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MEASUREMENT OF TRACE AROMATIC AMINES IN SEAWATER USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

High-performance liquid chromatography with electrochemical detection is used for the determination of the four aromatic amines: aniline, methyl aniline, 1-naphthylamine and diphenylamine at trace levels in seawater. Their chromatographic and electrochemical behaviour is discussed with respect to the analytical separation and detection from naturally complex matrices. Detection limits of 15 and 1.5 n*M* were achieved using coulometric and amperometric electrochemical cells respectively. Various parameters relating to signal recovery at trace levels are discussed.

INTRODUCTION

The analysis of trace organic compounds in water has made tremendous progress in recent years as evidenced by the numbers of analytical methods and instrumentation reported in the recent literature[1,2]. While analysts generally accept that an improvement in signal level or a reduction in noise will result in lower detection limits, with a concommittant increase in the number of detectable compounds[3], many of the newly developed techniques are either applicable only to a narrow range of species, or suffer from limited sensitivity. There are very few existing methods that can easily and routinely measure organic contaminants at less than 1 ppb*. Developing methods to detect individual compounds below this limit is essential to the marine analyst because of the extremely low concentrations at which most compounds occur in seawater, and the high significance of their interactions and interrelations at these levels.

There is a wide range of organic compounds suitable for analysis by liquid chromatography with electrochemical detection (LC–ED), and the working potential ranges (or "windows") now possible with the different types of electrode materials enable both oxidative and reductive processes to be studied. LC–ED techniques have recently been successfully applied to the field of biomedical research[4–6] but as yet,

* The American billion (10^9) is meant.

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have remained relatively unexploited in the analysis of environmental samples[7,8]. The high sensitivity and selectivity of LC–ED does, nonetheless, offer considerable advantages in this area of analysis. As part of a general review of the applicability of LC–ED to the direct analysis of natural waters, the technique was further developed and optimised for the detection of selected aromatic amines in seawater.

Compounds investigated

Four compounds (aniline, methyl aniline, 1-naphthylamine and diphenylamine) were selected as representatives of a chemical series of aromatic amines. These compounds are important industrially, and their input to the marine environment may well be anthropogenically dominated. They are used variously in the production of dyestuffs, bacteriocides, antioxidants, etc, and may therefore be significant in industrial discharges to estuarine and coastal areas though as yet there do not appear to be any reports on their occurrance in such environments. Aromatic amines are significant to the study of marine nitrogen cycling since the four compounds are similar to some natural biological metabolic by-products, and to some precursors of complex marine products[9]. A number of recent studies have indicated the considerable importance of organo-nitrogen compounds as nitrogen sources to a wide variety of heterotrophic marine organisms[10–12]. Development of methods of analysis capable of use at sea, such as LC-ED avoids many of the complexities of maintaining sample integrity, and minimises the effects of biological activity which may necessitate corrections for metabolic use or degradation of target compounds over time. It was therefore thought appropriate to examine the potential usefulness of LC–ED to this area.

A number of analytical schemes for the concentration, separation and detection of various nitrogen and halogen substituted aromatic compounds in water have appeared in the literature (*e.g.*, refs. 13 and 14). These rely either on preconcentrating very large quantities of water (typically 100–500 ml) using non-specific adsorbents such as activated charcoal or on using much more selective but less efficient adsorbents to isolate specific groups of compounds. There are a number of limitations with both of these approaches which are ultimately reflected in rather poor detection limits or great complexity in the final chromatogram. Other procedures have been used to examine similar compounds but these have first required clean-up steps such as oxalate precipitation or ion exchange to remove ionic interferences[15]. The present work examines the use of alkyl-modified silica material for the on-line isolation and concentration of aromatic amines from seawater samples with subsequent separation by HPLC and detection at an electrochemical detector.

EXPERIMENTAL

Aniline, diphenylamine (BDH, Poole, U.K.; AnalaR grade), 1-naphthylamine (BDH, GPR grade) and *n*-methylaniline (Aldrich, Gillingham, U.K.; GPR grade) were used as supplied for the preparation of 0.1 m*M* and 1 m*M* stock solutions in redistilled acetone. Working standards were prepared daily by spiking aliquots of the stock standards into distilled water or seawater (Irish Sea water filtered through a GF/F filter; salinity = 32‰). Dilute solutions (*ca.* 0.01 m*M*) were found to be unstable over a 24-h period at room temperature if also exposed to light.

