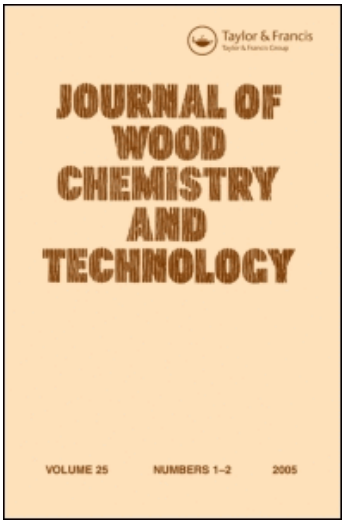

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Olof Dahlman; Karin Månsson

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ANALYSIS OF LOW MOLECULAR WEIGHT LIGNIN-DERIVED SULPHONATES BY CAPILLARY ZONE ELECTROPHORESIS

Olof Dahlman and Karin Månsson
Swedish Pulp and Paper Research Institute, STFI
Box 5604, S-11486 Stockholm, Sweden

ABSTRACT

A fast and simple analytical procedure, based on capillary zone electrophoresis (CZE), for separation of underivatized low molecular weight lignin-derived sulphonates has been developed. Optimal CZE-operation conditions for the separation and detection of several lignin-derived sulphonic acids was found when using a low pH (pH 1.7) phosphate buffer system, an applied voltage of 20 kV and UV-detection at the anode-side. At these conditions the electroosmotic flow is negligible and the analytes are transported through the capillary column by electromigration. The sulphonation and subsequent degradation of the lignin-model compound 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol has been studied by CZE-analysis of aliquots taken from a sulphite reaction mixture. No sample pretreatment was needed before analyzing the sulphite reaction mixture with CZE. The method has also been found to be useful for detection of sulphonic acids in samples from sulphite cooking. Crude extracts of a sulphite cooking liquor could be directly analysed by using the technique developed. Based on migration time data and comparison with an authentic sample, 1-(4-hydroxy-3-methoxyphenyl)-prop-2-ene-1-sulphonate was identified as one of the sulphonic acids present in the sulphite cooking liquor.

INTRODUCTION

During sulphite pulping and chemi-thermomechanical pulping (CTMP), sulphonic acid groups are introduced in the lignin by replacement of hydroxyl or ether functions at the α-carbon of the phenyl propane unit[1,2]. Depending on the

pulping conditions used, the lignin is sulphonated to various degrees and subsequently partially degraded due to sulfitolytic cleavage of β-aryl ether bonds. These reactions give rise to water-soluble lignosulphonates with molecular size ranging from small monomeric compounds up to high molecular weight polydisperse materials. Several studies have reported the characterization of water-soluble lignosulphonates in process liquors derived from sulphite pulping operations[3-9]. The analytical techniques commonly used for separation of low molecular weight lignin-derived sulphonates are based on liquid chromatography[4] or high performance liquid chromatography[6-9] after pre-column derivatization (acetylation and/or methylation) of the sulphonic acids.

Recently, capillary electrophoresis has been reported to be a highly useful technique for analytical separation of underivatized alkyl aromatic sulphonates[10], aromatic sulphonates[11] and aromatic sulphonic acid dyes[12]. In comparison to high performance liquid chromatography, capillary electrophoresis generally offers superior separation efficiency and considerably faster analytical run times. Thus, we decided to evaluate the potential of using capillary electrophoresis for characterization of sulphonic acids in process liquors derived from sulphite pulping and from CTMP manufacturing. The aim of the present study was to develop a fast and simple analytical procedure, based on capillary zone electrophoresis (CZE), for separation of underivatized low molecular weight lignin-derived sulphonates. Taking in account the structure and strongly acidic properties of the compounds to be analysed, we proceeded to develop optimal CZE-operating conditions on suitable reference compounds available at our laboratory. The developed analytical procedure was then applied on samples originating from different sulphite pulping operations, including samples from laboratory model reactions as well as pulp mill samples.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical-reagent grade. Dichloromethane and n-butanol used for extractions were obtained from Merck, Darmstadt, Germany. The p-toluene sulphonic acid (**1**) used as internal standard was obtained from Eastman Kodak, Rochester, USA. Dicyclohexylamine used in the ion-pair extraction experiment was obtained from Merck, Darmstadt, Germany. The ion-exchange

