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Fingerprinting of natural organic matter by capillary zone electrophoresis using organic modifiers and pattern recognition analysis

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Abstract

Capillary zone electrophoresis was used for characterising nine samples of natural organic matter (NOM) using phosphate buffer (25 m*M*, pH 7) and various modifiers; methanol (50 m*M*), acetonitrile (10%,v/v), dimethyl sulfoxide (5%,v/v), and urea (5 *M*). Principal component analysis (PCA) was used to examine whether the electrophoretic profiles can be utilised as fingerprints for tracing the NOM samples to their source and/or type of location. It was found that all modifiers except methanol affect the electropherograms. Furthermore, it was found that the PCA analysis carried out on the electrophoretic profiles recorded in buffer solution modified by urea gave the best results for fingerprinting. The distribution of the fingerprints suggests a model for the humic substances in which all samples can be regarded as mixtures between two endmembers: autochtonous and allocthoneous NOM. © 2002 Published by Elsevier Science B.V.

Keywords: Principal component analysis; Water analysis; Humic substances

1. Introduction

Aquatic natural organic matter (NOM) includes all dissolved organic compounds. They range from the molecules, such as polysaccharides, peptides, *N*-acetylamino sugars and polyphenols, the four main types of polymers encountered in the biosphere as well as the complex organic material evolved from the combination of these biopolymers during their residence in water. These compounds are collectively called humic substances.

The high structural complexity and the wide range of molecular masses of NOM are serious obstacles for separation of these natural compounds. Virtually every separation method available to the modern chemist have been applied to NOM and humic substance, but with limited success [1]. During the last decade capillary electrophoresis proved its superiority for separation of a wide variety of biomolecules such as peptides and proteins [2]. The success stories in separating these compounds inspired many of the NOM scientists to adopt these techniques to separate humic substances. However, the scientists soon realised that most of the electropherograms of humic substances exhibit a broad ''humic bump'' with few characteristic details. Therefore the terms ''characterisation'' [3–7] and ''fingerprinting'' [8–10] are now used more fre-

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quently than the term ''separation'' [11,12] in conjunction with capillary zone electrophoresis (CZE) of humic substances.

It has been demonstrated that humic substances give reproducible electropherograms, and these have been termed ''fingerprints'' [8–10]. The capacity to achieve fingerprinting of NOM samples has limited value in terms of understanding their composition, but may have several other interesting applications. For example, the fingerprints may be used as tracers of water masses. If other properties such as molecular size, hydrophobicity etc. can be related to the fingerprints, they may be used for optimisation of water treatment processes [13].

Organic modifiers have been added to the aqueous buffers used during CZE of humic substances (usually phosphate, borate, and acetate buffers) to improve the resolution of the electropherograms [6,11]. Improving the resolution enhances the information content of the electropherograms, and may give rise to more characteristic fingerprints.

The objectives of this study is (i) to investigate the effect of urea, acetonitrile, methanol and dimethyl sulfoxide on the electrophoretic behaviour of NOM samples, and (ii) to investigate the potential of the resulting electropherograms for use as fingerprints by pattern recognition analysis.

2. Experimental

2.1. Instrumentation and running conditions

The instrumentation consisted of a HP^{3D}CE capillary electrophoresis system (Hewlett-Packard, USA) equipped with a diode array detector. The uncoated fused-silica CE columns [80 cm (effective length= 75 cm)×75 μm I.D.] were purchased from Hewlett-Packard. The running conditions were: 25 °C, linear voltage gradient from 0 to 30 kV in 60 s, applied pressure of 10 mbar on the anodic side, total run time 25 min, detector wavelength 254 nm, hydrodynamic anodic injection (sample: 50 mbar, 5 s, running buffer: 50 mbar, 4 s).

The data (migration time and absorbance at 254 nm) were collected by means of the Hewlett-Packard Chemstation software.

2.2. Buffer and modifiers

The separation buffer, 25 m*M* phosphate (pH 7.0), was prepared from sodium dihydrogenphosphate and sodium hydrogenphosphate (analytical-reagent grade from Fluka, Buchs, Switzerland) in ''Milli-Q water'' (Millipore, Bedford, MA, USA). Benzyl alcohol (analytical-reagent grade from Fluka) was employed as marker for the electroosmotic flow (EOF). The organic modifiers were methanol, dimethyl sulfoxide (analytical grade from Fluka), urea (electrophoretic quality from Sigma–Aldrich, Steinheim, Germany), and acetonitrile (analytical-reagent grade from Merck, Darmstadt, Germany). The concentration of the separation buffer (25 m*M*) was kept constant when adding the modifiers.

