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**Characterisation of metal/humic acid systems  
through Capillary Electrophoresis**

J.J. van Staden, M.A.G.T. van den Hoop,  
R.F.M.J. Cleven

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This investigation has been performed by order and for the account of Directorate General, within the framework of project 518001, 'Environment / Method Development'.

## Abstract

Metal-humic acid systems have been characterised applying Capillary Electrophoresis (CE). Appropriate experimental conditions for carrier electrolyte, pH range, salt and humic acid concentrations and the applied potential were optimised to allow the influence of multivalent metal ions on the electropherogram of humic acid to be examined. These included like copper, calcium, iron, aluminum en cadmium. After these examinations, a selection of electropherograms of river samples has been interpreted. Upon application of a pH of 6, and an acetic acid based carrier electrolyte, the electropherograms of the metal/humic acid systems were seen to be well developed, and the humic acid concentration and peak area linearly related. Additions of heavy metals such as  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  provoked extra peaks. The performance of the humic acid electropherograms is shown to be strongly correlated with the metal/humic acid concentration ratio. River samples 'high' in DOC and 'low' in  $\text{Ca}^{2+}$  concentrations show electropherograms comparable to the basic form seen in metal/humic acid systems. The results demonstrate the potentiality of CE for characterizing humics in aquatic systems in relation to the binding of metal ions.

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## Samenvatting

Het rapport beschrijft de karakterisering van metaal/humuszuur systemen met Capillaire Elektroforese (CE). Eerst zijn de experimentele condities geoptimaliseerd. Met een humuszuur-oplossing is de geschiktheid van draag-elektroliet-oplossingen voor pH-traject van 4 tot 9 onderzocht. Ook is de invloed van de pH, de zoutconcentratie, de humuszuurconcentratie en de opgelegde potentiaal op elektroferogrammen van koper/humuszuur systemen onderzocht. Vervolgens zijn de effecten van de concentraties van diverse metaalionen zoals die van koper, calcium, ijzer, aluminium en cadmium op het elektroferogram van humuszuur bestudeerd. Op basis van de resultaten van bovengenoemde experimenten is een aantal elektroferogrammen van oppervlaktewatermonsters afkomstig van verschillende Europese rivieren geïnterpreteerd. Uit het onderzoek is gebleken dat draag-elektrolieten op basis van azijnzuur geschikt zijn voor metingen bij pH 4 en 6, en een boorzuur elektroliet geschikt is voor metingen bij pH 8. Elektroferogrammen van koper/humuszuur systemen vertonen bij hogere pH-waarden meer details dan bij lagere. Verhoging van de zoutconcentratie met 25 mmol/l natriumnitraat veroorzaakt een toename van de migratietijd en een vermindering van het aantal pieken in de elektroferogrammen van koper/humuszuur systeem. De humuszuurconcentratie in het onderzochte gebied van 0.6 tot 11.2 mmol/l C heeft geen grote invloed op de migratietijd, en blijkt een lineair verband te vertonen met het piekoppervlak. Addities van meerwaardige metaalionen hebben een of meer extra pieken tot gevolg. Oppervlaktewatermonsters met relatief hoge DOC- en lage calciumgehalten tonen elektroferogrammen die afgeleid kunnen worden geacht van die voor metaal/humuszuur systemen. De resultaten geven een beeld over de toepasbaarheid van CE voor dit type speciatie-onderzoek.

## Summary

The report describes the characterization of metal/humic acid system systems applying Capillary Electrophoresis (CE). Initially, experimental conditions have been optimised with respect to the electropherograms of a humic acid solution. Different carrier electrolytes, in a pH range from 4 to 9 have been tested. The influence of pH, salt concentration humic acid concentration and the applied potential on electropherograms of copper/humic acid systems have been investigated. Subsequently the effects of the concentration of different multivalent metal ions, such as copper, calcium, iron, aluminum en cadmium on the electropherograms of humic acid have been examined. On the basis of the results, a selection of electropherograms of river samples from European rivers have been interpreted. Acetic acid based carrier electrolyte appear to be suitable for measurements at pH 4 and 6, whereas carrier electrolytes based on boric acid appears to be appropriate at pH 8. Electropherograms of copper/humic acid systems showed more details at higher pH values than at lower pH values.

An increase of the salt concentration with 25 mmol/l sodium nitrate causes an increase in the migration times and an decrease in the number of peaks in the electropherograms of copper/humic acid systems. The humic acid concentration does not show a large influence on the migration time in the range 0.6 -11.2 mmol/l C, and appears to be linearly related to the relevant peak area. Additions of multivalent metal ions cause the occurrence of one or more extra peaks. Surface water samples with relatively high DOC and low calcium concentration show electropherograms comparable to those of metal/humic acid systems. This research shows potentialities of Capillary Electrophoresis for metal speciation.

## 1 INTRODUCTION

Humic substances are formed by decomposition of plant material. Humics are one of the most important resources of organic carbon in the environment. They constitute about 75% w/w of the organic matter in most mineral soils and about 50% of organic carbon in surface waters. The functional groups in humics, mainly carboxylic and phenolic, show the ability to interact with metal ions, oxides, hydroxides, minerals and other organic substances to form water-stable associations [1]. Humic material consists of many fractions. Each fraction expresses several distinct and important properties. Three main fractions are: humic acids, fulvic acids, and humin. These fractions are separated by the pH at which they are soluble. Humin is insoluble at any pH, while fulvic acids are soluble at any pH. Humic acid is soluble only at pH above 2. Each fraction is a heterogeneous mixture of organic substances having a wide range of molecular weights and negative charges. Humic acids tend to be larger, more fluorescent, and more polymerised than fulvic acids. The percentage of carbon in fulvics is lower, but the percentage of oxygen is higher than in humic acids. Although the fine structures of humic and fulvic acids are not completely known, a number of hypothetical structures have been proposed that account for the main characteristics [2]. Figure 1 shows a model structure of fulvic acid.

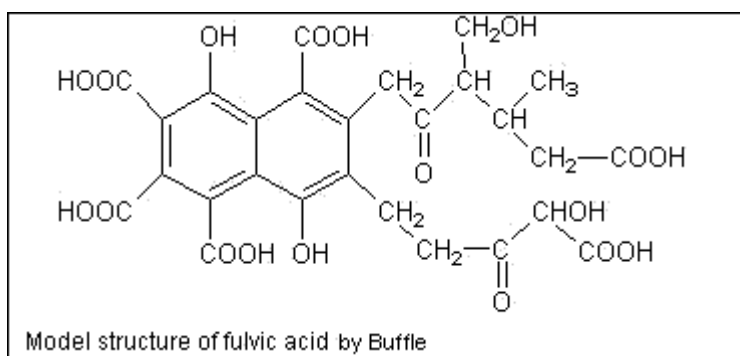


Figure 1. Hypothetical structure of fulvic acid

The distributions of metal ions over 'bound' and 'free' forms ('speciation pattern') in the environment, and thus possible ecotoxic effects of heavy metals, is mainly controlled by the complexing properties of humic material. Many studies have examined mechanisms of metal/humic acid complexation as a function of the pH or the ionic strength. Capillary Electrophoresis (CE) has already delivered very useful environmental applications for anions and cations. However, with respect to the complexation of metals by humics, the number of publications is rather scarce [3-7]. The main purpose of the present study is to explore potentialities of CE as an analytical tool for investigation of metal/humic acids interactions. It has been performed as part of European project 'Microbentic communities in European Rivers used to assess effects of land-derived toxicants'. The study has been carried out according to working plan 97/LAC/502501/EU-11/00.

## 2 MATERIALS EN METHODS

All chemicals used to prepare metal and carrier solutions were of pro analysis quality. Fluka humic acid has been used. Solutions were prepared using water purified with a Milli-Q system (Millipore, Bedford, MA, USA). In the experiments, the humic acid solutions and the carrier electrolytes were at the same pH value.

### 2.1 Apparatus

Electropherograms have been obtained using a Waters Quanta 4000 CE system equipped with a UV detector, a positive power supply and an automatic sample changer. For data acquisition, an Acer powermate 60 MHz Pentium was connected to the CE system via a SAT/IN interface. Data have been acquired using a 5 Hz data acquisition rate. Millennium 2010 (Version 2.10, Millipore) software has been used for data collection and treatment. Separations have been carried out using an AccuSep (Waters) fused silica capillary (60 cm x 75 µm ID; effective length 52 cm) at a voltage of 30 kV and a temperature of 30° C. Injection was performed hydrostatically by elevating the sample at 10 cm for 60 s at the anodic side of the capillary unless specified otherwise. Direct UV detection at 254 nm has been used. The time constant of the UV detector was 1 s. Before each sample injection, the capillary was rinsed successively with 0.1 mol/l NaOH (0.4 min.), deionized water (0.1 min.) and carrier electrolyte (1.8 min.).

