Selective determination of trace levels of phenol in river water using electrochemical concentration modulation correlation chromatography

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SUMMARY

Electrochemical concentration modulation (ECM) was used as a sample introduction technique in the correlation chromatographic (CC) trace determination of phenol in water. The linearity and sensitivity of the method were tested and detection limits were calculated. The selectivity of the technique was confirmed by comparison with loop injection experiments using several detection methods. In preliminary experiments is was found that ECM–CC, in combination with fluorescence detection, is selective and sensitive enough to be used for the monitoring of the phenol concentration in river water at the draining points for drinking water production.

INTRODUCTION

Water from polluted rivers, such as the Rhine, is used to feed buffer reservoirs as a first step in the production of drinking water. As the quality of river water is not constant, continuous monitoring at the draining point is necessary to maintain an acceptable initial purity. Phenols are important pollutants of river water, being degradation products of many organic compounds, such as pesticides used in agriculture, and waste products from a variety of chemical industries[1]

A large number of high-performance liquid chromatographic methods for the determination of phenols have been reported. However, most of them circumvent phenol itself and otherwise detection limits for phenol are relatively high. In general, this is caused by the inability to effect a satisfactory preconcentration of the polar phenol on apolar materials. Additionally, the fast-eluting phenol will often coelute with other polar compounds. EEC regulations[2] require phenol concentrations in water intended for human consumption to be less than $5.3 \cdot 10^{-9}$ mol/l. A method for phenol monitoring at the draining point in a river should therefore have a sufficiently low detection limit in addition to allowing continuous analysis. Because of the complexity of environmental water samples it should also be selective.

Nielen *et al.*[3] reported a sophisticated method involving two successive preconcentration and clean-up steps on a large hydrophobic precolumn and an ion-

exchange column, after which the actual separation took place on a reversed-phase column employing fluorescence detection. The limit of detection (LOD) for phenol was $1.1 \cdot 10^{-10}$ mol/l. Although the experimental setup used was fairly complicated (two pumps, three electrically actuated valves, three precolumns, a switching unit, a programmer), it was fully automated and reasonably selective. Laeven et al.[4] reported a correlation chromatographic method using fluorescence detection for the determination of phenol, achieving an LOD of $3.2 \cdot 10^{-11}$ mol/l in a 80-min experiment. The equipment was not very suitable for routine analysis, however, and the selectivity was only determined by the fluorescence detector.

Rennie and Mitchell[5] proposed an elegant method involving dual-electrode electrochemical detection without preconcentration, with a detection limit of $3.6 \cdot 10^{-10}$ mol/l for phenol. Here the detection method provides the selectivity and the method can be automated. Borra et al.[6] combined preconcentration on a graphitized carbon black cartridge column and not very selective UV detection at 280 nm, which produced an LOD for phenol of $2.7 \cdot 10^{-9}$ mol/l. Bigley and Grob[1] used a selective post-column reaction with 4-aminoantipyrine with UV detection at 509 nm and reported an LOD of $5.3 \cdot 10^{-6}$ mol/l without preconcentration. Another reaction–detection method was proposed by de Ruiter and co-workers[7,8], involving off-line dansylation, post-column photolysis and fluorescence detection of the reaction products, which gave a 10-fold gain in fluorescence intensity compared with phenol itself. However, the method is difficult to automate.

This paper describes a method that can be used for the continuous selective monitoring of phenol in river water. A relatively simple experimental set-up is needed and the method has sufficient sensitivity and high selectivity compared with the methods mentioned above. Further, the method can be automated without much effort.

