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EVALUATION OF AMPEROMETRIC DETECTORS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: ANALYSIS OF BENZODIAZEPINES

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SUMMARY

The design of amperometric detectors for high-performance liquid chromatography is discussed. A simple flow cell with interchangeable working electrodes made from glassy carbon, carbon paste and mercury is described, and its performance is compared with commercially available cells, using both constant-potential and pulse-measuring techniques.

The analysis of nitrazepam, diazepam and chlordiazepoxide was used as a model system; the detectors were used in the reduction mode and the mobile phase was methanol–water (60:40) containing 0.05 *M* ammonium acetate. The effects of various experimental parameters are reported. The detection limit was found to depend strongly on the reduction potential: at −0.93 V *vs.* Ag, 3 ng of nitrazepam could be detected, whereas at −1.30 V the detection limit was 30 ng, owing to the high background current at this potential. A potential more negative than −1.1 V must be used for the detection of diazepam and chlordiazepoxide; at −1.30 V the detection limit was 300 ng for these compounds.

INTRODUCTION

High-performance liquid chromatography (HPLC) with amperometric detection offers certain advantages over UV detection in terms of selectivity, sensitivity and cost[1]. A simple potentiostat with low-level current-measuring capabilities can easily be constructed[2]; however, the success of the whole system depends critically on the design of the electrochemical flow-through cell. Thus, preliminary experiments revealed that some commercial detectors did not function properly, and the reason was found to be the uncompensated resistance within these flow cells.

In this paper a simple detector cell is described in which there is negligible uncompensated resistance; the cell has a satisfactory response also when the mobile phase has a low electrical conductivity. The cell is equipped with interchangeable working electrodes made from glassy carbon, carbon paste and mercury, so that a relevant comparison of these electrode materials can be made. The analysis of benzodiazepines served as a model system; the detector was mainly operated in the

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reduction mode, but the anodic range of the detector was also tested. The reduction of oxygen, hydrogen ions and trace metals may interfere with the determination when the detector is used in the reduction mode, and it was of particular interest to ascertain the magnitude of these interferences. Normally, amperometric HPLC detectors are operated in the oxidation mode in order to avoid the above interferences.

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-pressure liquid chromatograph, incorporating a U6K injector and a Model 6000 A solvent delivery system, was used. The column (250 × 2.6 mm I.D.) was packed with 5-μm Spherisorb ODS; the number of theoretical plates of the newly packed column was 3800. A Perkin-Elmer LC-55 UV detector was used in some of the experiments. For electrochemical detection, a Princeton Applied Research 174A Polarographic Analyzer and a Radiometer Servograph REC51/REA112 recorder were used.

The construction of the electrochemical flow-through cell is shown in Fig. 1. The cell is based on the "wall-jet" principle[3] with the flowing stream from the chromatographic column directed perpendicular to the working electrode surface. The cell was made from Teflon, a silver wire served as a quasi-reference electrode and a platinum wire or the stainless-steel exit tube was used as a counter electrode.

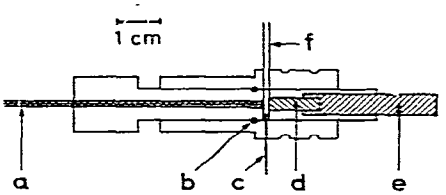

Fig. 1. Construction of the flow-through cell (Teflon). a = Stainless-steel inlet from chromatograph; b = O-ring; c = reference electrode, silver wire; d = working electrode, glassy carbon rod; e = brass rod; f = stainless steel exit tube, counter electrode.

The glassy carbon electrode was made by pressing a 3-mm rod (Tokai Electrode Mfg. Co. Ltd., Tokyo, Japan) into Teflon. The electrode mounted in a Plexiglass block was polished first with silicon carbide papers (No. 400 and 600), then with alumina suspensions (1 μm) and finally with diamond paste (1 and 0.25 μm) on rotating discs.

The carbon paste electrode was made from a Teflon rod with a 3-mm diameter well drilled in one end. The well was filled with a carbon paste prepared by mixing 5 g of graphite powder (Koch-Light, Colnbrook, Great Britain) and 3 ml of nujol as described by Adams[4]. Electrical connection was made via a platinum wire. The electrode surface was polished by rubbing the electrode against a smooth paper.

