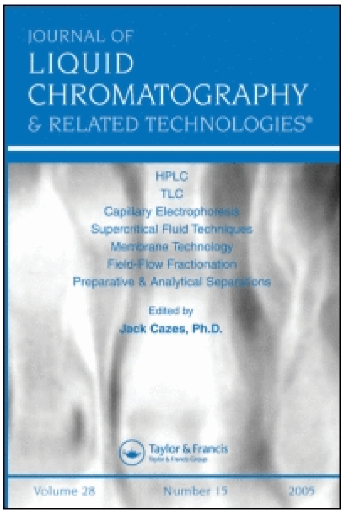

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Trojánek, A. and Křesťan, L.(1983) 'A New Polarographic Flow-Through Detector', Journal of Liquid Chromatography & Related Technologies, 6: 10, 1759 — 1776
To link to this Article: DOI: 10.1080/01483918308064889
URL: http://dx.doi.org/10.1080/01483918308064889

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(10), 1759-1776 (1983)

A NEW POLAROGRAPHIC FLOW-THROUGH DETECTOR.

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ABSTRACT

The construction of a flow-through polarographic detector is described, based on introduction of the test solution into a space filled with mercury. Two variants of the detector have been developed, with the inlet jet placed vertically and horizontally. The dynamic properties of the two detector variants, the linear dynamic range and the response reproducibility were tested using o-nitrophenol as a model substance. The detector with the vertical jet has a wide linear dynamic range and that with the horizontal jet exhibits an extremely rapid response with a time constant of the order tenths of a second.

INTRODUCTION

Most practically used flow-through voltammetric detectors operate with solid electrodes and respond to anodically oxidizable substances. However, there exist many important compounds containing functional groups that can be cathodically reduced under certain conditions (nitro-, nitroso- and azo-compounds, aldehydes, oximes, hydrazones, etc.). To detect these compounds in flow-through instruments, a number of polarographic detectors have been constructed over last twenty years, employing the advantages of mercury electrodes and sup-

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pressing to a varying extent their undesirable properties. All the polarographic detectors that have so far been described (1,2) are basically flow-through versions of a common polarographic cell, constructed so that the internal volume is as small as possible. They all contain a dropping mercury electrode consisting of a glass capillary, which is the most sensitive and most expensive part of the detector.

This paper describes the construction and properties of a flow-through polarographic detector based on a new principle, without a glass capillary.

EXPERIMENTAL

Chemicals

Methanol, acetic acid, sodium acetate, o-nitrophenol and 2,6-dinitrophenol (all from Lachema, Brno, Czechoslovakia) were of p.a. purity and were not further purified. The samples and the base electrolyte were deaerated prior to measurement by passage of argon.

Instruments

The detector was tested in a system consisting of a high-pressure linear pump (HPP 4001, Laboratorní Přístroje, Prague, Czechoslovakia) and a sampling valve whose construction is described below. For the connections, a stainless-steel capillary, 0.4 mm I.D., was employed.

The chromatographic measurements were carried out on an LC-XP liquid chromatograph (Pye Unicam, Cambridge, Great Britain), with a Separon C_{18} column, 25 cm long, 0.8 cm I.D., 10 µm (Laboratorní Přístroje, Prague, Czechoslovakia). Samples were injected through a 20 µl sampling loop.

A three-electrode polarograph with circuits for handling the current signal and the circuit for the measurement of electrolytic conductance were assembled from operational amplifiers. The conductance was measured with a large-area platinum electrode in the working electrode compartment at a frequency of 1 kHz.

The output signal was recorded using a TZ 4200 line recorder (Laboratorní Přístroje, Prague, Czechoslovakia) or a Tektronics 5103 storage oscilloscope (Tektronics, Beverton, USA).

Sampling Valve

One requirement of the sampling valve is to introduce the sample into the flowing stream as a zone with a sharp front edge. As the detectors tested do not cause a perceptible pressure gradient in the liquid stream, the sampling valve was constructed as a low-pressure device. The rotating sandwich valve with a rotor between two stators is made of plexiglass. The adjacent surfaces are lubricated with graphite and the bored channels are 0.4 mm in diameter. Fig. 1 depicts the valve in position "LOAD", when the sampling loop is filled with the sample using pressurized argon. On turning the rotor by 60° the loop content, including the rectangular channels, is swept into the stream between the pump and the detector.

