HUMIC SUBSTANCES

Capillary Zone Electrophoresis

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Humic substances (HS) which are widespread in the soil, present structural complexity and polyelectrolyte properties. Many techniques and methods have been used to obtain more information about them, as reviewed by Davies and Ghabbour in 1999. The characterization of HS has been the focus of intense research for many years because the organic matter in soil contributes to the quality of the soil more than the other constituents. Nowadays, one of the main goals is the separation of HS into fractions which can then be studied independently. The aim is to determine HS structure, which remains unknown, despite the enormous efforts and remarkable progress in this field. HS exhibit properties similar to weak acid polyelectrolytes, presenting a wide range in molecular weights, solubilities and acid strength. They are sparingly soluble in acid and slightly acidic solutions, but solubility increases with pH and they are only fully soluble alkaline solutions. Many general hypothetical models of HS structure have been proposed, and most of these have been discussed by Shevchenko and Bailey.

As HS play an important role in environmental chemistry, it is important to know their properties. HS are very important as regards the quality and productivity of a soil and in retention of metalions and pollutants by the environment. Dissolved humic material has a tendency to interact with these compounds, complexing metal ions or pollutants, which may alter their fate and transfer in the soil. The mechanisms involved in the interaction of these compounds with HS are not clear and may vary depending on the physicochemical properties of the compounds, soil pH, redox status and the heterogeneous structure of HS. The effect of pH, for example, is related to the degree of humic R-COOH and R-OH dissociation; deprotonation increases the polarity of the humic material and altering its structure.

Electrophoretic methods have been applied for the purpose of HS studies since the 1960s. The first work on the separation of HS by a capillary technique was performed in 1991 using capillary isotachophoresis. However, few really suitable methods for the separation of HS exist.

Nevertheless, to understand HS behaviour, it is useful to perform separation into fractions and determine the structure of each fraction. Capillary zone electrophoresis (CZE) has been applied by Rigol *et al.* in 1994 for this purpose.

HS Properties Studied by CZE

A short overview will be given here because the understanding of the properties of HS is the key to their successful separation.

Adsorption of HS

In the literature, most of authors have used high HS concentrations in order to reach or observe CZE separation patterns; however, no explanation was given (**Table 1**). In fact, it has been observed that when using a background electrolyte that did not interact with HS (rimantadine, for example), high adsorption of HS on bare fused silica capillary wall occurs. This explains why previous authors used rather high concentrations of HS solutions for analysis, because a considerable part of the HS was lost from the solution during the separation process. This observation is in agreement with the fact that HS are highly adsorbed on to silica rocks and silicates or induce interactions with metal ions on clay surface. Such adsorption processes of HS have already been studied on different silica-based materials with other methods. Adsorption on the surface of the silica capillary complicates CZE separation. It was demonstrated recently that the adsorption can be reduced using additives such as magnesium(II) ($>14\ \mathrm{mmol\ L^{-1}}$) in the background electrolyte (BGE). In such magnesium-doped electrolytes, more fractions of HS can be observed, but also a secondary hump pattern. Via study of HS adsorption, a new method of CZE separation has been developed, which allows the concentration of HS in solution needed for CZE separation to be lowered to around $35\text{–}50\ \mathrm{mg\ L^{-1}}$.

Oligomerization of HS

It has long been known that HS aggregate in solution. Various authors describe this property differently. Von Wandruszka *et al.* described the

Table 1 Review of background electrolytes used for HS separation by CZE, reported by Fetsch *et al.* in 1997 and references therein

