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High-performance liquid chromatographic analysis with electrochemical detection for residues of explosives in water samples around a former ammunition plant

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Abstract

Electrochemical detection (ED) in the oxidative and reductive mode was applied to the analysis of nitroaromatics, nitramines, aminoaromatics and nitrophenols in groundwater samples from the surroundings of a former ammunition plant. Settings for working potential and eluent composition were optimized in terms of detection limit, linearity and selectivity. The results were compared with those obtained by UV detection and the advantages of combined UV–ED detection for the analysis of complex samples are shown.

Keywords: Water analysis; Detectors, LC; Explosives; Nitroaromatic compounds; Nitramines; Aminoaromatic compounds; Nitrophenols

1. Introduction

During the last few years the analysis of soil and water samples from the drinking water supply area nearby former ammunition plants has become of increased importance [1–4]. Even today, soil and groundwater in these areas are severely contaminated by toxic and cancerogenic explosives, their by-products and metabolites. Analysis of the groundwater around the former ammunition plant at Elsnig in Saxony, Germany is especially important because of its vicinity to a protected drinking water area. The contamination of this water is supposedly very high, not only because of the large production capacity of the former plant of 3000 tons on a monthly basis (unique in Germany also because of its extremely wide manufacturing range), but also because there used to be a filling station for explosives (up to 4050 tons per month) during World War II and, after 1945, an open-air ammunition incineration place [5].

First investigations with HPLC and GC showed a large number of pollutants of various classes of compounds such as nitroaromatics, nitroaminoaromatics, nitramines, nitrophenols, nitrobenzoic acids, chlorobenzenes and nitrochlorobenzenes in a wide concentration range (from ng/l up to mg/l) [6–9]. This led to separation problems and possible errors in HPLC analysis in spite of sample preseparation into a neutral/basic and an acidic fraction by extraction at pH 9 and pH 2. When analysing such complex environmental samples by HPLC it is, therefore, preferable to use methods which provide both a sensitive and a highly selective detection.

The objective of the work described here was to

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test the efficiency of electrochemical detection (ED) for this problem in addition to UV detection. ED conditions were optimized using standard samples, and the efficiency of combined UV–ED detection for the analysis of environmental samples in the oxidative and reductive mode was tested.

2. Experimental

2.1. Apparatus

The measurements were carried out with a Hewlett-Packard (Waldbronn, Germany) series HPLC system including a quaternary pump (1050), a Rheodyne Model 7125 injector with a 20-μl sample loop, a variable-wavelength detector (1050) operated at 254 nm and an electrochemical detector (1049 A) coupled in series with the UV detector. A glassy carbon electrode was used as working electrode and an Ag/AgCl electrode as reference electrode. Oxidative detection was permitted in a potential range from +0.4 V to +1.4 V and reductive detection at working potentials from −0.2 V to −1.4 V. Two HP 3395 integrators were used for data acquisition.

2.2. Chromatographic conditions

Separations were performed on a column (250×4 mm I.D.) packed with 5 μm Eurospher RP-18 (Knauer, Berlin, Germany) at a flow-rate of 1.0 ml/min at 27°C.

The mobile phase consisted of methanol–aqueous buffer (51:49, v/v); buffers used were either (a) phosphate buffer (0.01 *M* sodium dihydrogenphosphate solution adjusted to pH 3 with phosphoric acid), (b) Mc Ilovaine buffer (0.01 *M* citric acid and 0.02 *M* disodium hydrogenphosphate, pH 5.0), or (c) sodium perchlorate solution (0.01 *M*, pH 5.5). The reported pH values are those of the aqueous solution before mixing with methanol. The HPLC-grade methanol, sodium dihydrogenphosphate, citric acid, disodium hydrogenphosphate, sodium perchlorate and phosphoric acid (all of the highest purity available) were purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q purification system (Millipore, Milford, MA, USA).

