Journal of Chromatography, 125 (1976) 375–388

CHROM. 9269

DESIGN AND CHARACTERIZATION OF A COULOMETRIC DETECTOR WITH A GLASSY CARBON ELECTRODE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

J. LANKELMA and H. POPPE
Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (The Netherlands)
(Received February 27th, 1976)

SUMMARY

The use of a glassy carbon electrode for detecting electrochemically oxidizable compounds with high current yield in high-performance liquid chromatography is described.

The various properties of the detector, such as sensitivity, dynamic behaviour, linear working range, detection limit and selectivity are discussed on the basis of theoretical considerations and experimental results. The detector has a detection limit in the picogram range with good dynamic behaviour.

The application of the detector to the rapid separation of biogenic aromatic acids is described and its applicability to the analysis of neuroleptics is discussed.

INTRODUCTION

Voltammetry and coulometry are valuable techniques in the quantitative analysis of electroactive compounds. However, when in a mixture the half-step potentials lie too close together, a separation process is necessary and in such instances liquid chromatography combined with electrochemical detection can be a convenient method.

The adaptation of a measuring system to a high-performance chromatographic separation process is possible only if the measurement system fulfils certain requirements with respect to dynamic behaviour, linear working range and detection limit. For electrochemical detection with the dropping mercury electrode, this adaptation has been shown to be possible[1,2]. However, in the anodic range the mercury electrode is of only limited use because of the anodic dissolution of the metal at potentials above 0.2–0.4 V *vs.* S.C.E. Moreover, the noise generated by the drop time variations influences the detection limits of these devices considerably. These limitations can be circumvented by using a solid electrode. Because of its chemical inertness and its

* Presented at Euroanalysis II, Budapest, August 24–29th, 1975.

larger potential range compared with metals such as gold or platinum[3] (from about —1.0 V to + 1.0 V *vs.* S.C.E.), glassy carbon was chosen as the electrode material.

The possible deactivation of the electrode surface, due to adsorption, can be a disadvantage of these solid electrodes. Knowledge of these effects and of the electrochemical behaviour above + 0.4 V *vs.* S.C.E. is limited compared with the numerous detailed studies that have been made with the dropping mercury electrode.

Detection in liquid chromatography by means of solid glassy carbon electrodes has been reported[4,5], using electrodes with small surface areas, but the detectors have not been characterized with respect to dynamic behaviour, linear range, sensitivity and noise. In this paper, the use of a glassy carbon electrode with a relatively large surface area is described.

It is necessary to define the concept of coulometric yield as used throughout this paper; it is defined as $i/\dot{m}nF$, where i = current (A), $\dot{m}$ = mass flow (mole/sec), n = number of electrons transferred per molecule and F = Faraday constant (C/mole). The term coulometric yield should not be confused with the term current efficiency which is used in electrochemistry.

A large surface area has the following advantages, all of which result from the fact that nearly all molecules can react at the electrode:

(a) an increase in the oxidation yield and therefore an improvement in the chromatographic signal will be obtained;

(b) small variations in the flow-rate will not affect the peak area, as the cell is acting effectively as a mass flow-sensitive device;

(c) the amount of compound can be calculated from the peak area directly if the number of electrons involved in the reaction is known;

(d) small variations in the temperature will have little effect; and

(e) partial deactivation of the electrode surface by adsorption of reaction products will have a relatively small influence.

APPARATUS

Construction

The detection cell, presented in Fig. 1, consists of two glassy carbon plates (V25, Carbone Lorraine, Paris, France) of dimensions 15 × 100 × 1.5 mm. The plates are polished with carborundum powder. In one plate the inlet tube (3-cm stainless-steel tube, I.D. 0.25 mm) and the outlet tube (3-cm silver tube, I.D. 1 mm) are cemented in electrically insulating epoxy resign (Araldite AV129). The necessary holes in the plates were made by ultrasonic drilling with carborundum powder. The cell volume is formed by a Teflon spacer between the plates, the thickness of the spacer being the height of the cell; Teflon sheets of 100 and 50 μm were used. The length of the duct is 80 mm and the width 7 mm. The plates are pressed together by means of two stainless-steel plates (thickness 10 mm) and six screws (M6). The glassy carbon plates are insulated from the steel by means of a Teflon sheet (thickness 1 mm). Electrical contact with the plates is made by means of a silver contact at the top of a Teflon screw.