### 2.2 Preparation of the solutions

#### *Humic acid solutions*

To prepare a stock solution of humic acid, 10 g of Fluka humic acid was added to 1 liter of water. Subsequently, aliquots of a NaOH solution (about 3 mol/l) have been added until a constant pH of 9. After shaking the mixture for 24 hours, the pH was reduced, by slowly adding a HNO<sub>3</sub> solution (about 3 mol/l) under continuous stirring of the mixture, to pH 3. After leaving the mixture for 24 hours, the precipitate was centrifuged at 18,000 rpm for about an hour. The pH of the decanted supernatant was brought to pH 7 with NaOH solution, to prevent further coagulation. The solution obtained was dialysed against Millipore water, using a Spectrapore tubing with a molecular mass cut-off of 3,500. Dialysis was continued until the conductivity of the refreshed dialysate remained constant at about 7 µS.cm<sup>-1</sup>. The dialyzed solution was subsequently treated with an ion exchanger of the type AG 50W-X4, to transfer the material into the acid form. After dilution, the DOC content of the solution was 112 mmol/l C.

For the experiments at pH 8, 10.5 ml NaOH 0.01 mol/l has been added to 10 ml of the humic acid stock solution. The mixture was diluted with water to 100 ml. Subsequently, 100 ml carrier electrolyte solution with pH 8 has been added. For the experiments at pH 6 or pH 4, 10 ml of the humic acid stock solution has been diluted with water to 100 ml. Subsequently, 100 ml carrier electrolyte solution with pH 6 and 4 respectively has been added. The final solutions contained 5.6 mmol mmol/l C.

#### *Metal stock solutions*

Stock solutions of 0.01 mol/l copper(II)-, calcium-, aluminium-, iron(III)- and cadmium(II) nitrate have been prepared by dissolving in 100 ml water: 2.42 g Cu(NO<sub>3</sub>)<sub>2</sub>, 2.36 g Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O, 3.75 g Al(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O, 4.04 g Fe(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O or 3.08 g Cd(NO<sub>3</sub>)<sub>2</sub>, respectively.

### *Carrier electrolytes*

The carrier electrolyte stock solution with pH 4 was prepared by adding 100 ml 0.1 mol/l acetic acid (HAc) to 25 ml sodium acetate (NaAc) solution of 0.1 mol/l. The carrier electrolyte stock solution with pH 6 was prepared from 100 ml NaAC 0.1 mol/l NaAc and 1 ml 0.1 mol/l HAc.

The carrier electrolyte stock solution with pH 8 was prepared from 100 ml boric acid containing 0.1 mol/l  $\text{H}_3\text{BO}_3$  and 25 ml sodium tetraborate solution containing 0.1 mol/l  $\text{Na}_2\text{B}_4\text{O}_7$ . Prior to use all carrier electrolytes solutions have been diluted with water by a factor of 10.

### **2.3 Samples**

A set of samples from various European rivers in Spain, Sweden, Finland and the Netherlands has been used for the experiment with real world samples. All samples are registered in the laboratory information system (LIMS). In this report the corresponding unique LIMS-code is used to identify the various sample solutions.



### 3 RESULTS AND DISCUSSION

#### 3.1 The pH and carrier electrolyte composition

Figure 2 shows the electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) obtained with acetate carrier electrolytes (pH 4 and 6) and borate carrier electrolyte (pH 8). The carrier electrolytes all produce the same basic pattern: first a small peak and subsequently one or more sharp peaks followed by a very broad peak. The peak at a migration time of about 3 minutes for carrier electrolyte pH 4, 2 minutes for carrier electrolyte pH 6 and 1.7 minutes for carrier electrolyte pH 8 is the neutral peak produced by non-ionic components in the sample matrices. The borate carrier electrolyte with a pH of 8, develops a fast electro-osmotic flow (EOF) so that the complete electropherogram takes only 6 minutes. An advantage of this high pH carrier electrolyte is that most functional groups are ionized. Thus, the chemical composition of the carrier electrolyte has a large influence on the electropherograms of humic acid solutions.

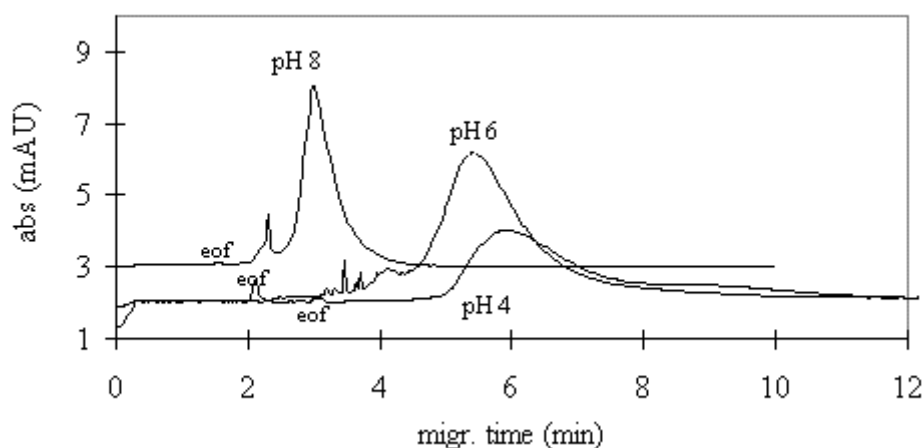


Figure 2. Electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) with different carrier electrolytes (for experimental details see chapter 2).

Figure 3 shows electropherograms of humic acid solution ( $[HA] = 5.6$  mmol/l C) in the presence of  $[Cu(II)] = 0.75$  meq/l obtained with acetate carrier electrolytes (pH 4 and 6) and borate carrier electrolyte (pH 8). As compared to the electropherograms in Figure 2 (without Cu-addition), the electropherograms with 0.75 meq/l Cu-additions show with carrier electrolyte pH 4, 6 and 8 an additional peak at 9.5, 4.5 and 3.5 minutes, respectively. Figure 4 shows electropherograms of humic acid solution ( $[HA] = 5.6$  mmol/l C) in the presence of a higher copper concentration,  $[Cu(II)] = 3$  meq/l. At this Cu-concentration, the solutions show signs of coagulation. The electropherograms with 3 meq/l additions show a peak of a positive ionic component at about 2 minutes. This peak is probably caused by a positive copper complex. As electropherograms without humic acid (blancs) show exactly the same peaks before the EOF-peak, it's evident that the peak is not to be attributed to copper humate.

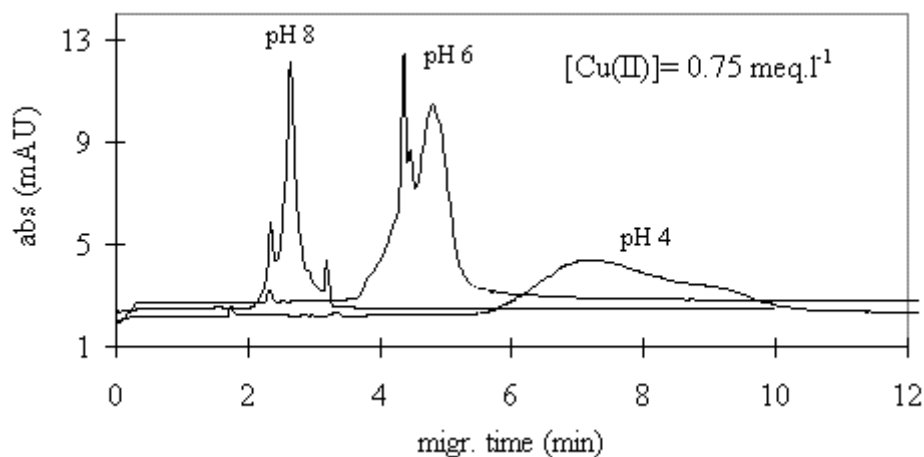


Figure 3. Electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) and  $[Cu(II)] = 0.75$  meq/l with different carrier electrolytes (for experimental details see chapter 2).