2.3. Sample preparation

Standard compounds (with the exception of 3,5-dinitrophenol) were obtained either from Promochem (Wesel, Germany), Merck (Darmstadt, Germany), Aldrich (Milwaukee, WI, USA) or Fluka (Buchs, Switzerland) in purities between 95% and 99%. 3,5-Dinitrophenol was prepared by the method of Hantsch [10]. Solutions of the standard mixtures were prepared by dissolving reference compounds in high-purity methanol or methanol–water (1:1), respectively.

Water samples were taken from a borehole (depths 25–27 m) in the area of the former ammunition plant at Elsnig. Samples were filled into 2.5-l brown glass bottles. If the samples were not analysed immediately 0.5 g/l sodium azide was added (according to Ref. [11]) and the sample was stored at 4°C for up to 1 month.

For sample preparation 0.5 l of the water was extracted three times with 20 ml dichloromethane at pH 9 for 30 min, giving the 'neutral/basic fraction'. In a second step the aqueous phase was extracted continuously with 150 ml dichloromethane at pH 2 for 20 h in a heavy-phase rotary perforator (Normag, Hofheim, Germany), giving the 'acidic fraction'. The solvents were evaporated and the residue filled up to 1.0 ml with the HPLC eluent.

3. Results and discussion

3.1. Oxidative electrochemical detection

The applicability of this detection mode was tested for sixteen nitrophenols and six nitroaminoaromatics, which are listed together with the used abbreviations in Table 1.

Among the three buffer solutions mentioned above the phosphate buffer was chosen, as it provided the lowest background currents and the best signal-to-noise ratio at all potentials. To determine the optimum and selective working potentials, hydrodynamic voltammograms were detected by repeated injection at increasing potential from +0.4 V to +1.4 V in steps of 100 mV using the autoincrement mode. Half-wave potentials ($E_{1/2}$) and optimum detection potentials are given in Table 1. Fig. 1

Table 1
Detection limits for ED and UV detection, half-wave potentials ($E_{1/2}$) and optimum detection potentials (E_{opt}) in the oxidative mode

Compound	Abbreviation	Detection limits (ng/ml)		$E_{1/2}$ (V)	E_{opt} (V)
		ED (+1.2 V)	UV (254 nm)		
2-Amino-4,6-dinitrotoluene	2-A-4,6-DNT	10	25	1.05	1.30
4-Amino-2,6-dinitrotoluene	4-A-2,6-DNT	10	25	1.05	1.30
2-Amino-3-nitrotoluene	2-A-3-NT	5	30	0.95	1.25
2-Amino-4-nitrotoluene	2-A-5-NT	5	15	0.95	1.20
2-Amino-6-dinitrotoluene	2-A-6-NT	5	15	0.95	1.20
2,6-Dinitrophenol	2,6-DNP	4	6	1.05	1.25
2,4-Dinitrophenol	2,4-DNP	7	7	1.00	1.20
3,4-Dinitrophenol	3,4-DNP	8	10	0.95	1.20
3,5-Dinitrophenol	3,5-DNP	5	5	0.95	1.10
2,2′,4,4′,6,6′-Hexanitrodiphenylamine	Hexyl	6	25	>1.40	1.40
4-Methyl-2,6-dinitrophenol	4-M-2,6-DNP	7	6	1.00	1.20
2-Methyl-4,6-dinitrophenol	2-M-4,6-DNP	7	5	1.05	1.20
2-Methyl-3-nitrophenol	2-M-3-NP	11	10	0.65	0.80
3-Methyl-2-nitrophenol	3-M-2-NP	10	25	0.65	0.80
3-Methyl-4-nitrophenol	3-M-4-NP	5	20	0.90	1.25
4-Methyl-2-nitrophenol	4-M-2-NP	9	10	0.85	1.15
4-Methyl-3-nitrophenol	4-M-3-NP	5	25	0.65	0.80
5-Methyl-2-nitrophenol	5-M-2-NP	12	8	0.85	1.15
2-Nitrophenol	2-NP	3	8	0.90	1.20
3-Nitrophenol	3-NP	8	12	0.80	1.00
4-Nitrophenol	4-NP	5	10	0.95	1.20
2,4,6-Trinitrophenol	PA	25	6	>1.40	1.40