Electrical part (Fig. 2)

A three-electrode system was used in order to minimize the influence of the

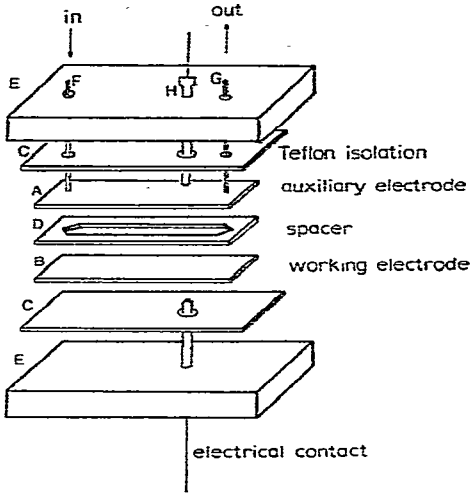


Fig. 1. Cell construction. A and B, glassy carbon plates constituting auxiliary and working electrode, respectively; C, Teflon isolation sheets; D, Teflon spacer; E, steel plates; F and G, inlet and outlet capillaries, respectively (the outlet tube is the reference electrode); not shown, six screws for pressing steel plates together.

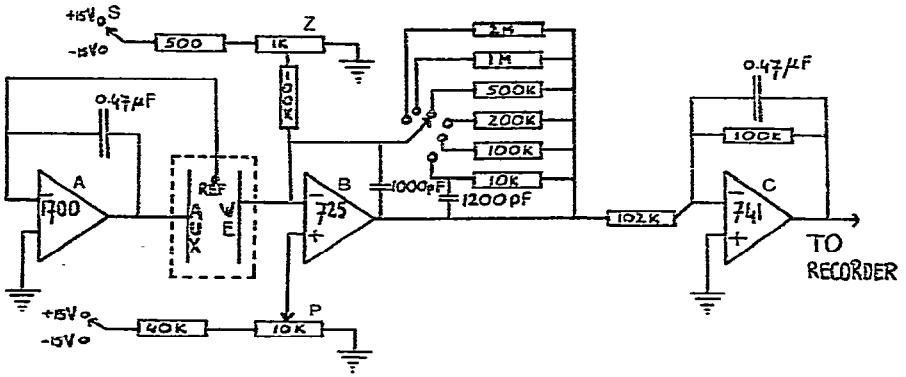


Fig. 2. Detector electronics. A, Amplifier (Philbrick 1700); B, Fairchild 725; C, Fairchild 741 (the current range can be chosen by switching the resistors at the second amplifier); P, potential control; Z, zero control, S, polarity of zero control.

resistance of the solution (*iR* drop) on the potential of the working electrode. Silver was used for the outlet tube because a reference electrode can be obtained with it. Two different electrode arrangements were used:

(a) both glassy carbon plates as the working electrode and downstream from the silver tube a stainless-steel tube (I.D. 2 mm) as the auxiliary electrode; and

(b) the upper plate as the auxiliary electrode and the opposite one as the working electrode.

The potential is controlled by a low-noise chopped operational amplifier (Philbrick 1700) and the current is amplified by a low-noise operational amplifier (Fairchild 725), followed by an active first-order filter (RC time 48 msec).

Chromatographic equipment

For testing the detector under chromatographic conditions, a pump (Labotron LDP 13A or Orlita DMP 1515), a stainless-steel column, a manometer with stainless-steel connecting tubes, a syringe injection device and an injection valve (Chromatronix HP SV 20) were used.

Chemicals

Potassium chloride, acetic acid, sodium acetate and L-ascorbic acid (pro analysi grade, E. Merck, Darmstadt, G.F.R.) and doubly distilled water were used. Amines and acids used as solutes were of different origin, most of them being of analytical-reagent grade.

THEORETICAL

General

When the eluent flows through the cell, electrochemically active compounds will react at the electrode surface and an electric current, proportional to the mass flow, will result at a constant flow-rate of the eluent. The liquid flows over the surface as a thin film of thickness about 0.1 mm and width 0.7 cm. As a result of the reaction at the electrode, transport by diffusion will start from the bulk towards the electrode surface. With the flow-rates encountered in chromatography and with dimensions of the cell used, the flow in the cell will be laminar[6]. Therefore, transport of electrochemically active material from the bulk towards the electrode surface can occur by molecular diffusion only.

In this paper, an expression is derived for the current as a function of the flow-rate and the surface area of the electrode. Mathematically, the problem has an analogy with heat conduction by flowing fluids. Graetz[7] was the first to describe the combination of heat conduction and laminar flow. Since then, several workers have dealt with this problem under different conditions and using different mathematical methods[8–10].

For the theoretical discussion the following assumptions are made:

(a) the cell is a rectangular duct in which an instantaneous reaction takes place at one wall of the cell;

(b) the flow is laminar;

(c) in the entrance rectangular cross-section the velocity is constant and the concentration is constant;

(d) all fluid properties are constant;

(e) diffusion in the direction of the flow is neglected; and

(f) mass transfer is two-dimensional, which means that the boundary effects at the vertical walls are neglected.

