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Analytical study of biomass pyrolysis oils II. Optimization of analytical conditions for the phenolic fraction using micellar electrokinetic chromatography

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Abstract

Biomass carbonization oils constitute an important source of chemicals and more recently an alternative energy source to fossil oils, and numerous studies have been carried out in order to establish their composition. As phenol derivatives (alkyl- and methoxyphenols, alkyldihydroxybenzenes, hydroxybenzaldehydes) and naphthol derivatives are products of high value, they are important components of these complex organic matrices. A study is reported of the electrophoretic behaviour of these compounds using micellar electrokinetic chromatography and, using a mixture of 22 model phenolic derivatives, the possibility of analysing such a complex matrix with such a broad lipophilic range in one run is demonstrated.

1. Introduction

Various world energy crises, involving supply difficulties and large increases in crude oil prices, environmental concerns and the agricultural over-production have led to renewed interest in alternative energy sources to fossil oils. Studies have led to greater interest in biomass as an energy source on the one hand and as a supply source for chemicals on the other. Unfortunately, pyrolysis oils, which are very complex matrices, change with time and this instability is a handicap in their exploitation and consequently their valorization. The stabilization of pyrolysis oils requires the chemical and structural characterization of this complex matrix. In 1986, Elliott [1,2], using capillary gas chromatography (GC) and mass spectrometry (MS), characterized for the first time the tars resulting from wood carbonization. This fundamental study constitutes the first overall approach to the analysis of wood carbonization tars, as previously only a detailed analysis of polyaromatic hydrocarbons had been reported [3]. However, because of the method used by Elliott to simplify the organic matrix prior to analysis by GC–MS, *i.e.*, ex-

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traction with dichloromethane, the results obtained, although very interesting, give an overestimation of low polarity compounds to the detriment of high-polarity compounds. In 1991 we completed this interesting study by Elliott using a set of liquid chromatographic techniques to fractionate the complex organic matrix of pyrolysis oils prior to GC–MS analyses [4]. The combination of ion-exchange chromatography and size-exclusion chromatography with coupled capillary GC–MS appeared to be suitable, and the strategy developed allowed the accurate characterization of the volatile part of these pyrolysis oils, *i.e.*, about 70% of tars condensed during the pyrolysis.

In order to increase the part of pyrolysis oils that is characterized, we considered replacing capillary GC with high-performance capillary electrophoresis, an emerging technique allowing performances close to those of capillary GC without any limitations due to the possible lack of volatility or thermodegradability of the analyte compounds. Among the polar compounds present in wood pyrolysis oils, alkyl- and methoxyphenols, alkyldihydroxybenzenes, hydroxybenzaldehydes and substituted naphthols are important classes of biomass pyrolysis oil components [4], and we report in this paper the optimization of the conditions for their analysis using micellar electrokinetic chromatography (MEKC). Although different studies have demonstrated the potentials of capillary zone electrophoresis (CZE) for the separation of chlorophenols as industrial pollutants [5] and electrokinetic chromatography, using either sodium dodecyl sulphate (SDS) micelles [6–8] or a β-cyclodextrin derivative [9] as a pseudo-stationary phase for the separation of phenolic isomer mixtures, no study has been reported on the analytical optimization of a complex mixture of phenolic derivatives with such a wide polarity range as encountered in biomass pyrolysis oils. Nevertheless, interesting recent studies are those by Masselter *et al.* [10] concerning the separation of three cresols and six xylenols using CZE with a dynamically coated fused-silica capillary and by Zemann *et al.* [11] on the separation of phenolic compounds from biomass solvolysis also using CZE with a dynamically coated fused-silica capillary.

2. Experimental

2.1. Reagents

The water used for the preparation of buffers was purified by reverse osmosis and filtration, using a Milli Ro + Milli Q System (Millipore, Molsheim, France).

The organic solvents used, methanol and acetonitrile of RS HPLC grade (Carlo Erba, Rueil Malmaison, France), were used as received.