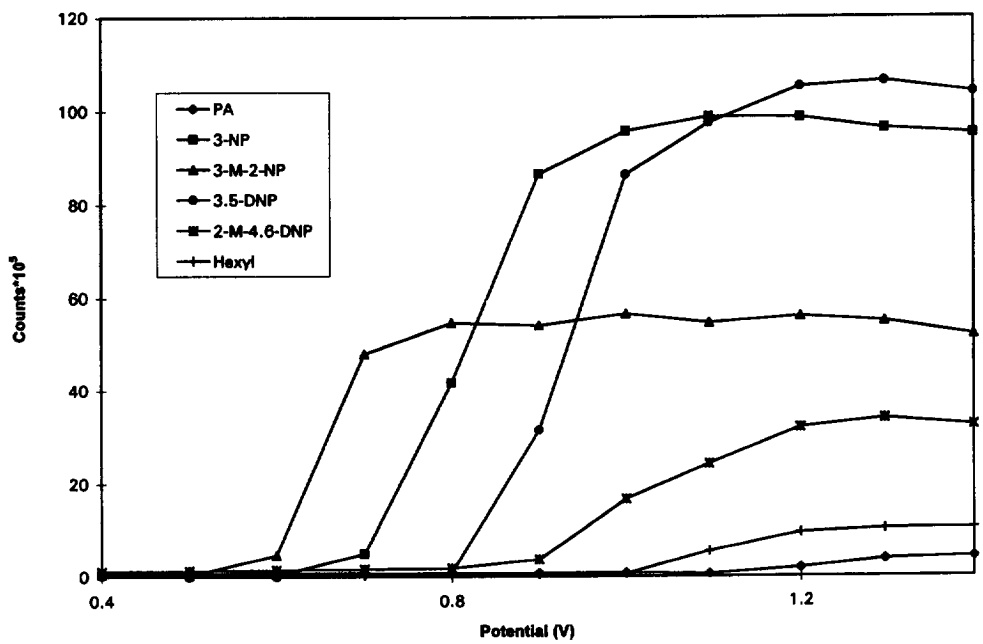

Fig. 1. Hydrodynamic voltammograms for selected nitrophenols in the oxidation mode.

shows hydrodynamic voltammograms for six selected compounds.

The half-wave potentials ($E_{1/2}$) and the responses of the nitrophenols and the nitroanilines largely depend on the type and position of additional substituents and reflect the electronic effects of these substituents. For instance, the $E_{1/2}$ of the phenols increase in the following order:

methylnitro-<nitro- ($m<o<p$)<dinitro-≤ methyldinitro-<trinitrophenol.

Thus, 2-methyl-3-nitrophenol, 3-methyl-2-nitrophenol and 4-methyl-3-nitrophenol can be detected selectively at potentials of <+0.7 V and the mononitrophenols together with the monomethylphenols at a potential of about +0.8 V. Most of the compounds can be detected simultaneously at +1.2 V with a relatively good signal-to-noise ratio.

The linearity of the detector was checked from 0.1 to 100 μg/ml at different potentials. Good linearity was observed in each case.

A comparison of the detection limits of the ED (at +1.2 V) and the UV detector is also summarized in Table 1.

The reproducibility was tested by five injections at two different concentrations. The relative standard deviations obtained for the ED (average 3% at 2.5 μg/ml and 8% at 0.1 μg/ml) were somewhat higher than for UV detection (average 1% at 2.5 μg/ml and 5% at 0.1 μg/ml). To sustain the detector sensitivity, the electrode was cleaned electrochemically by cyclic change of the potential from −1.2 to +1.5 V (10 cycles, 50 mV/s). This was done every 3 days when analysing standard mixtures and daily when real samples were investigated.

