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NEW ELECTROCHEMICAL CELL FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A new electrochemical detector of low dead volume allowing the use of various solid electrodes as well as a dropping mercury electrode has been developed for high-performance liquid chromatography. The electroactivity range has been determined in typical chromatographic effluents so as to select the appropriate working electrodes, and the main parameters of the detector (linear range, detection limits, reproducibility) examined using oxidizable or reducible organic molecules. Some applications involving the different chromatographic modes are presented. This new detector is very simple to use and gives satisfactory results with all kinds of chromatographic modes.

INTRODUCTION

Nowadays, electrochemical detection is a well-known detection mode in liquid chromatography. As early as 1950, the first studies were published on the use of the combination of polarography and liquid chromatography for separating and determining organic and inorganic compounds[1,2]. In 1969, a spectacular new development appeared in liquid chromatography: high-performance liquid chromatography (HPLC). This technique quickly became very successful owing to its high efficiency, and stimulated the development of improved detection methods[3–19].

In this paper we introduce a new type of cell which offers a unique flexibility, as it can be used with both solid electrodes and with dropping mercury electrodes. In the first case, one can use the "longitudinal" or the "wall jet" operating mode. Also it is possible to employ simultaneously two working electrodes and carry out a double detection for a particular solute. This cell can also be operated with a low dead volume mixer if it is desired to add a supporting electrolyte or a reagent at the column output.

We believe that thanks to this flow-through detector, using a single cell, it is

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possible to benefit from the many advantages of amperometric detection, in modern HPLC.

EXPERIMENTAL

Description of the measurement cell

The amperometric detection cell is based on a three-electrode system. A cross section can be seen in Fig. 1. The parallelepiped cell body is made of polychlorotrifluoroethylene (PCTFFE), and the electrodes are housed in PCTFE or PTFE stems, threated for ease of removal and interchangeability; fluorinated polymer O-rings ensure watertightness. The thin liquid layer circulating inside the cell is geometrically defined in a precise and reproducible way, by means of a 250-μm-thick PTFE gasket, sandwiched between the two half-bodies of the cell, and with a channel accurately positioned in its centre.

According to the way the electrodes are installed, the detection cell can operate in the "thin-layer" mode or in the "wall-jet" mode. Some other inlets are available either for another working electrode (bipotentiostatic configuration) or for simultaneous conductimetric detection. The inner and active element of the miniature reference electrode is Ag/AgCl/saturated KCl. The electrolyte junction, made of a porous ceramic plug, is very close to the working electrode in order to make measurements easier in low conductivity media. When using the dropping mercury electrode, the central PTFE gasket is replaced by a thicker one, adapted to the capillary shape and size.

The dead volume is variable, depending on the selected configuration (Table I).

Operation of the detection cell

This detection cell can be used with any HPLC system. We have carried out a large number of tests with the following equipment: Orlita AE 104 or Chromatem 380 pumps, fitted with a pulse damping system; Rheodyne or Valco injection valve; stainless-steel columns (1/4 in. I.D.) of various lengths. The associated electronic instruments were: P.A.R. 174 polarographic analyser; Tacussel PRG 5, PRG-MOD and PRG-E polarographic analyser; Leeds and Northrup recorder; Tacussel EPL 1/TV 11 GD recorder. Chemicals were of analytical grade from Prolabo (Paris, France) and E. Merck (Darmstadt, G.F.R.).

Selection of working electrode

The tests of the various materials available for the working electrode were conducted under the usual voltametric conditions with rotating disc electrodes (Tacussel Type EDI). Boron carbide, tungsten, molybdenum, zinc and nickel were tested, in addition to the usual materials, platinum, glassy carbon and mercury.

The determination of the electroactivity ranges in usual chromatographic solvents (water, methanol and water–acetonitrile mixtures containing 20% or 80% of water) indicated the possibility of using different electrodes. Since it is necessary to add a supporting electrolyte in electrochemical methods, all the tests were carried out with 0.1 *M* $LiClO_4$. The reference electrode was Ag/AgCl in aqueous saturated KCl.