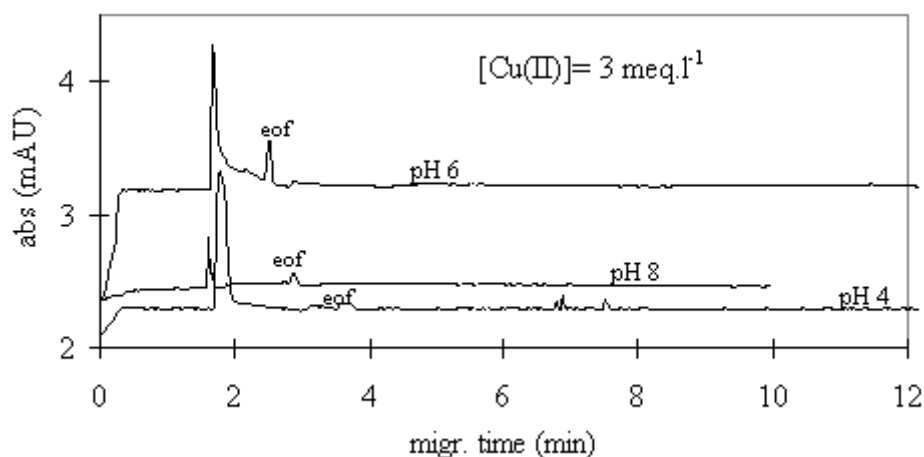


Figure 4. Electropherograms of humic acid solution ( $[HA] = 5.6$  mmol/l C) and  $[Cu(II)] = 3$  meq/l with different carrier electrolytes (for experimental details see chapter 2).

### 3.2 Salt concentration

For the determination of the influence of the salt-concentration in the carrier electrolyte, experiments with borate carrier electrolytes (pH 8) have been carried out with various  $\text{NaNO}_3$  concentrations. For  $\text{NaNO}_3$  concentrations larger than 50 mmol/l, the capillaries got blocked, due to high currents. Figure 5 shows electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) obtained with borate carrier electrolytes with 0 and 25 mmol/l  $\text{NaNO}_3$ . Figure 6 shows electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) in the presence of  $[Cu(II)] = 0.75$  meq/l, applying the borate carrier electrolytes with 0 and 25 mmol/l  $\text{NaNO}_3$ .

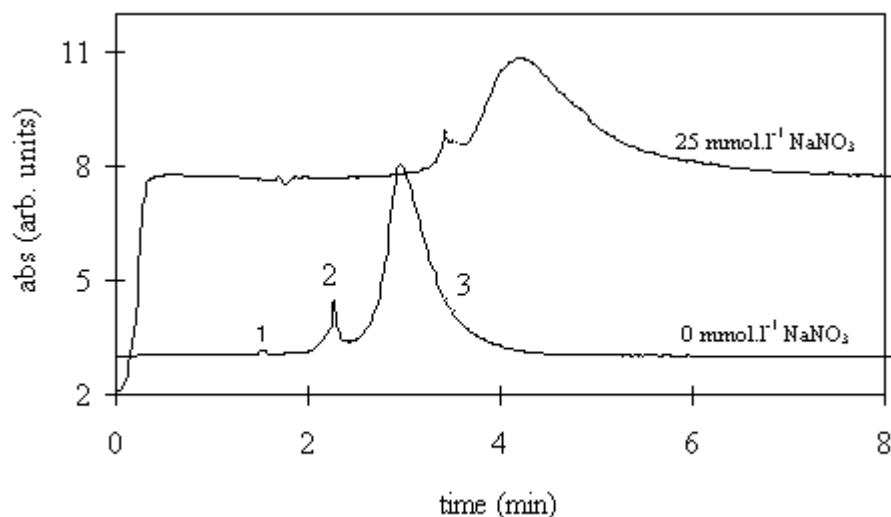


Figure 5. Electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) obtained with borate carrier electrolyte with 0 and 25 mmol/l  $NaNO_3$ . Carrier electrolyte: [boric acid]=10 mmol/l; pH=8; (for experimental details see chapter 2).

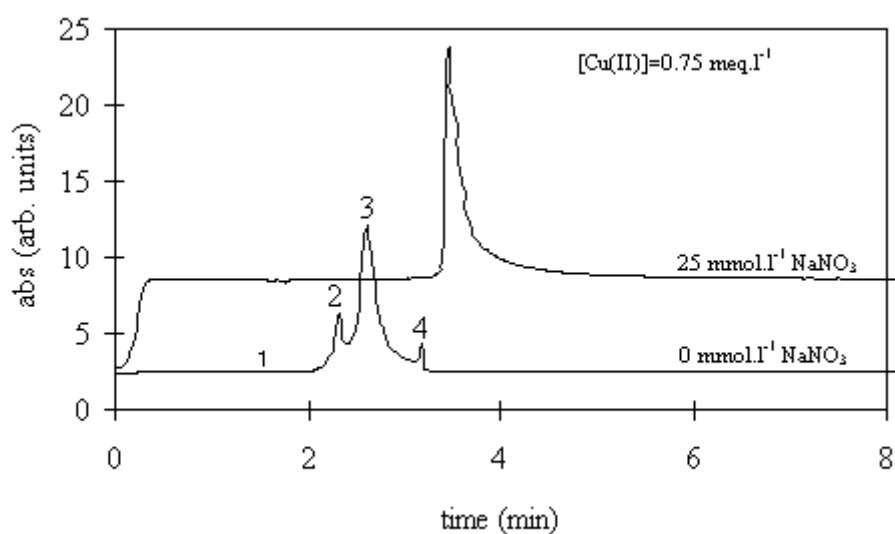


Figure 6. Electropherograms of humic acid solution ( $[HA]=5.6$  mmol/l C) and  $[Cu(II)] = 0.75$  meq/l obtained with borate carrier electrolyte with 0 and 25 mmol/l  $NaNO_3$ . ccarrier electrolyte: [boric acid] = 10 mmol/l; pH = 8; (for experimental details see chapter 2).

The addition of 25 mmol/l  $\text{NaNO}_3$  to the carrier electrolyte caused a less pronounced electropherogram of humic acid (without the presence of copper) with 1.5 min longer migration times for peak 2 and 3. The addition of 25 mmol/l  $\text{NaNO}_3$  to the carrier electrolyte causes a decrease in the number of peaks in the electropherogram of humic acid in the presence of copper, to only one peak. Additionally, a change has been observed with respect to the coagulation in copper/humic acid systems: addition of  $\text{NaNO}_3$  to the samples caused a visible shift to a higher  $\text{Cu(II)}$ -concentration at which coagulation occurred. A reduction of the effective charge of the macromolecular humic acid structure due to the presence of 'excess salt' may account for these phenomena.

### 3.3 Humic acid concentration

The influence of the concentration of humic acid on the response in CE has been investigated with respect to migration time, height and area of the humic acid peaks. Figure 7 shows the migration time and figure 8 shows the peak area for the main peak in electropherograms of the humic acid solution as a function of the concentration, in the concentration range from 0.6 to 11.2 mmol/l C. Over this range, there is virtually no change in migration time. The peak areas (and also: peak heights) for the main peaks show a fairly linear relationship with the humic acid concentrations.

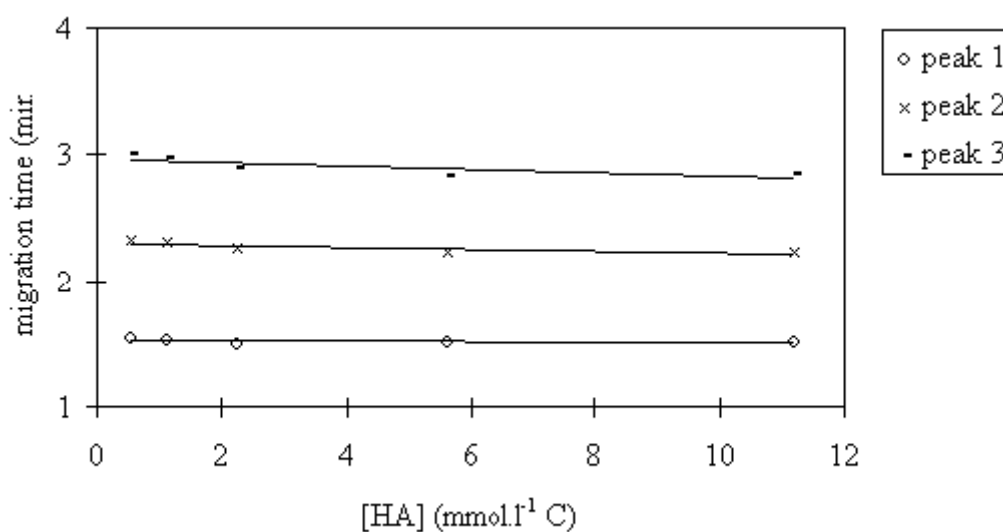


Figure 7. Average migration time ( $n = 3$ ) for the main peaks in electropherograms of humic acid solution with different humic acid concentrations. Carrier electrolyte: [boric acid] = 10 mmol/l;  $\text{pH}=8$ ; (for experimental details see chapter 2).