Background electrolyte	*Number of peaks*	*Hump*	*pH*	*[HS] (g L^{-1})*	*Reference*
10–200 mmol L^{-1} tetraborate	1–4	+	8.3–10.0	0.01–1	All authors
6 mmol L^{-1} tetraborate–3 mmol L^{-1} dihydrogenphosphate	3–6	+	8.9	0.05–0.5	Pompe *et al.* Fetsch *et al.*
10 mmol L^{-1} tetraborate–5 mol L^{-1} urea	3–5	+	7.4	High	Dunkelog *et al.*
10 mmol L^{-1} tetraborate–10% v/v acetonitrile	1–2	+	9.0	1	Norden *et al.*
10 mmol L^{-1} tetraborate–10% v/v acetone	1	+	9.0	1	Norden *et al.*
10 mmol L^{-1} tetraborate–10% v/v isopropanol	2–3	+	9.0	0.01–0.1	Norden *et al.*
10 mmol L^{-1} tetraborate–10% v/v propanol	2–3	+	9.0	1	Norden *et al.*
10 mmol L^{-1} tetraborate–10% v/v 2-propanol–5 mmol L^{-1} urea	2–6	+	9.0	1	Norden *et al.*
10 mmol L^{-1} tetraborate–10% v/v tetrahydrofuran	2–3	+	9.0	1	Norden *et al.*
20 mmol L^{-1} tetraborate–5 mmol L^{-1} CDTA	2–4	+	8.6	0.01–0.1	Norden *et al.*
20 mmol L^{-1} tetraborate–100 mmol L^{-1} boric acid	2–5	+	8.45	0.1–0.25	Fetsch *et al.*
8 mmol L^{-1} L-Alanine	2–4	+	3.17	High	Dunkelog *et al.*
HCl–59.8 mmol L^{-1} L-Alanine	3	+	3.17	0.1	Rigol *et al.*
HCl–60 mmol L^{-1} DL-Alanine	3–4	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–60 mmol L^{-1} DL-Alanine–10% v/v methanol	2–5	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–60 mmol L^{-1} L-Alanine	3	+	3.2	0.1–0.5	Fetsch *et al.*
HCl–60 mmol L^{-1} D-Alanine	4	+	3.2	0.1–0.5	Fetsch *et al.*
HCl–β-Alanine	1–2	+	≈ 3	0.1	Fetsch *et al.*
HCl–60 mmol L^{-1} β-Alanine	1–2	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–30 mmol L^{-1} β-Phenylalanine	1	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–30 mmol L^{-1} L-Cystine	1–2	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–L-Leucine	1–2	+	3.17	0.1	Rigol *et al.*
HCl–L-Lysine	1–2	+	≈ 3	0.1	Rigol *et al.*
HCl–L-Serine	1–2	+	3.17	0.1	Rigol *et al.*
HCl–10 mmol L^{-1} DL-Serine	1–2	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–15 mmol L^{-1} DL-Proline	1–2	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–L-Aspartic acid	1–2	+	≈ 3	0.1	Rigol *et al.*
HCl–60 mmol L^{-1} glycoclic acid	1	+	3.2	0.1–0.25	Fetsch *et al.*
HCl–100 mmol L^{-1} boric acid	5–15	–	3.15	0.5	Fetsch *et al.*
HCl–350 mmol L^{-1} boric acid	10–30	–	3.15	0.25–0.5	Fetsch *et al.*
HCl–500 mmol L^{-1} boric acid	3–8	+	3.38	0.15	Fetsch *et al.*
25–50 mmol L^{-1} dihydrogenphosphate	5–15	–	9.2	0.01–0.5	Fetsch *et al.*
50–100 mmol L^{-1} phosphate	5–15	–	6.3–9.2	0.1–1	Garrison *et al.* Fetsch *et al.*
100 mmol L^{-1} dihydrogenphosphate–5 mmol L^{-1} phosphate–250 mmol L^{-1} boric acid	3–8	+	3.3	0.15	Fetsch *et al.*
67 mmol L^{-1} dihydrogenphosphate–3.3 mmol L^{-1} phosphate–167 mmol L^{-1} boric acid–3.3 mmol L^{-1} wolframate	3–8	+	5.0	0.15	Fetsch *et al.*
20 mmol L^{-1} rimantadine hydrochloride	1–3	+	3.40	0.01–0.5	Fetsch *et al.*
20 mmol L^{-1} rimantadine hydrochloride–2 to 50 mmol L^{-1} $MgCl_2$	2–9	+	3.40	0.01–0.5	Fetsch *et al.*
50 mmol L^{-1} carbonate	1–4	+	9.0–11.4	≥ 0.05	Schmitt *et al.*
HCl–glycylglycine	1–2	+	≈ 3	0.1	Rigol *et al.*
HCl–glycine	1–2	+	3.17	0.1	Rigol *et al.*
Citric acid–citrate	1–2	+	≈ 3	0.1	Rigol *et al.*
HCl–imidazole	1–2	+	≈ 3	0.1	Rigol *et al.*
5 mmol L^{-1} imidazole–acetic acid–20 mmol L^{-1} boric acid	2–5	+	4.5	0.01–0.1	Norden *et al.*
50 mmol L^{-1} acetate	5–10	+	4.6–5.15	0.01–1	All authors
103 mmol L^{-1} urea	5–12	+	3.65–6.6	0.15	Fetsch *et al.*
20 mmol L^{-1} 2-(*N*-morpholino)ethanesulfonic acid (MES)	1–3	+	6.15	High	Dunkelog *et al.*
2-(*N*-morpholino)ethanesulfonic acid (MES)–NaOH	1–3	+	≈ 3	0.1	Rigol *et al.*
20 mmol L^{-1} *tris*(hydroxymethyl)aminomethane (TRIS)	1–3	+	8.30	High	Dunkelog *et al.*
HCl–*tris*(hydroxymethyl)aminomethane (TRIS)	1–3	+	≈ 3	0.1	Rigol *et al.*
20 mmol L^{-1} 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS)	1–2	+	10.4	High	Dunkelog *et al.*
20 mmol L^{-1} 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES)	1–2	+	9.5	High	Dunkelog *et al.*

