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Liquid chromatography–electrochemistry procedure for the determination of chlorophenolic compounds in pulp mill effluents and receiving waters

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ABSTRACT

An isocratic, reversed-phase liquid chromatography procedure is described for the separation and determination of chlorophenolic compounds, including several priority pollutant chlorophenols. The separation was experimentally optimised, and selective and sensitive detection was obtained by dual electrode amperometry. The most suitable combination was a silica-based column with phenyl functional groups, and a mobile phase of acetonitrile–acetate buffer (40:60, pH 5). Dual glassy-carbon electrodes were used in a parallel configuration in a thin-layer flow cell, with differential current measurement (+0.90 V relative to +0.60 V).

A solid-phase extraction scheme using a polymeric sorbent (Chromosorb 102) was devised for the extraction and preconcentration of selected chlorophenols, chloroguaiacols, and a chlorosyringol from aqueous samples. Use of tribromophenol as an internal standard was also investigated. The total procedure —preconcentration and chromatography— was applied to the analysis of effluents discharged by a paper mill, and a pulp mill.

INTRODUCTION

The industrial discharge of chlorinated organic compounds into natural waters, and the environment in general, has caused concern now for several decades [1,2]. It should be noted that not all of these organochlorines are necessarily harmful [3]; indeed such compounds are also produced by natural processes [4]. Therefore, it is essential to develop sensitive analytical methods capable of detecting individual organochlorines in order to characterise toxicity or other harmful effects.

The pulp and paper industry, through the chlorine bleaching of pulp, are major producers of organochlorines. Some of the compounds derived from the chlorination of the organic components of wood are both toxic and persistent [5–7]. One such group of compounds are the chlorophenolics, which are derived from the action of chlorine, and its hydrolysed derivatives, on the phenolic units that arise from the breakdown of lignin in the pulping process. World-wide, there is a growing requirement to monitor organochlorines, including chlorophenolics, in receiving waters as well as effluents [8,9]. The US Environmental Protection Agency (USEPA) lists eleven phenols as priority pollutants —among these are the chlorinated compounds 2-chlorophenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol and pentachlorophenol [10]. In Australia, recent commonwealth guide-lines for bleached kraft pulp mills [11,12] state that total levels of chlorophenolics must be measured in effluent, water, sediments and biota, with the additional qualification that individual chlorinated phenols found to be important may be included in revised monitoring schedules.

Analysis of aqueous samples for phenolics has traditionally involved methods that provide a mea-

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sure of the total phenol concentration (phenol index). Spectrophotometric procedures with 4-aminoantipyrine [13] are examples of such procedures. More recently analytical methods have been evolved that can determine individual phenolic compounds. The gas chromatography procedure of Voss *et al.* [14], with its preliminary formation and extraction of the acetate derivatives, is a commonly used procedure for the determination of chlorinated phenolic compounds in pulp mill effluents. High-performance liquid chromatography (HPLC) is an alternative and complementary procedure for the measurement of chlorophenolics. Early work with this technique used spectrophotometric [15,16] and fluorimetric detectors [17,18], in most instances derivatisation steps were necessary. The combination of HPLC with electrochemical detection (ED) has proved to be well-suited for determining phenolic compounds, because the sensitivity and selectivity of the detector permit the use of a simple isocratic mobile phase [19–22] with minimal pretreatment and without the requirement of derivative formation.

In this paper we present the results of an optimisation of an LC–ED procedure to determine chlorophenolics (especially those with multiple chlorine substitution) in both pulp and paper mill effluents and natural receiving waters. Also considered are means of pretreatment and preconcentration of chlorophenolics from natural waters. Chromatograms are presented for total effluents from representative pulp and paper mills as an application of the recommended procedure.

EXPERIMENTAL

Instrumentation

The components of the LC–ED system were a Waters M45C pump, 0.2 μm in-line filter, a Rheodyne 7125 injector (with 200-μl loop and position-sensing switch), and an EG&G Princeton Applied Research Model 400 electrochemical detector. To reduce pulsation at the detector, a Waters high-pressure noise filter was installed between the pump and injector. The analytical column was a Waters Nova-Pak Phenyl cartridge (8NVPH4, 100 mm × 8 mm) mounted in a Waters RCM-100 Compression Module. A Brownlee PRP-1 guard cartridge (PRP-GU, 30 mm × 4.6 mm), in a Brownlee 30 mm MPLC cartridge holder, protected the main reversed-phase column. All tubing in the LC–ED system was conventional 316 grade stainless steel. Chromatograms were displayed on a Yew Type 3056 dual-pen recorder; the detector output was also fed, via an analog-to-digital interface, to an IBM-style personal computer for processing with integration and data analysis software (DAPA Scientific).

