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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ORGANIC EXPLOSIVES COMPONENTS WITH ELECTROCHEMICAL DETECTION AT A PENDANT MERCURY DROP ELECTRODE

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SUMMARY

The pendant mercury drop electrode, in conjunction with a 3-μm particle size column packing, gives detection limits for fourteen nitrate and nitro compounds in the range 7–49 pg per 20 μl of injected sample. The linear range extends in excess of four orders of magnitude. These detection limits are approximately a ten-fold improvement on those reported for a mercury film electrode technique, and are comparable with the detection limits of electron capture detection in gas chromatography. The electrode characteristics are highly reproducible, the electrode may be renewed during or at the start of a chromatogram, and it is not subject to the contamination problems of the mercury film electrode.

INTRODUCTION

Organic explosives components, carrying nitrate or nitro substituents, may be determined by high-performance liquid chromatography (HPLC) with reductive mode electrochemical detection at glassy carbon or mercury film (on amalgamated gold) electrodes, as reported in a recent paper by Bratin *et al.*[1]. The limits of detection given are in the range 65–400 pg, hence the technique seems opposite to forensic science work. For the nitrate esters, which are a commonly encountered explosives type, the mercury film electrode (MFE) must be used because the reduction potential required (*ca.* -1.0 V *vs.* Ag/AgCl) extends beyond the useful range of glassy carbon. However, the characteristics of the MFE can vary considerably with time, according to usage[2], consequently in the analysis of some kinds of sample the electrode frequently must be dismantled and its surface renewed. Under such circumstances the use of a dropping mercury electrode is an obvious remedy. But the noise level originating in the formation and displacement of the mercury drops severely degrades detection limits. For instance, Dębowski *et al.*[3] recently report limits in the region of 10 ng for aromatic nitro compounds at a horizontal mercury dropping electrode. Other similar results are quoted in Stulik and Pacakova's review[4].

The present paper describes a pendant mercury drop electrode (PMDE) tech-

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nique that overcomes the foregoing problems, and, in conjunction with 3-μm particle HPLC columns, provides a level of sensitivity that is an order of magnitude better even than that of the MFE results. (PMDE is used in preference to HMDE, for a hanging mercury drop electrode, to avoid confusion with the horizontal mercury dropping electrode.)

EXPERIMENTAL

Materials

Solvents were HPLC grade (Rathburn Chemicals). Nitrogen was Oxygen-free grade (British Oxygen Co.) passed through an Analabs oxygen scrubber. The explosives components EGDN, HMX, NG, PETN, RDX, TET and TNT (full names are given in Table I) were kindly given by Dr. J. D. Twibell. The other nitro compounds (Table I), mercury and all other chemicals were Aristar grade (BDH) or otherwise the purest grade available commercially.

The aqueous potassium phosphate, 0.025 *M*, pH 3.0, was prepared from 11.5 g orthophosphoric acid (85%) dissolved in 4 l water. This solution was adjusted to pH 3.0 with potassium carbonate: the solid carbonate (*ca.* 6 g) was slowly added to the rapidly stirred solution.

Instrumentation

The Bioanalytical Systems transducer TL-6A, with a control unit made by Dr. P. Byrom, was used for the MFE experiments.

For the PMDE work and EG&G Princeton Applied Research Model 310 detector was used, together with their Model 174A polarographic analyzer. A modification was made to the detector that enabled an increased mercury drop size to be retained on the capillary: the distance between the capillary tip, which carries the mercury drop, and the eluent outlet jet was slightly reduced to 0.9 mm (a waste capillary was ground, with fine abrasive powder, further into its seating in the flowcell), and the jet orifice was distorted to a cup shape with an outlet diameter of 0.8 mm by means of a steel probe. As a result the mercury drop was compressed between the capillary tip and the orifice when, typically, a $\times 4$ "large" drop obtained with four pulses from the control unit was used. This represents a mass of mercury of 21.6 mg[5]. The mercury drop was usually changed at the start of each chromatogram, and could be changed during a chromatogram except at extreme sensitivity settings. It is important that the flowcell should be very firmly attached to the capillary otherwise operation of the unit's drop displacement knocker can alter the position of the capillary tip with respect to the orifice. In the present case PTFE sleeving was used to increase the tightness-of-fit of the flowcell onto the capillary. It is also important that the nitrogen purge outlet in the eluent receiver vessel that encloses the flowcell is extended to the bottom of the vessel to enable its contents (of the same composition as the eluent) to be thoroughly purged. Contrary to the manufacturer's literature[6], detection limits are improved considerably by this: perhaps with the distorted drop shape used here oxygen diffusion to the mercury surface more readily occurs. The Ag/AgCl reference electrode compartment was filled with aqueous lithium chloride, 5 *M*. For most of the work, when all of the explosives components of interest were to be detected together, the potential of the PMDE was maintained at -1.0 V (direct current mode).