Acetic acid–sodium acetate buffer (0.01 *M*, pH 5.6) was prepared from analytical grade reagents and HPLC grade water (Rathburn Chemicals, Walkerburn, U.K.). Before use, the buffer solution was filtered (0.45 μm Oxoid membrane filter) and further purified by passage through a pellicular, reverse-phase column (Whatman, Maidstone, U.K.; Co-Pell ODS).

HPLC grade methanol (Rathburn Chemicals) was used as supplied as organic modifier to the mobile phase. All phases were thoroughly mixed and degassed before use under vacuum using a water-venturi evacuator.

Chromatography

Injection systems and columns. The chromatographic apparatus is shown diagrammatically in Fig. 1. The Model RR/065 double-heated, uncompensated rapid reciprocating pump system (HPLC Technology, Macclesfield, U.K.) was linked to an injection valve (A) with a 20-μl sample loop (Model 7125, Rheodyne) for the direct injection of standards onto the analytical column (25 cm × 4 mm, 5 μm C_{18} material, RP-18 LiChroSorb, Merck Hibar, BDH), and to another valve (B) connected with a 2-ml sample loop (Model U6K, Waters Assoc.) for the injection of milliliter quantities of sea water onto the pre-concentration column. Both valves were, in turn, linked to a Rheodyne 7010 valve (C) fitted with a "pre-concentration

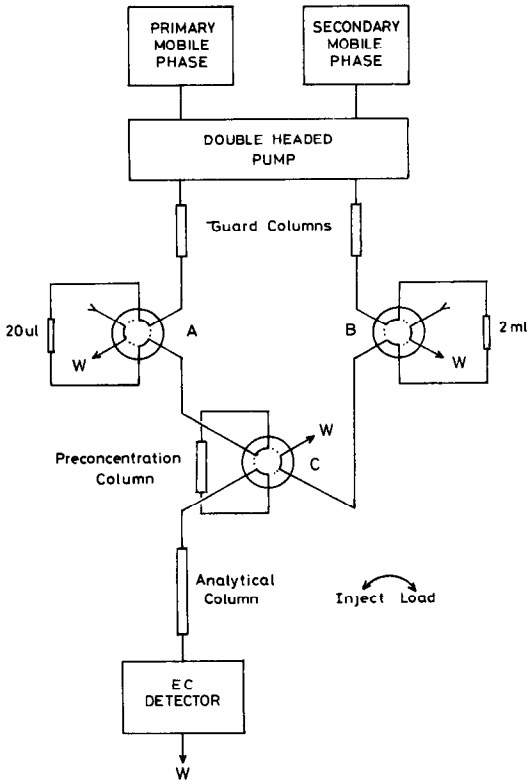

Fig. 1. Schematic diagram of the chromatography system (W = waste, and all valves are shown in the inject position).

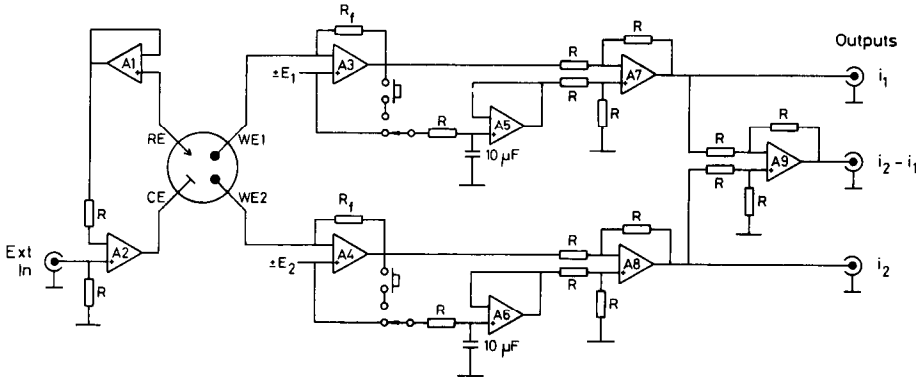

Fig. 2. Circuit diagram of the bipotentiostat used to control electrode potentials in the thin-layer dual electrode cell. Circuit description: the voltage follower (A1) and control amplifier (A2) buffer the reference electrode signal and counter electrode current. Potentials ($\pm E_1$ and $\pm E_2$) applied to the non-inverting inputs of the current followers A3 and A4 are the effective working electrode voltages, without change in sign. The output from A3 and A4 is therefore $\pm E + (-iR_f)$ where i is the value of the current flowing through the feedback resistor, R_f, and therefore through the working electrodes. Sample and hold voltage followers A5 and A6 can either subtract the value of the applied potentials [*i.e.* $\pm E + (-iR_f) - (\pm E) = -iR_f$], or zero the output to A7, A8 and A9 subtracting the total output signals from A3 and A4, thus reducing high background currents at the chart recorder. A1, A3 and A4 = OPA 100, A2 = LM11, A5 and A6 = OPA 103, A7, A8 and A9 = OP 07. R = 10K and all op-amps have their respective trimmer controls.