The following nomenclature is used:

b = width of the duct;
c_a = concentration of oxidizable compound a;
c_{a0} = concentration at $x = 0$;
$\langle c_{ax} \rangle$ = bulk concentration at x;
d = height of the duct;
D_a = diffusion coefficient of a in the eluent;

Gz = dimensionless Graetz number = $\frac{4d^2 v_x}{xD_a}$;

J = molar flux;

k_{in} = convective mass transfer coefficient, based on the log mean concentration; log mean concentration = $\frac{\langle c_{ax}\rangle - c_{a0}}{\ln \frac{\langle c_{ax}\rangle}{c_{a0}}}$;

Sh = dimensionless Sherwood number = $\frac{2k_{in}\, d}{D_a}$;

v_x = velocity in x-direction;
$\bar{v}_x$ = mean velocity in x-direction;
v_{x0} = velocity at $x = 0$;
v_y = velocity in y-direction;
x = coordinate in flow direction;
y = coordinate perpendicular to the plates.

By means of a mass balance in the xy plane, the following equation is obtained:

$$v_x \cdot \frac{\partial c_a}{\partial x} + v_y \cdot \frac{\partial c_a}{\partial y} = D_a \cdot \frac{\partial^2 c_a}{\partial y^2}$$

This equation must be solved for the following boundary conditions:

(a) $x = 0$: $-\frac{1}{2}d \leqslant y \leqslant +\frac{1}{2}d$; $v_x = v_{x0}$; $c_a = c_{a0}$

(b) $x > 0$: $y = +\frac{1}{2}d$; $v_y = v_x = 0$; $c_a = 0$

(c) $y = -\frac{1}{2}d$; $v_y = v_x = 0$; $\frac{\partial c_a}{\partial y} = 0$

The identical case for energy transport has been calculated numerically[9]. The dimensionless Nüsselt number has been given as a function of $1/Gz$; for mass transport, Sh is the analogous number. For the number of moles transferred per second to the electrode we have:

$$Jbx = \bar{v}_x db(c_{a0} - \langle c_{ax}\rangle) \tag{1}$$

The definition of the mass transfer coefficient gives[6]

$$J = k_{in} \frac{(c_{a0} - \langle c_{ax}\rangle)}{\ln \frac{c_{a0}}{\langle c_{ax}\rangle}} \tag{2}$$

and the definition of the Sherwood number for this geometry is

$$Sh = \frac{k_{in}\, 2d}{D_a} \tag{3}$$

From eqns. 1–3, we obtain

$$\ln \frac{c_{a0}}{\langle c_{ax}\rangle} = \frac{Sh\, D_a\, x}{2\bar{v}_x\, d^2} \tag{4}$$

As Sh is known to be a function of Gz, the number of moles transferred to the surface can be calculated from this formula. The result for the coulometric yield is

$$\frac{c_{a0} - \langle c_{ax} \rangle}{c_{a0}} = 1 - \exp\left(\frac{-Sh\, D_a\, x}{2\bar{v}_x\, d^2}\right) \tag{5}$$

Calculation of the coulometric yield under chromatographic conditions

Using the cell described under normal chromatographic conditions, the Graetz number will be less than 10. According to Stephan[9], Sh has then approached a constant value, which means that the mass transfer coefficient is no longer a function of the flow-rate. This value for Sh is 4.86.

Using eqn. 5, the yield can be calculated as a function of the electrode surface. This is illustrated in Fig. 3, and it can be seen that with reasonable cell dimensions it is possible to obtain nearly 100% coulometric yield (91%).

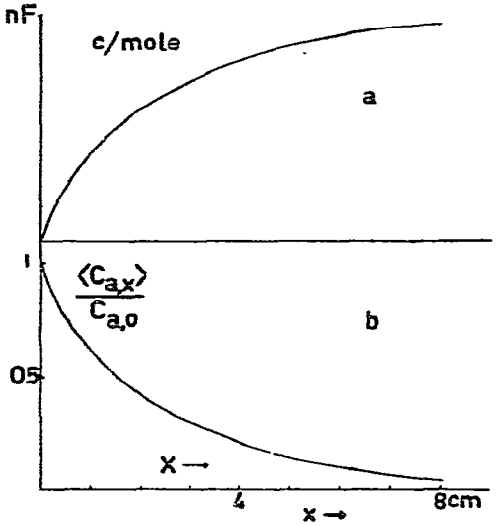


Fig. 3. Calculated percentage of reacted compound (a) and fraction of mean concentration from the beginning concentration (b) *versus* distance to the inlet point. Conditions: $\bar{v}_x = 0.8$ cm/sec; $D_a = 10^{-5}$ cm²/sec; height of the spacer = 100 μm; width = 0.7 cm.