A number of trials were carried out, first without then with nitrogen bubbling through the eluting solvent. This confirmed that oxygen, the presence of which is of

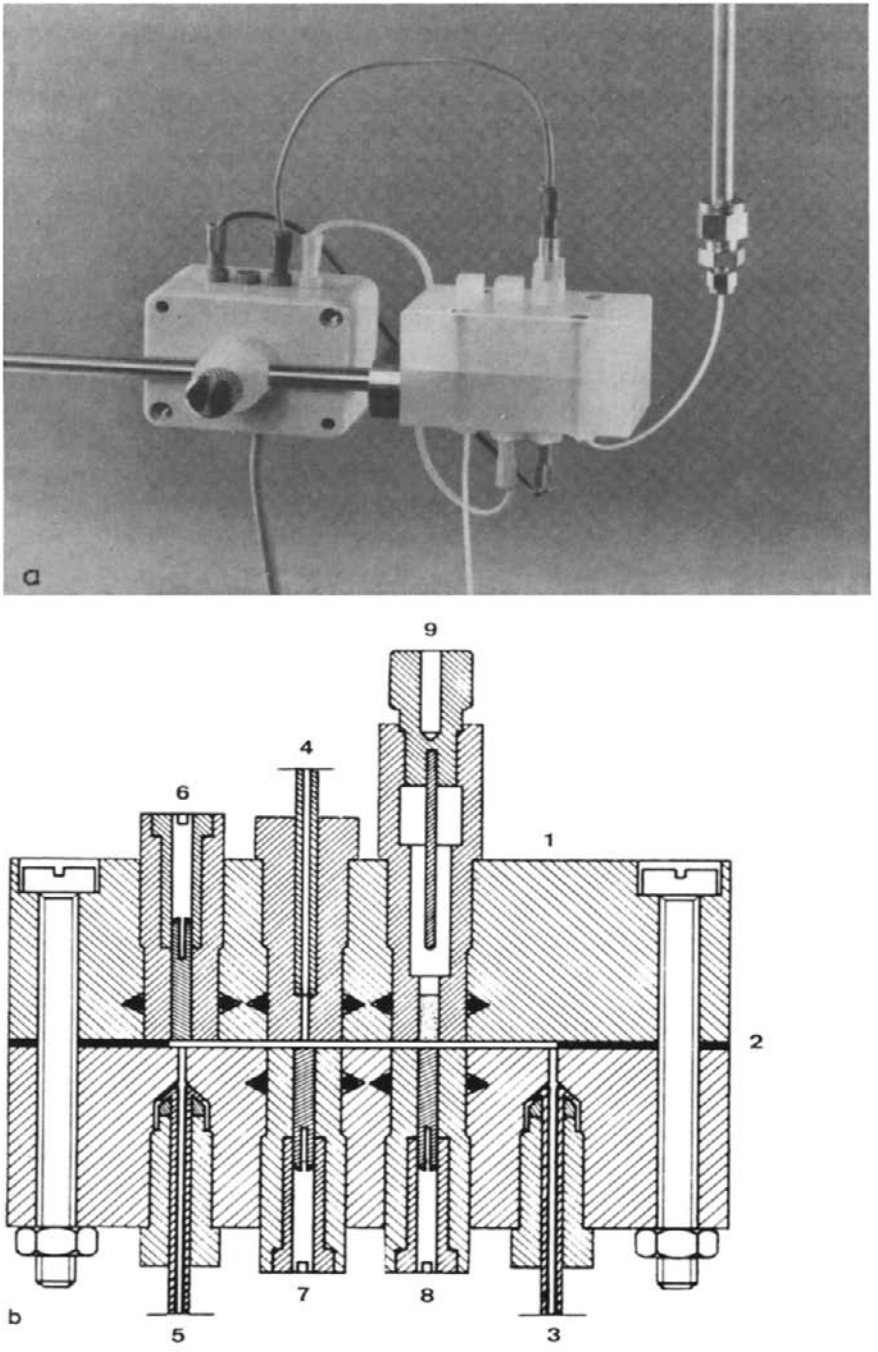

Fig. 1. (a), The new electrochemical cell for HPLC, where the operating volume is delimited by an opening in the central part of a PTFE sealing foil sandwiched between two PCTFE half-bodies. The electrodes are interchangeable by means of threaded PCTFE stems. (b), Cross-section of the cell: 1 = PCTFE cell body; 2 = PTFE (100–250 μm) sealing foil; 3 = solute input ("thin layer" mode); 4 = solute input ("wall jet" mode); 5 = solute output; 6–8 = solid electrodes (working, auxiliary); 9 = reference electrode.

