Journal of Chromatography, 90 (1974) 259–265

CHROM. 7281

DETERMINATION OF PURINE BASES BY MEANS OF CHROMATO-VOLTAMMETRY*

M. VÁRADI**
LABOR MIM Laboratory Instruments and Equipment Works, Budapest (Hungary)
Zs. FEHÉR**
United Works of Pharmaceutical and Dietetic Products, Budapest (Hungary)
and
E. PUNGOR
Institute for General and Analytical Chemistry, Technical University of Budapest, Budapest (Hungary)
(Received November 19th, 1973)

SUMMARY

A detector working on the voltammetric principle was used for the liquid chromatographic analysis of purine bases.

A new type of detector cell has been developed and its characteristic parameters were investigated. A linear correlation between the voltammetric sign and the concentration of the solution being analysed was obtained. The standard deviation was found to be 3% for 10 measurements. The lower limit of the determination was 10^{-10} mole for guanine.

The cell was employed as a specific detector cell for purine bases.

INTRODUCTION

One of the most sensitive methods of analytical chemistry is voltammetry (polarography), which has been used for liquid chromatographic detection for many years. In 1952, Kemula[1] was the first to apply a detector cell containing a dropping mercury electrode to liquid chromatographic systems, and he introduced the term for this method, chromatopolarography. Since then, various compounds, *e.g.*, isomers and homologues of nitroaniline, chloronitrobenzene, nitrophenol, DDT, alkaloids, amino acids[2] and pesticides[3] have been determined by this method.

The advantages of chromatopolarography were discussed in detail in earlier publications[2,3]. The most important are the high selectivity of the separation, in those instances when the polarographic half-wave potentials of the components to be measured are sufficiently different from each other, and the high sensitivity of the detection.

It is well known from the electrochemical literature that the mercury electrode applied in chromatopolarographic detectors behaves ideally as a polarographic

* A patent for the detector cell was applied for in January, 1973.

** Present address: Institute for General and Analytical Chemistry, Technical University of Budapest.

indicator electrode in the potential range −2.5 to +0.4 V (related to the normal hydrogen electrode). In the negative range, the polarizing potential is limited by the electrode reaction of the cation of the supporting electrolyte, or in aqueous solutions by formation of hydrogen, and in the positive range it is shown by the anodic dissolution of mercury. Most organic compounds are not electroactive in this potential range and their oxidation half-wave potential is usually found in a more positive range. In these instances solid electrodes, most frequently platinum or carbon[4], are used.

One of the solid electrodes, suitable for use in the positive potential range, is the silicone rubber-based graphite electrode developed by Pungor and co-workers at the Veszprém University for Chemical Engineering. Pungor and Szepesváry[5] reported on its characteristics and application and pointed out that numerous organic compounds can be reproducibly determined without the need to renew the electrode surface.

Joynes and Maggs[6] used the silicone rubber-based graphite electrode as the indicator electrode of a chromatographic detector. The aim of their work was to overcome the difficulties arising from the mechanical instability and sensitivity of the dropping mercury electrode. In order to control the operation of their measuring cell, they studied the compounds detected by Kemula with the dropping mercury electrode. The operation of the cell was found to be good but, in their measurements, the basic principles of voltammetry were not followed in all respects, *e.g.*, the ratio between the sizes of the indicator and reference electrodes and the voltages applied differed from those usually applied in voltammetry.

In this paper, we report on the development of a measuring cell containing a solid electrode that is applicable to voltammetric measurements carried out in the potential range from −0.3 to +1.5 V. In developing this detector cell, care has been taken that it should meet the requirements of modern chromatographic detectors, such as a small volume and a high sensitivity.

EXPERIMENTAL AND RESULTS

Reagents

The chemicals used were of analytical grade and were obtained from Reanal, Budapest, Hungary.

Construction of the chromatographic apparatus

The design of our experimental apparatus is shown in Fig. 1. In order to eliminate occasional interference from oxygen, nitrogen gas was bubbled through the solutions and the eluent was kept in a closed vessel under nitrogen gas. For transporting the eluent, a Type LS-204 micro-pump (Labor MIM, Budapest, Hungary) was used, ensuring a precision in the liquid flow-rate of ±0.5% in the range 20–250 $ml \cdot h^{-1}$ to a pressure of 50 atm.

For chromatographic separations, a 100×0.9 cm I.D. glass column filled with Sephadex G-10 was used.

For recording the voltammetric current and for ensuring a constant potential, a Radelkis Type OH 102 polarograph (Budapest, Hungary) was used. The sensitivity of this polarograph can be adjusted between 8×10^{-11} and 6.4×10^{-7} $A \cdot mm^{-1}$ in steps.