The thin-layer flow cell used in the electrochemical detector was that supplied by the manufacturer, as were the reference (Ag/AgCl) and auxiliary electrodes. However, we used a BAS MF-1000 dual glassy carbon electrode (3-mm diameter disks) as the working electrode. The channel in the flow-cell gasket was enlarged to a width of 7.5 mm to accommodate the electrodes mounted in the parallel configuration.

We used a syringe apparatus similar to that described by Renberg and Lindström[23] for preconcentration of phenolic compounds from aqueous samples. Solid-phase sorbents, not supplied in cartridges, were dry-packed into empty polyethylene cartridges (Dionex OnGuard) that contained filter inserts.

Reagents

All reagents salts were of analytical grade or better. We used 2,4,6-tribromophenol as supplied (99% pure; Aldrich). Standards for the chlorinated phenolics (Chem Service or Helix Biotech.) were dissolved in acetonitrile, with working standard solutions typically at a concentration of 10 μg/ml. Aqueous standards for preconcentration studies were prepared by diluting saturated aqueous (stock) solutions of the individual phenolic compounds with high purity water. The concentration of each aqueous stock solution (10–40 μg/ml) was determined by comparison with the detector response for standards dissolved in eluent. Whereupon working solutions of the aqueous standards (typically 2 μg/ml) were prepared by dilution of the stock solutions, and were refrigerated in borosilicate-glass, screw-cap vials with PTFE cap-liners. Acetonitrile in the eluents was of liquid chromatography grade. High-purity water was prepared by distillation, and then by passing the distillate through a three-cartridge Milli-Q water purification system (Millipore). All eluents were filtered under vacuum through an 0.45-μm Millipore HV Durapore membrane in an all-glass apparatus, and just prior to use a litre of eluent was degassed by ultra-sonicating under vacuum.

The recommended eluent was prepared in the following manner. 10 ml of a stock acetic acid–sodium acetate buffer solution (2.0 *M* sodium acetate adjusted to pH 4.15 with glacial acetic acid) was passed through a cartridge packed with XAD-4 resin (75–125 μm). The first 3 ml were discarded; the next 6 ml were added to pure water, and the volume made up to 600 ml. To this solution, acetonitrile was added to bring the final volume to 1 l (pH is 5.0).

Procedures

Eluents were pumped through the LC–ED system at 1.5 ml/min. Apart from dilute standard solutions prepared in the eluent that were injected directly onto the column, samples were pre-treated as described below before chromatographic analysis. In general, we attempted to ensure that the bulk composition of the injected analyte solutions was as similar as possible to the eluent. Chromatography was performed at 20 ± 2°C.

Preliminary surface treatment of the electrodes required only polishing with alumina paste (0.05 μm), and then washing with pure water. The electrochemical detector was operated in the dual potentiostat mode, with the lower potential (E_1) at + 0.60 V *vs.* Ag/AgCl, and the upper (E_2) at + 0.90 V *vs.* Ag/AgCl. Current sensitivity was set between 2 and 20 nA, and the filter time constant was 2 s.

Pretreatment of samples involved acidification to pH 2.8 with sulphuric acid. With pulp and paper mill effluents it was advantageous to centrifuge the sample (1500 g for 15 min) to deposit the flocculate of fine particles resulting from the initial acid treatment. The samples were cleaned up and preconcentrated by extraction onto solid-phase sorbents. Sorbents were activated by passing acetonitrile (5 ml) through the cartridge, and then washing with pure water (10 ml). The sample volume of 5 to 500 ml was injected through the sorbent cartridge by gas-tight glass syringe; flow-rates were between 1 and 2 ml/min. For volumes greater than 10 ml, we used an apparatus similar to Renberg and Lindström [23] for refilling the syringe, and collected the effluent in a measuring cylinder to monitor throughput. After loading the sample, the sorbent was rinsed with 5 ml of pure water, and dried by passing through 250 ml of air. The chlorophenolics were then eluted with 2.0 ml of acetonitrile. Before chromatography, the eluate from the sorbent was made up to 5.0 ml with 0.02 *M* acetate buffer (pH 4.15).

Effluent samples were collected by immersing a sample bottle in the mill's effluent discharge channel, and filling to the bottle's lip to avoid any air space above the liquid. The sample (and storage) bottle were either glass or high-density polyethylene with screw caps. These containers were typically pre-cleaned with methanol, and rinsed with pure water. To preserve effluent samples they were simply refrigerated at 4°C, or refrigerated after addition of anhydrous copper sulphate (3.25 g per 4 l). The copper salt serves as a bio-toxin to prevent biological transformation of effluent constituents [21], and it could also remove interference from sulphide in the LC–ED procedure.

