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Note

Electrochemical detection in high-performance liquid chromatographic analysis of aromatic amines

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Aromatic amines may enter the environment from a variety of sources: directly as industrial discharges, indirectly from pesticide degradation or from bacterial conversion of azo-compounds into the precursor amines. Many of these compounds are highly toxic, carcinogenic and/or mutagenic; it is known that benzidine, β -naphthylamine and 4-biphenylamine are responsible for most of the industrial bladder cancers^{1,2}. As they can be found in ambient air and water, efficient and rapid analytical procedures for these compounds are necessary.

The polar nature of aromatic amines and their low vapour pressures make difficult their analysis by gas chromatography because the amino groups may be adsorbed on the chromatographic support resulting in severe tailing or losses of components. It is therefore usually necessary to derivatize the amines which generally requires an anhydrous medium. One of us³ has recently reported a N-permethylation reaction in an aqueous solvent.

The advent of high-performance liquid chromatography (HPLC) and particularly the use of bonded phases in reversed-phase chromatography has permitted the development of various sensitive analytical procedures for the determination of aromatic amines in water without derivatization^{4–6}.

The aromatic amines are easily oxidized electrochemically and the analysis of some amines by use of an electrochemical detector (EICD) has been reported^{7–11}.

As an extension of our recent work¹² on the HPLC of hydroxy-aromatic compounds using EICD, we have studied the behaviour of a large number of aromatic amines of environmental interest with such a detector, emphasizing its selectivity properties.

EXPERIMENTAL

The equipment consisted of a Hewlett-Packard 1010 A liquid iso-chromatograph, a Rheodyne rotary injection valve with 20- μ l loop and a Partisil 5/25-ODS-3 Whatman steel column (250 \times 4 mm). The EICD was a Metrohm Model 656 equipped with a three-electrode detection cell, Model EA 1096/2, with an internal volume of *ca.* 1.3 μ l. A Metrohm VA 641 potentiostat was used and the detector output was displayed on a Houston Omniscrite recorder or on a C-RIA Shimadzu data processor.

The mobile phase consisted of water (triply distilled)–methanol (HPLC grade,

Carlo Erba) (1:1) containing 1 g/l LiClO_4 as supporting electrolyte. The aromatic amine standards were reagent grade and used without further purification.

The working electrode was polished daily using alumina powder (0.3 μm).

RESULTS AND DISCUSSION

Table I shows the elution order of the studied compounds; no further attempts (change of pH or eluent) to optimize the separation conditions were made because our interest was focused on the performance of the EICD.

TABLE I
CAPACITY FACTORS OF THE AROMATIC AMINES

For conditions, see text.

Compound		Capacity factor, k'
I	<i>m</i> -Phenylenediamine	0.05
II	<i>p</i> -Phenylenediamine	0.05
III	<i>o</i> -Phenylenediamine	0.28
IV	<i>p</i> -Anisidine	0.48
V	Aniline	0.76
VI	<i>m</i> -Anisidine	0.78
VII	Benzidine	0.86
VIII	<i>p</i> -Phenetidine	0.88
IX	<i>o</i> -Anisidine	1.20
X	<i>p</i> -Nitroaniline	1.31
XI	<i>p</i> -Toluidine	1.33
XII	<i>m</i> -Toluidine	1.38
XIII	<i>o</i> -Toluidine	1.67
XIV	<i>m</i> -Phenetidine	1.69
XV	Di(4-aminophenyl)methane	1.74
XVI	<i>o</i> -Chloroaniline	2.81
XVII	β -Naphthylamine	3.64
XVIII	α -Naphthylamine	3.81
XIX	1,8-Diaminonaphthalene	4.62
XX	N,N-Dimethylaniline	5.95
XXI	4-Aminodiphenyl	8.20

Figs. 1–5 illustrate the responses of the 21 aromatic amines as functions of the applied potential. The values of the responses depend on the anode conditions (age, polishing, previous history) and can vary somewhat between different experiments. This does not invalidate the “relative” response for a series of amines. By comparison of the various curves it is therefore possible to draw some conclusions of practical interest.

From Fig. 6, which compares the responses of a series of simple mono- and diamines without other functional groups, it is seen that the aromatic diamines have a significantly lower oxidation potential. This can be usefully exploited to “tune” in or out the monoamines. Fig. 7 shows chromatograms of the same series of non-substituted compounds at +1.10 and +0.4 V respectively. At the lower potential only the aromatic diamines are still detectable.