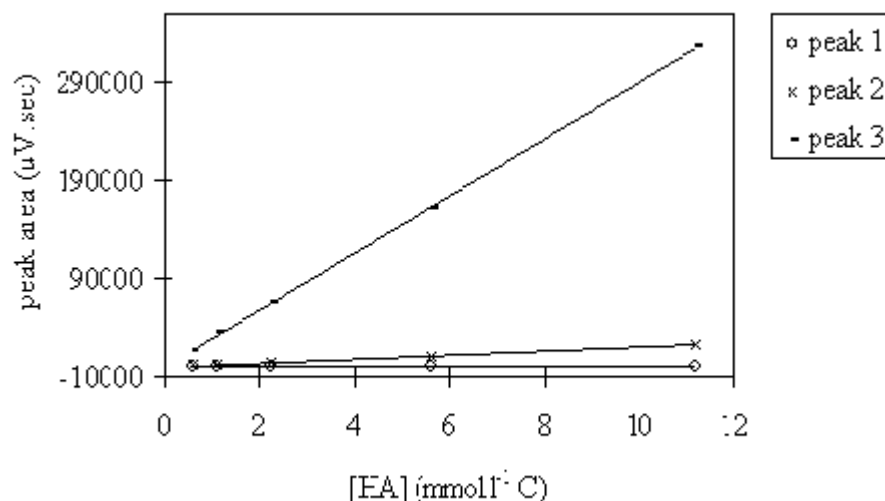


Figure 8. Average peak areas ( $n=3$ ) for the main peaks in electropherograms of humic acid solution with different humic acid concentrations. Same experimental conditions as for Figure 7.

### 3.4 Applied voltage

From a theoretical point of view, charged ions show a migration time behaviour which is linear with the reciprocal applied voltage. Electropherograms of humic acid solutions with and without the presence of copper nitrate, have been determined at various potentials. Figure 9 shows the migration time as a function of the reciprocal applied voltage for a humic acid solution with  $[HA]=5.6$  mmol/l C in the presence of  $[Cu(II)]=0.75$  meq/l. The reciprocal applied voltage shows good linear relationship with the migration time. Figure 10 and 11 show the peak height as a function of the applied voltage for the humic acid solution without and in the presence of  $[Cu(II)]=0.75$  meq/l, respectively.

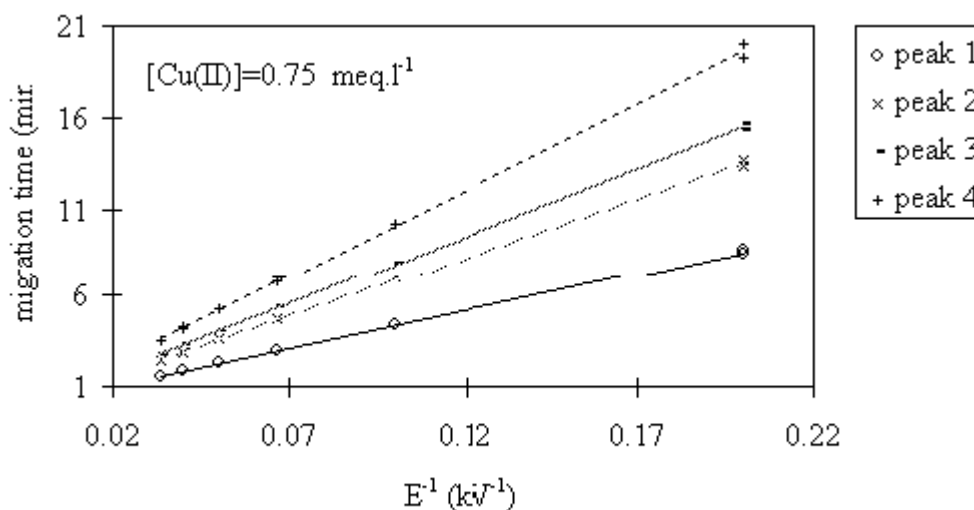


Figure 9. Migration time as a function of the reciprocal applied voltage for Cu(II)/humic acid system.  $[Cu(II)]=0.75$  meq/l;  $[HA]=5.6$  mmol/l C; Same experimental conditions as for Figure 7. Measurements in duplicate.

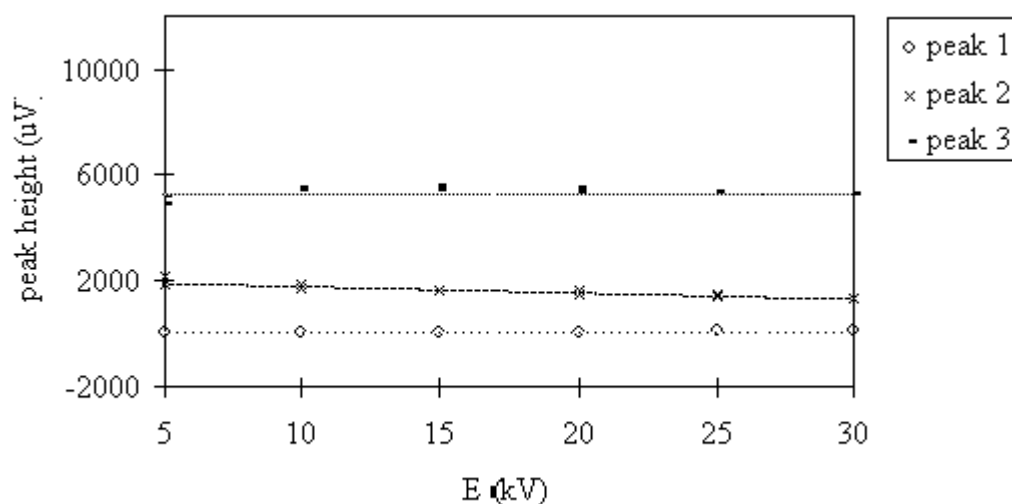


Figure 10. Peak height as a function of the applied voltage for humic acid.  $[HA]=5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7. Measurements in duplicate.

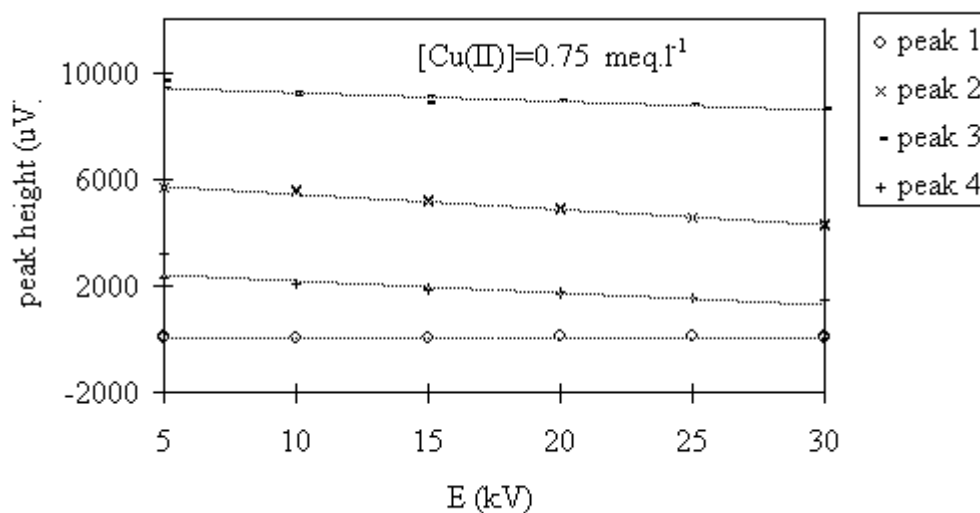


Figure 11. Peak height as a function of the applied voltage for the Cu(II)/humic acid system.  $[Cu(II)]=0.75 \text{ meq/l}$ ;  $[HA]=5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7. Measurements in duplicate.