resin, Amberlite 120 (H+) was obtained from BDH Chemicals, Poole, UK. For preparation of reagent solutions and buffer solutions, pure water (Millipore Milli-Q Plus) was used. The running electrolyte buffers were prepared by dissolving 85 % orthophosphoric acid (Merck) in water followed by addition of the appropriate amount of 1 M sodium hydroxide (Merck). Standard solutions containing sulphonates **1** - **8** and mixtures thereof were prepared in water.

Instrumentation

The CZE analyses were performed using a Dionex Capillary Electrophoresis system equipped with a 60 cm fused silica capillary column (i. d. 50 μm). The UV detection was performed at 214 nm with the detector placed at the anode-side 5 cm from the end of the capillary. Injections were performed in the hydrodynamic mode (gravity injection). The sample vial was elevated by 75 mm and the injection time was set at 10 s. The voltage applied was 20 kV.

Samples

The sulphonates **3** [4-hydroxy-3-methoxyphenyl-methane-sulphonate], **6** [3-(2-hydroxy-phenyl)-1-(4-hydroxy-3-methoxyphenyl)-3-oxo-propane-1-sulphonate], and **8** [1-(3,4-dimethoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-3-oxo-propane-1-sulphonate], shown in FIGURE 1, were references sample used at our laboratory. The lignin model compound 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol was also a reference compound at our laboratory.

The sulphonates **2** [1-(4-hydroxy-3-methoxyphenyl)-prop-1-ene-1-sulphonate], **4** [1-(4-hydroxy-3-methoxyphenyl)-prop-2-ene-1-sulphonate] and **5** [1-(4-hydroxy-3-methoxyphenyl)-prop-1-ene-3-sulphonate] were generous gifts from Prof. G. Gellerstedt at The Royal Institute of Technology in Stockholm.

Compound **7** [1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-3-hydroxy-propane-1-sulphonate] was obtained by heating an aqueous solution (1 ml) of 5 mg of 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol and 60 mg of sodium sulphite at 110 °C for 1 hr. The reaction product mixture was used without purification.

The sulphite cooking liquor used in this study was sampled at the acid delignifying stage in a mill using a two-stage sodium sulphite cooking process. The mill used fresh spruce-wood as raw material.

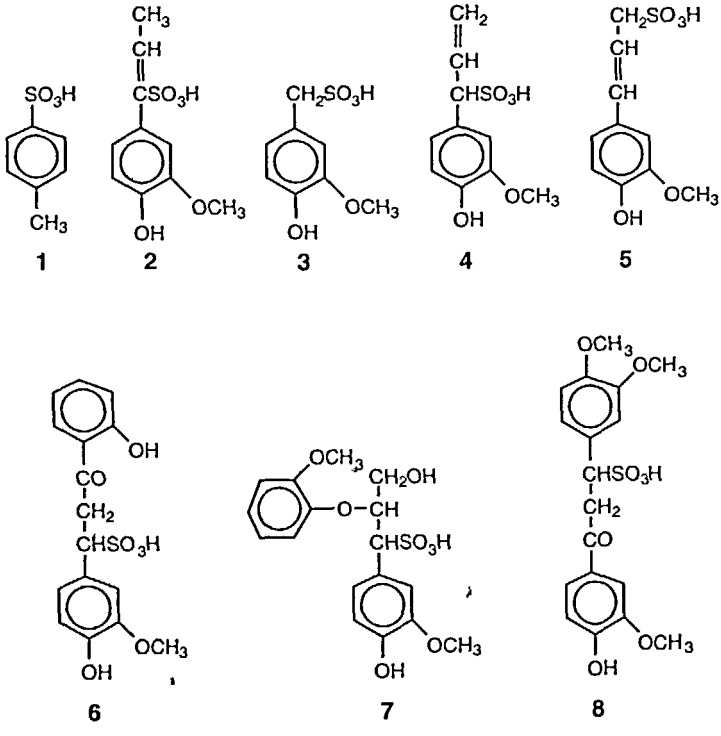

FIGURE 1. Chemical structures of compounds **1-8**.