TABLE I

EFFECT OF CONFIGURATION ON DEAD VOLUME

Working electrode		*Hydrodynamic configuration*	*Dead volume (μl)*
Number	*Nature*		
One	Pt, glass carbon, etc.	"Thin layer"	*ca.* 4
		"Wall jet"	*ca.* 0.2
	Mercury droplets	"Thin layer"	*ca.* 4
Two	Pt, glassy carbon, etc.	"Thin layer"	*ca.* 8

little importance in the positive range of potentials, drastically limits the negative range, whatever the working electrode; as soon as the applied potential becomes smaller than −0.1 V (platinum) or −0.25 V (other electrodes) one must eliminate oxygen from the solvent at the top of the column as well as from the sample before injection. Consequently, oxygen was removed from all the solutions before determining the electroactivity range, the limits of which are the potential values (anodic and cathodic) corresponding to a current density of 1 μA mm^{-2}. Table II shows the results obtained in a water–acetonitrile mixture: the larger ranges correspond to the use of a mercury electrode in the cathodic rangs, and nickel, platinum, boron carbide or glassy carbon (GC) electrodes in the anodic range.

TABLE II

ELECTROACTIVITY RANGE IN 80% ACETONITRILE–20% WATER AT DIFFERENT WORKING ELECTRODES

Units are volts *versus* the Ag/AgCl/KCl electrode.

Limits	*Electrode*							
	Pt	*GC*	*B_4C*	*Hg*	*Ni*	*W*	*Mo*	*Zn*
Anodic	1.3	1.1_5	1.3	0.4_5	1.2	0.8	0.1	0.4
Cathodic	-0.6_5	−0.9	-1.3_5	−1.9	−0.8	−0.9	-0.9_5	−0.6

Initially, a rather large number of materials seemed to be suitable, but a second series of tests reduced the available options. Thus, using 4-nitrophenol as solute, the stability of the diffusion current, its reproducibility and its dependence on the concentration of the solute were examined: only glassy carbon, platinum and mercury showed satisfactory results.

RESULTS AND DISCUSSION

Use of the cell with a glassy carbon electrode

Electrolysis output. Potassium ferrocyanide was selected a solute. The mobile phase was 0.05 *M* KNO_3, flow-rate 1 ml min^{-1}. The chromatographic column (200 × 4.6 mm) was filled with glass beads (100 μm). The applied potential was

+0.9 V *vs.* (Ag/AgCl/saturated KCl) and 10^{-7} mol was injected at the top of the column with a loop of 10 μl. The variation of the output with the flow-rate was normal and for 1 ml min^{-1} we obtained a yield of 2%.

Dynamic range. Three different compounds were used, two of which are oxidizable (aniline and phenol), and the other is reducible (nitrobenzene). The dynamic range is five decades in oxidation (10^{-12}–10^{-7} mol injected) and 3.5 decades in reduction ($5 \cdot 10^{-11}$–10^{-7} mol). The smaller dynamic range in reduction is probably due to O_2, H^+ and/or metallic compounds present as impurities.

Reproducibility of the detector response. The reproducibility is good, *ca.* 2–3%, as can be seen in Table III.