The same Teflon rod was also used as a support for the mercury pool electrode. The rod was placed in a vertical position and the well was filled with highly purified mercury.

The commercial cells were obtained from EDT Research (London, Great Britain) (type LC03, hereafter called the EDT cell) and Bioanalytical Systems (West

Lafayette, Ind., U.S.A.) (hereafter called the BS cell). The constructions of these cells have been described by Fleet and Little[3] and Kissinger *et al.*[2], respectively.

Reagents and solutions

The benzodiazepines, which were kindly supplied by A/S Apothekernes Laboratorium for Spesialpraeparater (Oslo, Norway), were dissolved in methanol. Methanol–water (60:40) containing 0.05 *M* ammonium acetate was used as the mobile phase; the salt served as a supporting electrolyte for the electrochemical detector. Oxygen was removed from the mobile phase by bubbling argon through the solution (1 l) for at least 4 h (normally overnight). The 10-ml samples were kept in 14-ml vials with plastic caps and deaerated for 5 min before the injection of 10-μl aliquots.

RESULTS AND DISCUSSION

Reduction of benzodiazepines

The polarographic reduction of nitrazepam, diazepam and chlordiazepoxide in aqueous solution is well documented[5,6]. However, no data were available for the particular medium used here, namely methanol–water (60:40) containing 0.05 *M* ammonium acetate (pH 7.25). The d.c. polarograms of the three benzodiazepines in this medium are shown in Fig. 2. The polarograms were recorded with a dropping mercury electrode in a quiescent solution. For chlordiazepoxide, three waves are observed with $E_{\frac{1}{2}}$ at −0.98, −1.24 and −1.62 V *vs.* Ag. For nitrazepam, two waves are observed with $E_{\frac{1}{2}}$ at −0.65 and −1.30 V *vs.* Ag (the first wave has a small maximum), whereas a single wave is obtained for diazepam with $E_{\frac{1}{2}}$ at −1.14 V *vs.* Ag. The wave heights indicate a 2:2:2 electron reduction of chlordiazepoxide, a 4:3 electron reduction of nitrazepam and a single 2-electron reduction of diazepam, in good agreement with the respective reduction mechanisms described by Oelschläger and co-workers[7–9]. The second wave of nitrazepam, which apparently corresponds to a 3-electron reduction, is probably a mixture of a 2- and a 4-electron reduction[8].

Voltammograms were also recorded using the glassy carbon and carbon paste electrodes which were subsequently used in the flow-through cells. For chlordiazepoxide no well defined waves were observed, but for the other two compounds the voltammograms were similar to the polarograms shown in Fig. 2; only the first wave of nitrazepam had the peaked shape characteristic of linear sweep voltammetry (see Fig. 4). On glassy carbon the "half-wave" potentials were −0.73 and −1.20 V for nitrazepam and −1.20 V for diazepam. The potentials varied slightly from day to day, probably as a result of changes in the condition of the electrode surface.

The effect of pH was studied by recording polarograms of the three benzodiazepines at different pH values; the pH was adjusted with acetic acid and ammonia solution. It was found that the half-wave potentials shifted slightly (less than 50 mV) towards more positive values when the pH was decreased from 7.7 to 5.8.

In all of the above experiments a silver wire was used as a quasi-reference electrode in order to reproduce the experimental conditions in the flow-through cell.