The CTMP-mill effluent tested was sampled at a mill producing bleached (hydrogen peroxide) spruce-wood CTMP. For impregnation of the wood-chips, the mill used about 35 kg sulphite per ton wood.

Isolation of Low Molecular Weight Lignin-derived Sulphonates in the Sulphite Cooking Liquor

After adjustment of the pH to 3 with 1 M sodium hydroxide, the cooking liquor sample (100 ml) was washed with 2x100 ml dichloromethane in order to remove wood-extractives and thereafter extracted with 5x75 ml n-butanol. The combined n-butanol phases were evaporated to dryness. The residue was dissolved

in 10 ml water and analysed without further purification (the monosulphonic acid fraction). The n-butanol-extracted cooking liquor was acidified to pH 2 by adding ion exchange resin, Amberlite 120 (H^+). The ion exchange resin was filtered off and washed with water (about 50 ml). The washings were combined with the acidified cooking liquor and the resulting solution was extracted with 110 ml of a 5:1 mixture of dichloromethane and n-butanol containing 10 ml dicyclohexylamine. This procedure was repeated twice. The resulting organic phases were combined and thereafter extracted with 4x75 ml alkaline water (pH 11, sodium hydroxide). The alkaline water-phases were combined and the pH was adjusted to 7 by adding ion exchange resin, Amberlite 120 (H^+). The neutralized water-phase was washed with 50 ml dichloromethane followed by 50 ml methyl-*tert.*-butyleter (MTBE) and then evaporated to dryness. The residue was dissolved in 10 ml water and analysed without further purification (the di- and trisulphonic acid fraction).

Isolation of Low Molecular Weight Lignin-derived Sulphonates in the CTMP-mill Effluent

The CTMP-mill effluent studied was subjected to the same extraction procedure as the sulphite cooking liquor. In addition, an aliquot of the effluent sample (dichloromethane-washed) was freeze-dried and thereafter leached with methanol. The resulting methanol leachate was diluted with acetone in order to precipitate the inorganic material. The precipitate was filtered off and the clear methanol solution was evaporated to dryness. The residue was dissolved in 1.5 ml water and analysed without further purification.

Degradation of Sulphonated 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol (Compound 7)

An aqueous solution (1 ml) of 5 mg of 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol and 60 mg of sodium sulphite was heated at 110 °C for 1 hr to give compound **7**. The temperature was then raised to 150 °C and the heating was continued for 1.5 h. An aliquot was removed for analysis and the heating was continued at 180 °C for 3 h. The resulting solution was cooled to room temperature and analysed without purification. The final pH of the solution was about 9.

RESULTS AND DISCUSSION

Capillary Zone Electrophoretic (CZE) Separation of Sulphonates 1-8

In the initial part of the study, suitable conditions for separation of the sulphonates **1-8** were developed. The CZE-conditions were chosen so that low molecular weight sulphonates could be analysed selectively in samples containing considerable amounts of other types of organic compounds, e.g. aromatic carboxylic acids, from the pulping of wood.