Chromatography conditions

The eluent reservoir was fitted with a nitrogen purge, and its contents maintained under reflux continuously, to eliminate oxygen. All eluent lines were of 316 stainless steel. The eluent flow from the pump, an Altex Model 102, was taken from the pump purge outlet in order to leave off-line the pressure transducer and the pulse dampener, which appeared to be a source of electroactive material that produced a rising baseline, particularly with the thin film electrode. A dump valve, connected through 1 m of 2 mm I.D. tubing to effect further dampening, enabled the off-line part of the pump to be purged separately from the remainder.

The injector was a Rheodyne Inc. Model 7125 set with its rotor axis vertically to enable samples to be purged with nitrogen in the syringe used for their transfer, as previously described[7]. Injector loop volumes were generally 20 μl. Samples were made up in the eluent.

Most of the chromatograms were run on ODS-Hypersil, 3 μm, conventionally packed into 15 cm × 4.5 mm columns, with an eluent composition of approximately 100 volumes of methanol added to 86 volumes of the aqueous potassium phosphate (0.025 *M*, pH 3.0). The exact eluent composition was adjusted to optimize the separation between PETN and 3NT, and between NB and TET. If the former pair was inadequately separated the aqueous component was increased by, *e.g.*, two volumes, whereas the proportion of methanol was similarly increased if the latter pair was unseparated. With an efficient system equal amounts of each of these amounts of each of these compounds should give clearly distinguishable peaks, as in Fig. 1. The flow-rate was 1.0 ml/min (ambient temperature).

RESULTS AND DISCUSSION

Chromatography

The separation of a standard mixture of 1 ng of each of the fourteen explosives components listed in Table I is shown in Fig. 1. Apart from the electrode, the conditions used were essentially those of Bratin *et al.*[1], but with some important changes in detail. The selectivity of different octadecylsilyl (ODS) adsorbents was found to vary appreciably. Thus, compared with ODS-Hypersil separations (*e.g.*, Fig. 1), on ODS-Spherisorb the elution order of RDX and EGDN was reversed, and other changes in selectivity occurred in the NG region. Overall, the ODS-Hypersil's selectivity was preferable. Also, a 3-μm particle size was available commercially in a loose form. This can be packed into columns just as readily and cheaply as the larger particle sizes, and its use increases sensitivity considerably (see below).

Although it is usual to include a chelating agent in the eluent for this type of work, to suppress effects due to contaminating reducible cations, a more stable baseline was obtained without such agents. Evidently, any beneficial effect they may have is more than offset by an enhanced dissolution of trace metal ions from the chromatograph. The buffer used (phosphate) was chosen to minimize the effect. As the buffer concentration is increased from 0.015 *M* to 0.05 *M* the retention time of PA increases from a coincidence with HMX to a coincidence with RDX. The separation of the three compounds is optimal at 0.025 *M*.

The methanolic eluent was preferred to an *n*-propanolic eluent[1] not only because of selectivity and viscosity considerations but also because of its lower reflux temperature. It was found that, despite the use of a nitrogen purge, traces of oxygen

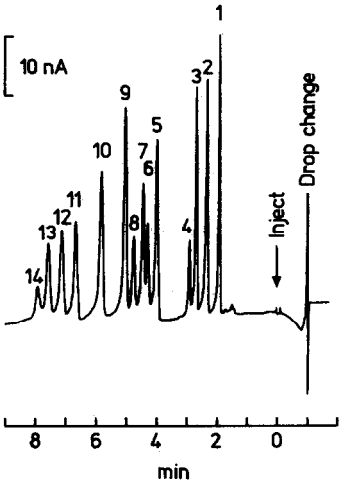

Fig. 1. Chromatogram of 1 ng each of HMX (1), PA (2), RDX (3), EGDN (4), DNB (5), TET (6), NB (7), NG (8), TNT (9), DNT (10), 2NT (11), 4NT (12), 3NT (13), PETN (14). Full names are given in Table I. The detector was a PMDE used under the conditions given under Experimental, as are the chromatographic conditions.

quickly became apparent in the eluent if refluxing ceased. A similar result was obtained with UV absorbance detection by Brown *et al.*[8].