Electrochemical concentration modulation

The principle of electrochemical concentration modulation (ECM) correlation chromatography (CC) was described previously[9,10]. A coulometric cell is placed in front of the separation column (Fig. 1). Using computer control, an alternating potential is applied to the working electrode while the sample–eluent mixture flows through the system continuously. When the upper and the lower potential are chosen to be above and below, respectively, the half-wave potential of the compound of interest, sample introduction takes place when the electrode potential is at the lower level, whereas the compound is completely oxidized at the upper level. The resulting detector signal will be a superposition of a number of chromatograms shifted in time. As the method is basically a correlation chromatographic method[11], the potential is modulated according to a pseudo-random binary sequence. In that case the detector signal can be deconvoluted by cross-correlating with the modulation pattern. The resulting correlogram resembles the corresponding chromatogram but will have a better signal-to-noise ratio because of the multiplex advantage[12,13]. The selectivity, however, is also enhanced as only compounds having half-wave potentials in the modulation region will appear in the correlogram. So far the selectivity of the method is the same as that of a dual-electrode determination. However, an additional selectivity dimension is provided by the detector. The only constraint is the detectability of the compound of interest or of an electrochemical reaction product. Amperometric and fluorescence detection were compared in this work.
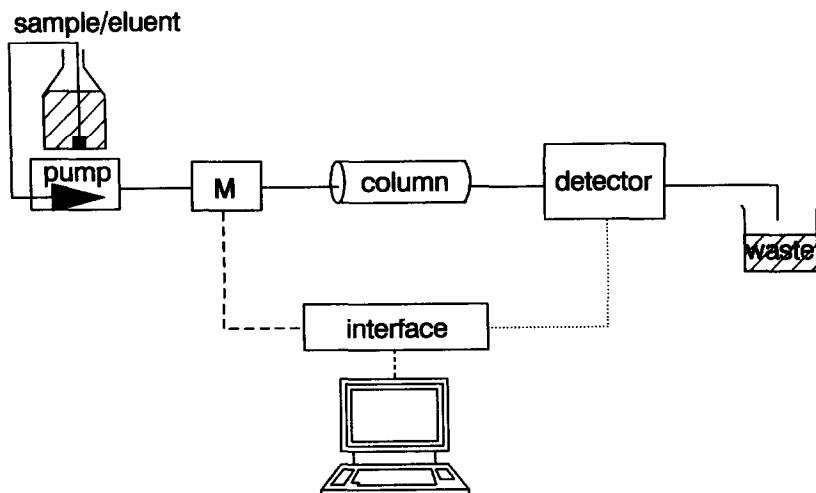
Fig. 1. Experimental set-up for ECM–CC. The eluent contains the sample. M is the modulator–potentiostat combination.

EXPERIMENTAL

Modulation CC

An ESA Model 5021 conditioning cell, capable of withstanding 80 bar backpressure, was used as the modulator cell. A potentiostat (Princeton Applied Research, Model 174A) supplied the potential to the porous graphite working electrode. The potentiostat was externally controlled by an intelligent ADC/DAC interface (Cambridge Electronic Design, Model 1401) connected to a microcomputer (Acorn, Model BBC Master). The detector signal was sampled synchronously with the same interface. The control software was developed in our laboratory. Cross-correlations were performed off-line on a Hewlett Packard HP 9000 Model 300 computer.

Injection sequences consisting of 63 or 511 clock periods (cp) of 10 s each were used to modulate the electrode potential. The modulation interval was selected by monitoring the fluorescence intensity as a function of the modulator cell working electrode potential after 20-μl loop injections of 10^{-6} mol/l phenol dissolved in the eluent. The fluorescence intensity decreased to half its original value when the electrode potential was 0.4 V with respect to the reference electrode of the modulator cell. A modulation interval of -0.1 to 0.9 V was used although a smaller interval was equally applicable.

Chromatography

An Applied Biosystems Model SF400 pump equipped with a liquid pulse damper delivered a constant eluent/sample flow of 0.60 ml/min. A Rheodyne injection valve with a 60-μl loop was used for the loop injection experiments. A 0.2-μm carbon filter (ESA) and a 0.5-μm metal in-line filter (Upchurch Scientific) preceded the modulator cell to prevent clogging of the porous working electrode. The metal frit

was placed between the carbon filter and the cell because the carbon filter was found to release small particles of carbon in an eluent containing acetonitrile.