Prediction of dynamic behaviour

For 100% coulometric yield, each molecule will reach the electrode within the residence time in the cell. The dynamic behaviour of the cell is described by the response to a concentration impulse, *i.e.*, the current *versus* time curve after an amount of substance in a very small volume has entered the cell. This current *versus* time curve will be exactly the same as if the oxidation took place after diffusion from a stagnant fluid film, as the chance of molecules reaching the electrode is independent of the movement alongside the electrode caused by the flow. Diffusion from a stagnant fluid film has been described[11,12]. The bulk concentration as a function of time is given as

$$\frac{\langle c_a \rangle}{c_{a0}} = \frac{8}{\pi^2} \sum_{v=0}^{\infty} \frac{1}{(2v+1)^2} \exp\left\{-\left[\frac{(2v+1)\pi}{2d}\right]^2 D_a\, t\right\}$$

The current caused by the impulse will be the derivative of this expression. In this way, a theoretical estimate of the peak broadening effect would be possible. However, comparison of theoretical with experimental values of the standard deviation, σ_d, caused by the detector is difficult, as the experimental value is based on measurement of the width at 0.6 maximal height of the peak. The same procedure applied to the theoretical curve would yield zero for σ_d, as the theoretical equation predicts an infinite value of the current for the moment that the substance enters the cell. A means of dealing with this problem is to consider the time interval in which a certain percentage of the substance reacts, and to compare it with the value expected for a gaussian curve. From Jost[11], it can be derived that when $\Delta t = 0.95\ 4d^2/\pi^2 D_a$, 68.5% of the substance has reacted. For a gaussian curve, the same percentage of the area is present between the boundaries $t_m - \sigma$ and $t_m + \sigma$, where t_m is the time of the maximum. When we equate 2σ to $\Delta t = 0.95\ 4d^2/\pi^2 D_a$, we obtain a rough estimate of σ, and accordingly we put

$$\sigma_d = \frac{0.95}{2} \cdot \frac{4d^2}{\pi^2 D_a}$$

These estimates are included in Table III.

Linearity

Although there may be other causes, non-linearity in electrochemical cells is caused mainly by the voltage drop in the electrolyte solution when a current flows. This so-called iR drop decreases the potential difference prevailing at the working electrode–solution interface. As a linear relationship between concentration and current can be expected only for a constant value of this potential difference, this iR drop must be kept as small as possible. There are several methods of accomplishing this requirement:

(i) Use of a solution of high conductivity (water with a high concentration of a conducting inert electrolyte). From both the chromatographic and electrochemical points of view, this procedure gives serious limitations.

(ii) Use of a cell of suitable geometric shape, in which the resistance between the working and reference electrodes is as small as possible. This procedure means a short distance and a wide cross-section between the electrodes.

(iii) Use of a three-electrode system. The two functions, conducting the current and serving as a reference point for the potential, normally performed by one electrode are separated in this instance, between a reference electrode and an auxiliary electrode which carries the current. A small reference electrode with high impedance can be used. These electrodes can be positioned very near to the working electrode (method ii).

A properly designed coulometric liquid chromatography detector should be capable of conducting fairly large currents, should have a small effective cell volume and should be compatible with different eluents. Therefore, a combination of methods ii and iii is the most suitable.

The relative positions of the working, reference and auxiliary electrode are important. The geometry should be such that the voltage drop that exists between the working and auxiliary electrodes as a result of the current is observed by the reference electrode to only a very small extent. This means that the current paths from the

auxiliary and reference electrodes to the working electrodes should have a common part of very small resistance.

Kissinger *et al.*[4] described an electrochemical detector with a three-electrode arrangement in which almost the whole of the voltage drop from the working to the auxiliary electrode is "seen" by the reference electrode. In this arrangement the eluent flows from the small cell through a high-resistance capillary into a larger vessel in which the reference and auxiliary electrodes are positioned. In this way, the essential advantage of a three-electrode system with respect to linearity is lost. The method used by Fleet and Little[5], where the auxiliary and reference electrodes are placed in separate outlets of the cell, is adequate. In the device described in this paper, the auxiliary electrode, having the same surface area as the working electrode, is placed directly in front of the latter. In this way, the total resistance is so small that large currents can be tolerated.

TESTING THE DETECTOR

During testing of the detector, the working electrode had a constant potential of + 0.75 V *vs.* Ag|AgCl|0.05 *M* Cl^-. The eluent was a solution 0.03 *M* in sodium acetate, 0.16 *M* in acetic acid (pH 4) and 0.05 *M* in potassium chloride. The silver tube was electrolytically covered with silver chloride. Together with the chloride added to the eluent, this arrangement results in a stable reference potential. L-Ascorbic acid was used as the test compound because it oxidises easily and no adsorption at the working electrode was observed. In order to reduce oxidation by air, the solvent for the samples was deaerated with nitrogen and cooled in ice before use. During the test, the temperature was held constant at 22°.