For humic acid, the peak height did virtually not change with an increasing applied voltage, whereas for the Cu(II)/humic acid system, the peak height slightly decreased with an increasing applied voltage.

### 3.5 Time effect of copper complexation

Initially, the experiments showed bad duplicates for electropherograms with addition of copper nitrate to humic acid solutions. Therefore, the effect of the time delay between addition of copper nitrate and the actual measurement has been investigated. Figures 12 and 13 show the corrected migration time (normalised on EOF peak) as a function of the time after addition of 0.9 and 1.3 meq/l Cu(II) respectively to a humic acid solution with a concentration of  $[HA] = 5.6 \text{ mmol/l C}$ . Figures 14 and 15 show the peak height as a function of the time after addition of 0.9 and 1.3 meq/l Cu(II) respectively to the humic acid solution.

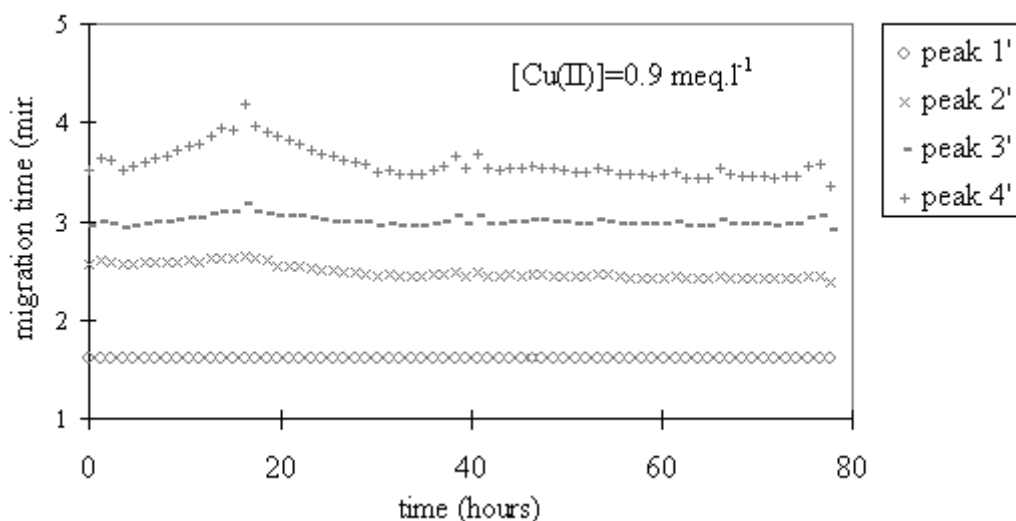


Figure 12. Corrected migration time as a function of the time after addition of Cu(II) to an humic acid solution.  $[Cu(II)] = 0.9 \text{ meq/l}$ ;  $[HA] = 5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7.

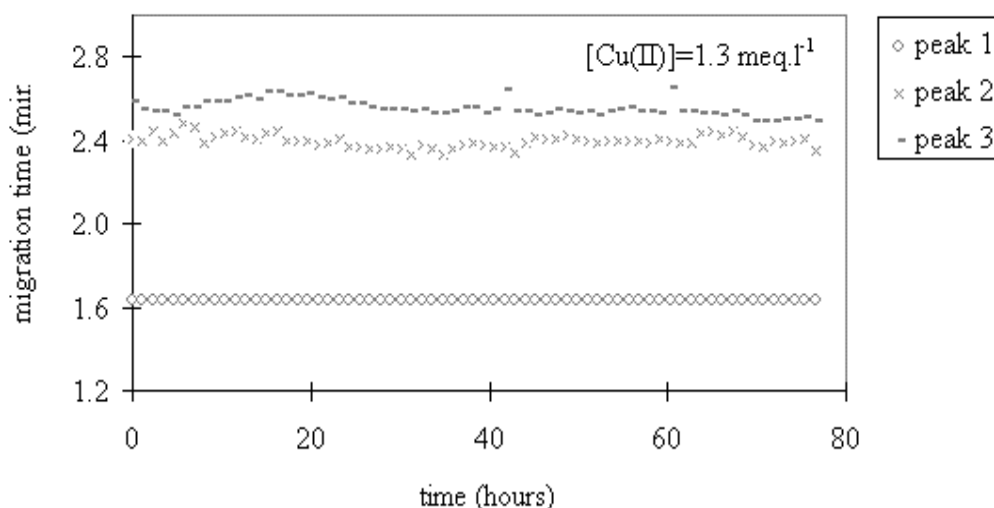


Figure 13. Corrected migration time as a function of the time after addition of Cu(II) to an humic acid solution.  $[Cu(II)] = 1.3 \text{ meq/l}$ ;  $[HA] = 5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7.

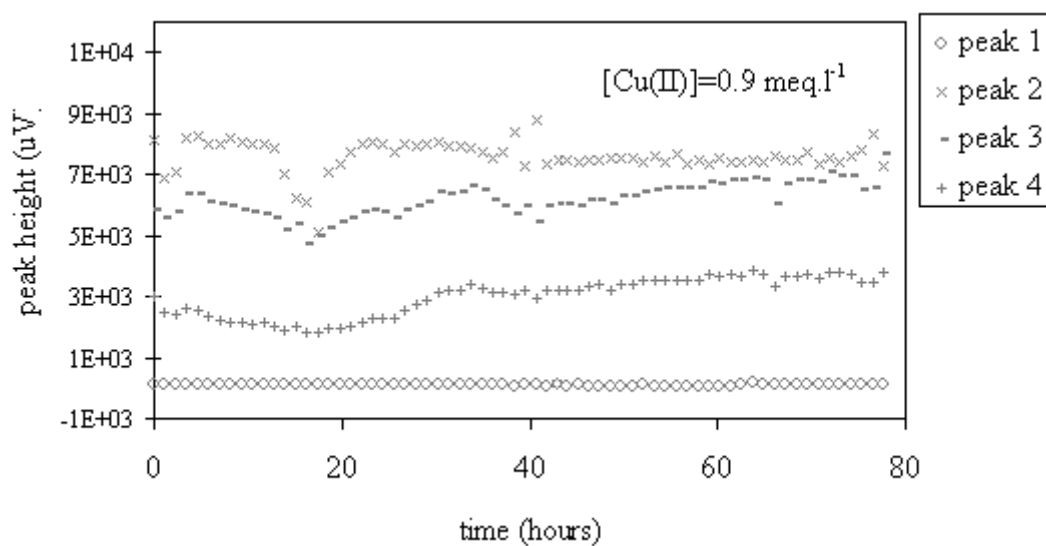


Figure 14. Peak height as a function of the time after addition of  $\text{Cu(II)}$  to a humic acid solution.  $[\text{Cu(II)}] = 0.9 \text{ meq/l}$ ;  $[\text{HA}] = 5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7.

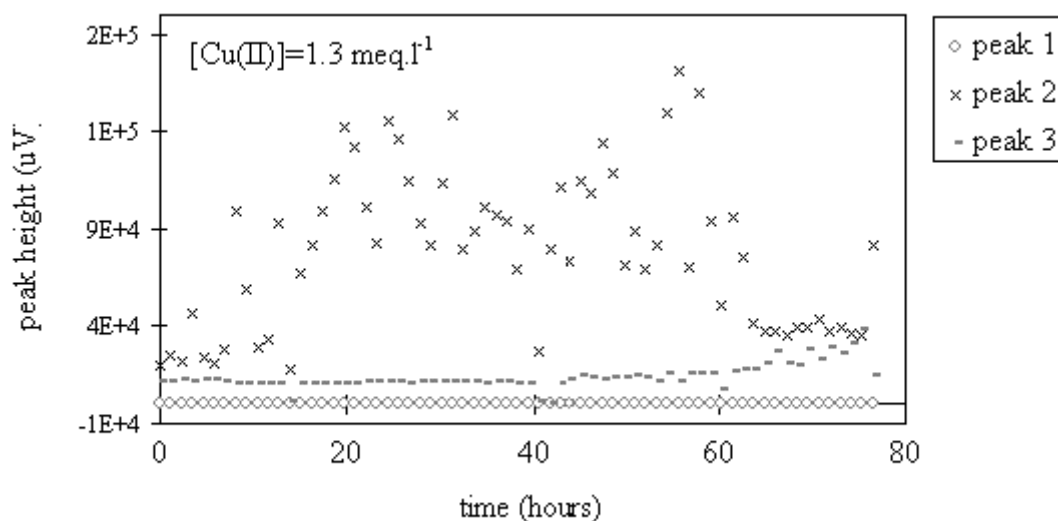


Figure 15. Peak height as a function of the time after addition of  $\text{Cu(II)}$  to a humic acid solution.  $[\text{Cu(II)}] = 1.3 \text{ meq/l}$ ;  $[\text{HA}]=5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7.