[HS], concentration of humic substances; + hump present; – no hump.

aggregates as pseudomicelles, whereas Wershaw and Chien *et al.* used humic membrane-micelle, Dachs *et al.* introduced the term fractal aggregates, Shevchenko *et al.* used polymers and finally, the formation of oligomers was proposed by Havel *et al.* in 1997.

The most interesting theory from the last decade is that of Wershaw, who introduced a model for humic materials which provides a mean of understanding the interactions of hydrophobic compounds and HS. This model considers humic material to be constituted of a number of different oligomers and simple compounds. The resulting structures are similar to micelles or membranes in which the interiors are hydrophobic and exterior surfaces hydrophilic. Thus, the enhancement in the solubility of DDT was observed by Wershaw in concentrated HS solutions which implies the formation of HS micelles. According to Karckhoff *et al.* and Wang *et al.*, the adsorption of hydrophobic organic compounds by organic matter involves weak mechanisms of adsorption such as hydrogen bonding. Few papers concerning aggregation of HS have been published as yet. Schmitt *et al.*, using micellar electrokinetic chromatography (MECK), observed that some HS behave like ionic micelles and defined a humic initial micellar concentration (CMC) around 30 mg L^{-1}. The micellar properties of HS are due to both hydrophilic and hydrophobic sites which are responsible for the enhancement of the solubility of organic compounds in aqueous media or the lowering of the surface tension of water.

In 1998, the aggregation properties of HS were studied by CZE. Oligomerization of HS was observed during CZE when the effect of HS concentration on CZE separation patterns was studied. The phenomenon was described for several BGE: DL-alanine, phosphate and rimantadine systems. When either concentration or sample injection time were increased, significant changes in HS separation patterns were observed. The CMC of humic acid was estimated to be around 35 mg L^{-1}.

Interaction of HS with Metal Ions and Organic Compounds

Metal Ions

The interaction of metal ions with natural soil particles is complex, involving various mechanisms Binding between trace metals and dissolved HS is important in controlling the chemical speciation, toxicity and bioavailability of trace metals. For example, it has been observed that, in the presence of HS dissolved in seawater, the accumulation rates of cadmium in organisms were faster in comparison to accumulation rates of cadmium from seawater without HS.

Different models of metal–HS interactions have been described. Due to the broad spectrum of binding sites reported by Ephraim, HS can interact with metal ions by adsorption, ion exchange, precipitation and/or surface complexation. Specific adsorption involves several heavy metal ions such as Cd^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+}. Coprecipitation of trace metals with carbonates is very important for semi-arid soils and in soils formed from limestone. Techniques like synchronous and time-resolved fluorescence, luminescence, anodic stripping voltammetry and modified carbon paste electrodes have been used in the study of the fate and distribution of inorganic pollutants in the environment. Many papers concerning the binding of metal ions to HS have been published.

In 1997, Nordén and Dabek-Zlotorzynska applied CZE to study Sr^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+} and Al^{3+}. Their interactions with HS were confirmed. Study of the influence of pH on the complexation showed that complexation can be better followed by direct rather than indirect detection. The following order of complexation was obtained by CZE: $Al^{3+} > Hg^{2+} > Cu^{2+} > Pb^{2+} > Sr^{2+}$. This result is in agreement with other work on metal ion–HS interaction strengths. Furthermore, when pH is increased, complexes are more stable and, ageing of HS solutions showed an increase of more than 25% in the amount Hg^{2+} bound to HS after 5 months. This increase is supposed to be connected to the possible time dependence of the reduction process, and/or to be related to the macromolecule nature of HS. Changes in HS separation pattern were also observed when adding iron(III) to HS and forming Fe^{3+}–HS complexes, when the study was performed at pH 12.

Radionuclides

The other important interactions which have been studied and are highly important with respect to environmental protection policies are those between HS and radionuclides like ^{60}Co, ^{85}Sr, ^{137}Cs, ^{237}Np, ^{241}Am, etc. For example, it has been shown that samarium(III) (10^{-10} to 10^{-5} mol L^{-1}) and americium(III) (10^{-11} mol L^{-1}) form 1 : 1 complexes with HS; their pH-independent complexation constants, log β, are respectively 7.1 ± 0.2 and 6.6 ± 0.2. Spiking actinide elements (Th, U, Np, Pu, Am) in HS fractions, it has been shown that these fractions vary greatly in their effectiveness and selectivity as ligands for early actinides.