Separation of benzodiazepines

Chlordiazepoxide, nitrazepam and diazepam are well separated by HPLC on

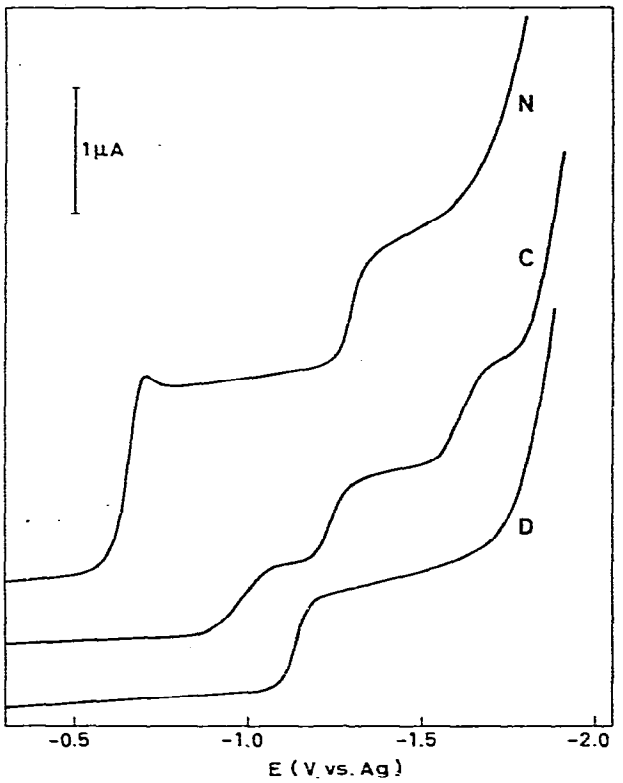

Fig. 2. Polarograms (d.c.) of 10^{-4} *M* nitrazepam (N), diazepam (D) and chlordiazepoxide (C) in methanol–water (60:40) containing 0.05 *M* ammonium acetate.

a reversed-phase column, using a mixture of methanol and water as the mobile phase. Methanol–water (60:40) containing 0.05 *M* ammonium acetate was used as mobile phase in this work; a typical chromatogram is shown in Fig. 3. Nitrazepam is eluted first, followed by chlordiazepoxide and diazepam; a similar result was obtained by Harzer and Barchet[10], whereas Knox and Pryde[11], who added ammonia to the mobile phase, reported a different sequence of the peaks. The separation depend on the methanol content of the mobile phase; with less methanol an inferior separation was obtained, and no separation was observed with methanol–water (40:60). Ammonium acetate, which gives a nominal pH of 7.25, serves as a supporting electrolyte for the electrochemical detector. Neither the separation nor the peak heights were improved when the pH was adjusted to higher or lower values with ammonia solution and acetic acid, respectively, in the pH range 5–8. The benzodiazepines are unstable in more strongly acidic and basic solutions, and the Spherisorb ODS packing of the column is unstable at pH values above 8.

Removal of oxygen

As shown in Fig. 3, the detector gives a signal for oxygen, which will interfere with the determination unless the concentration of oxygen is very low. Further, the solubility of oxygen is very high in a 60:40 methanol–water mixture[12]. For satisfactory detector performance in the cathodic region, it is essential that oxygen be

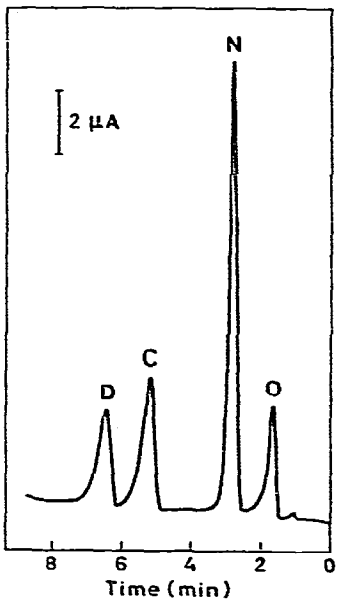

Fig. 3. Separation of 20 μg (0.07 μmole) of nitrazepam (N), 30 μg (0.1 μmole) of chlordiazepoxide (C), 28 μg (0.1 μmole) of diazepam (D) and trace amounts of oxygen (O) using amperometric detection. ODS column; mobile phase methanol–water (60:40) containing 0.05 *M* ammonium acetate; flow-rate 1 ml · min^{-1}; reduction potential −1.25 V *vs.* Ag.

removed from the mobile phase, as well as from the sample, prior to the analysis. Normally, the mobile phase was deaerated by bubbling argon through the 1-l container for at least 4 h (preferably overnight). Teflon tubing could not be tolerated in any part of the system, and stainless-steel tubing was used instead. The 10-ml samples were deaerated for 5 min; identical treatment of all samples was essential because of the high volatility of the solvent. Although a very thorough deaeration procedure was used, an oxygen peak was usually observed on the chromatograms.