When separating some alkyl-aromatic sulphonates by CZE, Desbéne et al[10] used a borate-boric acid buffer system at pH 9 containing up to 30 % of acetonitrile and a voltage of 30 kV. Similarly, Brumely[11] and Brumely and Brownrigg[13] analysed aromatic organic acids with CZE using a borate-boric acid buffer system at pH 8.3 and an applied voltage of 30 kV with detection at the cathode-side. Under such conditions both aromatic carboxylic acids and aromatic sulphonates migrate through the fused silica capillary to the detector[11,13]. To improve the selectivity of the CZE-analysis, we investigated the possibility of using low pH (1.5-2.0) buffer systems in which aromatic carboxylic acids are protonated and strongly acidic sulphonates are dissociated. When using a low pH buffer system the electroosmotic flow through the capillary column is very low (close to zero) since the silanol groups at the surface of the fused silica capillary become protonated at low (less than 2) pH[14]. By applying a voltage of about 20 kV and UV-detection at the anode-side, only negatively charged analytes in the low pH buffer system (e.g. sulphonates) migrate through the capillary column to the detector, whereas protonated aromatic carboxylic acids behave as neutral compounds with no electrophoretic migration. For example, p-toluene sulphonic acid (**1**) has a very low pKa-value (pKa about 1) compared to that of benzoic acid (pKa 4,2). Hence, by choosing a suitable phosphate buffer system with a pH of around 1.5, conditions were found where p-toluene sulphonic acid (**1**) migrate through the capillary column with a migration time of about 10 minutes, whereas benzoic acid exhibited no observable electrophoretic migration. This means that by using such CZE-operating conditions we can, more or less selectively, detect sulphonates in pulping liquor samples which also contain neutral compounds and aromatic carboxylic acids. However, it should be pointed out that some dibasic carboxylic acids such as oxalic acid and maleic acid will also migrate through the capillary and thus be detected under these conditions.

In FIGURE 2, electropherograms are shown corresponding to the CZE-analysis of a mixture of the sulphonates **1-8** using three phosphate buffer systems with slightly different pH and buffer concentration. As can be seen in FIGURE 2, only minor variations in migration times for the investigated sulphonates are observed between the electropherograms. In all three cases, the sulphonates **1-8** were well separated (base-line separation) within an analysis time of about 20 minutes or less. The order of CZE-detection of these sulphonates in the investigated phosphate buffer systems were determined by spiking the mixture with each of the compounds **2-8** respectively. According to FIGURE 2, the small molecular size sulphonate, p-toluene sulphonic acid (**1**), was detected first whereas the comparatively larger size sulphonates (**6-8**) were detected at the end of the electropherograms. In the 150 mM phosphoric acid buffer system (electropherogram A in FIGURE 2) the investigated sulphonates exhibited migration times from 10.1 to 17.9 minutes. When using a less acidic buffer system (pH 2) containing 60 mM sodium hydroxide and 150 mM phosphoric acid (electropherogram C in FIGURE 2), the migration times increased for all components in the sulphonate mixture. The pH 1.7 buffer system containing 150 mM phosphoric acid and 30 mM sodium hydroxide (electropherogram B in FIGURE 2) appeared to give the best separation of sulphonates **1-8** and a short analysis time (migration times from 9.5 to 18.0 minutes). This buffer system was therefore used in succeeding parts of this study.

CZE-analysis of Samples from Experiments with the Lignin Model Compound

The electropherogram in FIGURE 3 shows the CZE-analysis of the sulphonated products obtained by treatment of the lignin model compound 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol with aqueous sodium sulphite at 110 ^{o}C for 1 hr. As can be seen in FIGURE 3, the electropherogram exhibits a major peak at a migration time of about 17.5 minutes followed by a minor component at about 18 minutes. According to Gellerstedt[3,15,16], 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol is sulphonated under acidic or neutral conditions at the benzylic carbon to give a mixture of the *erythro* and *threo* forms of the α-sulphonic acid **7**. Independent of the diastereoisomeric form of the starting compound, treatment with weakly acidic sulphite solution (pH 4) was reported[15] to give a 2:1 mixture of the *erythro* and *threo* forms. Thus, the two peaks