The various phenolic compounds and the reagents used for buffer preparation, *i.e.*, borax, monobasic sodium phosphate and sodium hydroxide, were of 99+% purity from Aldrich (Strasbourg, France). SDS used in MEKC was of 99% purity from Sigma (La Verpillière, France).

2.2. Apparatus

We used a PACE 2100 system (Beckman, Fullerton, CA, USA), equipped with a UV detector and monitored with a PS/2 computer (IBM, Greenock, UK). Injections were performed hydrodynamically (injection time 1 s) and the detector was set at 214 nm. A 57 cm × 50 μm I.D. fused-silica capillary (Beckman) was utilized. The electrolyte pH was measured before each analysis using a Beckman Model ϕ pH meter.

Methanol was used as the electroosmotic flow marker. The micellar migration time was taken, according to Terabe *et al.* [12], as being equal to 4.5 times the migration time of the bulk solution, t_0, because of the co-elution of Sudan III (the marker for the micelle) with the most hydrophobic compounds under some analytical conditions.

3. Results and discussion

3.1. Study of the influence of fundamental thermodynamic parameters on the evolution of the resolution of an alkylphenol mixture

Among numerous phenolic derivatives constituting the phenolic fraction of wood pyrolysis oils, we first selected, in addition to phenol, the

following alkylphenols: *o*-, *m*- and *p*-cresol, 3,4-dimethylphenol, 4-*tert*.-butylphenol, 2-*tert*.-butyl-4-methylphenol, 2,6-di-*tert*.-butyl-4-methylphenol and 2,4,6-tri-*tert*.-butylphenol. In order to optimize the analysis of wood biomass oils, it appeared of interest to study the electrophoretic behaviour of these model compounds, which cover a wide hydrophobic range.

These phenolic derivatives, substituted with electron-donating groups, have very high pK_a values and are therefore difficult to ionize. Indeed, only phenol ($pK_a = 9.89$) and cresols (pK_a 10.01–10.20) [13] are partially ionized at pH 10, the limiting pH value above which the pyrolysis oils are unstable, the other alkylphenols being neutral at this pH. Therefore, as it was impossible to separate these phenolic compounds using CZE, we undertook their analysis on the basis of their lipophilic characteristics. To do so, we used MEKC, developed by Terabe *et al.* in 1984 [6], with SDS as a pseudo-stationary phase.

The micelles present a hydrophobic core and can interact differently with solutes of different lipophilic characteristics. According to Vindevogel and Sandra [14] and Terabe [15], the resolution of potentially ionizable molecules in MEKC depends on the SDS concentration, the nature and concentration of the organic solvent and the pH and ionic strength of the electrolyte. We therefore undertook to optimize each of these parameters successively.

Influence of surfactant concentration on resolution

When optimizing this parameter, we used a 25 m*M* borate–50 m*M* phosphate buffer (pH 9) in order to have a maximum electroosmotic flow and therefore a short analysis time. We used a voltage of 30 kV, which is the limit above which there is no longer good dissipation of the Joule effect induced in the capillary. This voltage was kept constant in order to maintain a constant electric field.

Under these conditions, the influence of increasing surfactant concentration from 10 to 100 m*M* was studied in order to optimize the capacity factors, which, according to Terabe [15], should have a value between 0.5 and 10.

A low SDS concentration (10 m*M*) gave acceptable capacity factors for the most hydrophobic alkylphenols, *i.e.*, 2,6-di-*tert*.-butyl-4-methylphenol and the 2,4,6-tri-*tert*.-butylphenol, and allowed a good resolution of these compounds, but led to too low capacity factors ($\ll 0.5$) for the most hydrophilic alkylphenols and non-resolution of the cresol isomers.

In contrast a high SDS concentration (100 m*M*) led to acceptable capacity factors for the most hydrophilic alkylphenols, allowing a good resolution of the three cresol isomers, but induced prohibitive capacity factors for the most hydrophobic alkylphenols ($k' \gg 10$). Under such conditions, the most hydrophobic alkylphenols, *i.e.*, 2,6-di-*tert*.-butyl-4-methylphenol and 2,4,6-tri-*tert*.-butylphenol, underwent such great interactions with the micelles that the electrophoretic system appears to be non-selective for these two compounds.