Detector parameters

Constant potential and pulse measurements. For amperometric detection the working electrode is normally kept at a constant potential. Obviously, this is the most practical approach, because a very simple potentiostat can then be employed. It has, however, been claimed that a pulse technique would give better sensitivity and electrode stability when solid electrodes are used[13]. In this work, these advantages of the pulse technique were not confirmed. On the contrary, the use of a normal pulse technique was found to give much higher background currents and poorer signal-to-noise ratios than constant-potential measurements, and even the differential pulse technique offered little improvement in detection limits (see below). The fast changes in the applied potential (*i.e.*, pulses) give rise to slow solid-state redox reactions which alter the surface of any working electrode made from carbon, and the pulses also result in significant capacitive currents[14–16]. These effects are illustrated in Fig. 4 for the reduction of nitrazepam on glassy carbon.

The claimed beneficial effect of the pulse techniques on electrode stability is supposed to be a result of the working electrode being kept at an initial "cleaning"

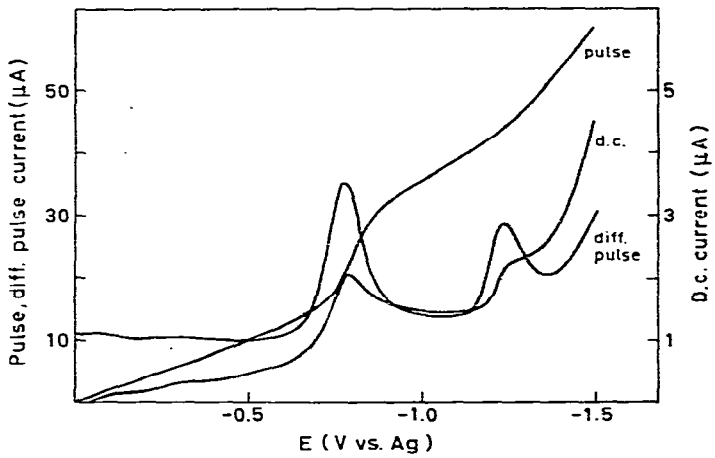

Fig. 4. Voltammograms of 10^{-4} *M* nitrazepam in methanol–water (60:40) containing 0.05 *M* ammonium acetate, obtained with a glassy carbon electrode and d.c., pulse and differential pulse techniques. Scan rate 5 mV · sec^{-1}; pulse repetition time 1 sec; modulation amplitude 50 mV.

potential between pulses, at which any unwanted electrode reactions cannot take place. Thus, the EDT detector incorporates a pulse potentiostat which is said to overcome the problems of adsorption and fouling of the electrode surface. However, in this work no improvement in electrode stability was observed when the pulse techniques were used. According to our experience, a poisoned electrode is best reactivated by mechanical cleaning (*i.e.*, polishing) of the electrode surface.

The long time constant of the memory circuits in the polarograph adds to the inconvenience of the pulse techniques; for narrow chromatographic peaks a low detector response must be expected.

No doubt differential pulse measurements at a constant initial potential will give a better selectivity than normal amperometric detection, because only species with half-wave potentials close to the initial potential will be detected. However, electrochemical detectors are selective in comparison with UV detectors for instance, and the additional selectivity offered by the differential pulse technique is therefore seldom required, unless the unknown compounds are very poorly separated by the column. The differential pulse technique is, however, useful for determining the "half-wave" potentials of unknown compounds from chromatograms recorded at different potentials, because it is simpler to locate the potential which corresponds to the maximum peak height than to construct the full d.c. voltammogram ($E_p = E_{\frac{1}{2}} - \Delta E/2$).

It has been pointed out[13,15] that the flow-rate dependence of the detector is minimized by using a pulse measuring technique. However, as will be shown below, the detector was found to have a very reproducible response when operated in the constant-potential mode, so again there should be little need for introducing the pulse approach, at least not for the type of liquid chromatograph used here.

Cell design. In order to avoid uncompensated resistance in any part of the flow-through cell, the three electrodes should be positioned as close as possible to each other. Of the various designs suggested in the literature, the wall-jet cell[3] is probably the best. The cell shown in Fig. 1, which is based on this principle, was

found to have a very satisfactory response. The outlet from the chromatographic column is directed perpendicular to the working electrode surface. A silver wire is used as a quasi-reference electrode; the electrode is positioned 1 mm from the working electrode. The stainless-steel exit tube, which is positioned 2 mm from the working electrode, serves as a mechanically robust counter electrode. In some experiments a platinum wire, positioned between the silver wire and the exit tube, was used as an alternative counter electrode; an identical detector response was observed in this instance. The working electrode shown in Fig. 1 is made from glassy carbon; the other working electrodes examined were made from carbon paste and mercury (pool). The characteristics of the different electrode materials are discussed below.