Sensitivity and detection limits

Shown in Fig. 2 are chromatograms, with PMDE detection, of the explosives components in quantities of 100, 20 and 10 pg. At 100 pg each compound is strongly detected. PETN cannot be seen at the 20-pg level; and at 10 pg the other nitrate esters together with the mononitroaromatic compounds and TET are in the region of their detection limits. The remaining compounds disappear slightly below this level.

TABLE I

EXPLOSIVES COMPONENTS USED —NAMES AND ABBREVIATIONS

Abbreviation	*Names*
DNB	1,3-Dinitrobenzene
DNT	2,4-Dinitrotoluene
EGDN	Ethylene glycol dinitrate
HMX	Octogen; cyclotetramethylenetetranitramine
NB	Nitrobenzene
NG	Nitroglycerine; glycerol trinitrate
2NT	2-Nitrotoluene
3NT	3-Nitrotoluene
4NT	4-Nitrotoluene
PA	Picric acid; 2,4,6-trinitrophenol
PETN	Pentaerythritol tetranitrate
RDX	Hexogen; cyclonite; cyclotrimethylenetrinitramine
TET	Tetryl; 2,4,6-trinitrophenylmethylnitramine
TNT	2,4,6-Trinitrotoluene

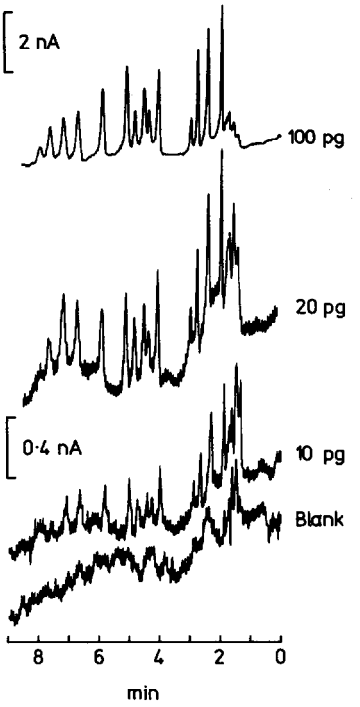

Fig. 2. Chromatograms of varied amounts of the explosives components shown in Fig. 1. The conditions are the same as in Fig. 1.

The detection limit calculated as three times the noise level for each compound is given in the second column of Table II. These results vary in accordance with the published HPLC–MFE results[1] but their absolute values are improved by an order of magnitude. A comparison of the two techniques in terms of peak current yield per ng of compound injected, with electrode potentials of −1.0 V, is shown in Table III. The MFE results were estimated from the published chromatograms; the PMDE results were calculated from experiments made over the whole linear range of the technique. Apart from HMX, to which the MFE is poorly responsive at this potential[1], the PMDE results are uniformly higher by a factor of about 10. If the different peak widths of the compared chromatographic conditions are taken into account (in the present work the half-widths are in the range 0.05–0.2 ml, compared with 0.25–1.25 ml from the published chromatograms), the actual increase in sensitivity due to the PMDE is by a factor of approximately 2. The remainder is consequent on the increased efficiency of the 3-μm column packing used here.

No significant difference was found in sensitivity due to any difference in eluent composition or pH between the two techniques, apart from effects due to baseline instability. Lower sensitivity occurred with sodium acetate–acetic acid buffers, which at pH 3.5 gave only half of the response observed in phosphate, citrate, or chloroacetate buffers.

In a well-deoxygenated system the background current of the PMDE was in the range 30–50 nA. The noise superimposed on this (Fig. 2) is due to pump pulsa-

TABLE II

PMDE–HPLC RESPONSE DATA FOR EXPLOSIVES COMPONENTS

C.L. = Confidence limit; C.V. = coefficient of variation. The compounds are listed in retention order. The data are from peak heights.

Compound	*Detection limit (pg)*	*Upper limit taken (ng)*	*Linear regression coefficient of slope (log–log plot) ± 95% C.L. (No. of points)*	*C.V. of 1-ng replicates (10 results from each compound) (%)*
HMX	7	27	0.955 ± 0.030 (8)	0.7
PA	7	81	1.004 ± 0.013 (9)	1.1
RDX	8	81	1.001 ± 0.017 (9)	0.8
EGDN	22	243	0.998 ± 0.016 (10)	1.5
DNB	10	243	0.990 ± 0.011 (10)	1.8
TET	24	243	1.011 ± 0.025 (8)	2.0
NB	13	243	1.011 ± 0.013 (10)	1.6
NG	19	243	1.003 ± 0.033 (10)	2.0
TNT	9	243	0.984 ± 0.012 (9)	1.3
DNT	12	243	1.003 ± 0.020 (10)	1.4
2NT	16	243	0.987 ± 0.017 (9)	2.7
4NT	18	243	0.982 ± 0.033 (9)	2.6
3NT	20	243	1.000 ± 0.014 (8)	3.8
PETN	49	243	1.002 ± 0.025 (9)	3.3