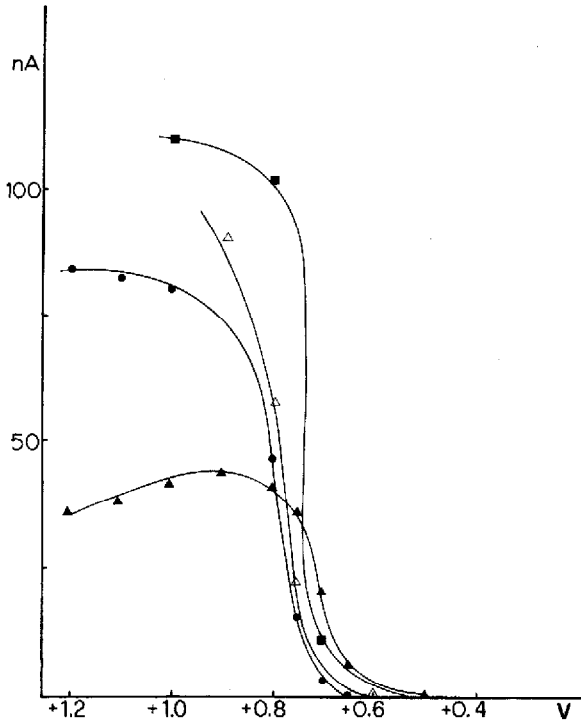


Fig. 1. Relationship between EICD response and applied potential (V versus Ag/AgCl) for 200 pmol of amine. Amines: ■, *m*-toluidine; ▲, *p*-toluidine; △, *o*-toluidine; ●, aniline.

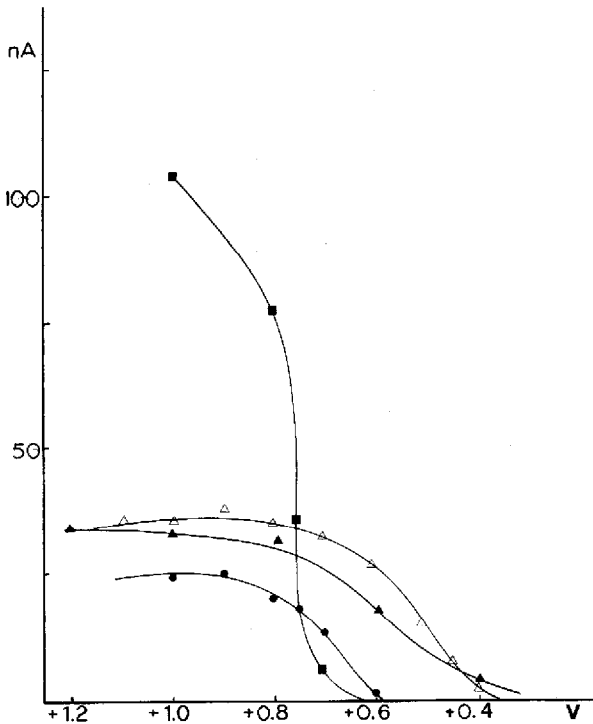


Fig. 2. As Fig. 1, except for amines: △, *o*-anisidine; ■, *m*-anisidine; ▲, *p*-anisidine; ●, N,N-dimethylaniline.

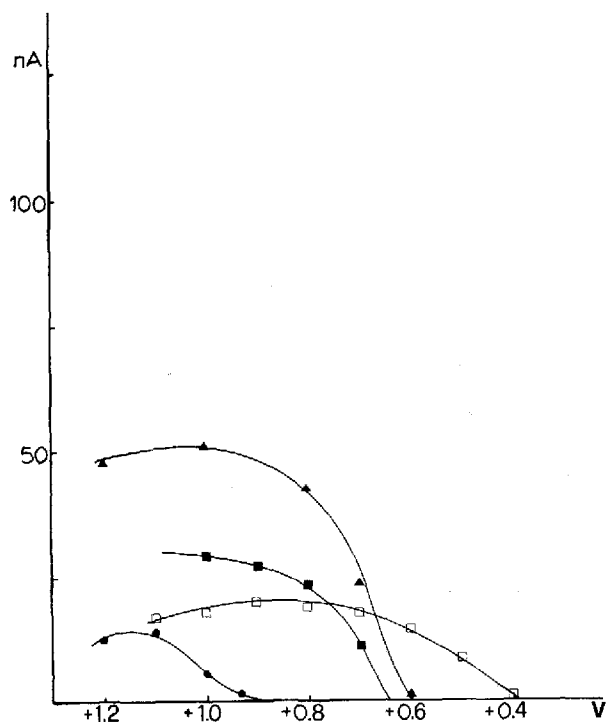


Fig. 3. As in Fig. 1, except for amines: ■, *m*-phenetidine; □, *p*-phenetidine; ▲, *o*-chloroaniline; ●, *p*-nitroaniline.