Normally, the volume of the flow cell is adjusted by altering the thickness of the thin layer of solution passing across the working electrode. For the wall-jet cells this is done by increasing the distance between the inlet tube and the working electrode. For the home-made cell a distance of 1 mm was mostly used; the effective cell volume (as defined by the area of the working electrode) was then 7 μl, and the total cell volume was 50 μl. As expected, a larger cell volume gave lower and broader chromatographic peaks, with marked tailing at the end. However, no significant increase in peak height was observed when the solution layer thickness was decreased below 1 mm.

The EDT cell was found to give results similar to those obtained with the home-made cell. However, at potentials more negative than -1.4 V a decrease in detector response was observed, which was probably due to uncompensated resistance within the cell; the counter and reference electrodes are positioned some distance from the glassy carbon working electrode. Further, only one of two reference electrodes tested was found to function properly.

The thin-layer cell designed by Kissinger *et al.*[2] and manufactured by Bioanalytical Systems was also tested, but it did not function properly under the experimental conditions used. In this cell the reference and counter electrodes are placed in a separate compartment far from the working electrode, and the electrical contact is made through a very thin layer of solution and thin tubing. Here a large uncompensated resistance cannot be avoided, particularly when a partly non-aqueous mobile phase is used. By making the connections between the electrodes shorter and wider, a better but still unsatisfactory response was obtained. No further use was therefore made of this cell.

Working electrodes. The ideal working electrode material should have large anodic and cathodic potential ranges with low background currents, and the properties of the electrode surface should not change with time. For practical reasons solid electrodes made from glassy carbon and carbon paste are more attractive than stationary or dropping-mercury electrodes. The EDT cell is equipped with a glassy carbon electrode, whereas the BS cell employs a carbon paste electrode. The cell shown in Fig. 1 was used with glassy carbon, carbon paste and mercury pool electrodes. A summary of our experience with these electrodes is given below.

The glassy carbon electrode must be polished very thoroughly in order to give a satisfactory voltammetric response. However, glassy carbon obtained from some manufacturers did not give a satisfactory background, irrespective of the polishing procedure. A poisoned or poorly polished electrode gives high background currents, and the reduction of the solvent appears at a relatively positive potential, whereas the "half-wave" potential of the electroactive compound is shifted in the

opposite direction owing to the low rate of the electrochemical charge-transfer reaction. The deactivation of the electrode was found to be much slower for a well polished than for a poorly polished electrode.

A residual wave was sometimes observed even on well polished electrodes at *ca.* −0.7 V. The wave disappeared when the electrode was kept at −1.0 V for some time, indicating that the residual wave is due to oxides present at the glassy carbon surface.

Background scans are shown in Fig. 5 for both a good and a bad glassy carbon electrode. The cathodic limit is determined by the reduction of hydrogen ions, and the anodic limit by the oxidation of the solvent. The voltammograms were obtained by scanning the potential from 0 V in the cathodic and anodic directions. It has been claimed that steady-state voltammetry would give much lower background currents than scanning voltammetry[14], but in the present work the two techniques gave comparable background currents in the potential region 0 to −1.0 V. At more negative potentials the steady-state currents were lower than those obtained in the scanning mode.