A 100 mm × 4.6 mm I.D. stainless-steel column was used, filled with Hypersil ODS (5 μm) particles. The plate number was *ca.* 2000.

Chemicals and solutions

Analytical-reagent grade chemicals were used unless indicated otherwise.

The eluent was acetonitrile (Rathburn, HPLC-S grade)–aqueous buffer (pH 4) (30:70, v/v). The buffer solution contained 0.05 mol/l acetic acid (Merck), adjusted to pH 4 with concentrated sodium hydroxide solution (Baker, Analyzed Reagent), and 0.05 mol/l potassium nitrate (Janssen Chimica). Acetonitrile was filtered before mixing with the buffer using a 0.5-μm PTFE membrane filter (Millipore, type FH). The buffer solution was filtered over a 0.2-μm membrane filter (Sartorius, cellulose acetate, type 11107).

Canal water, obtained from the Nieuwe Achtergracht in Amsterdam, was filtered over a 0.5-μm membrane filter (Millipore type AA), acidified with glacial acetic acid up to 0.05 mol/l and adjusted to pH 4.0 by addition of concentrated sodium hydroxide solution. Potassium nitrate was dissolved (0.05 mol/l) and the solution was filtered a second time using the 0.22-μm cellulose acetate filter. For the recovery experiment the canal water was spiked with phenol up to a concentration of $1.38 \cdot 10^{-8}$ mol/l. Stock solutions for the calibration experiments were made starting from a 10^{-3} mol/l phenol solution in acetonitrile. Subsequent diluted solutions were made using the standard eluent.

Detection

A Perking Elmer LS-4 fluorescence detector, equipped with a 3-μl flow cell, operating at an excitation wavelength of 270 nm and an emission wavelength of 299 nm with 10-nm slits, was used. Optimum excitation and emission wavelengths were obtained by measuring the excitation and emission spectra of a 10^{-6} mol/l phenol solution in the eluent.

Electrochemical detection was performed using a cell (Bioanalytical Systems) equipped with a glassy carbon working electrode and an Ag/AgCl reference electrode. The detection potential of 1.1 V was applied by a potentiostat (Bioanalytical Systems, Model LC-4). The optimum detection potential was obtained by measurement of a hydrodynamic voltammogram. The electrochemical detector was equilibrated overnight at the detection potential, allowing for the background current to settle. The UV detector (Waters Assoc., Model 441) used during the loop injection experiments was operated at 254 nm.

RESULTS AND DISCUSSION

Time constant and ghost peak

In a previous paper it was reported that the use of a chemical concentration modulator in CC can result in the appearance of ghost peaks in the correlogram[10]. Theoretically, ghost peaks are caused by reproducible injection errors resulting in erroneous injection patterns decomposable into shifted and/or inverted versions of the ideal injection pattern[14]. Physico-chemically the origin lies in the symbiotic action

of the time constant of the modulator and the position and size of the modulation interval relative to the response of a particular compound as a function of the modulation force.

In ECM–CC, the RC circuit constructed by the double-layer capacitance at the surface of the working electrode and the residual resistance between the working electrode and the reference electrode are responsible for the time constant of the modulator cell. The relative position of the half-wave potential of the analyte in the potential interval used is the other part of the potential ghost peak generator.

This problem can be solved in a number of ways[15]. In this work we used used a fast modulator cell and favourable eluent conditions such as a low viscosity (acetonitrile as modifier), a substantial salt concentration (0.1 mol/l) and a background electrolyte consisting of ions with a high mobility (potassium nitrate).

In the ESA Model 5021 conditioning cell, counter and reference electrodes are placed on both sides of the porous graphite electrode, which results not only in a more homogeneous working electrode potential but also in a smaller residual resistance. This should lead to an appreciable decrease in the time constant of the cell relative to the ESA Model 5020 guard cell used in the previous investigation, where the counter and reference electrodes are positioned on the upstream side of the working electrode.