TABLE III

CURRENT YIELD PER ng AT HPLC MAXIMA OF EXPLOSIVES COMPONENTS: COMPARISON OF PMDE (PRESENT WORK) AND MFE[1] DETECTION RESULTS

Compound	*Peak current per ng (nA)*	
	PMDE	*MFE*
HMX	45	1.3
PA	34	5.7
RDX	28	2.3
EGDN	11	
DNB	27	
TET	11	1.7
NB	15	
NG	10	1.0
TNT	30	2.8
DNT	19	2.3
2NT	15	
4NT	15	
3NT	11	
PETN	4.7	0.5

tion, despite the heavy dampening applied. Even greater sensitivity would be obtained with a totally pulse-free chromatograph. Both the background current and the pulsation sensitivity of the electrode were increased by the described modification, made to increase the size of the electrode drop, but these disadvantages were more than compensated by an increased sample signal, and by an increased baseline stability.

Range and reproducibility

Table II includes data from PMDE-detected chromatograms of each compound (Table I) mostly taken in three-fold-increasing amounts from 10 pg to an upper limit of 243 ng except for the first three compounds, whose increase in response with concentration fell away rapidly in the 243-ng region. This is probably due to chromatographic factors, such as peak-broadening with increased sample size, rather than to the performance characteristics of the detector. The effect is particularly noticeable for the narrow (*ca.* 50 μl) peak of HMX, the slope of whose concentration dependence in double logarithmic co-ordinates (column 4, Table II) deviates significantly from the value of unity required for a direct linear dependence. Of the other compounds only TNT deviates to a significant extent. As the results represent a range in excess of four orders of magnitude in sample size, in most applications of the technique the deviations are unlikely to be important.

The coefficients of variation from replicate chromatograms of 1-ng amounts of the compounds are given in column 5, Table II. As expected, these are to some extent correlated with the detection limits. But the main implication of the results is the excellent reproducibility with which the new electrode drop required for each chromatogram was formed.

Electrode stability

In experiments with the MFE difficulties arose because of an extensive variation in the useful life of the electrode. Somtimes over a week's use could be obtained after the electrode surface had been renewed; on other occasions the noise level or baseline drift became intolerable within a day's use. This could be partly countered by the routine preparation of a new surface for each day's work, but compared with the PMDE this was inconvenient and time-wasting. In contrast, a new PMDE can be formed immediately prior to the injection of each sample. The slight depression of the baseline that results is restored in about 30 sec at usual levels of sensitivity (Fig. 1), *i.e.*, within the void time of a chromatogram, although at extreme sensitivities a stabilization period of 2–3 min may be necessary.

The poor performance of the MFE is though to be due to the deposition of reduction products such as reduced heavy metal cations, which accumulate with the passage of each sample as well as from the eluent. Although the effect of the latter can be minimized by the appropriate choice of eluent composition and pumping circuitry, as mentioned earlier, and the former can be minimized by clean-up procedures[9], the problem remains sufficiently severe to place the MFE at a considerable disadvantage in the analysis of the types of sample of interest in forensic science work.

A comparison between the two electrodes used for an extract of a swab from a soiled hand (explosives-free) is shown in Fig. 3. (The dried cotton-wool swab was extracted with eluent.) Each detector was adjusted to give the same response to 1 ng

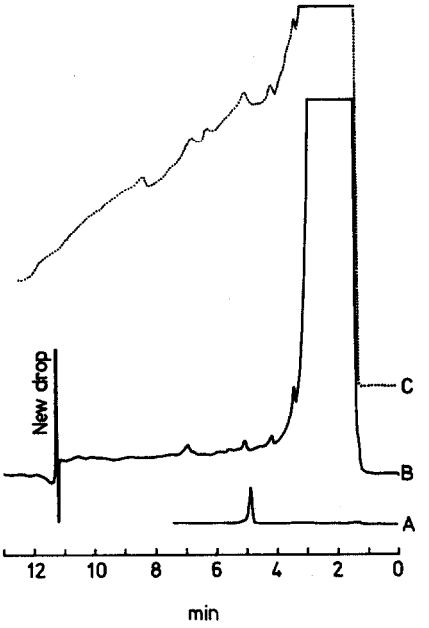

Fig. 3. Chromatograms detected at a PMDE (A, B) and an MFE (C), both maintained at −1.0 V *vs.* Ag/AgCl. Chromatograms B and C are from the same hand swab extract, both run at the same sensitivity relative to 1 ng TNT shown at A.