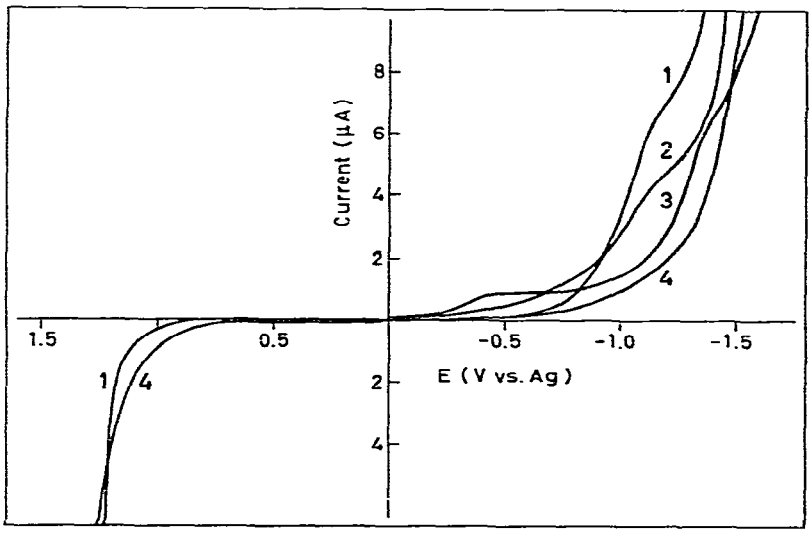

Fig. 5. Background voltammograms of the mobile phase for different working electrodes: 1 = carbon paste; 2 = glassy carbon, poorly polished; 3 = mercury pool; 4 = glassy carbon, well polished. Flow-rate 1 ml · min^{-1}; scan rate 5 ml · sec^{-1}.

For the carbon paste electrode the polishing procedure is relatively simple. After packing, the electrode surface is smoothed by rubbing the electrode across a glazed paper surface. A representative background scan is shown in Fig. 5, illustrating that a very good background is obtained in the anodic region, and also in the cathodic region below −0.6 V, but for more negative potentials an appreciable background is observed. The background was not improved by applying a potential of −1.0 V (or more negative) for some time, probably because trace amounts of oxygen are uniformly distributed throughout the paste and not only adsorbed on the electrode

surface. No problems with long-term stability were encountered on using methanol–water (60:40) as the mobile phase.

Glassy carbon and carbon paste electrodes are particularly well suited for detection in the anodic range. It is usually assumed that a mercury electrode would give a larger potential range and lower background currents than a carbon electrode in the cathodic region. Thus, a cathodic limit of *ca.* −1.8 V is indicated in Fig. 2. However, as can be seen from Fig. 5, the background for a mercury pool electrode was found to be similar to that of the carbon electrodes, probably because the stationary mercury surface is contaminated by metallic and/or organic impurities. Approximately the same cathodic limit was found by Rabenstein and Saetre[17] for their mercury pool detector. The residual wave in Fig. 5 at −0.3 V is probably due to trace amounts of oxygen within the system. The wave was not eliminated by applying a potential of −1.0 V for some time.

No doubt the use of a dropping mercury electrode would have extended the cathodic range of the detector (see Fig. 2). However, this electrode has obvious disadvantages; the current oscillations caused by the falling drops make the detection of narrow chromatographic peaks difficult, and the high charging currents give inferior detection limits (the detection limits given by Koen *et al.*[18] appear to be optimistic). According to our experience, a flow cell incorporating a dropping-mercury electrode is also a less reliable and practical detector for HPLC than cells with stationary electrodes. Finally, all cells with mercury electrodes have a very restricted anodic range.

The use of working electrodes made from platinum was not considered in this work as their potential range and background will be inferior to those of the carbon electrodes.

Choice of reduction potential. Normally, one would choose a potential near the cathodic limit of the detector in order to facilitate the determination of as many electroactive compounds as possible with a single setting of the detector. Because of the drastic rise in background current near the potential limit, a very negative potential cannot be used without some sacrifice in terms of the signal-to-noise ratio. Also, at a sufficiently negative potential the evolution of hydrogen may block the electrical contact between the electrodes. If a very selective detection is desired, the differential pulse technique may be used; in this instance the detector potential should be close to the half-wave potential of the compound in question.

The detection of nitrazepam is preferably carried out at −0.9 to −1.2 V using constant-potential amperometry, whereas −1.2 V or a more negative potential must be used for the detection of diazepam and chlordiazepoxide. When the chromatographic peak height of nitrazepam was drawn as a function of the detector potential, the "half-wave" potentials of the resulting voltammogram (which had no maximum) were found to correspond with those obtained using the same electrode and scanning voltammetry in a quiescent solution. This result confirms the absence of uncompensated resistance within the flow cell.