Static properties

Standing current. With eluent only there is a standing current due to impurities in the eluent. When using nylon connecting tubes only and hydrostatic syphoning, this current was 0.32 μA at the chosen voltage and using a spacer thickness of 100 μm.

Using stainless-steel tubes, a pump (Orlita DMP 1515), a manometer and a column (stainless steel), the standing current was 0.58 μA. The difference is probably due to metals dissolved from the tubes.

Sensitivity. The static mass flow sensitivity is defined as

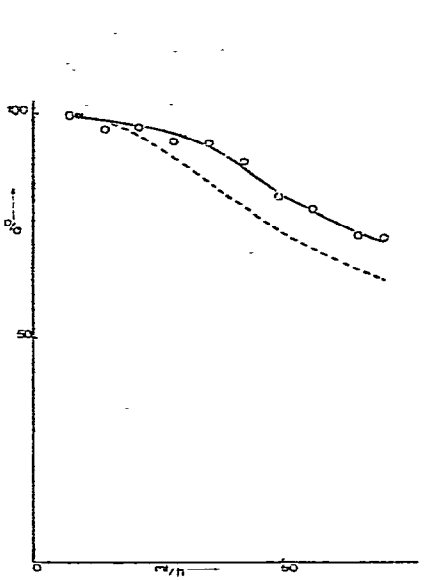
$$S = \frac{i}{w\,c}$$

where S = sensitivity (A sec/mole), i = signal (A), w = flow-rate (l/sec) and c = concentration (mole/l). It was measured using a sampling valve with a large loop[2].

When the coulometric yield is 100%, the sensitivity (C/mole) can be calculated by the equation

$$S = nF$$

where n = number of electrons transferred per molecule and F = Faraday constant. The dependence of the coulometric yield on the flow-rate is shown in Fig. 4, in which



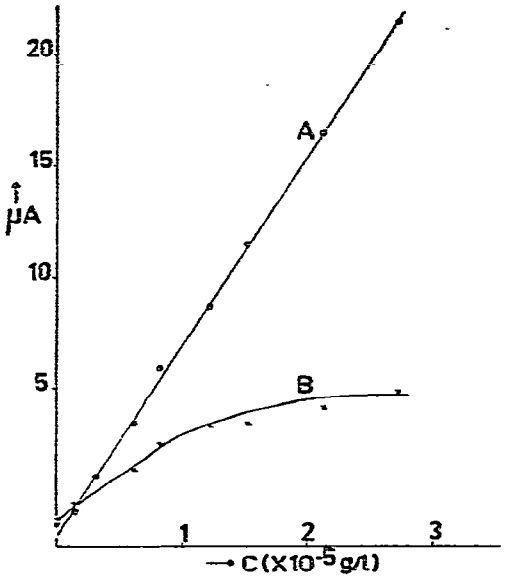


Fig. 4. Coulometric yield *versus* flow-rate; straight line, experimental; broken line, calculated. Conditions: voltage, + 0.90 V; spacer thickness, 50 μm; compound, ascorbic acid; concentration, $2.19 \cdot 10^{-4}$ mole/l.

Fig. 5. Linearity of the detector signal for two electrode arrangements: (A) working and auxiliary electrodes opposite each other; (B) glassy carbon plates as the working electrode and the auxiliary electrode downstream from the silver outlet. Conditions: Labotron pump; flow-rate, 20 ml/h; spacer thickness, 90 μm; voltage, + 0.75 V.

both experimental results and data calculated according to the theory described under Theoretical are plotted. The diffusion coefficient for ascorbic acid has been calculated to be $0.69 \cdot 10^{-5}$ cm^2/sec at 22° using Wilke's equation[13]. The coulometric yield of 100% corresponds to two electrons transferred per molecule, in agreement with the results in the literature[14]. As can be seen, the difference between the two lines is less than 10%. The concentration of the ascorbic acid solution was determined by titration with N-bromosuccinimide[15], which had been standardised with arsenic (III)[16].

Dependence of coulometric yield on spacer thickness. The coulometric yield, as can be calculated from eqn. 5, will increase when the term $Sh/4\bar{v}_x d^2$ increases. In Table I, the yield is given for various duct heights. From these data, it can be concluded that the coulometric yield is higher when using thinner spacers. Moreover, peak broadening will decrease under these circumstances. Spacers thinner than 50 μm should be avoided because of the limited flatness of the electrode material.

Linearity and linear dynamic range. The linearity was tested for the two different electrode arrangements described under *Electrical part*. The reason for using a downstream auxiliary electrode was to prevent reduction after the oxidation, which could occur for reversible reactions. However, as can be seen in Fig. 5, the arrangement of the working electrode opposite to the auxiliary electrode has a far greater linear range. The linear range is much larger than given in Fig. 5 (up to 10^{-3} A).