Rigol *et al.* used CZE to study humic fractions in organic soil and their relationship with radiocaesium mobility. The quantification of the radiocaesium confirmed that there may be some organic matter–radionuclide interactions other than those originated by HS and which may govern radionuclides retention in soils with a high content of organic matter.

Organic Compounds

It has been known for many years that organic compounds interact with HS. There have been studies on the interaction of pesticides (chlorodimeform and lindane) and herbicides (paraquat, 2,4-dichlorophenoxyacetic acid and atrazine) with HS. Later, the effect of HS was studied on solute transport in clays and it was suggested that humic and fulvic acids facilitate the transport of small organic molecules by encapsulation. Over the last 5 years, the effects of dissolved HS on the bioconcentration of xenobiotics have been studied by several authors. It was observed that dissolved HS can change the physicochemical properties of these pollutants in aqueous environments by modifying the hydrolysis kinetics, enhancing their water solubility or decreasing the toxicity of the organic chemicals. The binding of hydrophobic organic compounds to humic polymers has been studied, using a predictive thermodynamic HS–organic solute interaction model. A distributed reactivity model was studied for phenanthrene sorption and desorption equilibria by soils and sediments. Relationships were observed between the chemical and structural characteristics of associated organic matter and it was noted that hysteresis is important to the fate and transport of organic contaminants in environmental systems.

It was proposed to use the potential of dissolved HS to enhance the desorption of hydrophilic pollutants in remediation processes for soils and waters. Following this idea, some authors used a complexation–flocculation method to determine binding coefficients of phenanthrene, anthracene, pyrene and fluoranthene to dissolved HS. In some cases, pH dependence of the sorption was observed. While naphthalene binding to dissolved HS was not decreased at lower pH, dichlorodiphenyl trichloroethane (DDT) and polyaromatic hydrocarbons (PAHs) showed the opposite effect.

As reviewed in Table 1, the effect of organic solvents on the migration behaviour of HS in CZE was also studied. Migration time values are usually shifted to longer values compared to aqueous BGE. This effect is attributed to either a decrease in electroosmotic flow (EOF) or an increase in the electrophoretic mobility of HS compounds, or even to both effects together. Furthermore, some additional fractions were distinguished when applying mixed solvents. They are attributed to the formation of supramolecules between the organic solvent and HS.

Paramagnetic studies have been carried out in the study of atrazine solubilized by humic micellar solutions. This work suggests that atrazine is preferably present in the hydrophobic interior of HS micelles. Other techniques, such as light and X-ray scattering also give evidence of molecular aggregation in humic solutions. Using ultraviolet-visible (UV/Vis), Fourier transform infrared and electron spin resonance spectroscopy, a group of authors studied the mechanism of atrazine sorption on HS and confirmed that hydrogen bonding is responsible for the interaction. Proton transfer and possibly also hydrophobic bonding are involved in the interactions between atrazines, and HS and CZE studies of the binding between *S*-triazines and HS confirmed that interactions between ionizable pesticides and HS occur: differences in the electropherograms were observed.

Capillary Electrophoretic Separation of HS

The major advances in the separation of HS are listed in **Table 2**.

CZE separations of HS have been performed using UV/Vis, diode array or laser-induced fluorescence detection. In Table 1, the BGE used for this purpose are listed; mainly borates, amines and amino acids have been applied. Nevertheless, even if a large variety of BGE were used, four main types of electropherogram separation patterns can be observed and the three most important types of HS separation are shown in **Figure 1**. The first type is obtained in a tetraborate BGE and represents separation patterns with one or more broad peaks, called usually the humic hump.

Table 2 Important advances in HS separation

1960–70	First applications of electrophoretic methods to HS
1980–90	Application of size exclusion chromatography to HS
1991	First HS separation by capillary technique (Kopáček *et al.*)
1994	First application of capillary zone electrophoresis (CZE) to HS separation (Rigol *et al.*)
1996–1997	CZE study of HS interactions with some metal ions and organic compounds
1998	First separation of HS on to 15–30 peaks or fractions (Fetsch and Havel) Study of HS properties by CZE as adsorption on capillary wall and oligomerization process (Fetsch *et al.*) Combination of ultrafiltration and CZE (Rigol *et al.*)