RESULTS AND DISCUSSION

Initial experiments

Chromatography of the chlorophenol compounds and their derivatives may be effected by using their general phenolic character, and the subtle differences in chemical properties that result from changes in substituents. Another characteristic that is relevant to the chromatography of these compounds is the increase in acidity that is linked with increasing degree of chlorination (*cf.* phenol, $\text{p}K_a$ = 10.1; pentachlorophenol, $\text{p}K_a$ = 4.9) [24]. The former aspects of chlorphenolics favour the use of reversed-phase chromatography, whereas the varying acidity of these compounds suggest that ion-exclusion chromatography might be possible (by analogy with the separation of other weak organic acids).

We confirmed the work of Jupille *et al.* [19] [using a Brownlee Polypore-H column (250 mm × 4.6 mm), and phosphoric acid–acetonitrile eluents] that ion exclusion chromatography with amperometric detection could separate chlorinated phenolic compounds dissolved in the eluent. The order of elution was not in the order expected if the separation of the chlorophenolics was by an ion exclusion mechanism, but rather it was akin to a low-efficiency reversed-phase process. To test the procedure with a real sample matrix, pulp mill effluent samples were spiked with chlorophenolics, pretreated as above, and then injected directly onto the column. The result in each case was a total loss of chromatographic resolution; the chlorophenolics could not be detected. We suspected that the many other weak organic acids (such as resin acids and fatty acids) present in the effluents interfered strongly in the chromatography step. Therefore, a more practicable option was to improve the reversed-phase chromatographic method for chlorophenolics determination, and tailor it for analysis of pulp mill effluents and receiving waters.

In applying published LC–ED methods based on reversed-phase chromatography [20–22], the approach appeared quite feasible, but we identified a number of drawbacks for our intended application. These were: (i) a large background current at the detector, (ii) excessive baseline noise, (iii) lengthy retention of pentachlorophenol, and (iv) inadequate resolution of early eluting chlorophenols when effluent samples were analysed. It was anticipated that the first problem may contribute to the second.

Optimisation

We adopted an experimental approach to improve the performance of the reversed-phase LC–ED procedure for chlorophenolics determination. The presence of contaminants in reagents used for preparing the mobile phase was checked, sequentially, by solid-phase extraction of 100-ml volumes onto XAD-4 resin. The procedure was essentially that described above for the purification of eluents, with adsorbed contaminants being stripped from the resin with 1.5 ml of acetonitrile. Phenol, itself, proved to be a abiquitous trace impurity in all reagents. Clean-up of the mobile phase reagents by solid-phase extraction proved to be effective in both decreasing the magnitude of the background current at the detector, and suppressing baseline noise.

The composition of the eluent reported in published papers on LC–ED of chlorophenolics [20–22] seemed unnecessarily complex and of high ionic strength. Modern electrochemical detectors perform well with minimal supporting electrolyte. Therefore, we dispensed with the often used sodium perchlorate. A mixed pH buffer of acetic acid and citrate, was replaced with a simple sodium acetate–acetic acid buffer solution. The citrate may have been added previously to combat the formation of iron(III)–phenolate complexes. In analyses of natural waters and pulping effluents, iron(III) concentrations are unlikely to reach levels that would cause interference.

A Nova-Pak C_{18} column was used in initial experiments, but this was later replaced with the same type of column, but with phenyl functional groups. The phenyl packing proved to separate the early eluting chlorophenolics with at least equal efficiency to the C_{18} packing, but for the same eluent, retention times of the polychlorinated compounds were much shorter. The factor that has a strong impact on retention of the later eluting, polychlorinated phenols —2,4,6-trichlorophenol and pentachlorophenol— is the pH of the eluent (Fig. 1). The low pK_a values of these chlorophenols (6.5 and 4.9, respectively [24]) mean that, with the increased ionisation of their hydroxyl groups under more alkaline conditions, retention time declines sharply for pentachlorophenol and begins to fall for 2,4,6-trichlorophenol for pH > 6. Shoup and Mayer [20] made a similar observation, but here we have used these properties to enhance the performance of the analytical column so that separation of the full range of chlorophenolics is effected in under 25 min (Fig. 2).

To augment chromatographic resolution when complex samples are analysed, LC–ED procedures benefit from the selectivity of the electrochemical

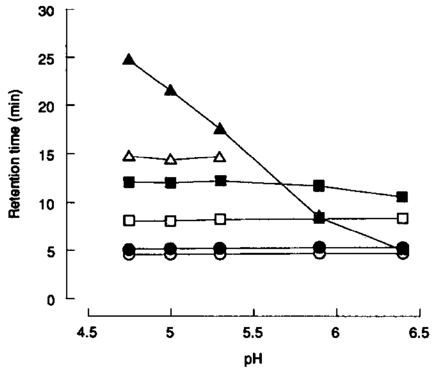

Fig. 1. Relationship of retention time of phenolic compounds with pH, under the chromatographic conditions described in the text. ○ = *m*-Cresol; ● = 2-chlorophenol; □ = 2,4-dichlorophenol; ■ = 2,4,6-trichlorophenol; △ = 3,4,5-trichloroguaiacol; ▲ = pentachlorophenol. Conditions: acetonitrile–sodium acetate buffer (40:60), flow-rate 1.5 ml/min.