The corrected migration time is calculated with:

$$t_{cn} = t_n * t_{en} / t_{e1}$$

with

$t_{cn}$  is the corrected migration time for the  $n^{\text{th}}$  measurement;

$t_n$  is the migration time for the  $n^{\text{th}}$  measurement;

$t_{en}$  is the migration time for the EOF-peak of the  $n^{\text{th}}$  measurement;

$t_{e1}$  is the migration time for the EOF-peak of the first measurement.

The corrected migration times for both Cu(II) additions stayed constant after about 30 hours. The peak heights for Cu(II) additions of 0.9 meq/l and 1.3 meq/l Cu(II) stayed constant after about 30 and 60 hours respectively. The peak due to the Cu(II) addition seemed to be the most unstable one. As a result of this experiment the measurements have been performed after waiting at least one day after additions of metal salt solution to the humic acid solutions.

### 3.6 Addition of $\text{Cu}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Fe}^{3+}$ , $\text{Al}^{3+}$ and $\text{Cd}^{2+}$ to humic acid solutions

Figures 16 to 20 show electropherograms of humic acid solution ( $[\text{HA}] = 5.6 \text{ mmol/l C}$ ) with  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$  additions, respectively, varying from 0 to 5 meq/l. The borate carrier electrolyte (pH 8) has been applied for these experiments.

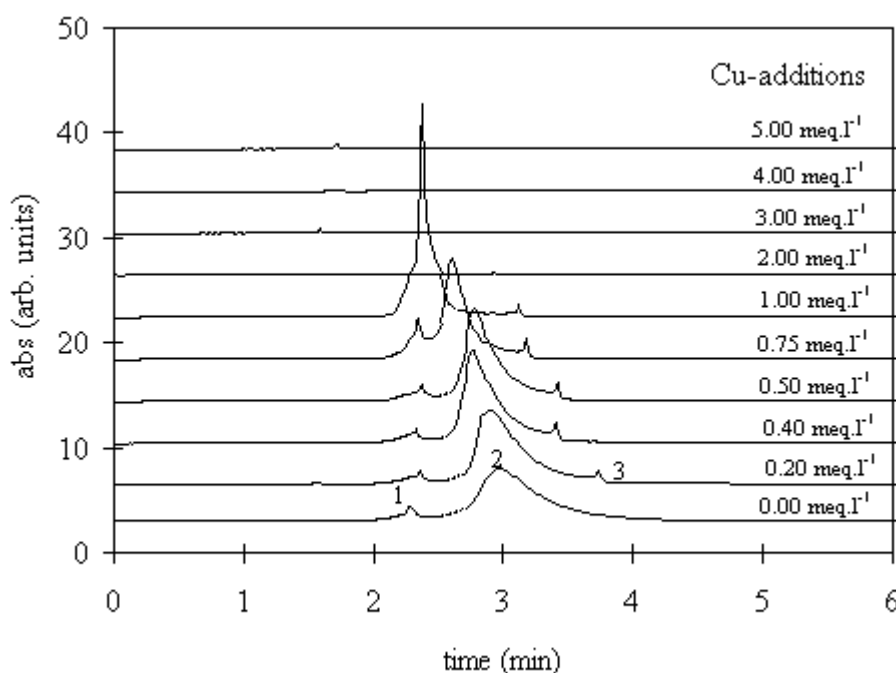


Figure 16. Electropherograms of humic acid solution with different Cu(II) additions.  $[\text{Cu(II)}] = 0$  to  $5 \text{ meq/l}$ ;  $[\text{HA}] = 5.6 \text{ mmol/l C}$ ; Same experimental conditions as for Figure 7.

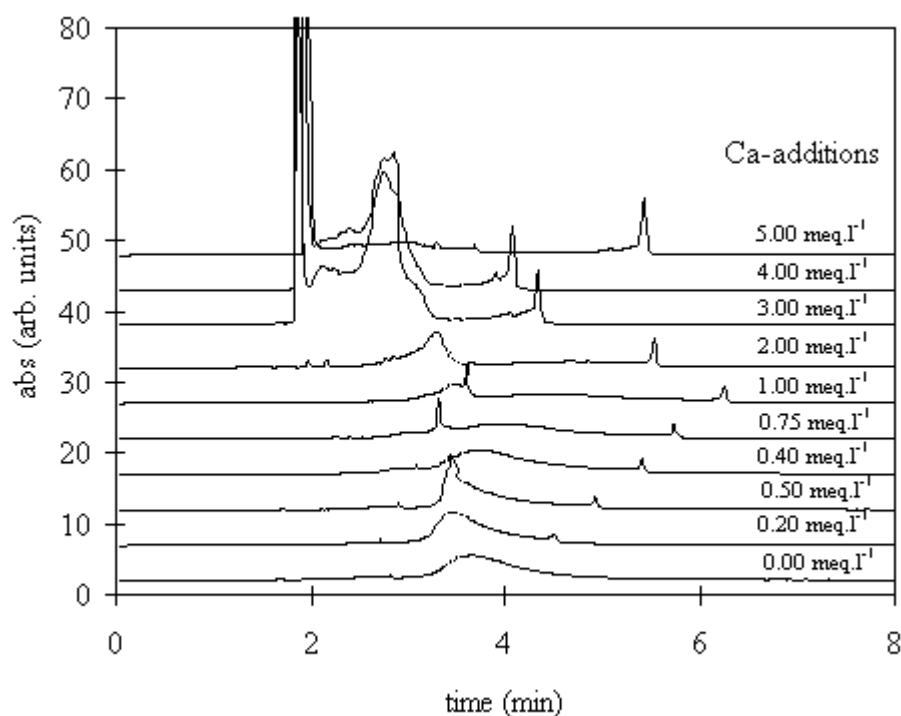


Figure 17. Electropherograms of humic acid solution with different Ca-additions, with  $[Ca]$  ranging from 0 to 5 meq/l;  $[HA] = 5.6$  mmol/l C; Same experimental conditions as for Figure 7.

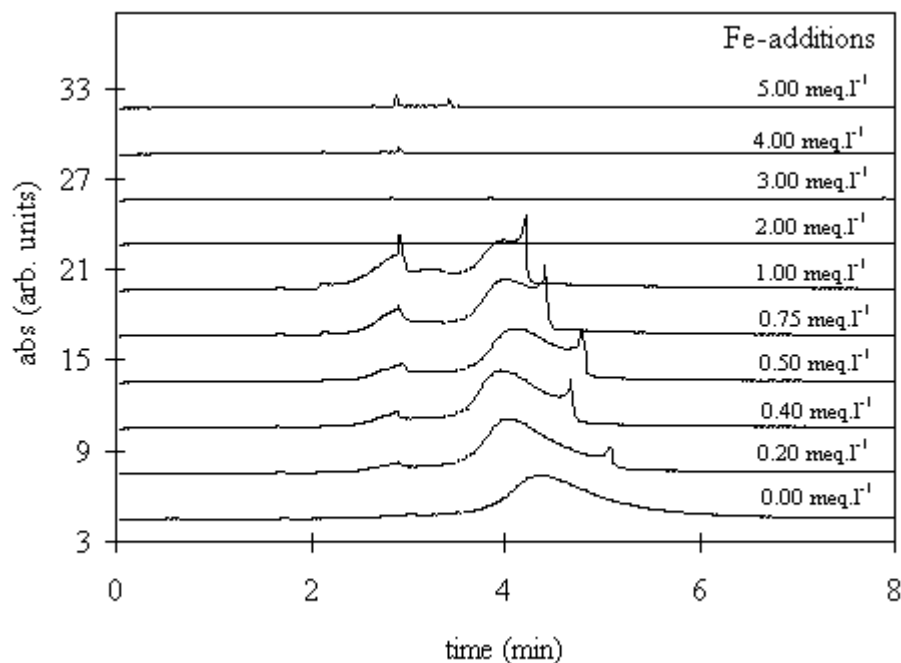


Figure 18. Electropherograms of humic acid solution with different Fe(III)-additions, with  $[Fe(III)]$  ranging from 0 to 5 meq/l;  $[HA] = 5.6$  mmol/l C; Same experimental conditions as for Figure 7.