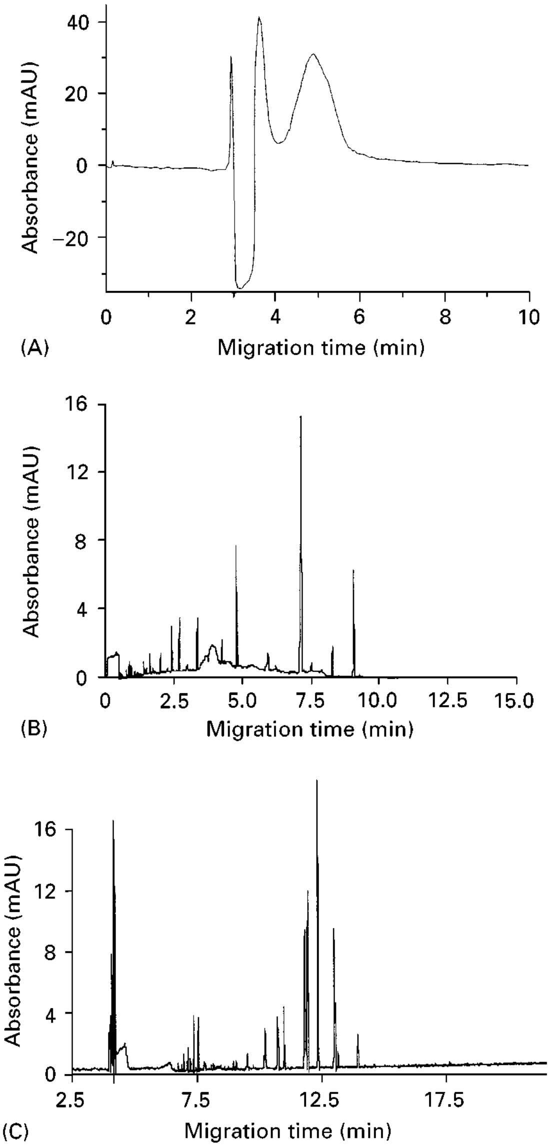

Figure 1 Three BGEs for coal-derived humic substances. Humic acid: Fluka no. 53680 (analysis number 38537/1 293) supplied by Fluka Chemika (Switzerland). Fused silica capillary: L = 43.5 cm (l = 35.5 cm) × 75 μm i.d. (A) BGE 1 and conditions: 60 mmol L^{-1} DL-alanine adjusted by HCl at pH 3.20; 15 kV, 20 s hydrodynamic injection, 40°C, 220 nm and C_{HA} = 100 mg L^{-1}. (B) BGE 2 and conditions: 50 mmol L^{-1} phosphate adjusted by NaOH at pH 9.20; 20 kV, 15 s hydrodynamic injection, 40°C, 220 nm and C_{HA} = 100 mg L^{-1}. (C) BGE 3 and conditions: 350 mmol L^{-1} boric acid adjusted by HCl at pH 3.20; 20 kV, 15 s hydrodynamic injection, 40°C, 220 nm and C_{HA} = 530 mg L^{-1}.

Sometimes, these humps do show shoulders and some resolution. It is usually suggested that the hump corresponds to the average electrophoretic mobility of the HS polymeric mixture.

The second type of electropherograms in L- or DL-alanine BGE consists of three fractions. These electropherograms can distinguish between HS of different origin via changes in migration times of the fractions and in the number of peaks. It was shown in these studies that temperature plays an important role in the patterns obtained. The best separation is obtained for temperatures in the range 30–40°C and the highest peak area and peak height are obtained at ≈ 40°C. An example of separation under optimized conditions is shown in Figure 1A. Three main fractions were found and, for the wavelength lower than 220 nm, a negative peak was observed. Separation also depends on pH and buffer concentration. As the EOF changes with pH, the migration times of the separated components are directly affected and generally decrease as pH increases. In the pH range 5.0–9.5, HS constituents are moving as anions. In the case of alanine-based BGE, it appears that one fraction is moving as a neutral molecule, while the others are moving as anions. Under the presence of HS, it is difficult to determine the EOF simultaneously with HS separation because common neutral markers used (mesityl oxide, methanol, acetone and water) interact with HS. Independent determination of the EOF is necessary.

In the case of borate BGE, when increasing BGE concentration, migration times increase but no important changes in separation patterns are observed.

The next type of HS separation gives a humic hump with small peaks on it. This effect is generally observed with dihydrogen phosphate BGE (Figure 1C) or dihydrogenphosphate combined with borate BGE.