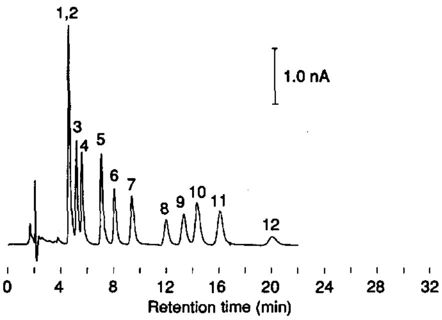

Fig. 2. Separation of standard suite of chlorophenolic compounds and *m*-cresol under the recommended chromatographic conditions described in the text. Peaks: 1 = *m*-cresol; 2 = 6-chlorovanillin; 3 = 2-chlorophenol; 4 = 4-chlorophenol; 5 = 4-chloro-3-methylphenol; 6 = 2,4-dichlorophenol; 7 = 4,6-dichloroguaiacol; 8 = 2,4,6-trichlorophenol; 9 = 4,5,6-trichloroguaiacol; 10 = 3,4,5-trichloroguaiacol; 11 = trichlorosyringol; 12 = pentachlorophenol.

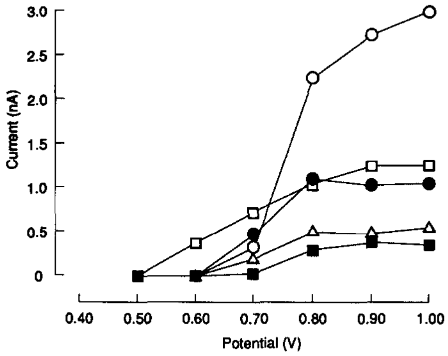

Fig. 3. Hydrodynamic voltammograms for the oxidation of chlorophenolic compounds 2,4-dichlorophenol (○), 2,4,6-trichlorophenol (●), 4,5,6-trichloroguaiacol (□) and pentachlorophenol (■), and tribromophenol (△) in eluent at a glassy carbon electrode (*vs.* Ag/AgCl).

detector. When a two-electrode flow cell is used the opportunities for specific detection are even greater [25]: with the electrode in a *series* configuration, analytes can be oxidised or reduced at the upstream electrode before detection at the second working electrode; in *parallel* configuration, the difference current can be monitored between the two working electrodes, set at different potentials (E_1, E_2). We chose the latter approach to discriminate against some of the more easily oxidised compounds (*i.e.* oxidised at potentials $<E_1$) in pulp mill effluents and natural waters that might co-elute with chlorophenolic compounds.

Hydrodynamic voltammograms are depicted in Fig. 3 for the oxidation of some of the chlorophenolic compounds at a glassy carbon electrode (*vs.* Ag/AgCl). There is anodic response from most chlorophenolics for potentials greater than 600 mV, with limiting currents at about 900 mV. These results are consistent with hydrodynamic voltammograms presented by Galceran and Santos [26]; their investigations were performed at neutral pH, which causes a general shift to slightly lower potentials. Therefore, using the eluent and pH recommended above, we should be able to accentuate the anodic detection of chlorophenolic compounds by monitoring the difference in output current (Δi) for dual parallel electrodes with E_1 = 600 mV and E_2 = 900 mV. The effectiveness of this approach is addressed in the applications discussed below.

Performance characteristics

A standard suite of 11 chlorophenolics and one alkylphenol were used for testing the optimised LC–ED procedure. This included the five chlorophenols in the USEPA designation of priority pollutant phenols, and five chlorophenolic compounds known to occur in pulp mill effluents [5,6] —6-chlorovanillin, 4,6-dichloroguaiacol, 3,4,5-trichloroguaiacol, 4,5,6-trichloroguaiacol and trichlorosyringol. 4-Chlorophenol and *m*-cresol were also added to the mixed standard solutions; the former to test the chromatographic separation of positional isomers, and the latter provided an example of a non-chlorinated simple phenol. Fig. 2 shows the separation of the standard compounds when injected in a solution identical to the eluent.