2.3. Sample handling

The NOM samples were separated by reverse osmosis (RO), followed by low temperature evaporation and freeze drying [14]. The powdered samples were dissolved (5 mg/ml) in the running buffer by magnetic stirring for 20 h and filtered through 0.2 μm glass fibre filters (Millipore, type F).

2.4. Data handling

Principal component analysis on the data matrix containing electrophoretic data profiles was carried out by SIRIUS (pattern recognition systems) software. The analysis was performed on data profiles normalised to unit area. MATLAB (MathWorks) was used for cubic spline interpolation and linear least squares fitting with non-negativity constraints.

2.5. NOM samples

The nine NOM samples used in this study (Table 1) were collected by Gjessing et al. [14] and subjected to a multi-method characterisation in the ''NOM-typing project'' [14]. Some additional information regarding these samples that are relevant for the pattern recognition analysis is provided below.

The samples from Maridalsvann (MAR), Aurevann (AUR) and Trehørningen (TRE) constitute a set of samples from clear water lakes (AUR and TRE

Table 1
Composition of the reverse osmosis isolates and reconstituted raw water, average electrophoretic mobilities, and average molecular mass

Sample	Ash[a] (%, w/w)	Cl[a] (mg L^{-1})	NO_3–N[a] (μg L^{-1})	SO_4 [a] (mg L^{-1})	Ca[a] (mg L^{-1})	Mg[a] (mg L^{-1})	Na[a] (mg L^{-1})	K[a] (mg L^{-1})	DOC[a] (mg L^{-1})	DOC/UV_{254} [a] (mg C L^{-1} cm^{-1})	AEM[b] (m^2 s^{-1} V^{-1}) 10^{-8}	Mr[c] (g mol^{-1})
Trehørningen (TRE)	57.5	1.1	82	3.1	0.69	0.28	3.09	0.39	4.8	25.5	−3.9	1400
Hellerudmyra May (HEM)	32.9	1.0	8	3.4	1.20	0.44	2.44	0.33	17.7	21.8	−3.9	2300
Aurevann (AUR)	56.0	1.2	117	3.4	0.30	0.11	3.88	0.28	4.8	25.1	−3.9	1500
Maridalsvann (MAR)	68.4	1.9	210	4.3	0.14	0.06	3.98	0.19	2.7	28.7	−3.8	700
Birkenes (BIR)	67.8	2.9	99	4.5	0.08	0.03	4.25	0.05	3.4	25.6	−3.9	1500
Humex B (HUM)	36.7	2.6	1	1.0	0.08	0.03	2.90	0.09	7.4	22.1	−3.9	2900
Gjerstad Limed (GJL)	60.6	1.5	52	3.8	0.14	0.07	3.21	0.11	4.2	21.8	−3.9	3400
Gjerstad Unlimed (GJU)	49.6	1.3	20	3.5	0.10	0.03	2.90	0.08	5.6	23.8	−3.8	2600
Hellerudmyra October (HEO)	26.8	1.5	24	4.8	0.12	0.03	4.59	0.05	21.9	24.3	−4.0	2500

(a) Ash content of the RO isolates and concentrations in reconstituted samples [14]. (b) Average electrophoretic mobility. (c) Average molecular masses determined by diffusivimetry [19].

are two lakes in the same water course). The samples from Humex B (HUM), and Hellerudmyra (HEM and HEO), constitute a set of samples from extremely bog-influenced locations (HEM and HEO were sampled in May and October, respectively). The sample from Birkenes (BIR) and the two samples from Gjerstad (GJL and GJU) are of intermediate nature (GJL and GJU were sampled from a limed catchment and an untreated control catchment respectively). Based on the size and characteristics of the catchment and water sources, the samples have been arranged previously in the following order: Hellerudmyra<Humex B<Birkenes<Gjerstad unlimed<Gjerstad limed<Trehørningen<Aurevann <Maridalsvann [15]. This order also reflects the sequence from extremely bog-influenced and dissolved organic carbon rich (DOC) water (Hellerudmyra) to DOC-poor clear water (Maridalsvann). The distribution of nitrogen in NOM size classes varies systematically in the same order [15].

3. Results and discussion

The migration of NOM molecules through the capillary is governed by the EOF and the charge/mass ratio of the molecules. At the pH of the separation buffer (7.0), the carboxylic groups of the NOM molecules give rise to a net negative charge [16]. Hence the molecules tend to migrate towards the anode. This tendency is counteracted by the EOF, which moves the bulk solution towards the cathode. At the experimental conditions used here (anodic injection) the molecules with the smallest charge/mass ratio reach the detector first. For very small and highly negatively charged molecules, the electrophoretic velocity may exceed the velocity of the EOF. These molecules will not be detected because they migrate away from the detector. In order to prevent this an external pressure of 10 mbar was applied at the anodic side. The observation of very low absorbances at the end of the electropherograms (25 min, Fig. 1) indicate that the applied pressure was sufficient to move the entire NOM samples past the detector, except in the 5 *M* urea experiments. This was also confirmed by recovery test [17].