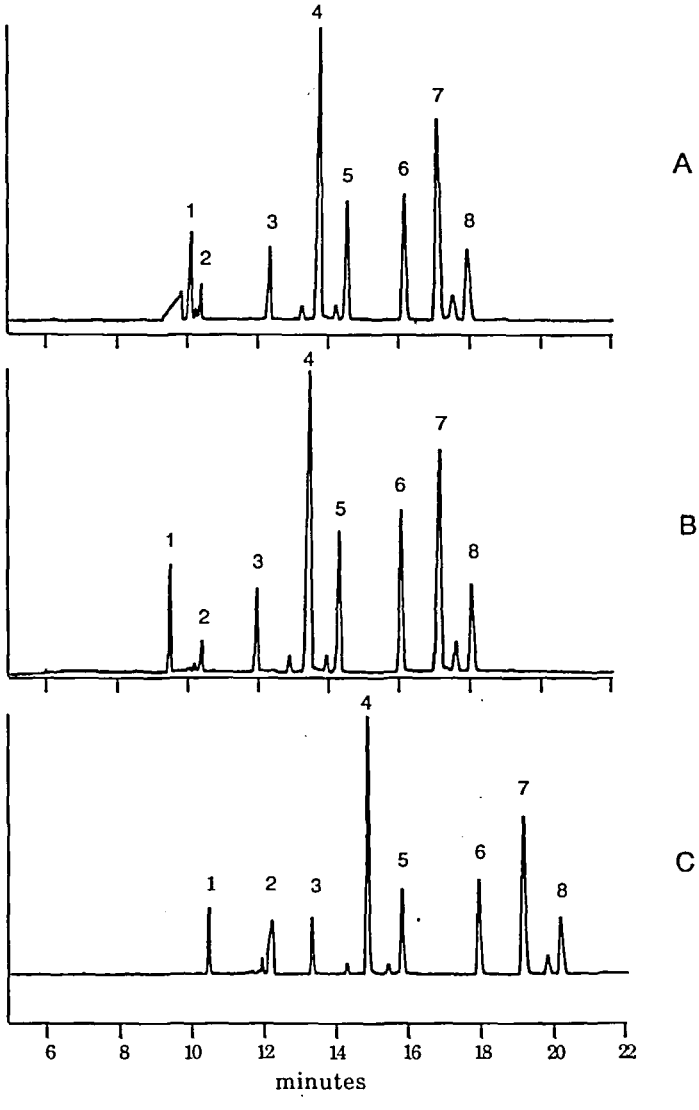

FIGURE 2. Electropherograms corresponding to the CZE-analysis of a mixture of the sulphonates **1-8** using three phosphate buffer systems with slightly different pH and buffer concentration: (A) 150 mM phosphoric acid buffer system (pH 1.5), (B) 150 mM phosphoric acid and 30 mM sodium hydroxide (pH 1.7), and (C) 150 mM phosphoric acid and 60 mM sodium hydroxide (pH 2).

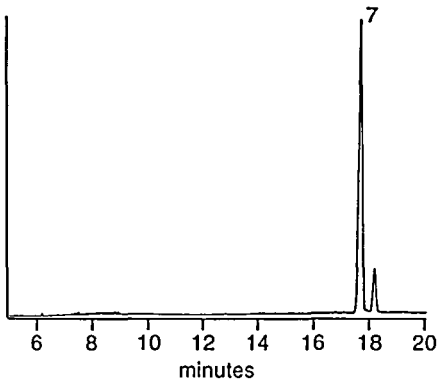

FIGURE 3. Electropherogram from the CZE-analysis of sulphonated products obtained by treatment of 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol with aqueous sodium sulphite at 110 °C for 1 hr. The running buffer was 150 mM phosphoric acid and 30 mM sodium hydroxide at pH 1.7.

appearing in the electropherogram in FIGURE 3 were tentatively assigned to the two diastereoisomeric forms of the α-sulphonic acid **7**.

CZE-analysis of the sample taken after treatment of the α-sulphonic acid **7** at 150 °C for 1.5 hr, gave an electropherogram identical with that in FIGURE 3. Thus, in accordance with earlier results[15], no further sulphonation reaction or decomposition of the α-sulphonic acid **7** occurs under these conditions. In contrast, the CZE-analysis of the sample obtained after treatment at 180 °C for 3 hr (FIGURE 4) indicated an extensive degradation of the α-sulphonic acid **7**, leading to a number of sulphonated products. In the electropherogram in FIGURE 4, strong peaks appear in the two migration zones 5-8 and 11-15 minutes. In the migration zone 11-15 minutes, the α-sulphonic acid **3** was identified (the peak with a migration time of about 12 minutes) on basis of migration time and by spiking the sample with the authentic reference compound. In addition, three components of unknown structure were detected in this migration zone (see FIGURE 4). The α-sulphonic acid **3** and several structurally similar monosulphonic acids have previously[15] been identified as the major products of neutral or alkaline sulphite treatment of 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol at