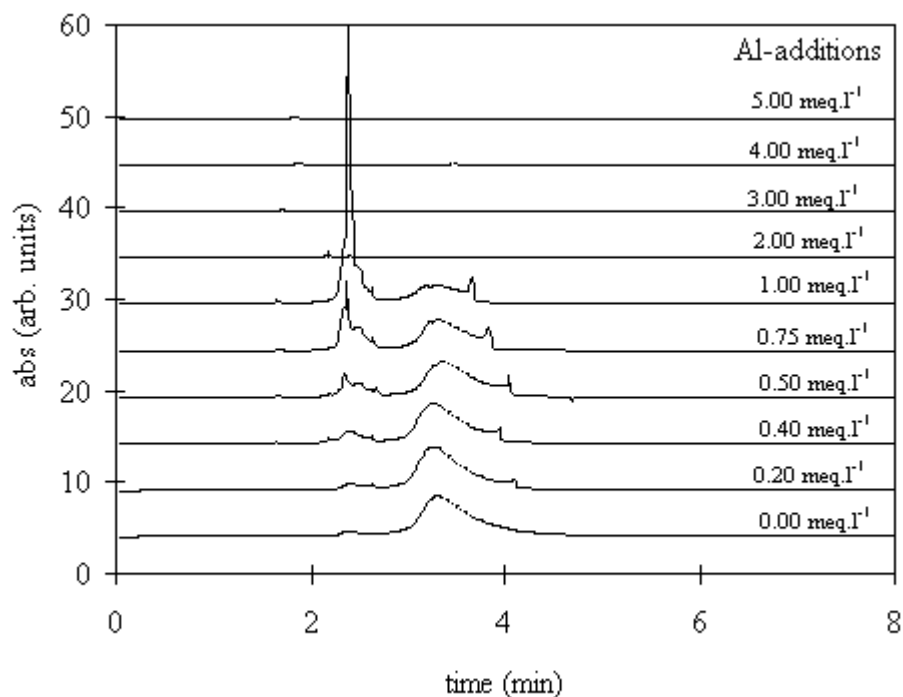


Figure 19. Electropherograms of humic acid solution with different Al(III)-additions, with  $[Al]$  ranging from 0 to 5 meq/l;  $[HA] = 5.6$  mmol/l C; Same experimental conditions as for Figure 7.

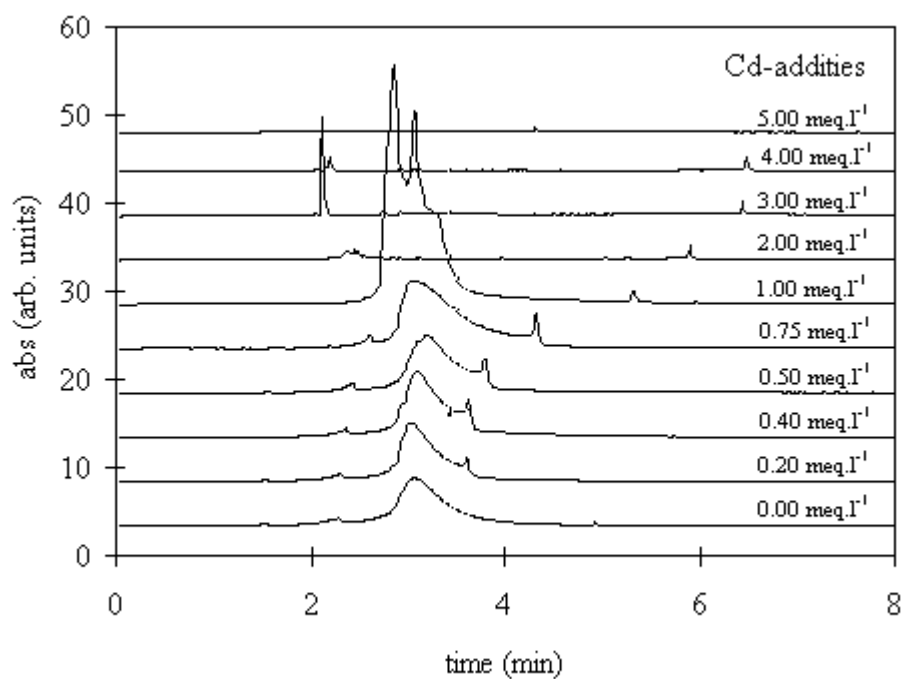


Figure 20. Electropherograms of humic acid solution with different Cd(II) additions, with  $[Cd]$  ranging from 0 to 5 meq/l;  $[HA] = 5.6$  mmol/l C; Same experimental conditions as for Figure 7.

Generally, metal additions caused one or more extra peaks in the electropherograms of humic acid, usually superimposed on the 'main' peak. Metal ion solutions without humic acid (blanks) showed electropherograms with straight baselines with only a small system-peak, strongly suggesting that the 'new' peaks will be evoked by interaction between the metal ions and humic acid. Upon adding increasing amounts of the multivalent heavy metal ions, in this study  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$ , to the humic acid solution, coagulation of metal humic complexes occurred. It seems that, as expected, the coagulation occurs at about 'charge neutralisation'. In the case of  $\text{Ca}^{2+}$ , the coagulation occurred at a somewhat higher metal/humic acid concentration ratio than in the case of the 'heavy metal' ions. The difference in complexing properties between earth alkaline and heavy metals will account for this observation.

### 3.7 Real world samples

Figures 21, 22 and 23 are the electropherograms of samples from river 'Pielisjoki' (Finland), 'Hültabäcken' (Sweden) and 'Riera Major' (Spain) respectively. The first two samples have been chosen from the available set of European river water samples, because of their relatively high DOC concentrations of 0.67 and 0.61 mmol/l C respectively. For comparison the third sample, low in DOC (0.08 mmol/l C), has been included in the series. The sample from 'Pielisjoki' shows an electropherogram quite similar to that of humic acid with metal additions: peak 1 is the system peak, peak 3 the 'bulk peak' and peak 2, 4 and 5 are attributed to metal/humic acid interactions. The second river sample, from 'Hültabäcken', shows a more flat electropherogram. The calcium content of the samples from 'Pielisjoki' and 'Hültabäcken' were 0.05 and 0.22 mmol/l, respectively. It is noted that all river water samples in the available sample set with both 'high' DOC and 'high' calcium concentrations show relatively flat electropherograms.

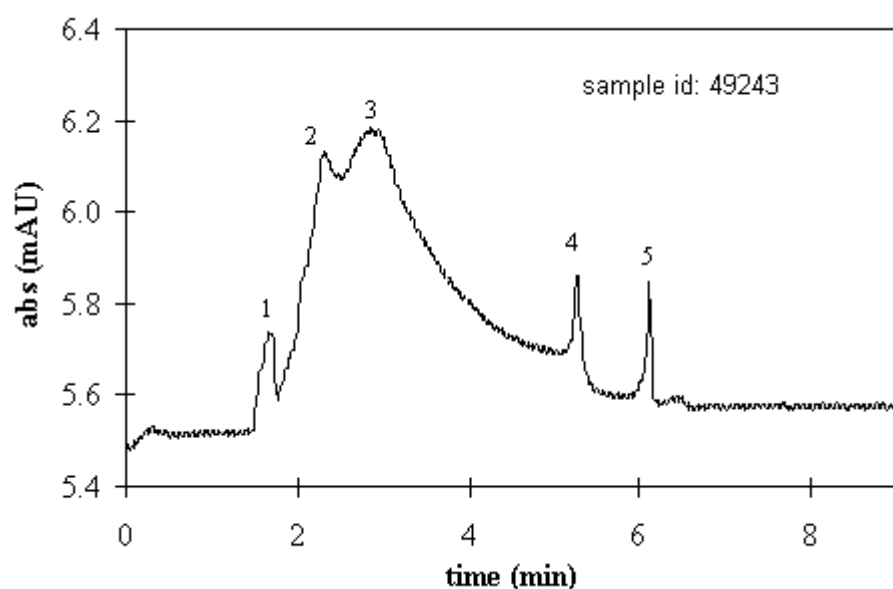


Figure 21. Electropherogram of river water sample from 'Pielisjoki'. DOC concentration: 0.67 mmol/l C. Carrier electrolyte: [boric acid] = 10 mmol/l; pH = 8; (for experimental details see chapter 2). Injection time: 240 s.