By studying the variation in peak height with potential a malfunctioning of the detector may be revealed. When this experiment was carried out with the EDT cell, a decrease in the peak heigh of nitrazepam was obtained for potentials more negative than −1.4 V.

Sensitivity and detection limits

The sensitivity of the HPLC technique depends on the chromatographic conditions, the detector design and the electrochemical behaviour of the compounds in question. For a given set of experimental conditions, the chromatographic peak heights depend on the retention time and the mechanism of the respective electrochemical reactions. Thus, the reason for the high sensitivity for nitrazepam which is indicated in Fig. 3 is that the reduction of nitrazepam involves seven electrons at −1.25 V (see Fig. 4), whereas only two electrons are involved in the reduction of diazepam and chlordiazepoxide at this potential. In addition, the short retention time of nitrazepam results in a large and narrow peak, with a width which is nearly half of that of the other two compounds.

As already mentioned, the magnitude of the background current depends on the potential chosen; at −1.3 V a marked drift and sudden shifts in the baseline were noticed, probably because of the evolution of hydrogen. At very negative potentials the detector also picked up some noise from the pumping system, as indicated in Fig. 6. Attempts to shield the detector from external electrical noise by placing it in a Faraday cage had little effect on the background.

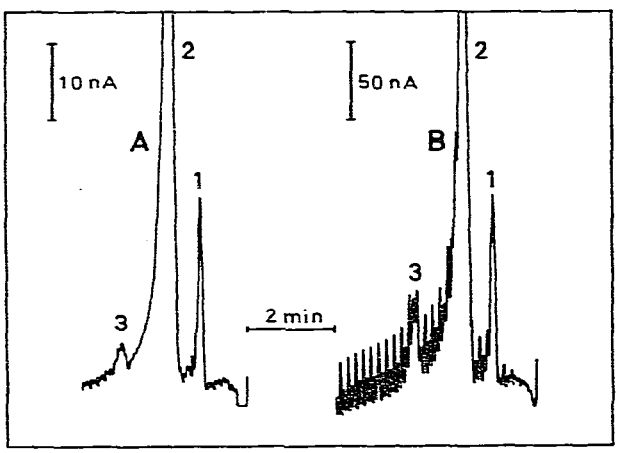

Fig. 6. Response of the detector to small amounts of nitrazepam. (A) 3 ng (10 μl) of nitrazepam detected at −0.93 V *vs.* Ag; (B) 30 ng (10 μl) at −1.30 V. Peaks: 1 = solvent front; 2 = oxygen; 3 = nitrazepam. Flow-rate 1 ml · min^{-1}.

As expected, the peak heights increased with flow-rate, and so did the background current; in the latter instance a linear dependence was observed. The results for nitrazepam are given in Table I.

In Table II the effect of various detector parameters on the detection limit (signal-to-noise ratio 2:1) is illustrated, using nitrazepam as the model compound. As expected, inferior detection limits are obtained for potentials near the cathodic limit. The use of the differential pulse technique is seen to give little improvement compared with the d.c. technique, except for the mercury pool electrode. The glassy carbon and carbon paste electrodes have similar detection limits. The mercury pool electrode appears to be inferior to the carbon electrodes at the more negative poten-

TABLE I

EFFECT OF FLOW-RATE ON PEAK HEIGHT, BACKGROUND AND RETENTION TIME FOR 30 μg OF NITRAZEPAM

Potential −1.2 V.

Flow-rate (ml · min^{-1})	*Peak height (μA)*	*Background (μA)*	*Retention time (min)*
0.7	8.8	11.5	11.2
1.5	9.7	17.0	5.2
2.0	12.3	20.5	3.9
2.5	16.8	24.5	3.1
3.0	18.8	28.3	2.6
3.5	20.5	32.7	2.2

TABLE II

DETECTION LIMITS FOR NITRAZEPAM, OBTAINED UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Working electrode	*Potential (V vs. Ag)*	*Technique*	*Detection limit (ng)*
Glassy carbon	−1.30	D.c.	30
	−0.93	D.c.	3
	−0.78	Differential pulse	2
Carbon paste	−1.30	D.c.	80
	−0.93	D.c.	3
	−0.78	Differential pulse	2
Mercury pool	−0.83	D.c.	30
	−0.68	Differential pulse	3

tials. When the EDT cell was used, detection limits identical with those given in Table II for the glassy carbon electrode were obtained. However, slightly different potentials were used for the EDT cell in order to compensate for the difference in the reference electrode potentials.