The reproducibility of the method was tested by dissolving five sub-samples of TRE. The results are presented below.

3.1. CZE of NOM in phosphate buffer

The results of the CZE analysis in pure phosphate buffer will be used as the frame of reference for discussion of the effects of modifiers. The most striking feature of the electropherograms is the characteristic unresolved humic peak (Fig. 1), previously termed humic hump [18]. The migration time of this hump (read at the maximum absorption) vary between 11.03 min (HUM) and 11. 81 min (BIR).

The effective electrophoretic mobilities were calculated by the expression:

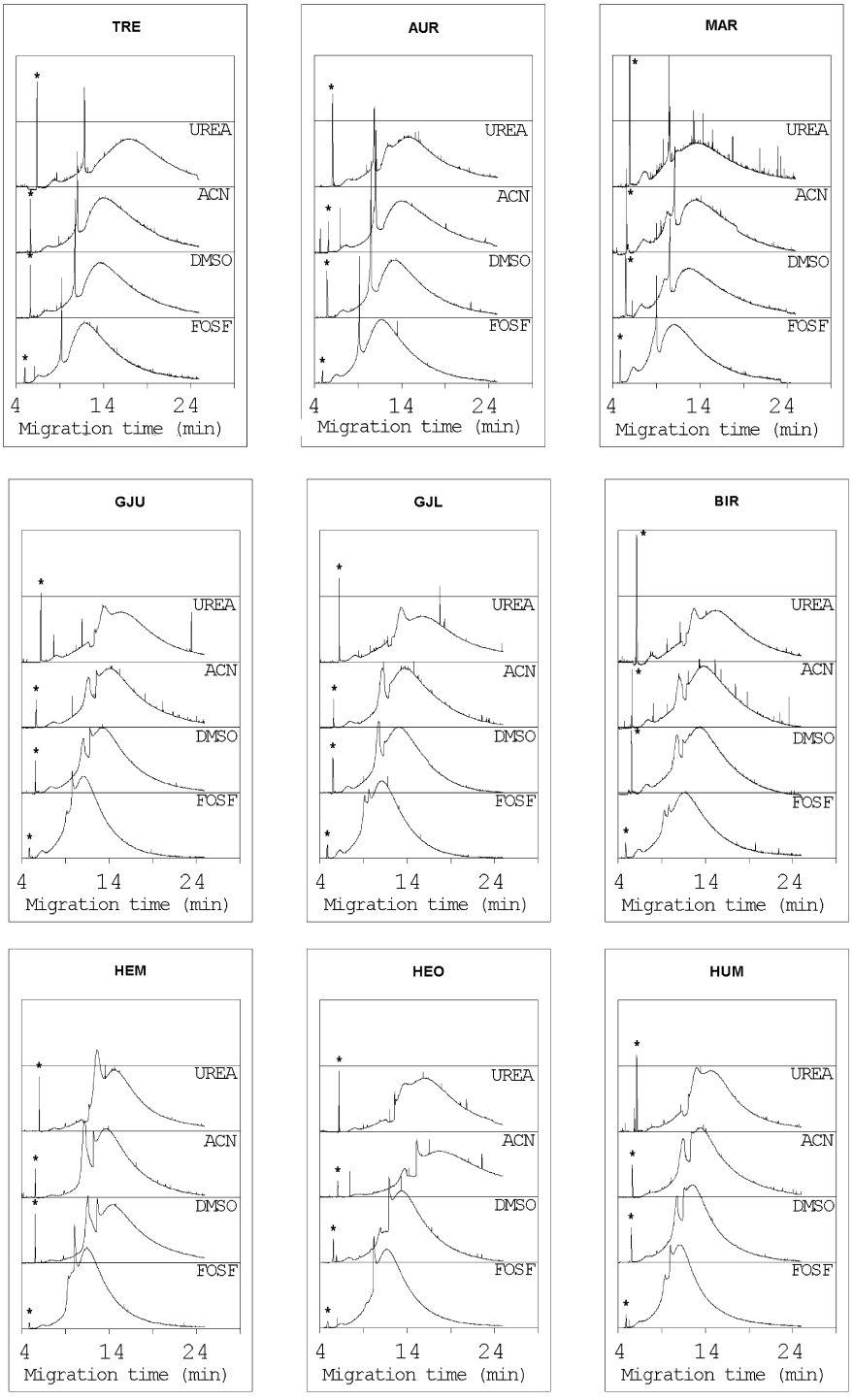

Fig. 1. Electropherograms recorded with pure 25 m*M* phosphate buffer (FOSF), and with the addition of 5 *M* urea (UREA), 10% (v/v) acetonitrile (ACN), and 5% (v/v) dimethyl sulfoxide (DMSO). The position of the EOF marker (benzyl alcohol) is indicated by a star.