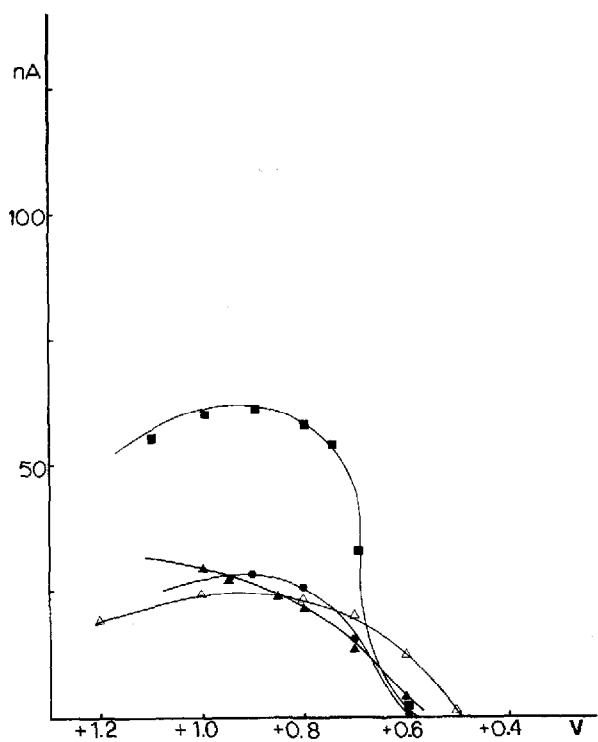


Fig. 4. As in Fig. 1, except amines: △, α -naphthylamine; ●, 4-aminodiphenyl; ▲, di(4-aminophenyl)methane; ■, β -naphthylamine.