In Table III the detection limits for the three benzodiazepines under optimal electrochemical conditions are compared with those obtained using a UV detector. For nitrazepam the same detection limit was obtained for the amperometric and UV detectors, whereas higher detection limits were obtained for diazepam and

TABLE III

DETECTION LIMITS FOR NITRAZEPAM, DIAZEPAM AND CHLORDIAZEPOXIDE, USING AMPEROMETRIC (GLASSY CARBON) AND UV (254 nm) DETECTION

Injection volume 10 μl.

Compound	*Amperometric detection*		*UV detection limit (ng)*
	Potential (V)	*Detection limit (ng)*	
Nitrazepam	−0.93	3	3
Diazepam	−1.30	300	6
Chlordiazepoxide	−1.30	300	4

chlordiazepoxide using electrochemical detection, because of the highly negative potential needed for the detection of these compounds. However, owing to the high sensitivity for nitrazepam, this compound has a lower detection limit, also at −1.3 V (30 ng, see Table II), than diazepam and chlordiazepoxide.

A typical chromatogram of 3 ng of nitrazepam is shown in Fig. 6. At the high current sensitivity used, the small traces of oxygen in the solution give rise to a relatively large peak.

Calibration graphs and reproducibility. The electrochemical detector was found to have a linear response over a wide concentration range. The results for nitrazepam are given in Table IV. A glassy carbon electrode was used here, but similar results were obtained for the carbon paste and mercury pool electrodes, and also for the EDT cell. As shown in Table IV, the relative standard deviation for repeated injections was of the order of 1–3%.

TABLE IV

VARIATION IN PEAK HEIGHT WITH CONCENTRATION OF NITRAZEPAM

Potential −0.93 V *vs.* Ag; flow-rate 1 ml · min^{-1}.

Concentration (ppm)	*n**	*Peak height***		
		$\bar{x}$ (μA)	s (μA)	s_R (%)
1400	5	8.0	0.31	3.9
700	5	4.28	0.09	2.1
350	5	2.12	0.02	0.9
280	5	1.66	0.03	1.8
140	4	0.803	0.010	1.2
70	5	0.412	0.008	1.9
35	4	0.201	0.002	1.0
28	5	0.163	0.002	1.5
14	4	0.082	0.001	1.2
7	5	0.040	0.001	2.5

* n = number of 10-μl injections.

** $\bar{x}$ = mean; s = standard deviation; s_R = relative standard deviation.

CONCLUSIONS REGARDING AMPEROMETRIC DETECTION

Electrochemical flow cells used as detectors in HPLC should have a design that minimizes any uncompensated resistance within the cells. Not all commercial cells are equally satisfactory in this respect. A simple yet satisfactory cell design is shown in Fig. 1; the cell can be used with different types of working electrodes. Electrodes made from glassy carbon were found to have a relatively large cathodic range, but needed a cumbersome polishing procedure, while carbon paste electrodes exhibited a particularly low background in the anodic region, and a fresh electrode surface could be obtained by simply removing the top layer of the carbon paste.

Constant-potential amperometry was found to be preferable to normal potential pulse measurements; the latter technique gave high background currents when solid electrodes were used. The differential pulse technique was better in this respect, but this technique only detects compounds with half-wave potentials close to the initial potential chosen.

The detection limits were found to depend strongly on potential, because the reaction rate, number of electrons and background current were all a function of this parameter. Compounds which are reduced at a potential more positive than *ca.* −1.0 V have much better detection limits than those reduced at a potential close to the cathodic limit of the detector. Thus, the detection limit of nitrazepam was found to be 3 ng, whereas chlordiazepoxide and diazepam had inferior detection limits. Because the detector was used in the reduction mode, oxygen had to be removed completely from the mobile phase as well as from the sample solution prior to the analysis.

Amperometric detectors are cheaper than most other high-sensitivity detectors. When used in combination with UV detection, for instance, the electrochemical detector may provide additional selective information on the electroactive components of complicated mixtures.

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