Therefore, an intermediate SDS concentration (50 m*M*) appears to be a good compromise, leading to acceptable capacity factors for the most hydrophilic alkylphenols ($k' \approx 0.5$) and capacity factors of the most hydrophobic alkylphenols just over 10. However, as evidenced in Fig. 1, with this optimum SDS concentration, although the resolution of the three cresol isomers is satisfactory, the most hydrophobic alkylphenols, *i.e.*, 2,6-di-*tert*.-butyl-4-methylphenol and 2,4,6-tri-*tert*.-butylphenol, are not resolved.

We next undertook a study of the influence of the addition of organic solvent on the resolution, the SDS concentration being kept at its optimum value of 50 m*M*.

Influence of cosolvent content on resolution

Among the different organic solvents miscible with water and allowing enhancement of the hydrophobic characteristics of the electrolyte (acetonitrile, 1-propanol, 2-propanol, tetrahydrofuran, etc.), we deliberately chose acetonitrile owing to its high dielectric constant value and its good hydrophobicity. With such a cosolvent, we should be able to modify the hydrophobicity, without altering the conductivity, of the electrolyte. The effect of the addition of acetonitrile in steps of 5% (v/v) on the electro-

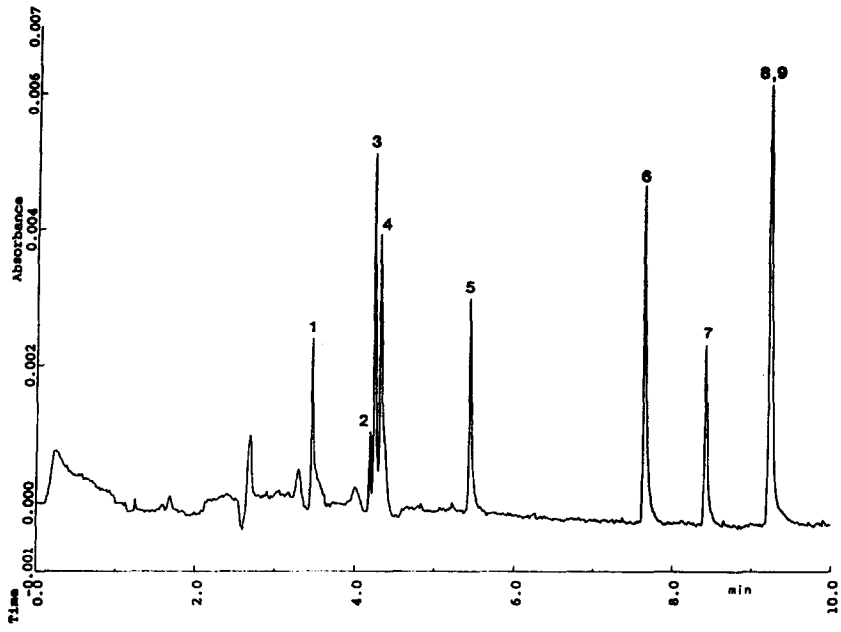

Fig. 1. Analysis of model alkylphenols by MEKC. Electrolyte, 25 m*M* borax–50 m*M* monobasic sodium phosphate (pH 9); [SDS] = 50 m*M*. Peaks: 1 = phenol; 2 = *o*-cresol; 3 = *m*-cresol; 4 = *p*-cresol; 5 = 3,4-dimethylphenol; 6 = 4-*tert.*-butylphenol; 7 = 2-*tert.*-butyl-4-methylphenol; 8 = 2,6-di-*tert.*-butyl-4-methylphenol; 9 = 2,4,6-tri-*tert.*-butylphenol.

phoretic behaviour of the model alkylphenols was studied.