column" (5 cm × 4 mm, 10 μm C_8 ODS material; Whatman). This arrangement permits small volume injections onto either column but will direct larger volume injections solely to the pre-concentration column thereby minimising contamination of the analytical column by the seawater samples. All solvents were, in addition, passed through two conditioned "guard" columns (C_{18} material) immediately after the pump stage to reduce the effects of residual trace contaminants in either of the two phases, and to stabilise the flow to the detector.

Detectors. The performance of two different types of electrochemical detector were compared in this study. The first was a thin-layer, dual, glassy-carbon electrode cell constructed in-house (Kel-F electrode block, stainless-steel counter electrode and Ag/AgCl/0.1 *M* LiCl reference electrode) and the second was a dual porous electrode system (Coulochem Model 5010 electrode assembly, 5100A voltage controller and 5020 guard cell: ESA Associates, Bedford, MA, U.S.A.). The potential on each thin-layer electrode could be independently controlled in the range (±)2.0 V by an in-house constructed bipotentiostat (Fig. 2). With the addition of a function generator the bipotentiostat could also be used to apply ramp voltages to both electrodes for the generation of stopped-flow d.c. cyclic voltammograms in the diagnostic interpretation of LC–ED peak data. Both cell assemblies were fully enclosed in earthed Faraday cages and all cables were properly shielded in order to minimise extraneous electrical noise pick-up.

RESULTS AND DISCUSSION

Electrochemistry

Preliminary cyclic linear scan voltammograms of the four compounds under

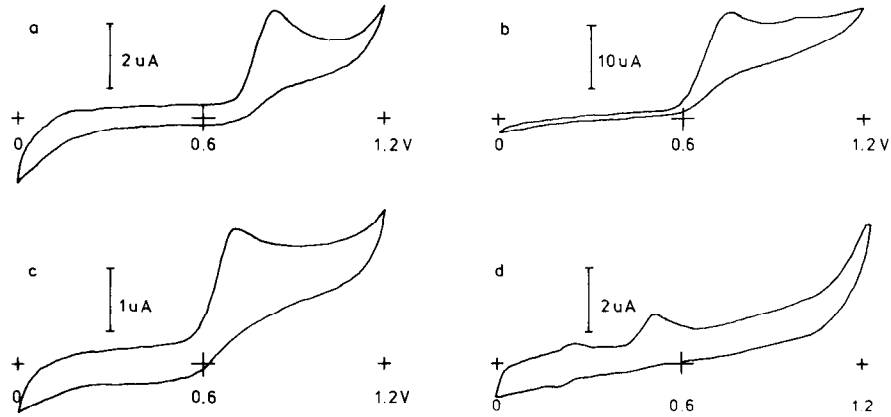

Fig. 3. Cyclic linear scan voltammograms of (a) aniline, (b) methylaniline, (c) 1-naphthylamine, (d) diphenylamine. Conditions: methanol–sodium acetate buffer (pH 5.6) (60:40), scan rate 50 mV/s.

investigation showed that they all undergo oxidation at a stationary glassy carbon electrode in the mobile phase used in the chromatography (Fig. 3). Each compound showed a well defined anodic oxidation step with no corresponding wave in the reverse, cathodic direction. Diphenylamine (Fig. 3d) exhibits two further waves which appear on anodic scans subsequent to the initial cycle which indicated the existence of electroactive oxidation products. This result is consistent with the reaction mechanism involving the removal of two electrons followed by the chemical coupling of the diphenylamine products at high positive potentials to form a benzidine-like molecule[16].