Precision was assessed by repeatedly injecting samples of the mixed standard solution, with each component at a concentration of 40 μg/l. For 50-μl injections, the relative standard deviation (n = 10) for the 12 standard phenolic compounds was 1–3%. Limits of detection (3 × baseline noise) for the same compounds using 50-μl injections ranged from 0.4 μg/l (or 4 nM, for *m*-cresol) to 6 μg/l (or 23 nM, for pentachlorophenol). In general, the limit of detection decreased for the chlorophenolics with longer retention times. Much lower limits of detection are possible with preconcentration, as discussed below.

The response index, r, defined in the power function:

$$y = Ac^r$$

(where y is detector output, c is solute concentration and A is a constant) is a measure of detector linearity [27]. For a truly linear detector $r = 1$, but the practical bounds for response linearity given by Scott [27] are 0.98–1.02. Linear regression analysis of peak area data in the concentration range 10–100 μg/l for all phenolic compounds in the mixed standard, generally produced results $0.98 < r < 1.02$. For low signal-to-noise ratios (*i.e.* at concentrations < 10 μg/l), artefacts arising from the peak integration can produce slight curvature; very occasionally this may extend to higher concentrations.

Calibration

Peaks in the chromatogram were identified from their co-elution with standard chlorophenolics added to the sample matrix. Further corroboration may be obtained from peak current ratios for individual peaks at different detector potentials, that are compared with ratios for likely standard compounds. This approach has been recommended by Shoup and Mayer [20]; it is a useful adjunct to the co-elution technique, but was not used in this study. Definitive characterisation of chlorophenolics may require collection of eluate fractions for subsequent gas chromatography–mass spectrometry, or possibly the use of a parallel LC–mass spectrometry procedure.

To quantify the concentrations of chlorophenolics in samples, the method of standard additions is appropriate, but laborious. An alternative procedure is to use an internal standard. If an analytical method involves a preconcentration step, an internal standard not only permits estimation of the analytes, but it also can obviate the need to continually monitor extraction recoveries. 2,4,6-Tribromophenol (TBP) has already been used as an internal standard in gas chromatography procedures, and it is suitable for our LC–ED system because it elutes in a vacant region of the chromatogram (retention time, $t_r \approx 17.8$ min with pH 5.0 eluent). Investigation of TBP as an internal standard is discussed in the following section on preconcentration.

Preconcentration

For analysis of natural samples, it is often necessary to both separate and preconcentrate the analytes. Chlorophenolics can be extracted from the sample matrix using either organic solvents or solid-phase sorbents. Extraction of acidified aqueous samples with one of the solvents, methylene chloride, diethyl ether or hexane, and then back-extraction into dilute alkali was unsuitable in each case because of incomplete recovery of the chlorophenolics. There was also the disadvantage that the solution for injection onto the chromatography column was quite unlike the eluent. We attempted to overcome this problem by re-acidifying the back extract, and passing it through a reversed-phase sorbent. However, this too was unsuccessful because traces of the original organic solvent remaining in the back-extract led to incomplete sorption of the chlorophenolics. Another problem with liquid–liquid extraction of complex natural samples, such as effluents, is the formation of emulsions among other factors that can cause incomplete phase separation. Therefore, no further investigations were made of liquid–liquid extraction as a means of preconcentration.

A range of solid-phase sorbents were tested as listed in Table I. Those marked with an asterisk in the table proved to be the most suitable in initial experiments. Recoveries of chlorophenolics (added to pure water) for these three sorbents —Sep-Pak C_{18}, Amberlite XAD-4 and Chromosorb 102— ranged between 70–90%, depending upon the individual compound, and the total concentration of adsorbed compounds. Other solid phases returned lower recoveries of the analytes.

After more detailed investigations, the Sep-Pak C_{18} phase was set aside because it bled contaminants that eluted late in the LC–ED chromatogram (see Fig. 4). The loading of weakly acidic samples (pH ≈ 3) seemed to generate these artefacts, particularly from fresh or little used Sep-Pak sorbents. At this pH there was also the added problem of the hydrolysis of the silica support. Of the polymeric phases, Chromosorb 102 was the more suitable. It did not have to be ground and sieved to produce the appropriate particle size; it was less prone to swelling and shrinking with changes in solution pH and ionic strength; and it contained fewer contaminants than Amberlite XAD-4. Otherwise, both polymeric

TABLE I

SOLID-PHASE SORBENTS TESTED FOR PRECONCENTRATION OF CHLOROPHENOLIC COMPOUNDS

For meaning of asterisks, see text.