According to Vindevogel and Sandra [14] and Terabe [15], not only does the addition of an organic solvent modify the hydrophobicity of the liquid medium and the selectivity of the electrophoretic system, but it also alters the electroosmotic flow velocity. Thus, under our experimental conditions, the migration time of the electroosmotic flow increased from 2.7 min without acetonitrile (Fig. 1) to 3 min when 5% of acetonitrile was added (Fig. 2) and to 3.2 min with 10% of acetonitrile, therefore allowing the migration-time window to be increased. However, this increase in the migration-time window, although favourable for the resolution of the most hydrophobic alkylphenols (2,6-di-*tert.*-butyl-4-methylphenol and 2,4,6-tri-*tert.*-butylphenol), leads, as mentioned earlier, to an increase in the hydrophobicity of the mobile phase, which leads to a decrease in the capacity factors of all the alkylphenols studied and in particular those of the most hydrophilic alkylphenols, *i.e.*, the three cresol isomers. Therefore, whereas an electrolyte containing 10% of acetonitrile leads to an excellent resolution of the most hydrophobic alkylphenols (2,6-di-*tert.*-butyl-4-methylphenol and 2,4,6-tri-*tert.*-butylphenol), it appears to be totally inadequate for the resolution of the most hydrophilic alkylphenols, *i.e.*, the cresol isomers, as the *ortho* and *meta* isomers comigrate because of a too low interaction with the micelles. Under such conditions, addition of 5% of acetonitrile to the mobile phase appears, as shown in Fig. 2, to be a good compromise leading to a satisfactory resolution of the most hydrophobic alkylphenols whereas the resolution of the most hydrophilic alkylphenols (cresol isomers) is still acceptable.

We then attempted to improve the resolution of the most hydrophilic alkylphenols, the cresol isomers in particular, when keeping the resolution of the most hydrophobic compounds constant. To do so, we studied the influence of pH on the electrophoretic behaviour of the model alkylphenols. As the compounds studied are

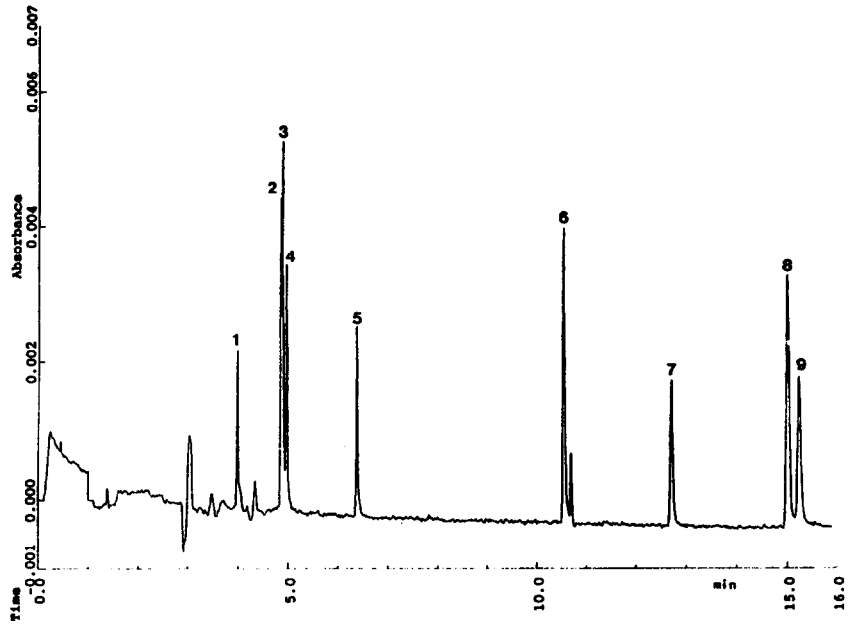

Fig. 2. Separation of the model alkylphenols by MEKC with a mobile phase containing 5% (v/v) of acetonitrile. Electrolyte and compounds as in Fig. 1.

potentially ionizable, it seems possible to adapt their polarity by modifying the pH of the electrolyte.