$$\mu = \left(\frac{l}{t_m} - \frac{l}{t_{nm}}\right) \cdot \frac{L}{V}$$

where l is the length of the capillary from the injection side to the detector, L is the total capillary length, t_m is the migration time, t_{nm} is the migration time of the neutral EOF marker, and V is the applied voltage. The average electrophoretic mobility was calculated by the equation:

$$\mu_{avg} = \frac{\sum \mu_i A_i}{\sum A_i}$$

where μ_i is the effective electrophoretic mobility of the group of NOM molecules passing the detector at time i, giving rise to an absorption of A_i. The average electrophoretic mobility vary within narrow limits ($-3.8 \cdot 10^{-8}$ – $-4.0 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}, Table 1). The effective electrophoretic mobility is proportional to the ratio charge to radius. The average Stokes–Einstein radius calculated from diffusion coefficients (Table 1) varies between $5.8 \cdot 10^{-10}$ m (MAR) and $10.7 \cdot 10^{-10}$ m (GJL) [19]. The average net negative charge calculated from these numbers range from 2.3 equivalents per mol (MAR) to 4.2 equivalents per mol (GJU).

The height of the peak at migration times of about 9 min separates the three samples from the clear water lakes (TRE, AUR, MAR) from the other samples (Fig. 1), but this is about the only obvious distinguishing character of the electropherograms.

3.2. The effect of modifiers

It has been suggested that the poor resolution of electropherograms of humic substances are due to the formation of humic molecular aggregates, and that certain additives may break these aggregates [6,11]. In the present study the effect of methanol, urea, dimethyl sulfoxide and acetonitrile was examined.

3.2.1. Methanol

Methanol was tested as an additive because of the results of Conte and Piccolo [20]. They reported pronounced effects with as little as $4.6 \cdot 10^{-7}$ *M* of methanol during high-pressure size-exclusion chromatography (HPSEC) of humic substances. They attributed the effect observed due to disruption of humic aggregates. We tested 5 m*M* and 50 m*M* methanol concentrations without observing any effects (results not shown). There are several explanations for this discrepancy. It is possible that the soil humic substances studied by Conte and Piccolo [20] have radically different properties than the aquatic humic substances studied here. It should be noted however, that the validity of the results reported by Conte and Piccolo have been questioned [21].

3.2.2. Urea

The bifunctional hydrogen donor/acceptor property of urea has been employed in CZE to break intra and/or intermolecular hydrogen bonds in biological molecules such as proteins and DNA [22]. Significant effects of 5 *M* urea on CZE of humic substances have been attributed to separation of complex humic molecular aggregates, and to folding or unfolding or aggregation/disaggregation of the humic substance molecules [6,11].

The addition of 5 *M* urea leads to reduced EOF and longer residence times of the NOM samples in the electrical field. Some changes in the electropherogram takes place in the front region of the humic hump (Fig. 1). The electropherograms of the samples from the clear water lakes (TRE, AUR, MAR) appears to be less affected by the addition of urea than the electropherograms of the other samples. It has been previously reported that the addition of 5 *M* urea to the running buffer facilitates the separation of the complex humic molecular aggregates [11]. The effects observed in the present study are far less pronounced.

3.2.3. Dimethyl sulfoxide (DMSO) and acetonitrile

These additives are discussed in the same section because they have very similar effects on the electropherograms (Fig. 1). Acetonitrile and DMSO have been used as additives to basic solutions used for extraction of humic substances from soils because of their ability to solvate polar substances [23]. Nordèn and Dabek-Zlotorzynska [6] observed similar electrophoretic profiles in the presence and absence of acetonitrile (10%, v/v) in 10 m*M* borate buffer. To the best of our knowledge DMSO has not been used previously as an additive for CZE of humic substances or NOM.