As most electrode reactions of interest for chromatographic applications are irreversible, the latter arrangement was chosen for all further experiments. The smaller

TABLE I

COULOMETRIC YIELD AT VARIOUS DUCT HEIGHTS

For D_a, a value of $0.69 \cdot 10^{-5}$ cm²/sec was taken, corresponding to the value calculated for ascorbic acid. Duct width, 0.7 cm; length, 7.6 cm; flow-rate, 20 ml/h.

Spacer height (μm)	*Coulometric yield (%) calculated*	*Measured*
50	96	97
75	88	—
107	75	64
200	57	—

linear range can be explained as a result of the electrode arrangement as described under *Linearity*.

The linear dynamic range is the ratio between the upper linearity limit and the noise of the standing current, and gives the useful working range of the detector. Its value is given in Table II. The very large dynamic range is noteworthy, especially when compared with those for other electrochemical detectors used in high-performance liquid chromatography[1-4].

Noise and detection limit. The method for measurement of noise has been described previously[1,2]. The integrated noise of the standing current was calculated for integration times corresponding to chromatographic peaks. The noise observed is present only when connecting the glassy carbon working electrode. Two explanations of the noise can be given.

(a) As the electrode material is porous, its complete wetting by the eluent takes place only after a very long time. Electrodes in use are therefore incompletely wetted. Changes in the area covered by the eluent will generate electric currents. If a new pore is filled with eluent, a spike will result; indeed, the noise observed seems to be composed of current spikes. A remarkable improvement was achieved by conditioning the working electrode in hot paraffin wax. After cooling, the solid wax was removed from the upper surface by wiping with tetrachloroethane. The improvement can be explained from the fact that part of the cooled wax stayed in the pores and a flat electrode surface was obtained.

(b) Voltage noise of the amplifier, which regulates the potential difference between the working electrode and the reference electrode at the desired value, will generate voltage fluctuations at the working electrode–solution interface. As this interface behaves like a large capacitor, this results in current fluctuations. The situation can easily be simulated. The result was that, with the amplifier used, a noise level of about 10^{-10} A can be expected from this source, assuming a capacity of 200 μF/cm² across the interface[3]. This is of the same order of magnitude as the observed noise and therefore this effect may give a significant contribution.

The standard deviations of the noise are represented in Table II.

When the amount of sample is limited, for instance in clinical and biochemical analysis, it is more relevant to give the detection limit as an absolute amount. The detection limit, expressed in moles, can be calculated using the equation

$$q_{det} = 3\sigma_{det} V_{peak}$$

TABLE II

STATIC NOISE OF THE DETECTOR AND LINEAR DYNAMIC RANGE

Conditions: spacer thickness, 100 μm; potential of working electrode, +0.75 V *vs.* Ag|AgCl|0.05 *M* Cl^-; temperature, 22°; the eluent was syphoned hydrostatically and nylon connecting tubes were used.

Integration time (sec)	*Standard deviation, integral (A sec × 10^{-9})*		*Standard deviation, current (A × 10^{-9})*		*Corresponding concentration (mole/l × 10^{-9})*		*Linear dynamic range*
	a^*	b^{**}	a^*	b^{**}	a^*	b^{**}	
1	0.27	0.48	0.27	0.48	0.40	0.32	> 10^{-6}
3	0.48	1.2	0.16	0.40	0.28	0.27	
10	0.90	2.4	0.09	0.24	0.15	0.16	
30	2.2	7	0.07	0.23	0.12	0.16	
100	10.5	22.5	0.10	0.23	0.17	0.16	
300	145	110	0.48	0.37	0.82	0.26	

* Flow-rate 13.5 ml/h.
** Flow-rate 53.5 ml/h.

where q_{det} = lowest detectable amount of component present in the sample, σ_{det} = the concentration corresponding to the standard deviation of the noise and V_{peak} = peak volume. In modern liquid chromatography, the peak volume is often of the order of 50 μl. Using the value for σ_{det} from Table II, q_{det} is found to be $5 \cdot 10^{-14}$ mole.

Dynamic properties

Peak broadening. A good detector must follow a chromatographic signal sufficiently rapidly, in other words, peak broadening caused by the detector must be small compared with that of the column. In order to measure the peak broadening of the detector, 0.5 μl of a $3.4 \cdot 10^{-3}$ *M* solution of ascorbic acid was injected by means of a precise syringe and a sampling device[17]. The peak broadening, as given in Table III, was determined by measuring half the width of the peak at six tenths of the maximal height. Calculated values, according to the method described under *Prediction of dynamic behaviour*, are also given. It can be seen that these values agree with the ex-

TABLE III

CONTRIBUTION TO PEAK WIDTH FOR ASCORBIC ACID EXPRESSED AS THE STANDARD DEVIATION, MEASURED AS THE HALF-WIDTH AT SIX TENTHS OF THE MAXIMAL HEIGHT OF THE PEAK.