Detector

The detector construction is schematically given in Fig. 2. The whole detector is also made of plexiglass and its body consists of two main parts, one containing the reference electrode and the other the mercury compartment. The two main parts are connected by a thread and the connection between them and between the jet holder and the front of the mercury compartment are sealed. The inlet channel (0.4 mm I.D.) and the jet are tightly and flexibly connected by a small seal at the channel circumference. The two

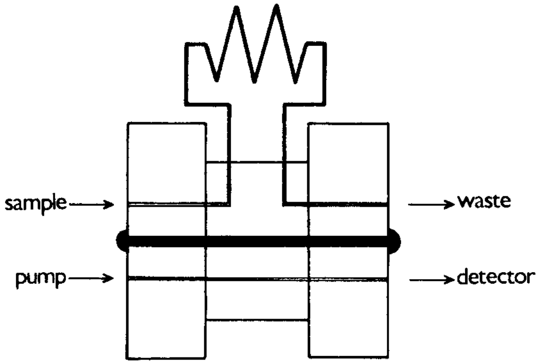

FIGURE 1. Sampling valve.

outlets in the mercury compartment permit the work with the detector in both vertical and horizontal position.

The most important part of the detector is the jet through which the test solution is introduced into the mercury. It is made of porous ceramics and pressed into the plexiglass holder. The jet has a channel 0.4 mm in diameter, and the jet is narrowed to 0.2 mm for 1 mm at the tip. The part of the jet that is in contact with mercury is covered with a protective lacquer (Brush-on Plastic Tape, No. 1672-2C, TechSpray, USA). Common epoxide materials are unsuitable for the jet insulation, because they cannot resist prolonged action of methanol. After drying of the lacquer, the jet tip was ground with a fine emery paper, thus obtaining a non-insulated contact area with a diameter of about 0.7 mm with the 0.2 mm jet and about 1 mm with the 0.4 mm jet.

An Ag/AgCl reference electrode with 1M LiCl in 50% methanol was used.

RESULTS AND DISCUSSION

The principle of the detector is introduction of the test solution into a space filled with mercury.

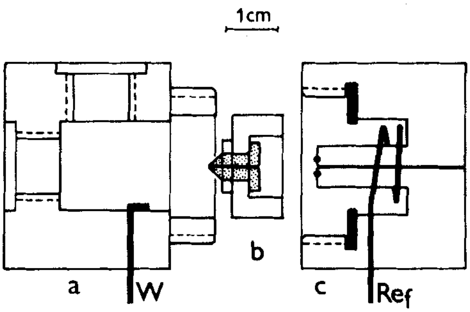

FIGURE 2. Schematic diagram of the detector. a) mercury compartment, b) porous jet in the plexiglass holder, c) reference electrode compartment. Contacts to the working (W) and reference electrodes (Ref) are depicted.

The working electrode is the mercury surface surrounding the drop or layer of the test liquid and can be connected with the other electrodes in various ways. To test the properties of this detector, we used the inlet stainless-steel capillary as the auxiliary electrode and the working and reference electrodes were connected by the jet made of a porous material.

Among the properties important for practical use, the response stability and rate, the linear dynamic range (the concentration range within which the response is linear) and the dependences of the measured quantities on the flow-rate were determined. As the model substance, o-nitrophenol (ONPh) was selected in a medium of 0.1M acetate buffer of pH 4.6 containing 50% vol. methanol. Thus the detector was intentionally tested under difficult chromatographic conditions with a low electric conductance. When the detector contained this electrolyte, a resistance of ca. 200 kΩ was measured between the tip of the jet and the auxiliary electrode. In the measurement, 400 µl of the sample was usually injected into the detector, thus ensuring that the amount injected was sufficient for the attainment of a steady-state current signal.

The axis of the detector jet can basically be oriented in two directions, vertically or horizontally. As will be seen below, different directions of the jet axis with respect to the direction of the gravitational force and buoyancy lead to detectors with different properties and thus they are discussed separately.

Detector with Vertical Jet Axis

Due to the formation of droplets of the liquid at the jet tip and their disconnection caused by buoyancy, the current signal obtained oscillates similar to that measured with a dropping mercury electrode. The oscillations cause difficulties in the evaluation of the current signal, especially at low concentrations of the electroactive substance (Fig. 3). The oscillations can be suppressed by using the sampling method based on a peak detector that does not require synchronization with the drop disconnection (3).