Addition of acetonitrile (10%, v/v) and DMSO (5%, v/v) have very little effect on the electrophoretic profiles of the three clear water lake samples (TRE, AUR, MAR). A slightly improved definition of the shoulder in front of the peak at about 9 min is observed for the MAR sample (Fig. 1). For the other samples, addition of acetonitrile and DMSO result in splitting of the humic hump at the front side [the molecules with lowest q/r (charge to radius)]. Whether this is due to disruption of aggregates or unfolding of molecules is not clear.

3.3. Pattern recognition analysis

Principal component analysis (PCA) was chosen as the tool for pattern recognition analysis because of the robustness of the method towards highly correlated variables, and the large number of variables (about 2500) compared to the number of samples (9). The primary variables are the absorbances recorded at the migration times that constitute the electropherograms. However, in order to correct for small variations in EOF between samples, the effective electrophoretic mobilities were used rather than the migration times. An unwanted effect is that the absorbance readings are recorded at different electrophoretic mobilities for samples that have different EOFs. The PCA analysis compares absorbances for molecular groups with the same effective electrophoretic mobility (μ). Thus, all absorbances were interpolated by cubic spline interpolation onto a common μ-grid prior to PCA. Pretreatment of the data involved subtraction of the baseline and normalisation of the electropherograms to unit area (arbitrary units).In PCA no distinction is made between dependent and independent variables. The principle of PCA is best illustrated for a two-variable situation. Fig. 2 illustrates a set of data in which 6 samples (P1–P6) are described by two variables (X1, X2). The first principal component (PC1) is constructed (by least square technique) as the line of best fit. Note that PC1 is a linear combination of the original variables (X1, X2). Each sample is then orthogonally projected onto PC1. The distance from origin to the projected sample along PC1 is the score of this sample on PC1. Note that the scores may be negative ($t_{2,1}$ the score of P2 on PC1) or positive ($t_{4,1}$ the score of P4 on PC1). Samples that have similar

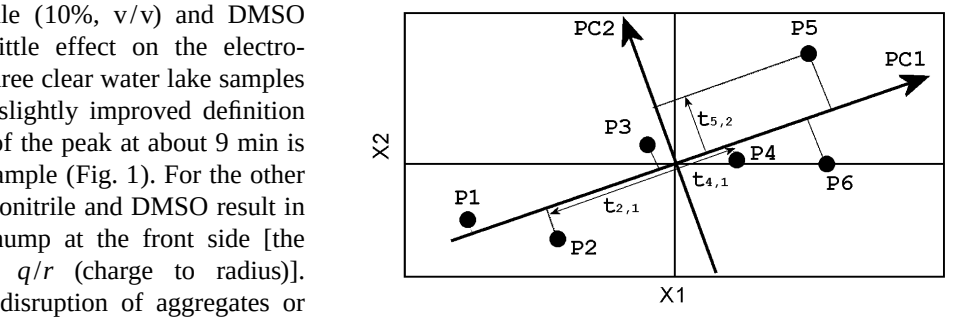

Fig. 2. Illustration of the principle of principal component analysis for a set of six samples (P1–P6) characterised by two variable (X1, X2). PC1 and PC2 are the first and second principal components. The scores of sample i on principal component j is denoted $t_{i,j.}$.

scores on PC1 are regarded as similar with respect to the combination of variables represented by PC1 (e.g. P5 and P6). The next steps in the PCA are to construct the second principal component PC2 orthogonally to PC1, and to determine the score of the samples on this second principal component. For the presented example, P5 and P6 have almost identical scores on PC1 and would be judged similar if the analysis stopped here. However, they are separated by scores of opposite sign on PC2. Samples that have similar scores on all principal components are identical.

In principle one may construct as many principal components as there are variables in the set of data. However, only noise is modelled as the number of principal components that exceeds a threshold.

The new variables (the principal components) are linear combinations of the original variables. One of the main advantages of PCA is that the number of variables required to adequately describe the set of samples is strongly reduced.

One of the objectives of this study was to determine the experimental conditions that produce the most characteristic fingerprints for tracing the NOM samples to their source and/or type of location. For this purpose, the following assumptions were made:

1. NOM from bog influenced sources is dominated by allochtonous organic matter.
2. NOM from clear water lakes is dominated by autochtonous organic matter.
3. Allochtonous and autocthoneous organic matter produce distinct electropherograms.

4. NOM from nearby locations with similar topography, vegetation and hydrology should produce electrophoretic profiles that are more similar than NOM from locations with contrasting conditions.