Fig. 2 and Fig. 3 show ED and UV chromatograms of the neutral/basic and the acidic fraction of a chosen groundwater sample from Elsnig at +1.2 V and 254 nm. These chromatograms reflect the complexity of this sample. Nitroaromatics, nitroaminoaromatics and nitramines are the main components in the neutral/basic fraction being generally common contaminants for aquifer water from former ammunition production sites [3,4,6,8]. The origin of the chloro- and the chloronitrobenzenes can still not be explained completely. It may be that these compounds are intermediates or byproducts of explosives or they were used instead of toluene at the end of the war [9].

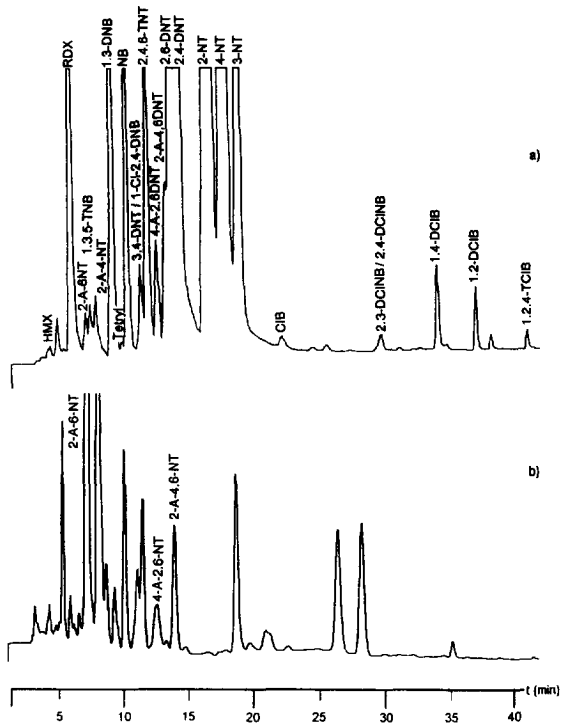

Fig. 2. Chromatograms of the neutral/basic fraction of a groundwater sample from Elsnig: (a) UV detection (254 nm); (b) electrochemical detection (+1.2 V).

The acidic fraction contains some nitrophenols, nitramines and 2,2′,4,4′,6,6′-hexanitrodiphenylamin (hexyl) already found in the water samples from Elsnig [4,7,9,12]. The occurrence of hexyl in this fraction is due to its acidic behaviour (pK_a=4.38 [13]) caused by the strong electron acceptor properties of the six nitro groups.

Additionally we found 3,5-dinitrophenol and 2-, 3- and 4-nitrobenzoic acid (NBA) as well as 2,4-dinitrobenzoic acid (DNBA) in this fraction. According to our knowledge these compounds were detected in water samples around Elsnig for the first time. The structures of these contaminants were unambiguously identified or confirmed by ^{1}H NMR spectroscopy [7]. The reliable determination of the nitrobenzoic acids by HPLC required the optimization of the chromatographic and the detection conditions, which is the object of our present work. The nitrophenols and the nitrobenzoic acids can be assumed as products of the oxidative degradation of