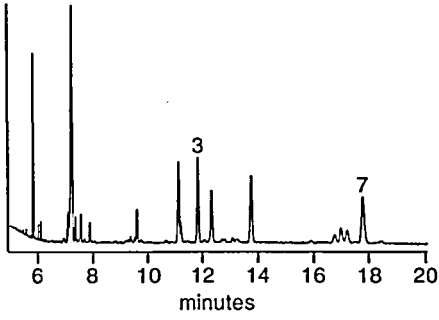

FIGURE 4. Electropherogram from the CZE-analysis of sulphonated products obtained by treatment of 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol with aqueous sodium sulphite at 180 °C for 3 hr. The running buffer was 150 mM phosphoric acid and 30 mM sodium hydroxide at pH 1.7.

180 °C. Thus, the unidentified peaks appearing at the migration zone 11-15 minutes in the electropherogram in FIGURE 4, probably belong to monosulphonic acids with structure similar to the α–sulphonic acid **3**.

In addition to monosulphonic acids, Gellerstedt and Gierer[15] also identified some di and trisulphonic acids as products originating from alkaline sulfitolytic cleavage of the β–guaiacyl ether bond of the α-sulphonic acid **7**. Such di and trisulphonic acids have a higher charge-density and thus migrate much faster than the corresponding monosulphonic acids (e.g. compound **3**) in the buffer system used. Therefore, it is reasonable to assume that the peaks in the first migration zone (5-8 minutes) in the electropherogram in FIGURE 4 belong to di or trisulphonic acids originating from the α-sulphonic acid **7**.

CZE-analysis of Sulphite Cooking Liquor Samples

The extensive extraction and complexation/decomplexation procedure[6,8] used in work-up of the sulphite cooking liquor divides the lignin-derived sulphonates into two fractions. A less polar fraction (the butanol extract) containing mainly low molecular weight monosulphonic acids, and a more polar fraction (the dicyclohexylamine complexation/ decomplexation extract) containing low molecular weight

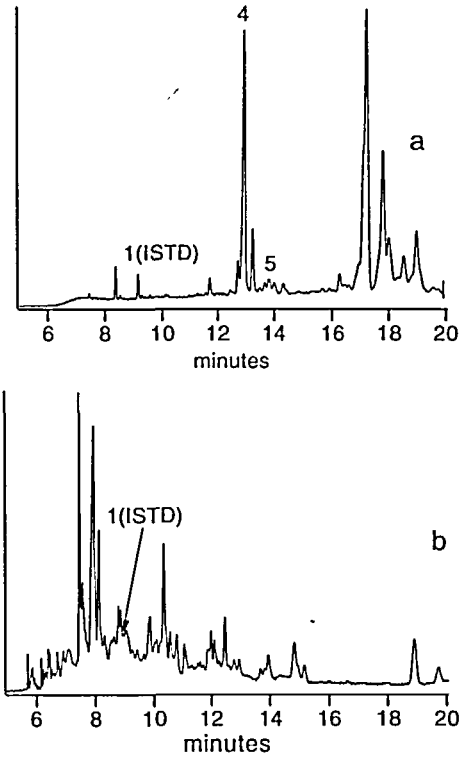

FIGURE 5. Electropherograms from the CZE-analysis of the sulphite cooking liquor, (a) the butanol-extract and (b) the dicyclohexylamine complexation/ decomplexation extract. The running buffer was 150 mM phosphoric acid and 30 mM sodium hydroxide at pH 1.7.

di and trisulphonic acids. High molecular weight lignosulphonates do not complex with dicyclohexylamine under the conditions employed[6] and thus remain in the sulphite cooking liquor after the work-up procedure.