Based on the size and characteristics of the catchment and water sources, the samples have been previously arranged [15] in the order: Hellerudmyra<Humex B<Birkenes<Gjerstad unlimed<Gjerstad limed<Trehørningen<Aurevann <Maridalsvann. This order also reflects the sequence from extremely bog-influenced and DOC-rich water (Hellerudmyra) to DOC-poor clear water (Maridalsvann). According to the first three assumptions the samples from the clear water lakes (TRE, AUR, MAR) should have similar fingerprints that are different from the strongly bog influenced samples (HEM, HEO, HUM). The samples from GJU, GJL and BIR should have fingerprints of intermediate character. TRE and AUR were sampled from two lakes in the same water course. GJU and GJL were sampled from two sub catchments within the same catchment. HEM and HEO are from the same locations, sampled in May and October respectively. According to the fourth assumption the pattern recognition analysis should group these samples in the pairs: (TRE, AUR), (GJU, GJL), and (HEM, HEO).

3.3.1. Phosphate buffer

The first two principal components (PCs) explain 98.8% of the variations in the set of data constituted by the electrophoretic profiles recorded in 25 m*M* phosphate buffer without modifiers (Fig. 3A). The five samples (S1–S5) are five separate injections of the TRE sample and were included in the PCA to illustrate the reproducibility of the method. In the variable space spanned by the first two principal components (PC1 and PC2), the samples from the clear water lakes plot in the upper left hand corner. Two of the three strongly bog-influenced samples (HEM and HUM) plot in the lower right hand corner. GJL and GJU have quite similar co-ordinates, and BIR is located between the clear water lakes and the bog influenced samples. Hence, several of the criteria for a successful distribution of fingerprints were achieved by the use of phosphate buffer. However, there are some deviations. AUR appears to be more similar to MAR than to TRE, and the fingerprint of HEO makes it an outlier with little resemblance to the other two bog influenced samples (HEM, HUM). It should be noted here that the deviation of HEO could be attributed to the fact that this sample was taken in October, whereas the other samples were collected in May. It does not seem unreasonable to assume that the relative proportion of allocthonous and autocthononous organic matter show some seasonal variation.

3.3.2. Dimethyl sulfoxide

The first two principal components explain 97.8% of the variation in the set of data constituted by the electrophoretic profiles recorded with addition of 5% DMSO to the running buffer (Fig. 3B). The three clear water samples constitute one group in the centre of the plot, and the three samples of intermediate nature (BIR, GJL, GJU) have very similar co-ordinates. However, the three strongly bog-influenced samples (HEM, HEO, HUM) are distributed over the entire variable space. Addition of DMSO to the running buffer has a negative effect for the use of the electropherograms as fingerprints. The reason for this is that the splitting of the humic hump caused by DMSO (Fig. 1) actually makes the electrophoretic profiles of the bog-influenced samples more similar to the electrophoretic profiles of the clear water lakes than when using pure phosphate buffer. The pattern recognition analysis regards the new ''peak'' at the frontal side of the humic hump as a contribution to the characteristic clear water sample peak at migration times of about 10.5 min (Fig. 1).

3.3.3. Acetonitrile

The first two principal components explain 98.5% of the variation in the set of data constituted by the electrophoretic profiles recorded with addition of 10% acetonitrile to the running buffer. Addition of acetonitrile has the same effect on the electrophoretic profiles as the addition of DMSO (Fig. 1). The PCA of the electropherograms (Fig. 3C) produces about the same distribution of the samples (Fig. 3B), except that the samples from Hellerudmyra (HEM, HEO) have interchanged positions, and that HUM and HEM plot together in the lower right hand corner. The distribution of the samples is quite similar to the distribution obtained by pure phosphate buffer. However, addition of acetonitrile has a