Influence of pH on resolution

If the pH of the electrolyte is changed from 9 to 10, *i.e.*, a value close to the pK_a of the cresol isomers (10.01, 10.17 and 10.20 for *m*-, *p*- and *o*-cresol, respectively [13]), not only will the partition coefficient between the pseudo-stationary phase and the mobile phase of these compounds be modified but also their electrophoretic mobility is altered. As the experimental mobilities of the different constituents of the analysed mixture depend not only on the electrophoretic mobility of the micelles and on the electroosmotic flow, but also on their own electrophoretic mobility as anions [8], an important change in the migration orders occurs when the pH is increased from 9 to 10. Indeed, an inversion occurs for the *m*- and *p*-cresol isomers (compare Figs. 2 and 3).

Moreover, as shown in Fig. 3, with this pH of the electrolyte and the optimized concentrations of SDS and acetonitrile, a satisfactory resolution is obtained for all of the alkylphenols with an analysis time not much longer than at pH 9.

To achieve the optimization of the separation of the model alkylphenols, we studied the influence of the ionic strength on the resolution per unit time.

Influence of ionic strength on resolution per unit time

As a decrease in ionic strength should lead to an increase in electroosmotic flow [16], such a modification of the analytical conditions should induce a shorter analysis time, the apparent migration speed increasing. This is effectively what was observed, as shown in Fig. 4.

The analysis time was decreased from 16 to 10 min when the ionic strength was halved. Unfortunately, this improvement in the analysis time leads to a decrease in resolution, irrespective of the hydrophobicity of the solutes. Therefore, a decrease in the analysis time by decreasing the ionic strength of the electrolyte does not seem realistic and the analytical conditions used previously, *i.e.*, a 25 m*M* borax buffer adjusted to pH 10 with 100 m*M* sodium hydroxide, an

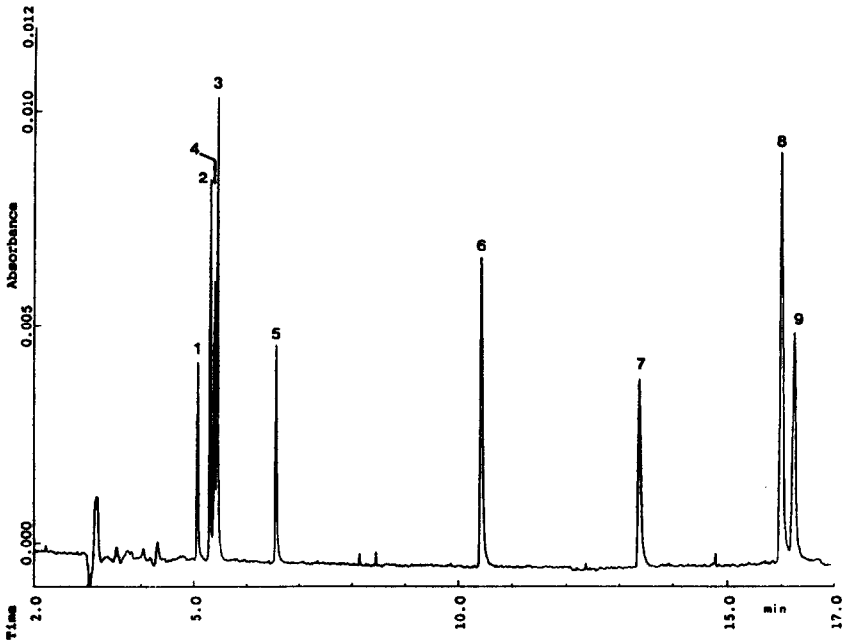

Fig. 3. Analysis of the alkylphenols at pH 10. Electrolyte, 25 m*M* borax–100 m*M* sodium hydroxide (pH 10); [SDS] = 50 m*M*; [ACN] = 5% (v/v). Compounds as in Fig. 1.