Solid-phase sorbents	Particle size (μm)
*Sep-Pak C_{18} (Waters Associates)	55–105
Sep-Pak Accell QMA (Waters Associates)	37–55
*Amberlite XAD-4 (Rohm and Haas)	75–125
BN-X4 (Benson)	50
Chromosorb 101 (Johns-Manville)	45–75
*Chromosorb 102 (Johns-Manville)	45–75
Bond-Elut -PH, -C_8, -C_{18}, -CN, -CBA and -NH_2 (Analytichem)	40

sorbents behaved similarly; they were more efficient extractants than Sep-Pak C_{18} for phenol and early eluting phenolics, but at the same time tended to adsorb a wider spectrum of organic compounds (as also noted recently by Hennion [28]). We found that salt (NaCl) addition was unnecessary for the efficient adsorption of the chlorophenolics used in this study.

A thorough evaluation of recoveries for a selection of chlorophenolic compounds was made using the recommended preconcentration procedure with Chromosorb 102 as sorbent. The compounds chosen for the study generally covered the range of polarities for multiple chlorine substitution. Detector response, *after* preconcentration, for an individual analyte dissolved in pure water was compared with the response for the same level of analyte spiked in a paper mill effluent. All results were related to the detector response for direct injection of standard solutions of the chlorophenolics made up in the aqueous acetonitrile eluent. The investigation was run over a range of concentrations to permit regression analysis. In parallel experiments, the spike was added to (i) the effluent sample before loading onto the Chromosorb 102 cartridge, and (ii) the acetonitrile eluate resulting from extraction of the effluent sample. Hence, it should be possible to discern the efficiency of adsorption/desorption of chlorophenolics on Chromosorb 102 in contrast to the interference from co-extracted organic compounds at the detector's working electrode. To assess TBP as an internal standard, it was added initially to all sample and standard solutions.

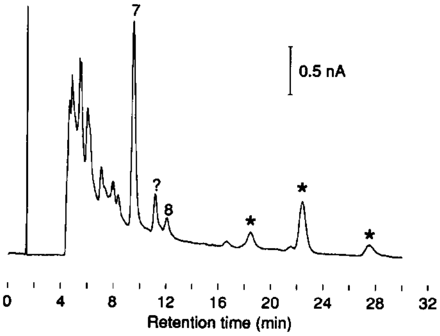

Fig. 4. LC–ED chromatogram of a total effluent from Paper mill A using the recommended chromatographic procedure after extraction on a Sep-Pak C_{18} cartridge. Peaks as in Fig. 2; the peaks marked with an asterisk are artefacts introduced by the Sep-Pak C_{18} sorbent, and the one with a question mark is unidentified.

Calculated recoveries for selected chlorophenolics and TBP preconcentrated on Chromosorb 102 are presented in Table II. When the standard spike was added to the effluent extract after the preconcentration step, there appeared to be some diminution of peak currents for all compounds, apart from pentachlorophenol. Since the decrease in detector response varied for each chlorophenolic, interference in the LC–ED chromatography from other extracted compounds is more likely than depressed working electrode performance. For additions of the chlorophenolics to the paper mill effluent prior to the preconcentration, recoveries ranged from 84 to 100%. Recoveries increased with increasing degree of chlorine substitution and more non-polar character. This trend is more obvious when recoveries are calculated on the basis of peak areas obtained from preconcentration of aqueous standards on Chromosorb 102 (figures in parentheses in Table

TABLE II

RECOVERIES OF CHLOROPHENOLIC COMPOUNDS AND TRIBROMOPHENOL AFTER PRECONCENTRATION ON CHROMOSORB 102 SORBENT

DCP = 2,4-dichlorophenol; DCG = 4,6-dichloroguaiacol; TCP = 2,4,6-trichlorophenol; TCG = 4,5,6-trichloroguaiacol; PCP = pentachlorophenol; TBP = 2,4,6-tribromophenol. Recoveries are related to standard solutions for each compound that were made up in eluent and injected directly onto the analytical column, apart from figures in parentheses that are related to standards that were put through the preconcentration procedure.

Experiment	Recovery (%)					
	DCP	DCG	TCP	TCG	PCP	TBP
Standards	109.4	96.7	101.0	95.2	102.9	102.2
Effluent (post-)[a]	97.2 (88.9)	86.6 (89.6)	92.1 (91.2)	92.3 (97.0)	101.6 (98.8)	92.2 (90.2)
Effluent (pre-)[b]	91.9 (84.0)	84.2 (87.0)	86.3 (85.5)	84.4 (86.6)	101.0 (98.8)	87.0 (85.2)

[a] Spike added to effluent extract after preconcentration.

[b] Spike added to effluent sample before preconcentration.

II). Some sources of random error are removed from these latter data, because additions of standards were made from the one spiking solution for all preconcentration trials.