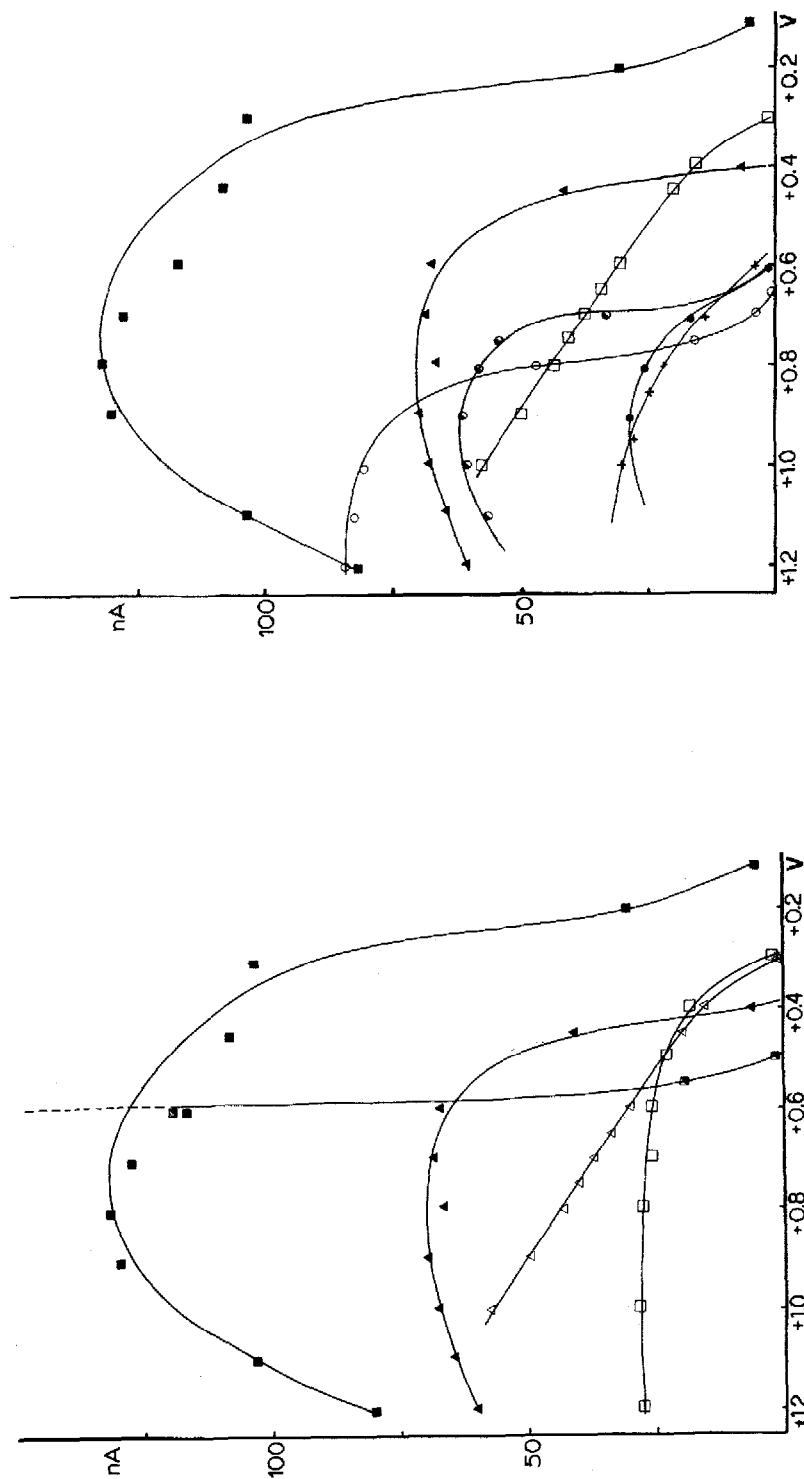


Fig. 5. As in Fig. 1, except amines: \square , *o*-phenylenediamine; \blacksquare , *m*-phenylenediamine; \blacktriangle , *p*-phenylenediamine; \blacktriangle , benzidine; \blacksquare , 1,8-diaminonaphthalene.

Fig. 6. Comparison between the EICD responses for simple mono- and di-amines at various potentials. Amines: \circ , aniline; \blacktriangle , benzidine; \blacksquare , *p*-phenylenediamine; \bullet , 4-aminodiphenyl; $+$, di(4-aminophenyl)methane; \circ , β -naphthylamine; \square , 1,8-diaminonaphthalene.

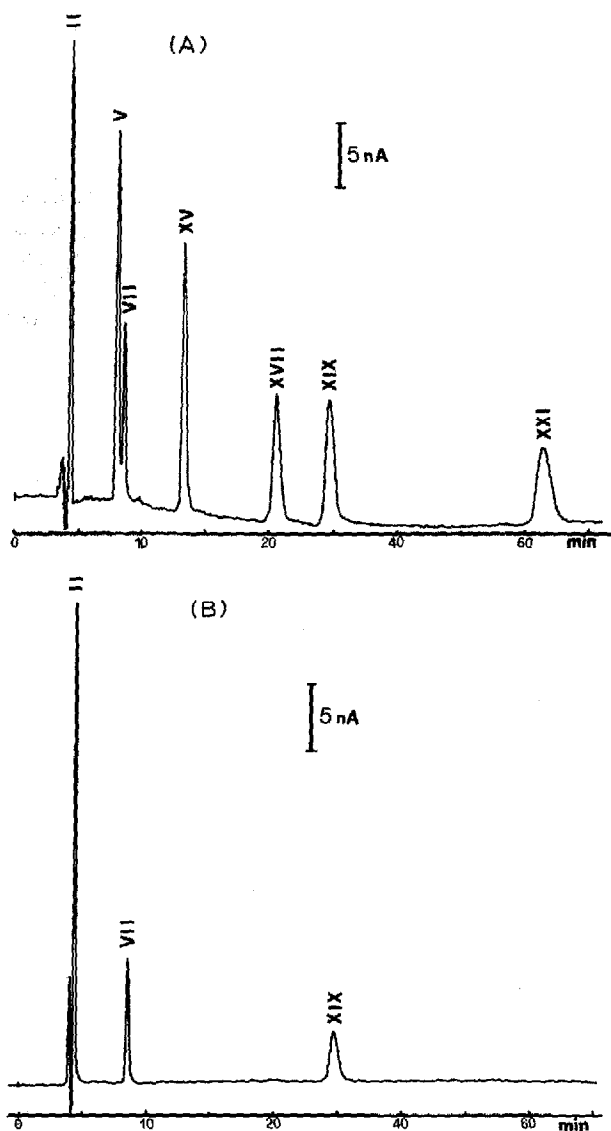


Fig. 7. Chromatograms of a mixture of non-substituted aromatic amines at potentials of +1.10 V (A) and +0.4 V (B). Separation conditions as in the experimental section. Sample injected: 20 μl of 10^{-5} M solution. For identification of the peaks, see Table I.

Another significant application is shown in Fig. 8. Under our experimental conditions, aniline and benzidine are nearly completely separated but in a 1000-fold greater concentration of aniline the carcinogenic benzidine is practically undetectable at a potential of +1.00 V because of the high response and concentration of aniline; on the other hand, at a potential of +0.45 V the response of aniline is practically negligible whereas the peak of benzidine is still perfectly detectable.

Fig. 9 shows calibration curves for five of the well known toxic or carcinogenic amines; in the examined range of concentration all give a linear response.

