravuori, R. Koivikko, K. Pihlaja, Water Res. 36 (2002) 4552.