In our previous work, the best fit to the decay rate of the current response after the application of a potential step was accomplished using the sum of two exponentials as a fit function. Although the physical meaning of the two associated time constants is not clear, their values can be used as a measure of the speed of the modulator cell.

The time constants for both cells were determined using the same experimental conditions. We found for the conditioning cell values of 0.11 and 0.98 s and for the guard cell 0.16 and 3.9 s. The weight factors of the corresponding exponentials were almost the same for both cells. The lowest time constants are probably limited to some extent by the electronic circuits used in collecting the data. It is clear that the conditioning cell is considerably faster. Fig. 2 shows the charging currents for both cells as a function of time after the potential step. In the correlogram only a tiny, so-called λ_3 ghost peak could be detected at a "retention time" of 170 s when a 63-unit injection sequence was used. This type of ghost peak disappears after subtraction of a correlogram originating from the inverted (high-level potential becomes low-level potential and *vice versa*) modulation pattern. This is shown in Fig. 3. Fine tuning of the modulation interval would also result in the disappearance of this ghost peak[10]. The standard modulation pattern results in a negative phenol peak because a high electrode potential corresponds to a negative injection.

Sensitivity

Calibration graphs were recorded for loop injection and ECM–CC experiments using both electrochemical and fluorescence detection. The standard deviation of the baseline noise in the resulting chromatograms and correlograms was used as a measure of the uncertainty in the determinations. The calibration graphs are shown in Fig. 4. The dashed lines represent three times the standard deviation (3σ) of the baseline noise. The crossings of the dashed and solid lines are a measure of the detection limit according to the 3σ criterion.

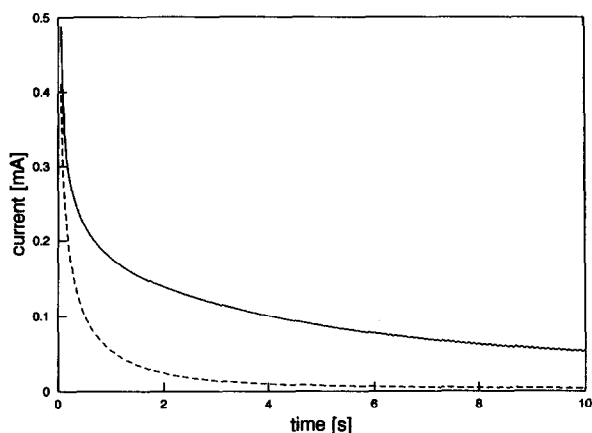All points on the calibration graph for the ECM–CC experiment with electro-

Fig. 2. Decay curves of the charging current after the application of a −0.2 to 0.8 V potential step to the Model 5020 guard cell (solid line) and the Model 5021 conditioning cell (dashed line). Experimental conditions as described.