Whilst the oxidation mechanism for each compound clearly involves the removal of electrons from the nitrogen moiety, followed by quasi-reversible or irreversible chemical follow-up reactions, the ease of oxidation of the individual com-

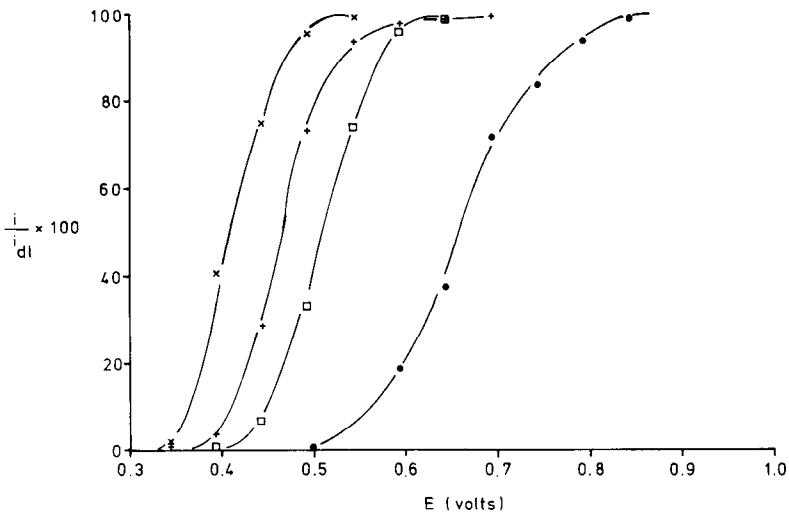

Fig. 4. Hydrodynamic voltammograms of aniline (●), methylaniline (□) naphthylamine (+) and diphenylamine (×) plotted as normalised peak height against applied potential.

TABLE I

ELECTROANALYTICAL DATA FOR THE FOUR AROMATIC AMINES OBTAINED USING HYDRODYNAMIC VOLTAMMETRY

Compound	$E_{1/2}$ *(mV)*	*n*
Aniline	665	0.58
Methylaniline	520	0.90
Naphthylamine	459	0.94
Diphenylamine	413	0.95

pounds under the given conditions varies considerably. This point is illustrated by the cyclic voltammograms shown in Fig. 3, where the peak potentials (taken as a general measure of the difficulty of oxidation) increase from 450 to 750 mV in the order diphenylamine, naphthylamine, methylaniline, aniline. If required, it would therefore be possible to discriminate in favour of the detection of the more easily oxidisable compounds by maintaining a lower electrolysis potential (*i.e.* 500–550 mV) than would be necessary for the simultaneous detection of all four.

Hydrodynamic voltammetry supported the results obtained by cyclic voltammetry. In addition, it indicated that the oxidation of aniline proceeds as a two-stage process with $E_{1/2}$ values of 665 and 940 mV respectively. Analysis of the data according to the Nernst equation,

$$E = E_{1/2} + Q \ln \frac{i_{dl} - i}{i}$$

where $E_{1/2}$ is the standard electrode reaction potential for the electrochemical system, and $Q = RT/nF$ where R, T, n and F have their normal electrochemical meanings, and i_{dl} is the diffusion-limited plateau value of the current, i, again shows the same trend in oxidation potentials (Table I). The reactions of aniline are consistent with a "chain polymerisation" involving radical ions to form "aniline black". The formation of aniline black was experimentally observed at the electrode surface in static conditions when relatively concentrated solutions were subjected to high and continued anodic potentials. Methylaniline, naphthylamine and diphenylamine oxidation mechanisms under the same conditions are thought to be consistent with the hypothetical oxidation pathways for aromatic amines.

The hydrodynamic voltammograms of the four compounds measured using the thin-layer cell have the same positional relationship but with the voltage axis displaced anodically by approximately 220 mV from those obtained with the porous electrode system due to the potential difference between the standard Ag/AgCl/LiCl reference electrode and that fitted in the Coulochem system (of unknown identity).

Chromatography

LC–ED without pre-concentration. Initial chromatographic experiments with standard 1 ppm mixtures indicated that adequate resolution of the four compounds could be achieved using methanol–0.1 *M* sodium acetate buffer (60:40). The use of different organic modifiers (for instance, 1–5% of dimethylsulphoxide, acetone or

isopropanol) made only minor differences to the peak currents, but sometimes substantial differences to the background currents (the addition of 5% acetone, for example, doubled the baseline current).