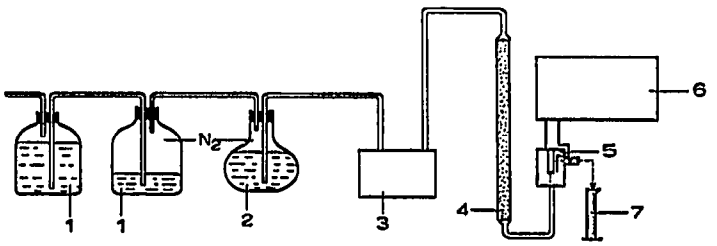

Fig. 1. Schematic diagram of the apparatus for chromatographic analysis. (1) Deville vessel; (2) eluent reservoir; (3) pump; (4) chromatographic column; (5) voltammetric detector cell; (6) polarograph; (7) calibrated receiver.

Construction of the detector cell

The construction of the voltammetric detector cell is shown in Fig. 2. The cell is made of Plexi-glass and contains a silicone rubber-based graphite electrode 1.5 mm in diameter as an indicator electrode, which is applicable to concentration measurements in streaming solutions[7].

The electrode is fixed in a screwed holder so that it is easy to change. A silver wire coated with silver chloride is used as the reference electrode, immersed in the streaming solution containing chloride at constant concentration. The eluent flows to the indicator electrode through a constriction 1 mm in length and 0.3 mm in diameter. The liquid flow increases at the constriction before the electrode and the electrode is placed perpendicular to the direction of the flow of the liquid stream, thus causing turbulent flow surrounding the electrode.

As the liquid flows perpendicular to the electrode, the interfering effect caused by air bubbles entering the system is eliminated or at least decreased, because the air bubbles pass through the constriction and rapidly reach the surface of the liquid, thus causing no interference on the surface of the electrode.

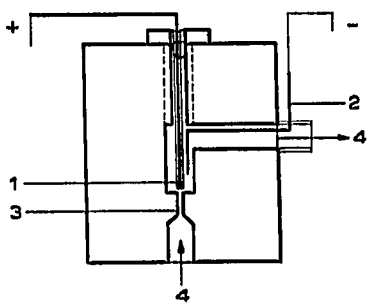

Fig. 2. Construction of the voltammetric detector cell. (1) Indicator electrode; (2) reference electrode; (3) choke; (4) direction of liquid flow.

Operation of the detector cell

As is well known from hydrodynamic voltammetry[8], the transport of material in a streaming liquid is achieved in two different ways. On the one hand, molecular diffusion takes place as a result of the concentration gradient in the liquid, and on the other hand the particles dissolved in the liquid move from one place to another

as a consequence of the flow. These two processes together are called convective diffusion. In voltammetric measurements carried out in streaming solutions, the current intensity corresponding to the amount of material transported by convective diffusion also depends on the hydrodynamic parameters.

The differential equation describing the convective diffusion for a solution flowing perpendicularly to the disc electrode was solved by Matsuda[9] and Marchiano and Arvia[10], and the equation describing the limiting current is as follows:

$$i_L = knFAD^{\frac{2}{3}}\nu^{-\frac{1}{6}} \cdot \left(\frac{U}{L}\right)^{\frac{1}{2}} \cdot c \qquad (1)$$

where

k = numerical constant
i_L = limiting current
n = number of electrons taking part in the electrochemical reaction
F = Faraday constant
A = electrode surface area
D = diffusion coefficient
ν = kinematic viscosity
U = flow-rate
L = characteristic size of the electrode
c = concentration of the electroactive component

According to eqn. 1, the current intensity measured at a constant potential (chosen in limiting current range) is proportional to the concentration and the square root of the flow-rate. This linear correlation was proved experimentally in both the laminar and turbulent regions by several workers[11,12]. According to Wranglén and Nilsson's experience, the slope of the straight line i_L *versus* $\sqrt{U}$ is greater in turbulent flow than in laminar flow[13].

The equation describing the diffusion mass transport:

$$j_{diff} = \frac{Dc}{\delta} \qquad (2)$$

where δ is the thickness of the diffusion boundary layer, shows that the decrease in the diffusion boundary layer (*e.g.*, by producing turbulence) causes an increase in the sign of the current and so also in the sensitivity of the measurement.

Determination of the characteristic parameters of the detector cell

In order to determine the different parameters of the cell, a Tygon tube, 1 mm in diameter, was used instead of the chromatographic column (Fig. 1) into which the sample was injected.

Experiments were carried out in order to determine the reproducibility of the measurements with the cell. A 0.5-ml volume of $5 \cdot 10^{-4}$ M guanine solution was injected into phosphate buffer and the voltammetric current was recorded as a function of time. The area under the current intensity curves was determined by weighing. The standard deviation was found to be 3% for 10 measurements.