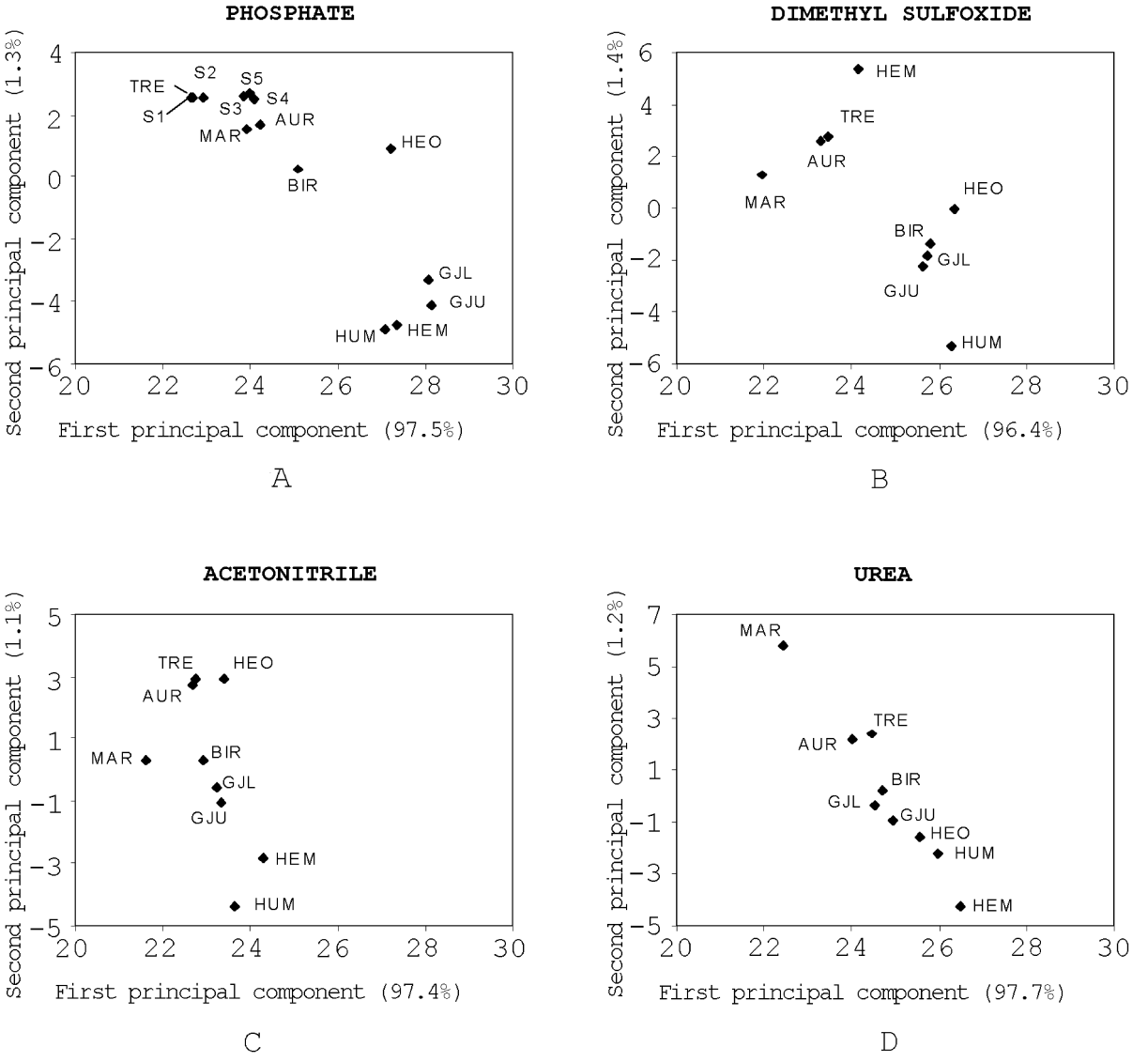

Fig. 3. Results of PCA analysis of the electropherograms presented in Fig. 1 after pretreatment (see text).

positive effect by the fact that TRE and AUR now have very similar co-ordinates. The displacement of HEO with respect to the other bog-influenced samples (HEM, HUM) is the same as observed in Fig. 3A.

3.3.4. Urea

The first two principal components explain 98.9% of the variation in the set of data constituted by the electrophoretic profiles recorded with addition of 5 *M* urea to the running buffer (Fig. 3D). The distribution of the fingerprints comes very close to what one would expect based on the assumptions made above. The three clear water lakes plot in the upper left hand corner, and TRE and AUR have almost identical co-ordinates. The three samples of intermediate nature (BIR, GJL, GJU) occupy the central positions in the diagram, and GJU and GJL plot close together. The lower right hand corner is occupied by the three bog-influenced samples (HEO,

HUM, HEM). The only sample that does not comply with the assumptions made above is HEO. This sample appears to be more similar to HUM than to HEM. Again, this may be caused by seasonal effects.

One may suspect that the distribution of fingerprints in Fig. 3D is influenced by noise in the data. The electropherogram of MAR in urea was particularly noisy (Fig. 1). However, principal component analysis of the electrophoretic profiles after averaging every 22 data points (reducing the number of data from 2200 to 100) produce the same distribution as shown in Fig. 3D. The outcome of the principal component analysis is robust towards noise. The results obtained in this study are somewhat different from the results obtained by Bergli [17] who found that electropherograms recorded with the addition of acetonitrile provided the best fingerprints. The reasons for this discrepancy are that Bergli [17] did not correct the electrophoretic profiles for differences in the EOF, and that he used centred electropherograms for the PCA.