TABLE III

REPRODUCIBILITY OF THE DETECTOR RESPONSE WITH GLASSY CARBON

Compounds introduced	*Mean peak height (nA)*	*S.D.*		*No. of measurements*
		nA	%	
Aniline, 10^{-8} mol	2583	26.09	1	20
Nitrobenzene, 10^{-8} mol	5534	193.7	3.5	10
Mixture of aniline and chloroaniline	1st peak 766.4	17	2.22	
	2nd peak 221.6	4.03	1.82	10
	3rd peak 200.4	2.94	1.47	
Mixture of two phenolic compounds	1st peak 1185	57.37	4.84	58
	2nd peak 738	34.75	4.7	

Detection limits. With well defined chromatographic conditions (pump, solvent, flow-rate), the results are 10^{-12} mol (93 pg) for aniline and $5 \cdot 10^{-11}$ mol (6 ng) for nitrobenzene.

Applications in organic analysis. The performance of the detector was tested by injecting a mixture of chlorophenols. Fig. 2a shows the reversed-phase chromatographic separation of five phenols. This type of chromatography is well adapted to electrochemical detection. Indeed, the eluents are made up of a mixture of water, with different kinds of solvents, *e.g.*, methanol, acetonitrile, tetrahydrofuran. We had to add a supporting electrolyte to conduct the current, and the pH and ionic strength were adjusted to obtain the best conditions for electrochemical reactions. Most of the separations were carried out by this mode of chromatography. However, adsorption chromatography is still used in many cases and we tried to work with this mode of separation for the analysis of a mixture of chloroanilines on a column of Partisil (5 μm). The electrochemical detection is inoperative in solvents of low dielectric constant such as dichloromethane or hexane, even when they contain a few percent of isopropanol or acetic acid. We added a supporting electrolyte, either directly to the eluent (for example 0.1 *M* $(C_4H_9)_4NClO_4$), or by mixing at the output of the column; in the last case, a new solvent containing the supporting electrolyte (methanol + 0.2 *M* $LiClO_4$) was added to the eluent. The two flow-rates were adjusted to about the same value. The introduction of the mixture did not disturb the separation.

Glassy carbon seems to be fully satisfactory in all solvent mixtures.