The relationship between the current intensity and the concentration of

the solution injected was investigated. By injecting equal volumes of guanine solutions of different concentrations, a calibration graph (Fig. 3) was obtained, which showed that the voltammetric current intensity (area under the peak) is directly proportional to the amount of the substance injected in the concentration range examined. The lower limit of the determination was measured, and it was found that when 10^{-10} mole of guanine was injected, a reproducible signal, well separated from the background noise, was obtained.

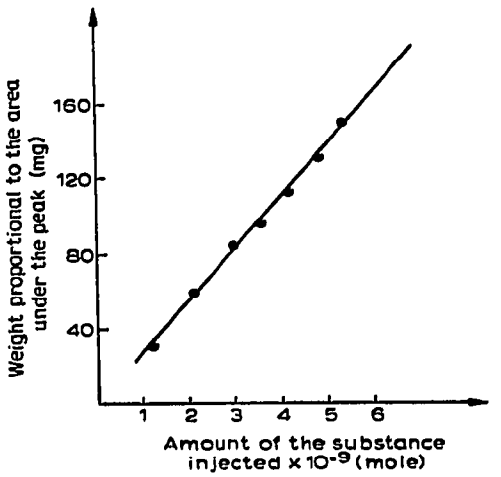

Fig. 3. Relationship between the amount of substance injected and the area under the peak. Potential, +0.90 V; sensitivity, $2 \cdot 10^{-10}$ A·div^{-1}; flow-rate, 50 ml·h^{-1}, amount of sample injected, 0.3 ml.

Determination of purine bases by use of the detector cell

Dryhurst[14] examined the electrochemical behaviour of purine bases and some nucleosides on a pyrolitic graphite electrode, and reported measurements made on these compounds and also the mechanism of electrochemical oxidation.

By means of the silicone rubber-based graphite electrode, these compounds similarly proved to be measurable, and our results obtained in a stationary system are given in Table I.

Our detector cell was used to detect purine bases separated on a Sephadex column. The chromatographic separation was performed with the apparatus shown in Fig. 1, using a chromatographic column and eluents described by Sweetman and Nyhan[15]. The sample (0.1 ml) contained $1.25 \cdot 10^{-7}$ mole of each of the four purine bases. The elution was first carried out with phosphate buffer and, at a flow-rate of 30 ml·h^{-1}, the results shown in Fig. 4A were obtained. The separation of the first two components was incomplete.

When the flow-rate was decreased to 15 ml·h^{-1}, the separation lasted for 18 h, and during this period the last component was completely spread on the column (Fig. 4B).

When the initial elution rate was also 15 ml·h^{-1}, but after the xanthine left the column this rate was increased to 90 ml·h^{-1}, the analysis lasted for 9 h and a complete separation was achieved (Fig. 4C).

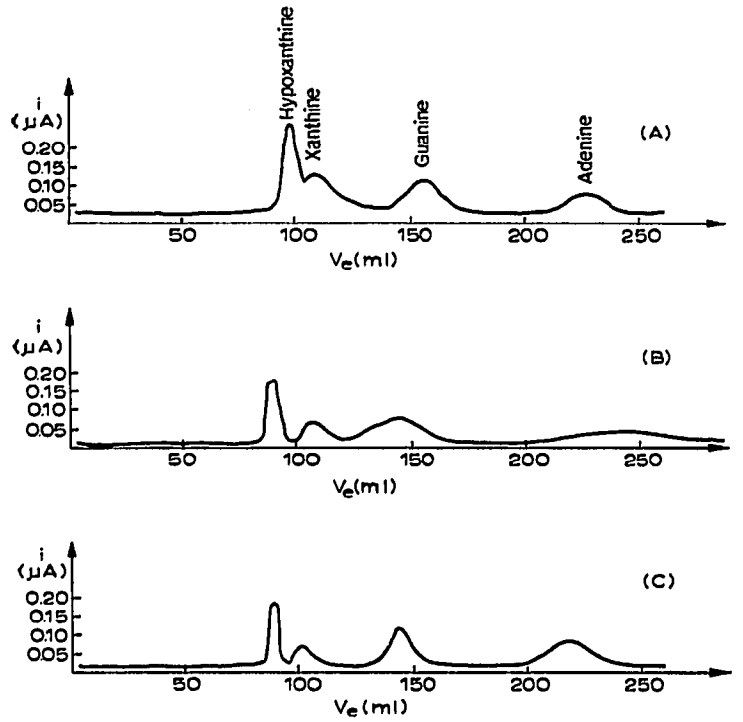

Fig. 4. Separation of purines on a 100 × 0.9 cm Sephadex G-10 column. Eluent: 0.066 *M* Na_2HPO_4, 0.1 *M* NaCl. Sample: $1.25 \cdot 10^{-7}$ mole of each purine per 0.1 ml. Potential: 0.96 V. Flow-rate: A, 30 ml·h^{-1}, 0–8 h; B, 15 ml·h^{-1}, 0–18 h; C, 15 ml·h^{-1}, 0–8 h, and 90 ml·h^{-1}, 8–9.3 h.