The limit of detection, based on the quantity required to give a response twice the noise level, is in the range of 0.1 ng, obviously depending on the applied voltage as

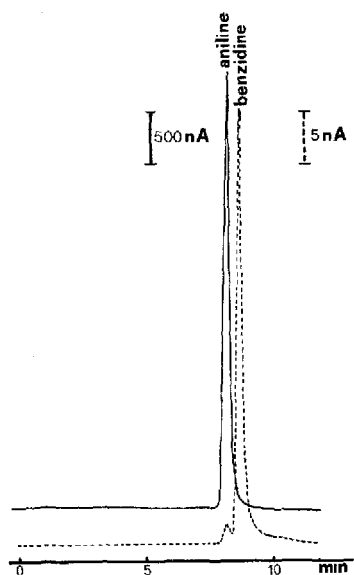


Fig. 8. Chromatograms of a mixture of 10^{-3} M aniline and 10^{-6} M benzidine. Electrode potential: +1.0 V (—); +0.45 V (---).

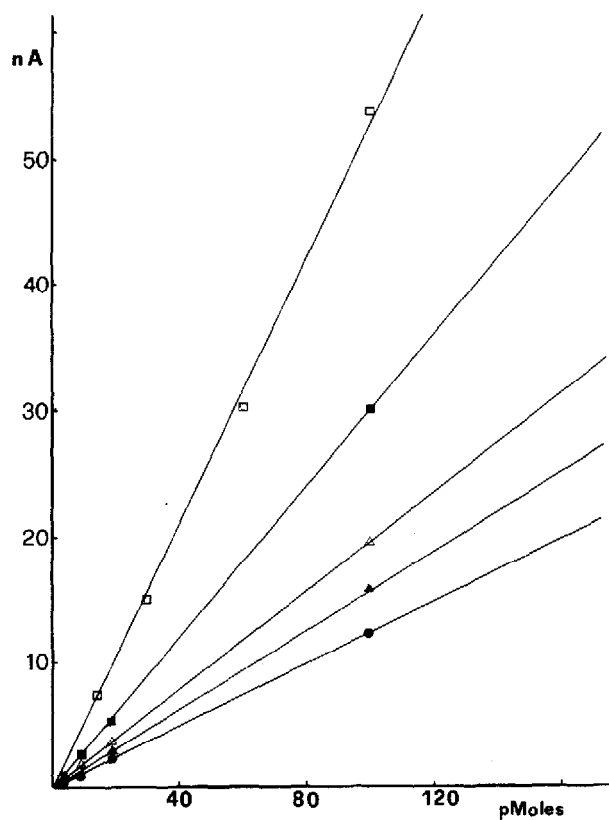


Fig. 9. Calibration curves at a potential of +0.88 V. Amines: \square , *p*-phenylenediamine; \blacksquare , benzidine; \triangle , β -naphthylamine; \blacktriangle , *o*-phenylenediamine; \bullet , 4-aminodiphenyl.

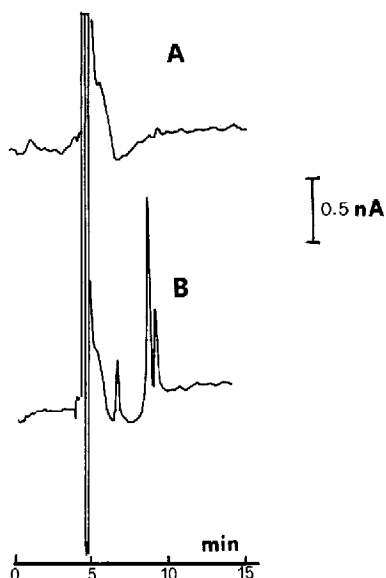


Fig. 10. Chromatograms of Bologna drinking water, before (A) and after (B) spiking with $5 \cdot 10^{-7} M$ of *o*-phenylenediamine, $10^{-7} M$ aniline and $10^{-7} M$ benzidine. Sample injected: $20 \mu\text{l}$. Electrode potential: $+1.0 \text{ V}$.

above and on other not optimized factors such as the eluent and flow-rate.

Fig. 10 shows a chromatogram of Bologna tap-water and the same water fortified with some amines. Apart from peaks due to the solvent which disturb the determination of amines with low capacity factors (*p*- and *m*-phenylenediamine), it is clearly possible to evaluate quantities of the order of 1 pmol.

The possibility of direct analysis of such compounds without preliminary clean-up or derivatization procedures indicates the suitability of electrochemical detection in the trace analysis of easily oxidizable compounds.

ACKNOWLEDGEMENT

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