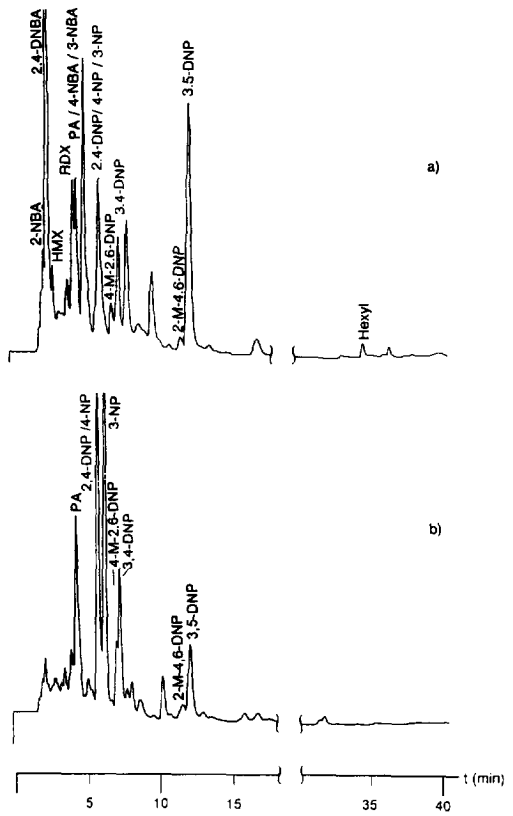

Fig. 3. Chromatograms of the acidic fraction of a groundwater sample from Elsnig: (a) UV detection (254 nm); (b) electrochemical detection (+1.2 V).

the nitro-, chloro- and aminoaromatics, respectively [3,14].

The chromatograms in Fig. 2 and 3 also illustrate that the ED, especially with the acidic fraction, provides some advantages, as it allows the selective detection of nitrophenols in the presence of the nitramines octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX, Octogen) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, Hexogen) and some nitrobenzoic acids. Furthermore, ED chromatograms show several unknown peaks, not detectable with the UV detector. The identification of these compounds is under investigation.

The results of the quantitative ED analysis in the oxidative mode for the acidic and the neutral/basic fraction of the groundwater sample, together with the results of HPLC–UV analysis for substances detectable with both detection principles are given in Table 2. For each of the substances except 3-methyl-4-nitrophenol, similar results were obtained. The higher detected amount of this compound was probably caused by an UV-active chromatographic co-elution. The values were also compared with the results obtained with ^{1}H NMR spectroscopy and GC-NPD (nitrogen–phosphorous detection) analysis [7,12]. As these results show, the ED is also suitable for quantitative analysis.

Table 2
Quantitative analysis of the neutral/basic and the acidic fraction of the groundwater sample for substances detectable with both ED and UV detection (μg/l)

Compounds	HPLC–ED (+1.2 V)	HPLC–UV (254 nm)
Neutral/basic fraction		
2-A-6-NT	21	18
2-A-4-NT	16	15
4-A-2,6-DNT	10	9
2-A-4,6-DNT	30	31
Acidic fraction		
Hexyl	–	13
2,4-DNP	38	20
3,4-DNP	23	27
3,5-DNP	179	168
2-M-4,6-DNP	16	16
4-M-2,6-DNP	25	27
3-M-4-NP	4	22
3-NP	52	54
4-NP	97	84

Because of peak overlapping results were obtained under two different chromatographic conditions: methanol–buffer 51:49 (v/v), and 60:40 (v/v).

3.2. Reductive electrochemical detection

For sixteen nitroaromatics and nitramines, hydrodynamic voltammograms were obtained by repeated injection at increasing potentials from −0.2 V to −1.4 V (100-mV step).

The half-wave potentials obtained and the optimum detection potentials in sodium perchlorate solution are given in Table 3. It shows that the nitroaromatics can be reduced in a wide potential range and the half-wave potentials increase in the following order:

trinitro-<-dinitro-<mononitroaromatics
<nitroanilines<nitramines.

That means that trinitroaromatic compounds can

Table 3
Half-wave potentials ($E_{1/2}$) and optimum detection potentials (E_{opt}) in the reductive mode