On the other hand, quite important changes in the separation patterns and number of peaks, were observed using slightly acid BGE, which consists of a high concentration of boric acid. This gives the best HS separation resolving from 10 to 30 peaks depending on the type of humic acid sample. In this case, highly concentrated (100–350 mmol L^{-1}) boric acid BGE is used as a complexing BGE (Figure 1C). The possible explanation for this excellent separation pattern is as follows: functional groups of hydroxycarboxylic acids, oxalic acid and oligoalcohols are present in the HS structure. Boric acid interacts with phenolic and carboxylic groups and therefore also reacts in a similar way with HS. This reaction was also proved by spectrophotometric and potentiometric studies. It was suggested that the large number of peaks observed in highly concentrated boric acid BGE is due to the breaking of HS oligomers into real fractions of humic substances (**Figure 2**). Humic substances contain a limited number of chemical entities (even as low as perhaps only 3–7). However, when the concentration of HS is increased, these species do oligomerize. Complexation of boric acid with phenolic or carboxylic groups prevents the formation of oligomers and thus the individual chemical compounds can be separated by CZE. The species formed must be kinetically robust, which is the condition to observe separated peaks. If just three fractions, HA_1, HA_2 and HA_3, are considered, oligomerization might take place according to eqns [1–3]:

$$m(HA_1) \rightleftharpoons (HA_1)_m \qquad [1]$$

$$n(HA_2) \rightleftharpoons (HA_2)_n \qquad [2]$$

$$o(HA_3) \rightleftharpoons (HA_3)_o \qquad [3]$$

In addition, mixed oligomers can be formed according to the general reaction:

$$p(HA_1) + q(HA_2) + r(HA_3) \rightleftharpoons (HA_1)_p(HA_2)_q(HA_3)_r \text{ (oligomer)} \qquad [4]$$

If reaction [4] is quantitative, instead of the peaks of HA_1, HA_2, HA_3 species, just one peak (one hump) of the oligomer will be observed.

This oligomer will be broken down by reaction with boric acid

$$\begin{aligned}(HA_1)_p(HA_2)_q(HA_3)_r &+ sB(OH)_3\\ \rightleftharpoons pHA_1(B(OH)_3)_t &+ qHA_2(B(OH)_3)_u\\ &+ xHA_3(B(OH)_3)_v + (s - pt - qu - xv)B(OH)_3\end{aligned} \qquad [5]$$