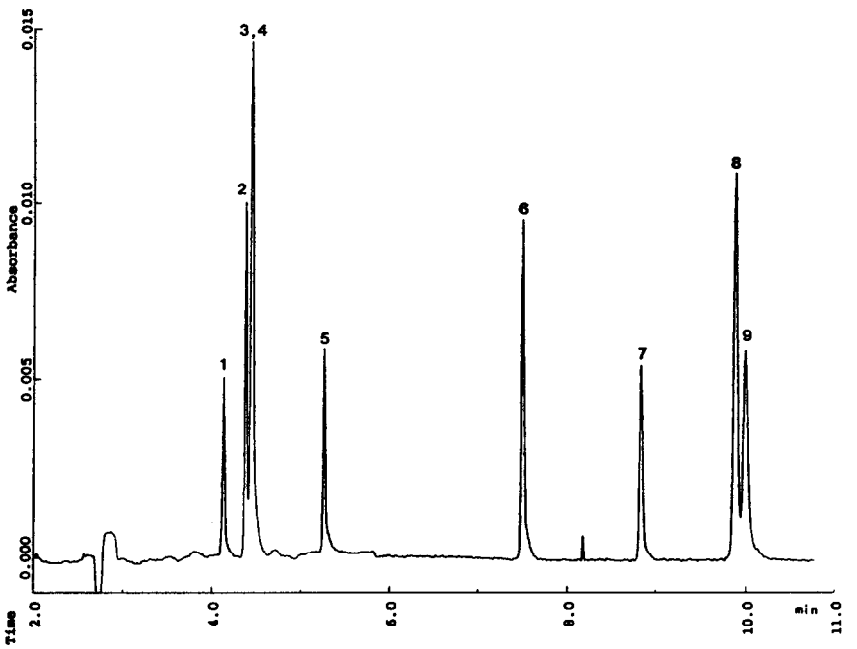

Fig. 4. Analysis of the alkylphenols at pH 10 with a lowered ionic strength. Electrolyte, 12.5 m*M* borax–50 m*M* sodium hydroxide (pH 10); [SDS] = 50 m*M*; [ACN] = 5% (v/v). Compounds as in Fig. 1.

SDS concentration of 50 m*M*, an acetonitrile content of 5% (v/v), an applied potential of 30 kV and a constant temperature of 30°C seem to be the optimum for achieving the separation of alkylphenols with a relatively broad lipophilic range in a reasonable amount of time.

As the acid fraction of the carbonization oils from biomass is not constituted of alkylphenols alone, we then tried to model more precisely these organic matrices using a more complex and more representative mixture.

3.2. *Modelling of the phenolic fraction of biomass carbonization oils*

As various classes of phenols constitute the phenolic fraction of this complex organic matrix, we enriched the model mixture. On the basis of a previous study using GC–MS [4], we selected thirteen other commercially available phenols, *i.e.*, 2-, 3- and 4-methoxyphenol, 2-methoxy-4-methylphenol, 3- and 4-hydroxybenzaldehyde, catechol, resorcinol, hydroquinone, orcinol, and 4-methylcatechol and 1- and 2-naphthol.

We therefore tried to analyse a mixture of 22 phenol derivatives known to be constituents of biomass carbonization oils. The electropherogram obtained using the conditions previously optimized on the simplified alkylphenol mixture is shown in Fig. 5.

A good resolution of the hydrophobic compounds is obtained, but not for the most hydrophilic compounds. It is important to point out that at pH 10 numerous hydrophilic compounds are partially ionized, which decreases their interactions with the micelles. In order to strengthen the interactions of these compounds and therefore to enhance the selectivity of the chromatographic system, we studied the influence of a decrease in pH on the resolution. The electropherogram obtained at pH 9 is shown in Fig. 6.

As can be seen, there is an increased resolution on decreasing the pH from 10 to 9. Indeed, under the latter conditions, the resolu-