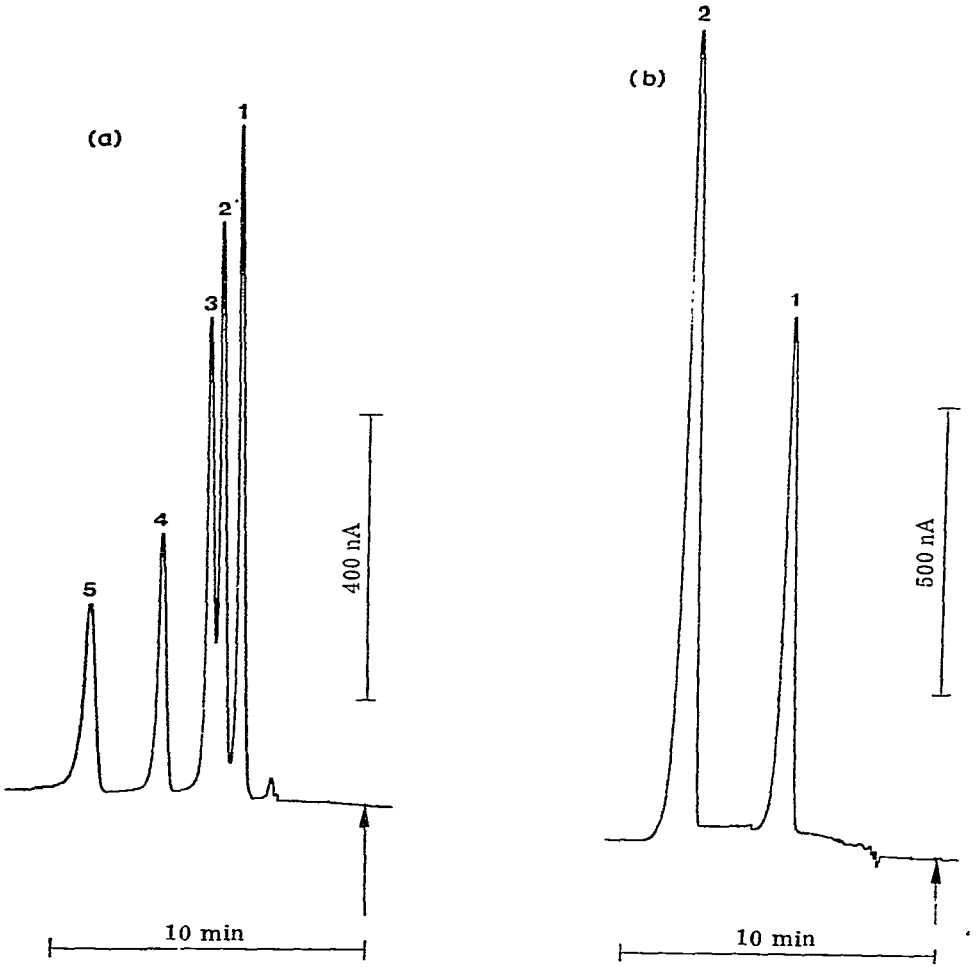

Fig. 2. (a), Analysis of mixtures by reversed-phase chromatography: 1 = phenol (200 ng); 2 = *o*-chlorophenol (200 ng); 3 = *p*-chlorophenol; 4 = dichlorophenol (200 ng); 5 = trichlorophenol (400 ng). Column: LiChrosorb RP-8, 5 μm, 20 cm. Eluent: methanol–water (70:30) + 0.05 *M* $LiClO_4$; flow-rate, 0.8 ml min^{-1}. Injection: 10 μl. Cell: Tacussel DELC. Polarographic analyzer: Tacussel PRG-E. Electrode: glassy carbon. Applied potential: + 1.20 V (Ag/AgCl/saturated KCl). (b), Separation of two nitrophenols at the dropping mercury electrode (DME): 1 = *p*-nitrophenol (10^{-8} *M*); 2 = *o*-nitrophenol (10^{-8} *M*). Column: Normatom C 18, 10 cm × 1/4 in. I.D. Eluent (deaerated): methanol–water (60:40) + 0.01 *M* H_3PO_4; flow-rate, 1 ml min^{-1}. Injection: 10 μl. Cell: Tacussel DELC. Polarographic analyzer: Tacussel PRG-E. Electrode: DME; drop time, 80 msec. Applied potential: −0.8 V (Ag/AgCl/saturated KCl).

Use of the cell with the dropping mercury electrode

The same cell may be used with a dropping mercury electrode after changing the joint and replacement of the glassy carbon. In this mode the background current decreases with the drop time while the peak current increases. The best compromise is reached with a drop time of *ca.* 0.08–0.1 sec.

Dynamic range. The dynamic range for nitrobenzene is three decades (10^{-10}–10^{-7} mol injected).

Detection limits. For the same compound, the detection limit was 10^{-10} mol injected under well defined chromatographic conditions.

Reproducibility. For the same compound, the following results were obtained: amount injected, 10^{-8} mol; mean peak height, 2250 nA; S.D. 130 nA (5.7%); number of measurements, 13.

Applications. Fig. 2b shows the separation of a mixture of nitrophenols (*o* and *p* isomers, 10^{-8} mol) after deoxygenation of the supporting electrolyte and of the sample. The oxygen peak, which is not present after deoxygenation, is confused with the peak of *p*-nitrophenol.

Use of the cell in flow injection analysis

In this case, separation was not necessary. This technique was used for measuring a series of the same samples with different concentrations. We used the same chromatographic system with a small column of glass beads: the eluent flowed continuously in the cell and from time to time the sample was injected by means of the 10-μl loop. The analysis of a phenolic compound is possible at a rate of twenty samples per hour.

Broading of chromatographic peaks

The response time of the working electrodes and of the associated electronics is less than 0.5 sec; however, the dead volume is lower, especially in the "wall jet" mode, than that of most other types of HPLC detectors. For different reasons, care must be taken that the band broadening due to the detector itself is very small; we have checked this experimentally by using alternately a UV detector (Varian 4, 300–254 nm) and an electrochemical detector for separating a mixture of phenol (0.94 μg), cresol (1.08 μg) and *tert.*-butyl-4-catechol (1.6 μg); the two detectors have also been set up in series for certain trials.