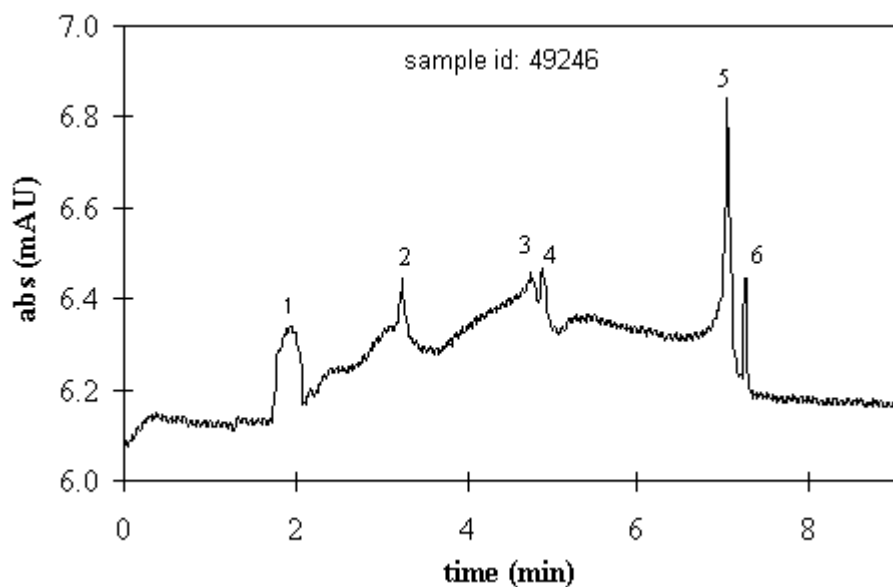


Figure 22. Electropherograms of the river water sample from 'Hültabäcken'. DOC concentration: 0.61 mmol/l C. Carrier electrolyte: [boric acid] = 10 mmol/l; pH = 8; (for experimental details see chapter 2). Injection time: 240 s.

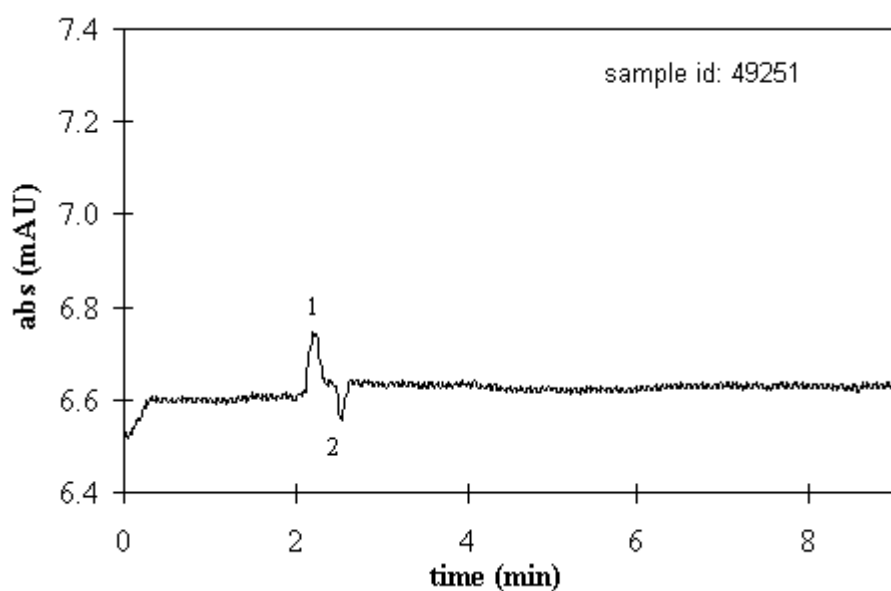


Figure 23. Electropherogram of the river water sample from 'Riera Major'. DOC concentration: 0.08 mmol/l C. Carrier electrolyte:

*[boric acid] = 10 mmol/l; pH = 8; (for experimental details see chapter 2). Injection time: 240 s.*

All river samples with both 'high' DOC and 'low' calcium concentration show electropherograms that correspond in many aspects with the 'basic' form shown by metal/humic acid systems. The river water sample 'Riera Major', low in DOC (0.08 mmol/l C) shows an electropherogram with only the system peak. Due to the low amount of DOC, the CE system is, under the experimental conditions adopted, insufficiently sensitive for humic acid-like entities.

## 4 CONCLUSIONS

Humic material, and thus naturally occurring organic matter, consists of a heterogeneous mixture of numerous macromolecular compounds, differing in structure, in size and in composition. Still, humic material usually shows a fairly consistent 'mean' structure and a rather constant overall composition and size distribution. That's why humic material is often referred to as humic acid, suggesting as it was only one compound. In spite of the heterogeneity of humic matter, metal binding characteristics of the 'mixture' can be studied and the results treated as 'sum parameters' of humic acid. The results can be applied to describe metal speciation patterns in the environmental samples.

In applying different analytical tools, each of them elucidating specific aspects of the metal binding characteristics, a consistent picture of the fundamentals of metal speciation, and thus of ecotoxicity of heavy metals, may be attained. In this respect, the contribution of CE is predominantly focussed on the 'charge' and 'size', as these two parameters of a component primarily control the electrophoretic velocity, and thus the migration time. In humic matter the 'net charge' and the 'effective size' of the discernable 'molecules', usually represented as spherical random coils, are predominantly controlled by pH and the presence of inert salts due to their capability to reduce charge effects by counterion binding to the charged functional groups.

Due to the heterogeneity of humic acid, the electropherograms are quite broad. Although the possibilities for quantification will be limited, the linear functionality between peak area and concentration for humic acid is remarkable. In the presence of multivalent metal ions, the occurrence of a broad main peak persists. However, one or more additional peaks show up, upon additions of  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$ . The formation of additional peaks must be attributed to (complexation) products of interaction between the metal ions and the functional groups.

The chemical environment in the vicinity of the humic acid coils appears to have a substantial influence on the peak performance. Electropherograms of copper/humic acid systems showed more details at higher pH values. Increasing the salt concentration with 25 mmol/l sodium nitrate caused an increase in the migration times and a decrease in the number of peaks in the electropherograms of the copper/humic acid system. Charge effects, due to changes in pH or concentrations inert salt, will account for these effects. However, on the basis of the limited observations in this study, it is not possible to discriminate between direct effects on the 'net charge' and the indirect effect on the 'effective size' of the entities involved.

A change of the humic acid concentrations from 0.6 to 11.2 mmol/l C caused virtually no change in the migration times, and the applied potential showed a reciprocal function with the migration time. In this respect the humic acid molecules behave in a regular way, supporting the idea that observed changes in the peak performance as a result of the addition of metal ions, originate from interactions with the metal ions.

An important feature observed is the 'disappearance' of the CE response in those cases where the concentration ratio metal/humic acid rises beyond a certain limit. Simultaneously, coagulation is observed. It is well known that binding of multivalent metal ions to humic material often results in bridging humic acid molecules, and in a shrinkage of the

macromolecular coil due to the strong reduction of the net charge. The concentration ratio at which coagulation is effectuated, will be strongly dependent on the pH.

The fact that the real world samples from a number of European rivers, sufficiently high in DOC, yielded electropherograms that correspond to those of metal/humic acid systems, supports the idea that organic matter in river water is a master parameter in the speciation of metals. Moreover, as duplicate electropherograms of the natural samples appeared to be fairly identical, CE provides fingerprints that can be applied as tool in the identification of samples.

This research shows some potentialities of capillary electrophoresis in the characterisation of humics in aquatic systems in relation to the binding of metal ions, confirming its applicability as an analytical tool in the study of metal speciation.



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**Appendix 1: MAILING LIST**

- 1 Deputy Director-General and Director Public Health RIVM, dr. G. Elzinga
- 2 Director Environment RIVM, prof. ir. N. D. van Egmond
- 3 prof. dr. W. Admiraal, University of Amsterdam
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- 5 dr. H. Blanck, University of Göteborg
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- 22 Mw. A.W. Hoegee-Wehmann (RIVM/LAC)
- 23 ing. L. Fokkert (RIVM/LAC)
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