chemical detection in Fig. 4c result from a 63 cp injection sequence. Each injection period lasts 10 s. The result for the standard solution containing the highest phenol concentration ($8.48 \cdot 10^{-6}$ mol/l) is not plotted on the calibration graph because during the measurement the surface of the glassy carbon working electrode became
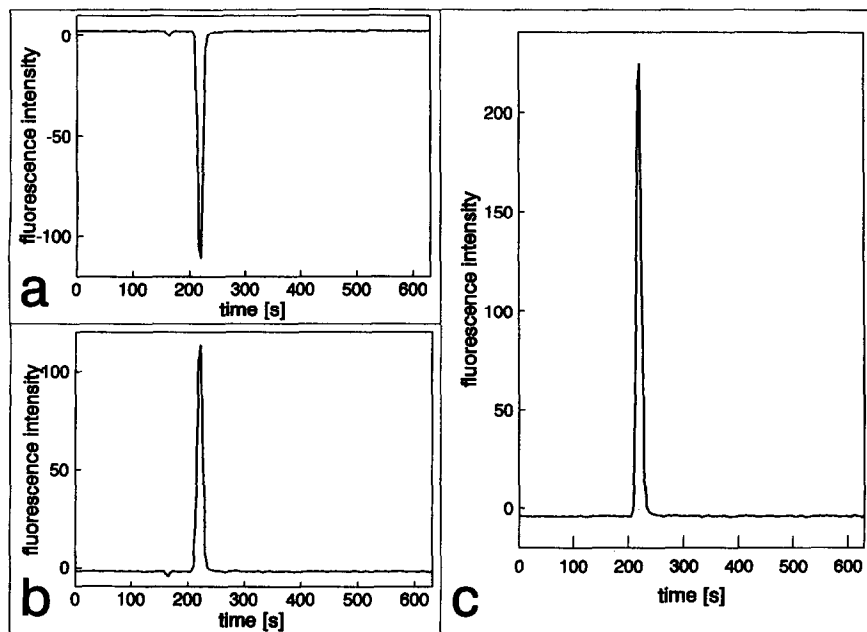

Fig. 3. Correlograms for a $4.2 \cdot 10^{-6}$ mol/l phenol solution in the eluent. The small peak at 170 s in the λ_3 ghost peak. Correlograms were obtained using (a) the standard modulation pattern and (b) the inverted modulation pattern. (c) Subtraction of (a) from (b); the ghost peak disappeared.
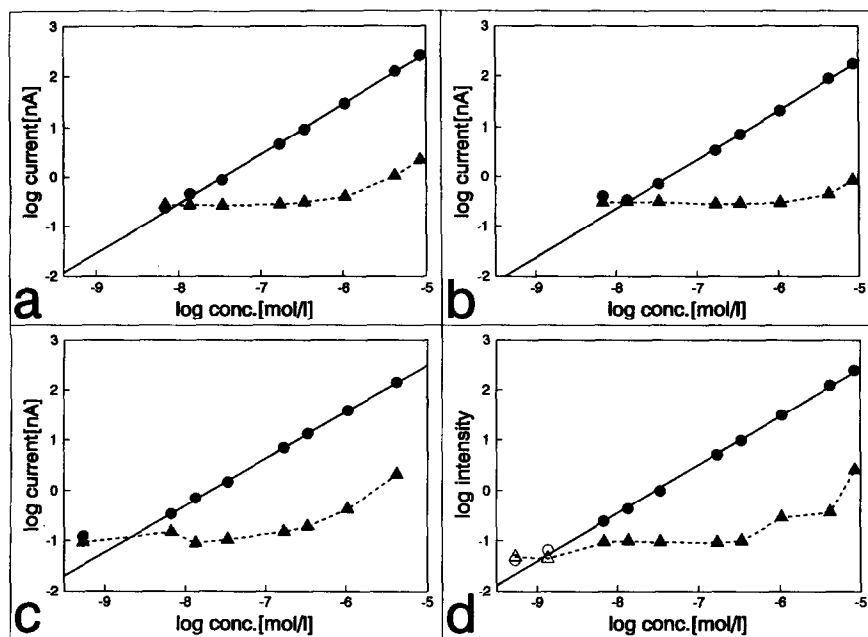
Fig. 4. Log–log calibration graphs using (a) loop injection and electrochemical detection; (b) loop injection and fluorescence detection; (c) ECM–CC and electrochemical detection; (d) ECM–CC and fluorescence detection. Solid lines are the calibration graphs. Dashed lines are $3\sigma_{baseline\ noise}$ curves. Solid symbols in the ECM–CC experiments were from 63 cp injection sequences (11 min); open symbols from 511 cp sequences (80 min). Statistical data are given in Table I.

covered with a polymeric phenol film, causing a rapid decrease in sensitivity. The polymeric film had to be removed by polishing the surface with a diamond powder slurry.