The distribution of samples in the variable space spanned by PC1 and PC2 is very interesting, because linear distributions indicate that the samples are mixtures of two endmembers. The fact that it is the clear water sample MAR and the bog water sample HEM that occupy opposite positions in the linear distribution may indicate that the two endmembers are autochtonoues and allocthonous organic matter, respectively. It is not suggested here that MAR and HEM represent the pure endmembers. Nevertheless, the hypothesis that the samples are mixtures of two endmembers was tested by fitting the observed electrophoretic profiles with linear combinations of the electrophoretic profiles recorded for MAR and HEM by linear least squares fitting. The correlation between observed and predicted electrophoretic profiles using this two endmember mixing model (Table 2) range between 0.92 (TRE) and 0.99 (GJU). According to this model all samples may be modelled as mixtures between MAR and HEM. For example, AUR is composed of 54% MAR and 46% HEM (Table 2). Much remains to be learned about processes governing production of autochtonous dissolved organic matter. However, excretion by planktonic grazers is believed to be important both in limnic [24,25], and marine [26,27] environments. Hence, it seems reasonable to assume that the concentration of autochtonous dissolved organic matter is related to the eutrophic status of the waters. The fact that the four water sources with highest modelled proportion of MAR (MAR, AUR, TRE, BIR, Table 2) are also the sample with highest concentrations of NO_3^- (Table 1) and hence supposedly the most euthrophic, further support the hypothesis that MAR contain predominantly autochtonous NOM.

Table 2
Results obtained by the two endmember mixing model that considers all samples to be mixtures of MAR and HEM, and correlation between observed and modelled electropherograms

	MAR (proportion)	HEM (proportion)	Correlation coefficient
MAR	1.00	0.00	1.00
AUR	0.54	0.46	0.96
TRE	0.52	0.48	0.92
BIR	0.37	0.63	0.97
GJL	0.32	0.68	0.98
GJU	0.27	0.73	0.99
HEO	0.19	0.81	0.95
HUM	0.18	0.82	0.97
HEM	0.00	1.00	1.00

The samples MAR and HEM are probably not pure endmembers. Nevertheless, a closer inspection of the electrophoretic profiles may throw some light on the (q/r) properties of autochtonous and allochtonous NOM. Fig. 4 shows that the MAR sample contains more materials with short migration times (i.e. less than 12 min) than the HEM sample. This could be due either to lower molecular charge (q), or larger radii (r). The fact that MAR has the lowest molecular mass of all the samples included in this study (Table 1) suggests that the difference is caused by lower molecular charge.

This study was based on fingerprints produced by CZE. It is possible that PCA of chromatograms produced by other techniques, such as HPSEC, may also be used for tracing the NOM samples to their source and/or type of location.

4. Conclusions

This study shows that CZE of NOM using phosphate buffer results in reproducible electropherograms. Addition of methanol up to 50 m*M* has no

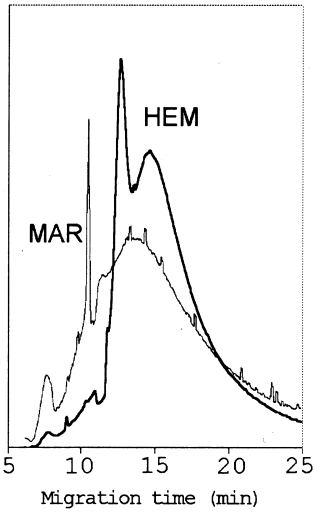

Fig. 4. Electropherograms of MAR (thin line) and HEM (heavy line) recorded by the use of 25 m*M* phosphate buffer (pH 7) and 5 *M* urea.

effect on the electrophoretic profiles. Addition of DMSO (5%, v/v) and acetonitrile (10%, v/v) affects the electrophoretic profiles of NOM samples from bog-influenced sources by splitting of the humic hump at the front side (the molecules with lowest q/r). Addition of 5 *M* urea leads to reduced EOF and longer residence times of the NOM samples in the electrical field. Some changes in the electropherogram take place in the front region of the humic hump.

The electropherograms recorded in the various buffer systems may be used as fingerprints for tracing the NOM samples to their source and/or type of location, with variable accuracy. Only the electrophoretic profiles recorded with urea (5 *M*) added to the running buffer produce fingerprints that fulfil the assumptions that: (1) NOM from bog influenced sources are dominated by allochtonous organic matter, (2) NOM from clear water lakes are dominated by autochtonous organic matter, (3) allochtonous and autocthoneous organic matter produce distinct electropherograms, and (4) NOM from nearby locations with similar topography, vegetation and hydrology should produce electrophoretic profiles that are more similar than NOM from locations with contrasting conditions.

The auotochtonous endmember is characterised by lower molecular charge and lower molecular radius than the allocthonous endmember.

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