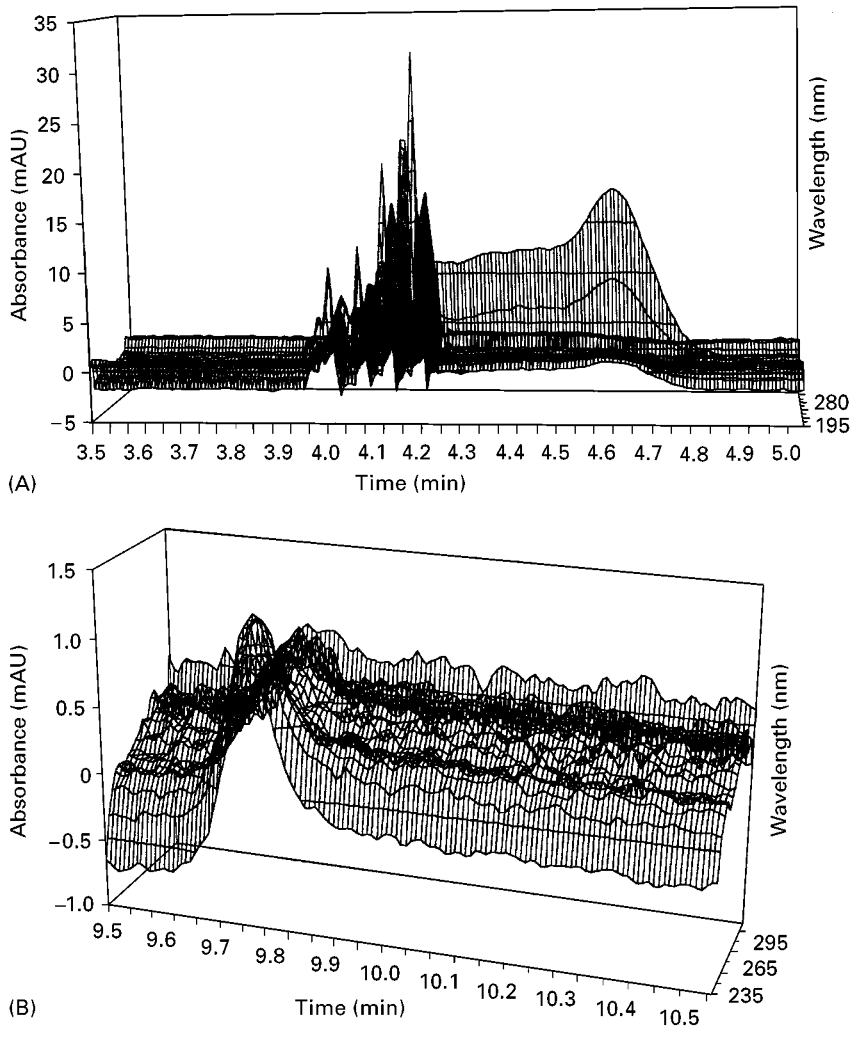

Figure 2 Fluka humic acid three-dimensional electropherogram at higher HS concentration in boric acid BGE. Humic acid: Fluka no. 53680 (analysis number 38537/1 594) supplied by Fluka Chemika (Switzerland). Fused silica capillary: L = 43.5 cm (l = 35.5 cm) × 75 μm i.d. BGE: 350 mmol L^{-1} H_3BO_3 adjusted by HCl at pH 3.15. Conditions. 20 kV, 40°C, 15 s hydrodynamic injection and C_{HA} = 533 mg L^{-1}. (A) HA Cathedrale; (B) HA Volcano; (C) HA Alpes; (D) HA Mt Everest (Reproduced with permission from Fetsch and Havel, 1998.)

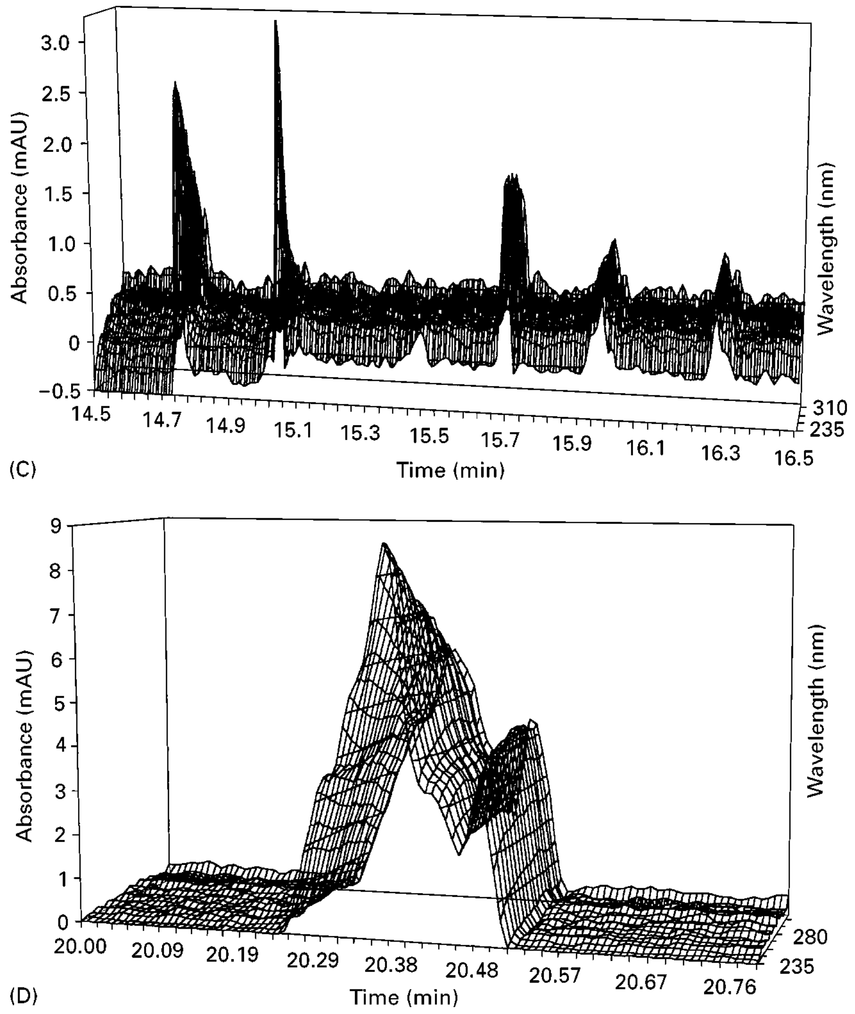

Figure 2 *Continued*

Oligomers are broken down and three monomers will be observed in this theoretical case. Thus, it seems that for the first time separation of HS into the real fractions, real chemical entities forming the humic substances mixture, has been achieved in highly concentrated boric acid BGE. Some other examples of fingerprints are presented in **Figure 3**, showing the separation of humic acids of peat, soil, oxyhumolite and tschernozem origin.

In conclusion, different CZE separations have been presented with very different results; the most interesting and efficient were the results of Fetsch and Havel in highly concentrated boric acid solution.

Future Developments

CZE has been shown to be the most powerful tool for the separation and characterization of HS due to their ionic and/or polyelectrolyte properties. The CZE separation patterns of HS obtained may, in the future, find a direct application in forensic science.