A 20-μl injection of spiked seawater containing 100 pmoles of each compound (Fig. 5) highlighted two major problems associated with the direct injection of such a sample onto a reversed-phase column. Firstly, a severe baseline disturbance occurred immediately after the solvent peak which limited the maximum amplification of the signal that could be used. This effect was attributed to a disturbance of the charge equilibrium of the electrode surface charged sites resulting from the high ionic strength, and initially polar, seawater sample matrix. This effect is prominent because of the high current sensitivities used for this trace analysis. It was of particular significance because of the length of time the electrode took to recover before the emergence of the first target compound, aniline. The second notable effect was the obvious presence of an unresolved baseline "hump". This feature was found to vary somewhat between samples of different environmental origin but was always present as a limiting factor in sensitivity and ultimate performance of the technique. It is presumably due to the residual background concentration of marine organic material, since the effect disappears when samples of the compounds are diluted in distilled water. Using direct injections, and having regard to the baseline interferences, on-column detection limits of approximately 30 pmoles for each amine were obtained for the porous electrode assembly, and approximately 3 pmoles for the thin-layer cell (equivalent to bulk concentrations of 1.5 μM and 0.15 μM respectively). As the anticipated concentrations of these compounds in seawater are in the low nm range, the necessity of a concentration step was clear.

LC–ED with pre-concentration. The direct in-line pre-concentration of large volume samples was achieved by passing 1–2 ml of seawater through the preconcentration column and subsequently back-flushing it onto the main analytical column with the primary mobile phase. Chromatograms of seawater spiked with the four compounds again exhibited substantial baseline deflections resulting from the simultaneous accumulation of the background marine organic material. So as to try to reduce the extent of this interference, experiments were performed whereby a series of secondary mobile phases of increasing methanol concentration were passed through the pre-concentration column after the accumulation of the sample and prior to its transfer to the analytical column. This removes the more polar components of the natural organic fraction from the pre-concentration column and in addition, reduces transference of any interfering components of the original water sample onto the main analytical column that might otherwise lead to poor reproducibilities[14]. The results indicated that the optimum recovery of each compound could be obtained with a maximum methanol concentration of approximately 40–45% without significantly interfering with the chromatography of the compounds of interest. The use of this procedure allowed good linearity of response to be observed over the range of injection volumes of 10 μl to 2 ml, and for concentrations ranging from 1.5 nM to 1 μM. Due to slight changes in chromatographic behaviour as column loadings increase peak area is a more reliable measure for quantitation than peak height if samples with widely different concentrations are to be compared.

Under these conditions, sample volumes of 1–2 ml could be injected without loss of resolution or sensitivity (Fig. 6). On-column detection limits of 30 and 3