Compound	Abbreviation	$E_{1/2}$ (−V)	E_{opt} (−V)
2-Amino-4,6-dinitrotoluene	2-A-4,6-DNT	1.15	1.20
4-Amino-2,6-dinitrotoluene	4-A-2,6-DNT	1.15	1.20
2-Amino-4-dinitrotoluene	2-A-4-NT	1.15	1.20
2-Amino-6-dinitrotoluene	2-A-6-NT	1.15	1.20
1,3-Dinitrobenzene	1,3-DNB	0.80	1.00
2,4-Dinitrotoluene	2,4-DNT	1.00	1.15
2,6-Dinitrotoluene	2,6-DNT	1.00	1.15
3,4-Dinitrotoluene	3,4-DNT	0.85	1.00
2-Nitrotoluene	2-NT	1.15	1.20
3-Nitrotoluene	3-NT	1.10	1.20
4-Nitrotoluene	4-NT	1.10	1.20
N-Methyl-2,4,6,N-tetranitroaniline	Tetryl	0.77/0.92	1.00
1,3,5-Trinitrobenzene	1,3,5-TNB	0.62/0.80	0.90
2,4,6-Trinitrotoluene	2,4,6-TNT	0.70/0.87	1.00

be detected selectively in a potential range from −0.6 to −0.7 V, while dinitroaromatics together with trinitroaromatics are detected at −0.8 to −0.9 V. A signal for RDX appears only at potentials >−1.1 V. The best potential for the nonselective, simultaneous detection of all components is −1.2 V. This detection selectivity was similar for the McIlovaine buffer and the phosphate buffer.

Fig. 4 shows ED chromatograms of the neutral/basic fraction of the groundwater sample at different potentials.

The linearity of the detector was tested in the concentration range 0.1–100 μg/ml using different potentials and the three buffers. Good linearity was observed in each case.

In spite of good degassing of sample and mobile phase (helium degassing and additional use of vacuum degasser; Knauer) and the use of stainless-steel mobile-phase transfer lines [15], a high background current, a relatively high noise and a system peak at a short retention time were observed. Therefore, higher relative standard deviations (average 5% at 5 mg/ml) compared with UV detection (average 1%) and higher detection limits (5 to 10 times, because of the high background current, which required working in the nonsensitive 500 μA mode) were obtained.

Moreover, early eluting compounds such as HMX and RDX partly overlapped with the system peak.

The employment of a gold and a mercury film (Au/Hg) electrode, home-made according to Bratin et al. [15] and Maskarinec et al. [16], did not improve the sensitivity of detection.

However, lower detection limits were achieved by Lloyd [17] using a pendent mercury drop electrode (PMDE). A different way to solve this problem could be the use of electrochemical detectors with

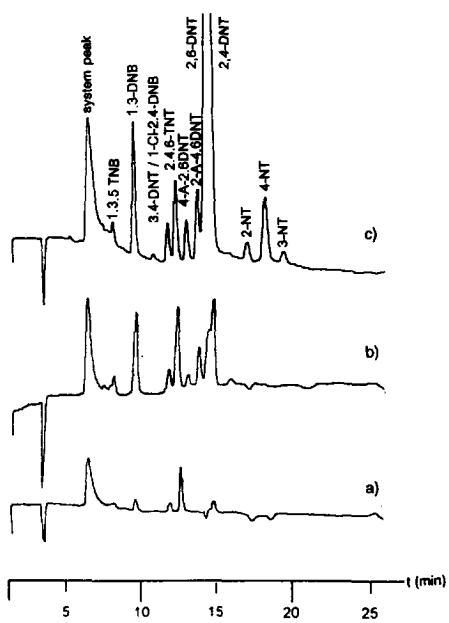

Fig. 4. ED chromatograms of the neutral/basic fraction of groundwater at different potentials: (a) −0.8 V; (b) −1.0 V; (c) −1.2 V.

modified cell design (for instance wall jet cells) or micro-electrodes.

4. Conclusions

The ED allows for selective and sensitive detection of substances with either amino or phenol groups in the oxidative mode and a selective detection of substances with a nitro group in the reductive mode. The sensitivity of the detection of nitroaromatics in the reductive mode is lower than with UV detection.

In both modes selectivity can be adapted to the analytical problem by changing the working potential.

Using both detectors in series, one single HPLC run yields more information in the investigation of complex environmental samples. In this way, co-elutions were found and additional pollutants detected, giving an improvement of reliability of the analytical results.

Acknowledgements

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