In FIGURE 5, electropherograms are shown corresponding to the CZE-analysis of the butanol-extract (a) and the dicyclohexylamine complexation/ decomplexation extract (b). The electropherogram of the butanol-extract exhibits peaks within the migration zone 12-20 minutes. By spiking the sample with the reference compounds listed in FIGURE 1, two peaks in the electropherogram were

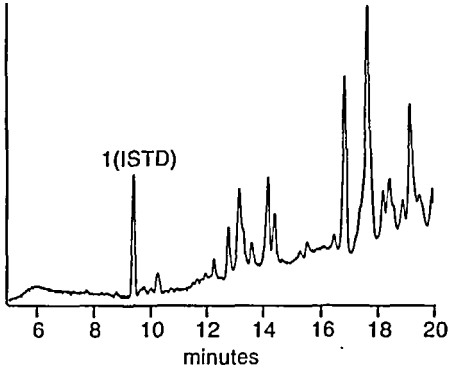

FIGURE 6. Electropherogram of the methanol-leachate of the freeze-dried CTMP-effluent sample. The running buffer was 150 mM phosphoric acid and 30 mM sodium hydroxide at pH 1.7.

found to have migration times identical to those of compounds **4** and **5**. The monosulphonic acids **4** and **5** have previously been found in liquors from sulphite pulping operations[6,7,17,18]. These compounds, which can be obtained by sodium sulphite treatment of coniferyl benzoate[18] or coniferyl alcohol[9], are believed to be formed from coniferyl alcohol groups in the lignin during the initial stage of the sulphite deligninfication[7].

As expected, the electropherogram of the dicyclohexylamine complexation/ decomplexation extract (electropherogram b in FIGURE 5) exhibits strong peaks within a fast migrating zone where di and trisulphonic acids are expected to be found.

CZE-analysis of CTMP-mill Effluent Samples

The electropherogram corresponding to the CZE-analysis of the butanol-extract of CTMP mill effluent exhibited some small peaks within the migration zone between 15-20 minutes (not shown) whereas the electropherogram of the dicyclohexylamine complexation/decomplexation extract showed a number of small peaks originating from components with much faster migration times (not shown). However, we where not able to safely identify the peaks in these two electropherograms with

those of the reference compounds listed in FIGURE 1. The CTMP-effluent probably contained too much organic material to enable a proper isolation, by the extraction procedure used, of the small amounts of lignin-derived sulphonic acids present. Instead, a freeze-dried sample of the CTMP-effluent was leached with methanol and the leachate was analysed with CZE. The electropherogram obtained of the methanol-leachate is shown in FIGURE 6. In this electropherogram, several small peaks appeared within the migration zone between 10-15 minutes together with strong peaks appearing at longer migration times. The peaks were, however, rather broad (probably due to a high content of inorganic material in the sample) which makes a identification of the peaks difficult. Hence, were not able to safely match any of the peaks in the electropherogram with the reference compounds investigated. Monosulphonic acids, e.g. compound **2**, **4** and **5**, have previously been found in spent liquor from mild sulphite and hydrolysis treatment of spruce wood under conditions simulating chemical pretreatments in high yield pulping [9].

CONCLUSIONS

Capillary zone electrophoresis using fused silica capillary with on-column UV-detection and a phosphate buffer system with pH of 1.7 has been found to be a useful technique for analysing low molecular weight lignin-derived sulphonates. Within a quite short analysis time (about 20 minutes) a number of lignin-derived sulphonic acids were separated with base-line separation. In contrast to liquid chromatography commonly used, it is possible to analyse these types of compounds by capillary zone electrophoresis without any prior derivatization (methylation or acetylation).

The sulphonation and subsequent degradation of the lignin-model compound 1-(4-hydroxy-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-glycerol into a number of mono, di or trisulphonic acids could be monitored simply by analyzing aliquots of the sulphite reaction mixtures with capillary zone electrophoresis.

The usefulness and limitations of the developed capillary zone electrophoresis technique when analysing samples from sulphite cooking and CTMP-liquors has been demonstrated. Crude butanol-extracts and dicyclohexylamine complexation/ decomplexation extracts of sulphite cooking liquors can be directly analysed using the developed technique.

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