The calculated values correspond to 100% coulometric yield.

Flow-rate (ml/h)	*Duct height (μm)*	*Peak broadening*		
		Experimental		*Calculated (sec)*
		sec	*μl*	
40	50	0.65 ± 0.03	7.2	0.7
20	50	0.64 ± 0.4	3.5	0.7
10	50	0.7 ± 0.8	1.9	0.7
40	100	0.87 ± 0.02	9.7	2.8
20	100	1.44 ± 0.02	7.9	2.8
10	100	2.80 ± 0.08	7.7	2.8

perimental results provided that 100% coulometric yield is approached, *i.e.*, with a spacer thickness of 50 μm and for a spacer thickness of 100 μm at the lowest flow-rate. For the diffusion coefficient, a value of $0.69 \cdot 10^{-5}$ cm^2/sec was taken.

SELECTIVITY

When changing the potential of the working electrode, the sensitivity for some compounds can be changed, depending on their half-step potentials. When the separation of two peaks is bad, the peak of the compound with the higher half-step potential can be made to disappear by lowering the potential. The amount of both compounds can then be calculated from the two peaks areas. Information on the peaks can be obtained by varying the potential.

APPLICATION TO CHEMICAL ANALYSIS

At present, there is increasing interest in the analysis of endogenous metabolites and drugs and their metabolites in body fluids. As many of these compounds are oxidizable, liquid chromatography with electrochemical detection is of interest because of its low detection limit and its selectivity. Detection by means of anodic electrochemical oxidation has been applied by Kissinger *et al.*[4] and Fleet and Little[5] for the detection of ascorbic acid, uric acid and some catecholamines.

Some psychopharmaceuticals that are oxidizable at + 0.9 V are as follows: *neuroleptics:* chlorpromazine, thioridazine, periciazine, thiethylperazine, thioproperazine, perphenazine, fluphenazine, chlorprothixene, clopenthixol, flupenthixol and thiothixene; *antidepressants:* amitriptyline, nortriptyline, imipramine, desipramine and doxopine. The detection of the so-called "long-acting depot" neuroleptics such as the decanoic esters of fluphenazine, perphenazine, clopenthixol and flupenthixol is of special interest.

Reversed-phase chromatography using coulometric detection might be a useful method for analysis in blood serum. Fig. 6 shows a peak of 0.5 ng of fluphenazine, separated from the solvent peak by a column filled with modified silica[18].

The results of the separation of some biogenic amines are given in Fig. 7. Fig. 7a gives the result for UV detection; the phase system will be described elsewhere[18]. The coulometric detector was placed in series with the UV detector. Fig. 7b gives the result when the applied voltage is 0.75 V; only peaks 2 and 5 are detected. Fig. 7c gives the result when the applied potential is + 0.6 V. When the UV detector is placed in series after the coulometric detector, the peak belonging to 5-aminosalicylic acid appears to be much higher. This interesting increase in sensitivity is illustrated in Fig. 8, which should be compared with Fig. 7a.

CONCLUSION

The results show that the use of a large electrode surface area has a number of advantages, among which the most important are: the higher sensitivity and the smaller influence of electrode contamination on the sensitivity. The detection limit lies in the picogram range and the electrode arrangement results in a linear dynamic range of 10^6.

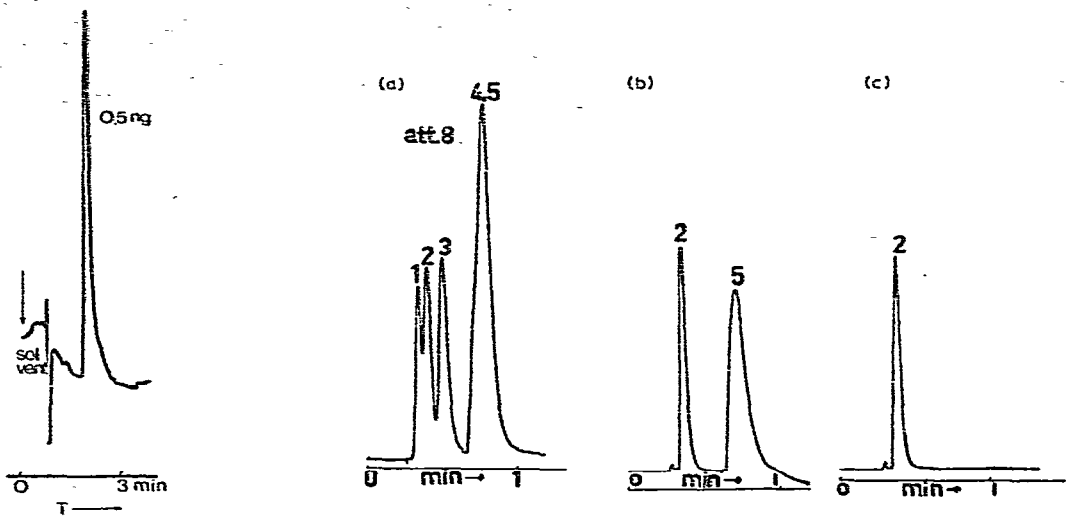


Fig. 6. Peak from 0.5 ng of fluphenazine. Conditions: voltage, 0.9 V; column, 3 cm stainless-steel tube, I.D. 2.8 mm, filled with modified silica (particle size 4–7 μm); eluent, 60% methanol, 40% water, 0.05 *M* phosphate buffer, pH 6.4; flow-rate, 3.5 μl/sec; pressure, 20 bar.