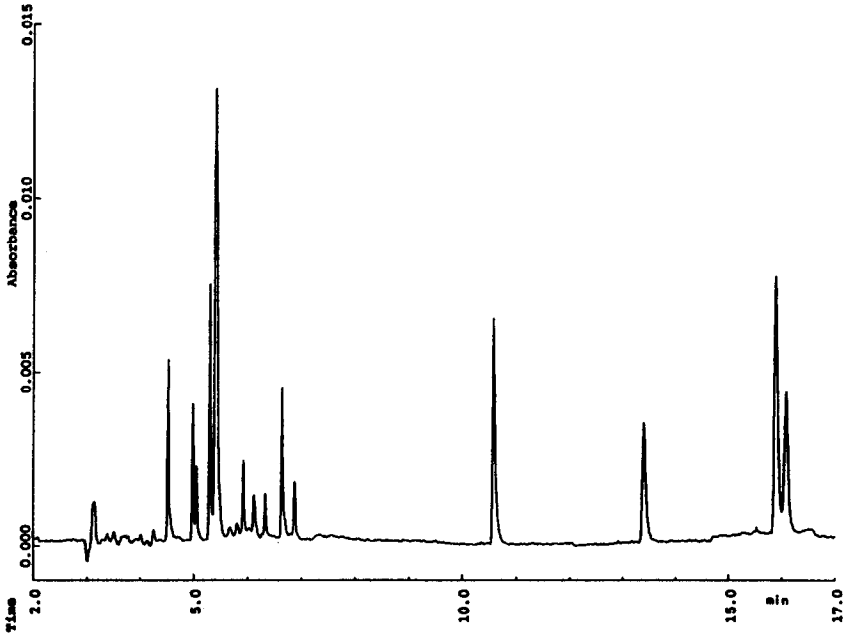

Fig. 5. Analysis of a mixture of 22 phenolic derivatives constituting the acidic fraction of wood pyrolysis oils using the optimized conditions for alkylphenols. Electrolyte as in Fig. 3.

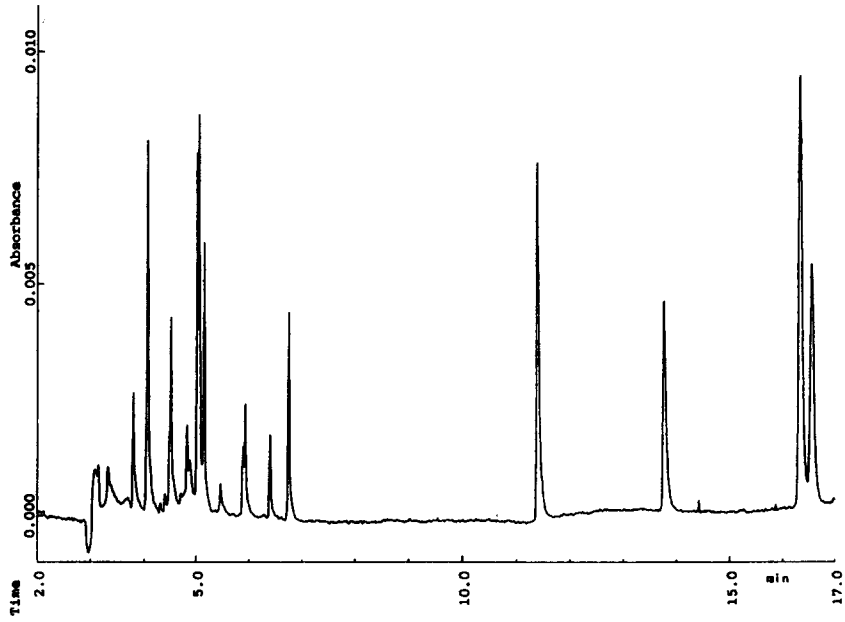

Fig. 6. Analysis at pH 9 of a mixture of 22 phenolic derivatives constituting the acidic fraction of wood pyrolysis oils. Electrolyte as in Fig. 2.

tion of the most hydrophilic compounds is clearly improved, even if not completely achieved, while the resolution of the most hydrophobic compounds remains almost constant and therefore acceptable.

In order to achieve this separation, we then undertook to reinforce the interactions between the most hydrophilic compounds and the micelles by reducing the hydrophobic characteristics of the electrolyte by decreasing the acetonitrile content in the electrolyte. The separation obtained at pH 9 and without acetonitrile is shown in Fig. 7.