Concerning band broadening, the results obtained are exactly the same for the two detectors; a greatly increased sensitivity, especially for *tert.*-butyl-4-catechol, was noticed in the electrochemical detection.

Pulse voltametry

When a solid electrode is used (platinum, glassy carbon, etc.) the flow can be either perpendicular (wall-jet) or tangential to the conducting surface. The electroactive material is brought to the electrode by convection, and if a laminar flow is assumed, this convection increases when the flow-rate is increased. One of the major points of interest in pulse polarography, for motionless solutions, is the importance of an instantaneous faradaic current following pulse application, due to the greatly concentrated gradient present in the electrode vicinity during the first few milliseconds. In the present case, the importance of this gradient depends on the flow-rate: the greater is the flow, the thinner is the hydrodynamic layer, δ, as pointed out by Levich[20].

It appears, therefore, that the pulse method will give higher currents for lower flow-rates than direct current methods. However, both methods will be equivalent when the flow-rate is increased because the hydrodynamic layers will become the same size. In spite of these remarks, the pulse method may prove of value for simultaneously eluted electroactive material, the redox potentials of which are quite different.

CONCLUSION

Simple in design but flexible in its use, the amperometric detection cell described can operate freely in the "thin layer" or "wall jet" mode. Many uses are offered by the possibility of rapidly modifying the number and types of electrodes, whether solid or dropping mercury electrodes. After thorough experimentation in the different modes of liquid chromatography, the reproducibility is excellent, the linearity range broad and the detection limits very satisfactory.

Operating in the pulse mode results in an improvement which is more appreciable in the selectivity than in sensitivity.

We think that this new cell can make a perceptible contribution to the progress of electrochemical detection, and constitute a valuable alternative to the conventional optical techniques primarily used in chromatographic analytical laboratories.

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REFERENCES

1 B. Drake, *Acta Chem. Scand.*, 4 (1950) 554.
2 W. Kemula, *Rocz. Chem.*, 26 (1952) 281.
3 P. L. Joynes and R. J. Maggs, *J. Chromatogr. Sci.*, 8 (1970) 427.
4 J. F. K. Huber, M. Poppe and G. Denbost, *J. Chromatogr. Sci.*, 8 (1970) 192.
5 R. Stillman and T. S. Ma, *Mikrochim. Acta*, (1973) 491.
6 B. Fleet and C. J. Little, *J. Chromatogr. Sci.*, 12 (1974) 747.
7 P. T. Kissinger, *Anal. Chem.*, 49 (1977) 447 A.
8 A. Mac Donald and P. D. Duke, *J. Chromatogr.*, 83 (1973) 331.
9 P. J. Kissinger, C. Refschauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
10 Y. Takata and G. Muto, *Anal. Chem.*, 45 (1973) 1864.
11 D. C. Johnson and J. Larochelle, *Talanta*, 20 (1973) 959.
12 J. Devynck, R. Pique and G. Delarue, *Analusis*, 8 (1975) 417.
13 K. Tanaka, Y. Ishihara and H. Sunahara, *Bunseki Kagaku (Jap. Anal.)*, 24 (1975) 235.
14 J. Lankelma and H. Poppe, *J. Chromatogr. Sci.*, 14 (1976) 310.
15 M. Lemar and M. Porthault, *J. Chromatogr.*, 130 (1977) 372.
16 J. L. Ponchon, P. Hutter, F. J. Pujol and P. Blond, *Analusis*, 7 (1979) 376.
17 L. Michel and A. Zatka, *Anal. Chim. Acta*, 105 (1979) 109.
18 H. B. Hanekamp, P. Bos and R. W. Frei, *J. Chromatogr.*, 186 (1979) 489.
19 W. Kutner, J. Debowski and W. Kemula, *J. Chromatogr.*, 191 (1980) 47.
20 V. G. Levich, *Physicochemical Hydrodynamics*, Prentice-Hall, Englewood Cliffs, NJ, 1962.