TABLE I

VOLTAMMETRIC DATA OF SOME COMPOUNDS DETERMINED WITH THE SILICONE RUBBER-BASED GRAPHITE ELECTRODE

Sensitivity: $6 \cdot 10^{-7}$ A·div^{-1}. Rate of polarization: 1.50 V·min^{-1}.

Compound	*Half-wave potential vs. SCE (V)*	*Current constant ($10^{-2}A \cdot mole^{-1}$)*	*Supporting electrolyte*	
			Buffer	*pH*
Uric acid	0.25	3.64	Phosphate	8.3
Adenine	1.04	20.80	Acetate	4.8
Guanine	0.81	20.80	Acetate	4.8
Xanthine	0.96	6.00	Phosphate	8.3
Hypoxanthine	1.12	3.42	Phosphate	8.3
Uracil	—	—	Acetate	4.8
Cytosine	—	—	Acetate	4.8
Thymine	—	—	Acetate	4.8
Adenosine*	1.12	25.76	Phosphate	8.3
Guanosine	0.80	10.76	Acetate	4.8
GMP*	0.98	8.50	Acetate	4.8

* Rate of polarization: 0.75 V·min^{-1}.

When the separation was repeated using 0.05 *M* sodium chloride solution as the eluent, a better separation was obtained in a shorter time (Fig. 5). When xanthine had left the column, the elution rate was increased from 30 to 90 ml·h^{-1}.

Naturally, the same conditions must be used in the calibration of the chromatovoltammetric system as in the subsequent analyses.

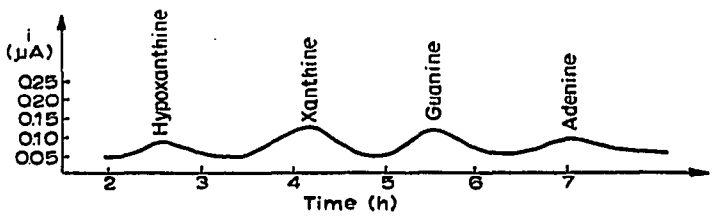

Fig. 5. Separation of purines on 100 × 0.9 cm Sephadex G-10 column. Eluent: 0.05 *M* NaCl. Sample: 1.25·10^{-7} mole of each purine per 0.1 ml. Potential: 0.96 V. Flow-rate: 0–6.5 h, 30 ml·h^{-1}; 6.5–9 h, 90 ml·h^{-1}.

In Table I, it is pointed out that while the purine bases can be measured satisfactorily on the silicone rubber-based graphite electrode, the pyrimidine bases do not show voltammetric activity in the polarization range examined. Therefore, these compounds do not cause any interference in the chromatovoltammetric detection of purine bases, that is, the detector cell operates as a specific detector. This is very advantageous in many analytical operations compared with the UV detectors generally used for the detection of these compounds.

CONCLUSION

The selective measurement of the purine bases can be performed satisfactorily by means of a voltammetric detector cell ensuring turbulent flow, provided that the constant conditions necessary for the voltammetric measurement are maintained.

REFERENCES

1 W. Kemula, *Rocz. Chem.*, *26* (1952) 281.
2 W. Kemula, *Pure Appl. Chem.*, (1970) 763.
3 J. G. Koen and J. F. Huber, *J. Chromatogr. Sci.*, 8 (1970) 192.
4 P. T. Kissinger, C. Refshauge, R. Driling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
5 E. Pungor and É. Szepesváry, *Anal. Chim. Acta*, 43 (1968) 289.
6 P. L. Joynes and R. S. Maggs, *J. Chromatogr. Sci.*, 8 (1970) 427.
7 E. Pungor, Zs. Fehér and G. Nagy, *Anal. Chim. Acta*, 51 (1970) 417.
8 V. G. Levich, *Physicochemical Hydrodynamics*, Prentice-Hall Inc., Englewood Cliffs, N.J., 1962.
9 H. Matsuda, *J. Electroanal. Chem.*, 15 (1967) 109.
10 S. L. Marchiano and A. J. Arvia, *Electrochim. Acta*, 12 (1967) 801.
11 J. S. Bazán, S. L. Marchiano and A. J. Arvia, *Electrochim. Acta*, 12 (1967) 821.
12 M. B. Bardin and A. J. Dikuszar, *Zh. Anal. Khim.*, 26 (1971) 1068.
13 G. Wranglén and O. Nilsson, *Electrochim. Acta*, 7 (1962) 121.
14 G. Dryhurst, *J. Electrochem. Soc.*, 116 (1969) 1411.
15 L. Sweetman and W. L. Nyhan, *J. Chromatogr.*, 32 (1968) 662.