In a second trial of the preconcentration technique with a total effluent from the paper mill collected at later date, the same chlorophenolics were added, but with trichlorosyringol in place of 4,6-dichloroguaiacol. Recoveries were quantitative in this instance, apart from 2,4-dichlorophenol which was marginally diminished at 96%. The discrepancy between these two recovery trials can be attributed to differences in the sorbent loading caused by variability in (i) effluent composition (from changes in feedstock, and variation of pulping and bleaching chemistry), and possibly (ii) slight changes in sample pre-treatment. The original mill effluent had a pH of 6; while effluent used in the subsequent trial had a pH of 9.5, and it also had a high clay particulate loading that necessitated filtration (0.5 μm, Whatman GF/F) in place of centrifugation. The former effluent was diluted 6% with pure water in pretreatment procedures whereas the latter was diluted by 16%. In monitoring effluents for chlorophenolic compounds it is recommended that extraction recoveries are monitored regularly.

Very similar recoveries were recorded for TBP as for the chlorophenolics. Hence, on the basis that TBP does not interfere in the chromatographic separation of the chlorophenolics, and that it behaves very similarly in the preconcentration procedure, it can function as an internal standard. The other important proviso is that the compound chosen as an internal standard cannot be present in samples to be analysed. TBP is not a natural compound, and it has not been observed in pulp mill effluents (although in one instance with mill effluent samples we have observed an unknown compound that overlapped with TBP). Even for a compound like pentachlorophenol which is often extracted with different efficiency than TBP, it is still possible to calibrate via the internal standard method provided that the analyte: internal standard ratio is calculated from data derived from the preconcentration procedure, rather than from comparison of detector response for standards that are injected directly onto the LC–ED column. For accurate assessment of recovery or use of an internal standard it should also be emphasised that complete dissolution of chlorophenolic compounds should be ensured in aqueous standards; many of these compounds have very low solubilities in water, and tend to remain as a surface film.

Application

Effluent samples were obtained from a paper mill processing softwood and hardwood pulps (hereafter referred to as Paper mill A), and a pulp mill that uses mostly hardwoods as feedstock (hereafter referred to as Pulp mill B). The chlorophenolics proved to be quite stable under the storage conditions described above, and we noticed no difference

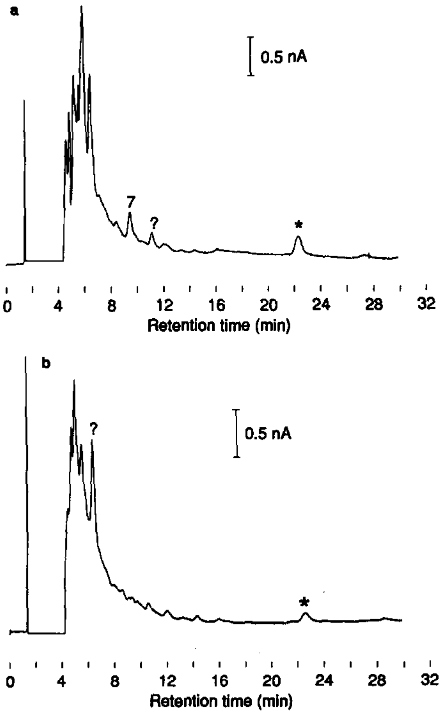

Fig. 5. LC–ED chromatograms of total effluent samples collected from Pulp mill B on two separate occasions (a and b), with different AOX levels: (a) 17 mg/l, (b) 11 mg/l. The analyses were performed on extracts from Sep-Pak C_{18} cartridges. Peaks as in for Fig. 4.

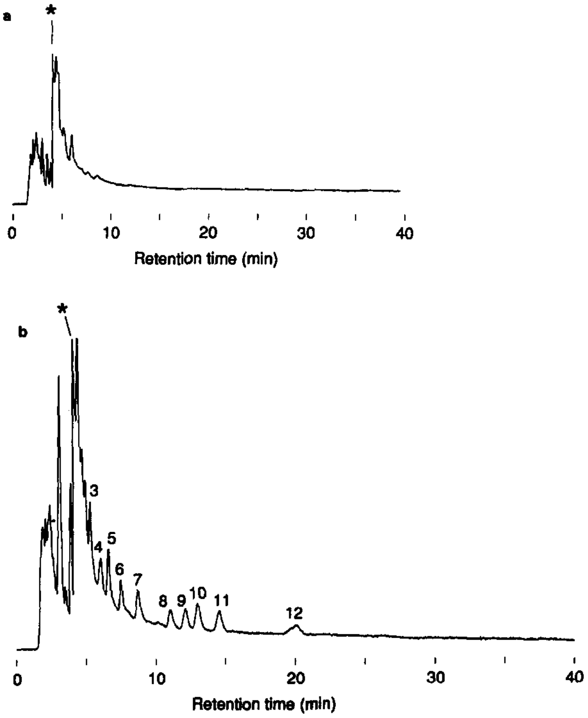

Fig. 6. LC–ED chromatograms of Fig. 5b effluent sample after spiking with the standard mixture of chlorophenolic compounds (chlorophenols: 7 μg/l, other chlorophenolics: 10 μg/l). Peaks as in Fig. 2; the asterisk indicates a change in current sensitivity from 100 to 10 nA f.s. (a) Single-electrode amperometry: current trace recorded at an applied potential of + 600 mV *vs.* Ag/AgCl (E_1). (b) Dual-electrode amperometry: differential current trace (Δi), for E_1 = + 600 mV *vs.* Ag/AgCl, E_2 = + 900 mV *vs.* Ag/AgCl.

between samples treated and not treated with the copper sulphate.