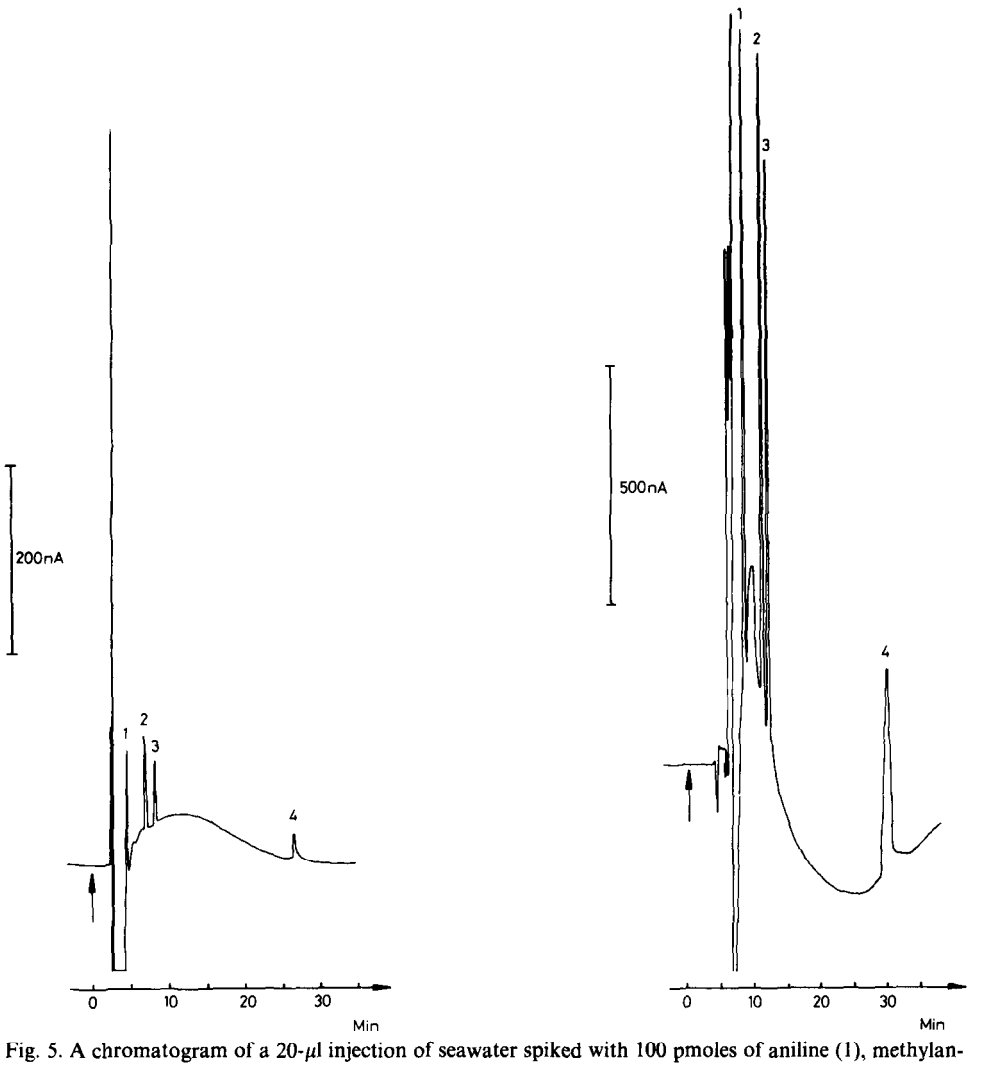

Fig. 5. A chromatogram of a 20-μl injection of seawater spiked with 100 pmoles of aniline (1), methylaniline (2), 1-naphthylamine (3) and diphenylamine (4).

Fig. 6. A chromatogram of a spiked seawater sample injection (1 ml; 100 pmoles each compound) after pre-concentration. Peak identities as for Fig. 5.

pmoles were observed for the porous and thin-layer cells respectively. However, taking account of the pre-concentration stage, these correspond to actual seawater concentrations of 15 and 1.5 n*M* for each compound. It is interesting to note that these injection volumes are approximately an order of magnitude less than similar published procedures for nitro- and chlorosubstituted aromatics, yet with slightly better detection limits[14,15]. At the highest sensitivities utilized this work, problems of baseline drift were apparent but could be adequately compensated by judicious use of the auto-zero feature of both instruments.

Thin-layer versus coulometric detection. It is noteworthy that operational difficulties restricted what would otherwise appear to be the optimum combination of preconcentration with coulometric detection. Whilst the electrolysis efficiency of the porous electrode assembly is approximately one order of magnitude higher than that of the thin-layer cell, the background current due to the 100% electrolysis of the mobile phase is similarly increased. At extreme sensitivities in particular, problems of noise and baseline drift are more acute than with the thin layer. As a result, the better signal-to-noise characteristics of the thin layer system permit lower detection limits to be obtained. However, in both cases, practical working detection limits were about one order of magnitude worse than the theoretical detection limits because of the problems of baseline drift at the highest sensitivities which necessitate frequent re-zeroing.

The multi-electrode detector option. Both detectors used in this study were dual electrode systems and therefore had the capability of operating with each working electrode held at different oxidation potentials. Whilst this has been shown to be of use in, for example, discriminating between easily oxidised and less easily oxidised compounds, or alternatively in reducing background currents by operating in differential mode, there was no distinct advantage in using two electrodes for these aromatic amines at trace levels. The range of the oxidation potentials for aniline, methyl aniline, naphthylamine and diphenylamine is so great that no useful discrimination could be achieved by operating the first electrode at a much lower potential than the second one. Whilst operating in differential mode was practicable, it was found experimentally that a better signal-to-noise ratio could generally be obtained by using the autozero function with only one electrode. Nevertheless, differential operation would be useful in dual electrode instruments without such a feature.

CONCLUSIONS

LC–ED has been shown to be a satisfasctory technique for the determination of the four aromatic amines aniline, methyl aniline, 1-naphthylamine and diphenylamine at trace levels in seawater. Detection limits of 15 and 1.5 n*M* were obtained using coulometric and amperometric electrochemical cells respectively when using an in-line pre-concentration step. These results compare favourably with previously published work. The experimental procedure combines the advantages of simplicity and ease of use with the exacting demands of sensitivity, selectivity and speed, and avoids many potential contamination problems associated with manual procedures. A consideration of various parameters relating to signal recovery at trace levels suggests that the thin-layer cell configuration is more appropriate for such applications.

Whilst it is accepted that the range of compounds available for detection by LC–ED is limited to those compounds that are electroactive the technique should be extendable to a variety of other compounds of environmental interest.

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