The points denoted by the open circles on the calibration graph for the ECM–CC experiments with fluorescence detection were measured using a 511 cp injection sequence to lower the detection limits. For higher concentrations it is not necessary to use long injection sequences and for the highest concentrations loop injections will do as well in terms of signal-to-noise ratio. The points with the solid symbols were recorded using a 63 cp injection sequence.

ECM–CC results were normalized to give peak heights comparable to those in loop injection experiments; only the noise is significantly reduced, as can be seen from the figures.

Table I lists the statistical data for the calibration graphs shown. The calibration graph obtained for the ECM–CC experiment using electrochemical detection is reasonably straight over three orders of magnitude. With fluorescence detection the linear range spans four orders of magnitude. Table II lists the detection limits for ECM–CC and loop injection experiments. Also, a factor describing the measured and theoretical values for the gain in signal-to-noise ratio at phenol concentrations near the detection limit is given for the correlation experiments with respect to the corresponding loop injection experiment.

TABLE I

STATISTICAL DATA ON LOG–LOG CALIBRATION GRAPHS

Regression analysis	Experiment[a]			
	L/EC	L/Fl	CC/EC	CC/Fl
Lowest concentration (mol/l)	$1.4 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$	$6.8 \cdot 10^{-9}$	$1.4 \cdot 10^{-9}$
Highest concentration (mol/l)	$8.5 \cdot 10^{-6}$	$8.5 \cdot 10^{-6}$	$4.2 \cdot 10^{-6}$	$8.5 \cdot 10^{-6}$
Number of points	7	7	7	9
Intercept	7.41	7.18	7.14	7.20
Slope	0.99	0.98	0.93	0.96
Coefficiënt of determination	0.999	0.999	0.999	0.998
Standard error of y estimate	0.04	0.03	0.02	0.05
Standard error of slope	0.02	0.01	0.01	0.01

[a] L = loop injection; CC = correlation chromatography; EC = electrochemical detection; Fl = fluorescence detection.

As the long-term baseline stability of the fluorescence detector was much better than that for the electrochemical detector, the former was chosen for the 511 cp ECM–CC experiment. As can be seen in Table II, an 80-min ECM–CC experiment with fluorescence detection results in a lowering of the detection limit by a factor 11, which is in agreement with the theoretical value.

The detection limit achieved is sufficient for the desired determination of phenol in river water. It is striking that the noise in both the loop injections experiments and the ECM–CC experiments increases when higher concentrations of phenol are used. This means that the signal-to-noise ratio becomes constant at higher phenol concentrations.

TABLE II

ESTIMATED DETECTION LIMITS[a] AND SIGNAL-TO-NOISE GAIN FACTORS[b]

Experiment[c]	Sequence length (cp)	No. of injections	LOD[d] (mol/l)	Gain factor	
				Experimental	Theoretical
L/EC	–	1	$1.0 \cdot 10^{-8}$	–	–
L/Fl	–	1	$1.4 \cdot 10^{-8}$	–	–
CC/EC	63	32	$2.3 \cdot 10^{-8}$	4	4
CC/Fl	63	32	$3.2 \cdot 10^{-9}$	4	4
CC/Fl	511	256	$1.2 \cdot 10^{-9}$	11	11

[a] Three times the standard deviation of the baseline noise, calculated over 300 points.
[b] Ratio of estimated detection limits of loop injection experiments and corresponding CC experimental values. Theoretical values are calculated using signal-to-noise gain = $\frac{1}{2}\sqrt{n}$, where n = sequence length[16].
[c] See Table I.
[d] Concentrations in the eluent.

Selectivity

The selectivity enhancement achieved when using ECM for sample introduction was tested using ECM–CC with fluorescence detection and comparing the results obtained for canal water with those obtained by loop injection using various detectors. As the canal water contained a trace of phenol there was no need to spike the sample solution at this point. Fig. 5a–c show the chromatograms after injection of 60 μl of filtered canal water prepared as described under Experimental, using (a) UV detection (b), electrochemical detection and (c) fluorescence detection. The last method is obviously the most selective but not the most sensitive. Fig. 5d shows a correlogram of the same sample–eluent solution, obtained using ECM–CC with fluorescence detection. Clearly the phenol peak at 225 s is enhanced with respect to the signal at 70 s when the intensities are compared with the chromatogram in Fig. 5c. Further, the decrease in baseline noise is clear.