of TNT, for which a chromatogram from the PMDE is shown at A (Fig. 3). Each of the other chromatograms is dominated by an intense initial peak from which the PMDE rapidly recovers (B), but the MFE (C) does not. A slight shift in the PMDE baseline occurs, but this is restored when a new drop is formed for the next analysis, as the chromatogram B shows. At the MFE the effect of a series of such samples is cumulative. It can, to some degree, be remedied if the electrode potential is briefly held at 0 V, when some deposited material is discharged, presumably. However, the electrode requires an appreciable period of time to stabilize after this treatment.

Although the PMDE may be changed during a chromatogram this is generally unnecessary as the baseline shift within a single chromatogram is normally tolerably small, as is any reduction in sensitivity due to electrode contamination. The great value of this reproducibly renewable electrode lies in its ability to eliminate the cumulative effects of successive contaminations.

Application to traces of explosives

Some chromatograms obtained from trace amounts (5–10 ng) of typical high explosives are shown in Fig. 4. These chromatograms were obtained from acetone extracts of μg amounts of sample, which were diluted with eluent and filtered through 0.2-μm membranes. The amounts represented by the chromatograms have been calculated from the dilution factors. The peak identities are as given in Fig. 1. These, and the relative amounts present, are in agreement with the known compositions of the samples.

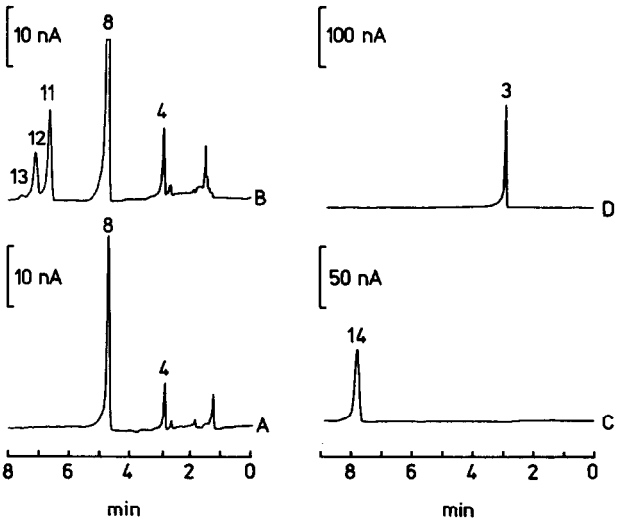

Fig. 4. Chromatograms of explosives traces: a gelignite, 10 ng (A); a blasting gelatine, 10 ng (B); Cortex fuse filling, 10 ng (C); an RDX-based explosive, 5 ng (D). The peak identities (numbers) are given in the caption to Fig. 1. Detection was at a PMDE under the conditions used in the other figures.

CONCLUSIONS

The described technique was developed primarily as a basis for the screening of samples such as hand swabs for trace amounts of explosives. Its specific development in this direction will be described later[9]. Capillary column gas chromatography (GC) with electron-capture detection (ECD) is also a valuable technique in this application[10,11] and gives detection limits from pure solutions in a similar range although uncorrelated with the HPLC–PMDE technique (mainly for chromatographic rather than electrochemical reasons, apparently). However, the quantities of solution taken for GC analysis are typically 1 μl whereas the HPLC results refer to 20-μl volumes, so that in terms of concentration sensitivity, for a number of compounds at least, HPLC–PMDE seems to be the more sensitive technique. An important outcome is that evaporative preconcentration steps can be more easily avoided in HPLC techniques.

The HPLC–PMDE technique is also the more selective in that its electron-transfer step is subject to a much greater degree of control than the process in the ECD. Indeed, many compounds to which the ECD is sensitive give no response at the PMDE operated under the described conditions. Hence, the only additional independent information that GC–ECD can provide is equivalent to another retention time, which might equally well be obtained with greater specificity under HPLC conditions of modified selectivity. Both techniques have similar turn-round times.

For individual compounds the PMDE selectivity may be increased considerably, *e.g.*, by the use of pulsed polarization techniques, with some sacrifice of sensitivity. If HMX is not sought the selectivity for the remainder of the compounds investigated can be increased without significant loss of sensitivity if the electrode potential is raised from -1.0 to -0.9 V. However, the principal objective at present

is to establish improved screening techniques in order that samples meriting further close attention can be rapidly identified.

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