Nevertheless, even if several different models of HS are proposed and various properties of HS intensively studied, the real structure of humic, fulvic, humin and hymatomelanic acids is still unknown. The latest results obtained by CZE showing separation into 10–30 fractions present an optimistic insight into the humic substances puzzle. On the basis of recent results, fraction collection in order to perform studies on individual fractions by gas chromatography–mass spectrometry, matrix assisted laser desorption–time of flight (MALDI-TOF) mass spectrometry and nuclear magnetic resonance is beginning to appear feasible. It is possible that the problem of HS structure is at last on the way to being resolved.

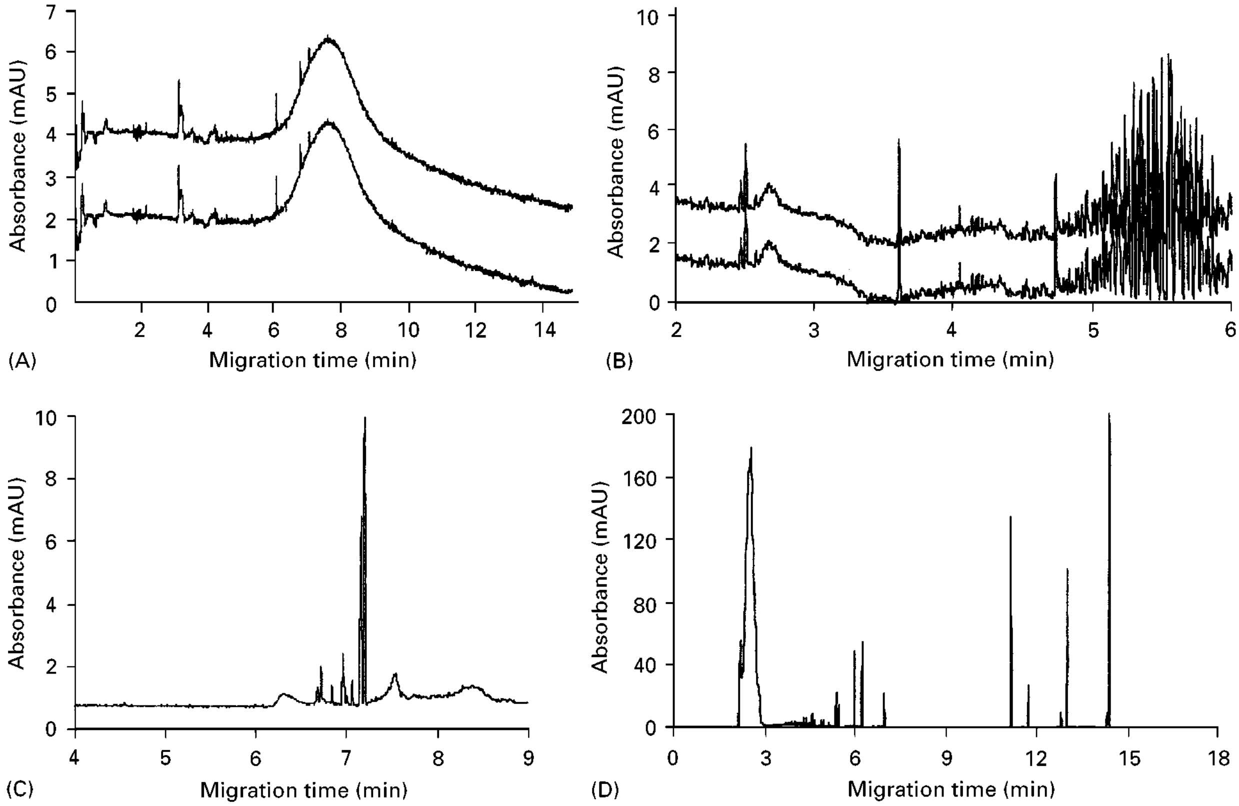

Figure 3 Electropherogram fingerprints of humic substances of different origin in boric acid BGE. (A) International humic substances society peat reference humic acid; (B) Desmonte soil humic acid, Argentina; (C) oxyhumolite-derived humic acid, Bílina, Czech Republic; (D) Tschemozem humic acid, Chotesov, Czech Republic. Fused silica capillary: $L = 43.4$ cm ($l = 35.4$ cm) $\times$ 75 μm i.d. BGE: 350 mmol L^{-1} H_3BO_3 adjusted by HCl at pH 3.20. Conditions: 20 kV, 40°C, 215 nm and 1 s hydrodynamic injection. Humic acid concentration: $C_{HA} \cong 600$ mg L^{-1}.

Further Reading

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