Phenol in canal water

As was observed previously, the canal water contained a trace amount of phenol. To check for the losses during the filtration steps, the canal water was spiked with phenol such that the additional concentration became $1.38 \cdot 10^{-8}$ mol/l. After the correlation experiments the difference between the peak heights of the unspiked and the spiked correlograms was used to calculate the phenol recovered. The recovery was 78% (single experiment). The phenol concentration in the sample–eluent when corrected for the recovery was $2.1 \cdot 10^{-8}$ mol/l. As only 70% of the eluent consisted of
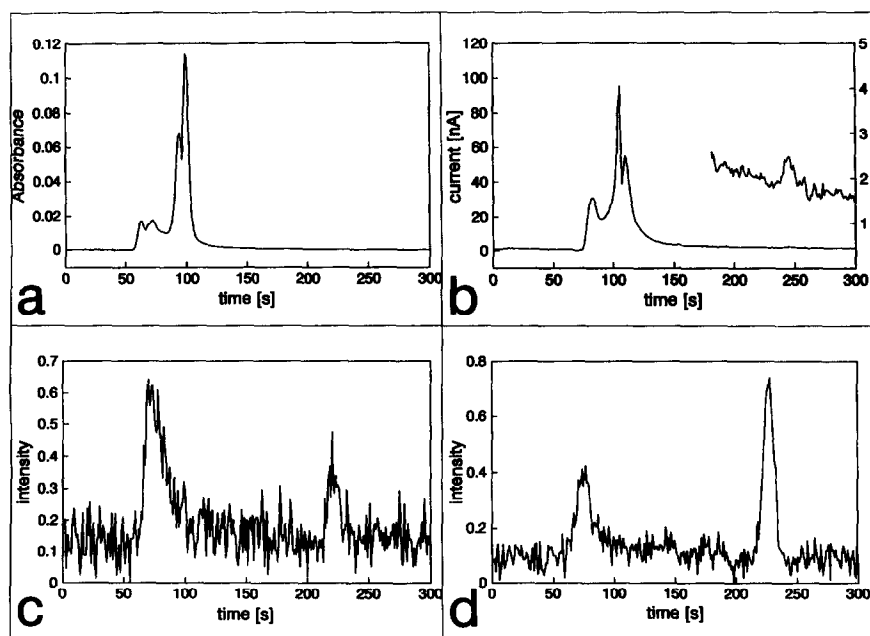

Fig. 5. (a–c) Chromatograms and (d) a correlogram for a canal water sample. Phenol elutes at 230–240 s. The selectivity increases in the order (a) UV detection, (b) electrochemical detection, (c) fluorescence detection and (d) ECM–CC with fluorescence detection.

canal water, the phenol concentration in the pure canal water sample was calculated to be $3.0 \cdot 10^{-8}$ mol/l.

CONCLUSION

The selectivity of the method compares well with those of the methods mentioned in the Introduction. The sensitivity is sufficient but certainly not the best. The reason for this is partly that almost no optimization was attempted. The column used was not very efficient (2000 plates), the excitation source of the fluorescence detector was old and noisy and the electrochemical detector used was not very sophisticated. A coulometric detector would be preferable in phenol determinations because of the formation of a polymeric layer on the electrode surface. However, in comparison with the loop injection experiments the gain in signal-to-noise ratio is according to the theory in most instances, so the use of more sensitive detectors may lower the detection limits even more. Although the ECM–CC technique was used here to determine only phenol, it can also be used for substituted phenols provided that their half-wave potentials are compatible with the modulation interval used.

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