Chromatograms for the total effluents from the mills are shown in Figs. 4 and 5. Chlorophenolics identified in the effluent from Paper mill A were 4,6-dichloroguaiacol and 2,4,6-trichlorophenol; their concentrations were estimated by standard additions technique to be 213 ± 20 μg/l and 9.9 ± 0.5 μg/l, respectively. Confirmation of peak identity in collected fractions from the HPLC column was made by gas chromatography–mass spectrometry [29], after derivatising chlorophenolics in the mill effluent by using essentially the method of Voss *et al.* [14]. Effluents from Pulp mill B contained lesser amounts of the polychlorinated phenolics. Only the 4,6-dichloroguaiacol was tentatively identified. Pulp mill B provided data from "adsorbable organohalogen" (AOX) measurements for the two effluent samples analysed here (Fig. 5). As expected, concentrations (by inspection) of polychlorinated phenolics diminish with a decrease in AOX level of the effluent.

The efficacy of using dual-electrode detection, and monitoring the differential current is demonstrated in Fig. 6. We added the standard suite of chlorophenolic standards to the effluent sample shown in Fig. 5b. The current trace recorded at an applied potential of + 600 mV (E_1) shows no response to the chlorophenolics (Fig. 6a). However, the differential current trace (Δi, for E_1 = 600 mV, E_2 = 900 mV) shows peaks for all the chlorophenolics apart from 6-chlorovanillin which is masked by other clearly eluting compounds in the mill effluent (Fig. 6b). We found that there was no need to frequently re-polish the electrodes, as there was no obvious deterioration in electrode performance over several days with the repeated injection of extracted effluent samples.

In a preliminary experiment, we attempted to remove some of the maze of early eluting peaks that can interfere with the determination of the monochlorophenolic compounds. Ultra-filtration was the method of choice. A sample of Paper mill A effluent was filtered through a GF/F filter (Whatman), and then a 15-ml aliquot was ultra-filtered using a centrifugation process (Millipore Ultrafree-20 filter unit; 10 000 NMWL polysulfone membrane). The ultra-filtration using the procedure as recommended by the manufacturer should yield a filtrate with 10 000 molecular mass cut-off. Comparative analysis of this filtrate with a sample that was subjected to primary filtration alone, with the GF/F filter, indicated that <3% of the chlorophenolics were recovered. Either the chlorophenolic compounds adsorbed to the membrane, or more likely they were mostly associated with > 10 000 molecular mass organic material in a hydrophobic interaction. Further investigations are required to find pretreatment procedures that will more selectively extract the early eluting chlorophenolics from samples of pulp mill effluents.

Although we did not analyse a natural receiving water sample, the capabilities of the analytical procedure were tested by successively diluting Paper mill A effluent with 1% NaCl solution. These diluted samples were preconcentrated by solid-phase extraction using the recommended procedure to give a final concentration equivalent to that in the original effluent. The recovery was gauged by comparing the concentration determined in the concentrate with that of the undiluted effluent. Within the precision of the analytical procedure, recovery was complete for at least a 50-fold dilution of the effluent. A lack of interference from natural organic compounds in individual receiving waters would need to verified for legitimate application of the preconcentration procedure.

The LC–ED method for determining chlorophenolics as described here, is not intended to supplant existing analytical procedures that definitively identify chlorophenolic compounds in aqueous samples, such as that of Voss *et al.* [14] when coupled with mass spectrometry. It is a complementary technique that with its simpler pretreatment steps is well suited to routine monitoring once an effluent or receiving water has been well characterised. As demonstrated by Rennie [30] with an earlier HPLC method for phenols, it could also be automated easily.

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NOTE ADDED IN PROOF

A very recently published paper (Tolosa *et al.*, *Mar. Pollut. Bull.*, 22 (1991) 603–607) reports on the occurrence of 2,4,6-tribromophenol in estuarine sediment extracts. Therefore, TBP might be disqualified as an internal standard for the analysis of some environmental samples by our LC–ED procedure. Some of the isomers of tetrachlorophenol could be suitable substitutes.

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