Fig. 7. Separation of biogenic amines. (a) Rapid separation of biogenic aromatics: 1 = 3-amino-toluenesulphonic acid; 2 = 5-aminosalicylic acid; 3 = 3-hydroxymandelic acid; 4 = 3,5-dihydroxybenzoic acid; 5 = 4-methylaminobenzoic acid. Conditions: UV detection at 265 nm; column, 10-cm stainless-steel tube, I.D. 2.8 mm, filled with modified silica (particle size 4–7 μm); eluent, 0.05 *M* perchloric acid, 0.05 *M* potassium chloride, 1.7% butanol in water; flow-rate, 28 μl/sec; pressure, 220 bar. (b) Coulometric detection at 0.75 V; peaks 2 and 5 are detected. (c) Coulometric detection at 0.60 V; only peak 2 is detected.

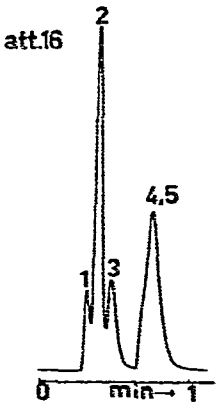


Fig. 8. UV detection after reaction in the electrochemical cell (potential + 0.75 V). The reaction products of peak 2 have a greater UV absorption at the chosen wavelength compared with Fig. 7a.

Other eluents having a lower electrical conductivity can be used. More compounds will be detectable with, *e.g.*, acetonitrile as the mobile phase because of its wider anodic range[19]. The possibility of using eluents of low conductivity permits a wider choice of chromatographic phase systems.

For 100% coulometric yield, the apparatus can also be used for the determination of the number of electrons transferred per molecule.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. G. den Boef for good advice during the preparation of the manuscript, Dr. U. R. Tjaden for his assistance with the application of his phase system, and Mr. K. Camstra whose technical skill made the construction of the cell possible.

REFERENCES

1 J. G. Koen, J. F. K. Huber, H. Poppe and G. den Boef, *J. Chromatogr. Sci.*, 8 (1970) 192.
2 J. Lankelma and H. Poppe, *J. Chromatogr. Sci.*, 14 (1976) 310.
3 H. E. Zittel and F. J. Miller, *Anal. Chem.*, 17 (1965) 200.
4 P. T. Kissinger, C. Refshauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
5 B. Fleet and C. J. Little, *J. Chromatogr. Sci.*, 12 (1974) 747.
6 R. P. Bird, W. E. Stewart and E. N. Lightfoot, *Transport Phenomena*, Wiley, New York, 1960.
7 L. Graetz, *Ann. Phys.*, 18 (1883) 79 and 25 (1885) 337.
8 E. M. Sparrow, *NACA Tech. Note*, No. 3331 (1955).
9 K. Stephan, *Chem.-Ing.-Tech.*, 32 (1960) 401.
10 W. E. Mercer, W. M. Pearce and J. E. Hitchcock, *J. Heat Transfer*, (1967) 251.
11 W. Jost, *Diffusion in Solids, Liquids and Gases*, Academic Press, New York, 1960, p. 37.
12 H. Dünwald and C. Z. Wagner, *Z. Phys. Chem. B*, 24 (1934) 53.
13 S. Bretznajder, *Prediction of Transport and other Physical Properties of Fluids*, Pergamon Press, Oxford, 1971, p. 372.
14 K. S. V. Santhanam and V. R. Krishnan, *Anal. Chem.*, 33 (1961) 1493.
15 M. Z. Barakat, M. F. A. El-Wahab and M. M. El-Sadr, *Anal. Chem.*, 27 (1955) 536.
16 A. Berka, J. Vulterin and J. Zýka, *Massanalytische Oxydations- und Reduktionsmethoden*, Akademische Verlagsgesellschaft, Leipzig, 1964, p. 47.
17 J. F. K. Huber, *J. Chromatogr. Sci.*, 7 (1969) 172.
18 U. R. Tjaden and J. F. K. Huber, in preparation.
19 A. J. Bard, *Electroanalytical Chemistry*, Vol. 3, Marcel Dekker, New York, 1969, p. 61.