As can be seen, it is possible under these conditions to achieve a baseline separation of 20 of the 22 model phenolic compounds. Only the two most hydrophobic compounds, i.e., 2,6-di-*tert.*-butyl-4-methylphenol and 2,4,6-tri-*tert.*-butylphenol, are not separated. Further, as expected, the electrophoretic behaviours of the geometric isomers of the same compound are very similar except for the dihydroxybenzene isomers. Such different behaviours probably result from the formation of a complex between the borate ion and catechol [17], which is impossible with resorcinol or hydroquinone.

4. Conclusions

MEKC has been found to be a well adapted technique for the resolution of complex mixtures of phenolic derivatives known to be constituents of biomass. In order to model most effectively the phenolic fraction of biomass during this study of the optimization of the analytical conditions, we used a mixture not only composed of phenolic derivatives with a wide hydrophobicity range but also of all the geometric isomers for one derivative family. Despite the complexity of this sample, it appears possible to resolve such a matrix with satisfactory resolution in only one run and in a very reasonable amount of time (13 min). Moreover, total resolution can be obtained by resorting to two runs, the first leading to the baseline resolution of the most hydrophilic phenolic compounds and the second allowing the

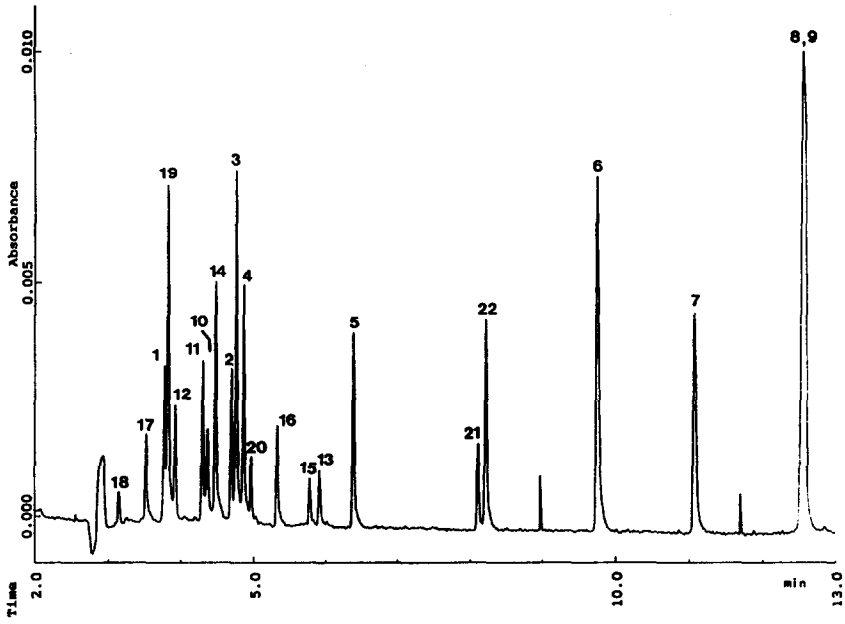

Fig. 7. Analysis of a mixture of 22 phenolic derivatives constituting the acidic fraction of wood pyrolysis oils at pH 9 and without acetonitrile. Electrolyte as in Fig. 1. Peaks: 1 = phenol; 2 = *o*-cresol; 3 = *m*-cresol; 4 = *p*-cresol; 5 = 3,4-dimethylphenol; 6 = 4-*tert*.-butylphenol; 7 = 2-*tert*.-butyl-4-methylphenol; 8 = 2,6-di-*tert*.-butyl-4-methylphenol; 9 = 2,4,6-tri-*tert*.-butylphenol; 10 = 2-methoxyphenol; 11 = 3-methoxyphenol; 12 = 4-methoxyphenol; 13 = 2-methoxy-4-methylphenol; 14 = 3-hydroxybenzaldehyde; 15 = 4-hydroxybenzaldehyde; 16 = catechol; 17 = resorcinol; 18 = hydroquinone; 19 = orcinol; 20 = 4-methylcatechol; 21 = 1-naphthol; 22 = 2-naphthol.

separation of the most hydrophobic alkylphenols with a satisfactory resolution ($R_s > 1.5$).

We envisage in the near future applying this method to the resolution of the phenolic fraction from wood carbonization oils.

5. Acknowledgement

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