Response Rate

The response rate is an important property that is often used as the main criterion for the comparison of detectors of various designs. However, it is practically impossible to compare detectors on the basis of the data given in the literature in terms of geometric, response, internal, effective, rinse, hold-up, wash-out, dead, etc., volumes, as the relationships among these quantities are not unambiguously defined and their values variously depend on the experimental conditions.

A relatively reliable dynamic parameter is the response time constant, whose value generally depends on the flow-rate and possibly on other experimental parameters. The finding of these dependences can be

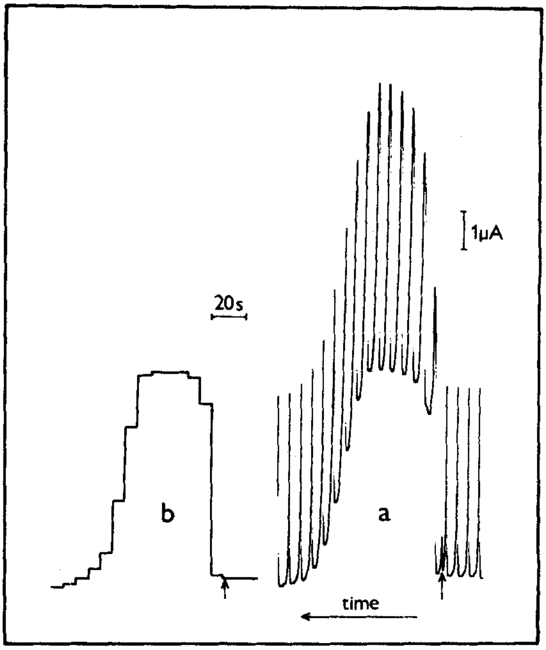

FIGURE 3. Response of the vertical jet detector; 400 µl of 2×10^{-5} M CNPh injected. Jet diameter 0.4 mm, flow-rate 0.5 ml/min. a) signal, b) sampled signal.

considered as a sufficient characteristic of the response rate of the detector or, more precisely, of the whole detection system. The time constant of the detector response can be determined from the output signal recorded after a stepwise change in the input concentration. It is often possible (and the present detectors are not an exception) to express the time--course of the output signal with a sufficient precision by the exponential equation, $S = S_M(1-\exp(-t/\tau))$, where $\underline{S}$ is the signal amplitude at time t, $\underline{S}_M$ is the maximal amplitude and $\underline{\tau}$ is the response time constant. Therefore, the $\underline{\tau}$ value can be found from the known signal amplitude at any time after the beginning of the

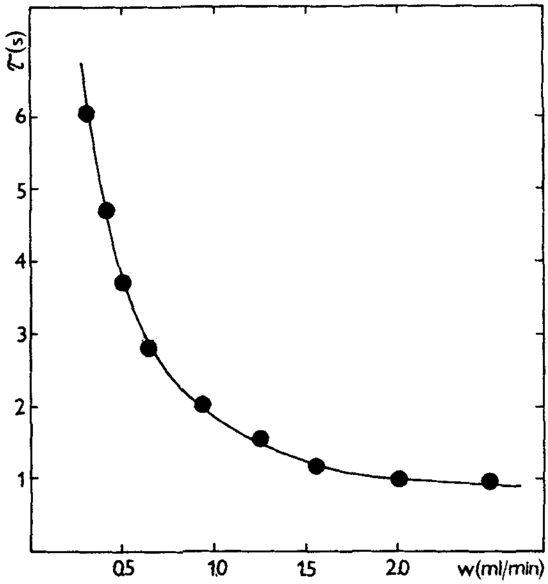

FIGURE 4. Dependence of the response time constant on the flow-rate. Vertical jet detector, jet diameter 0.2 mm. 400 µl of $2x10^{-5}$ M ONPh injected.

signal growth and it can be used to compare the performance of various detectors under the same experimental conditions.

An accurate determination of the response time constant is somewhat difficult for the detector with the vertical jet axis, because the steepest part of the curve must be approximated by a straight line. The time constant values given in Fig. 4 were thus obtained from the above equation by evaluating the part of the curve between the points at which the detector signal attained 50 and 90% of the steady-state value.

The product of the volume flow-rate and the time constant, the response volume, V_r (4), remained virtually constant over the whole range of the flow-rates studied and amounted on average to 31.7 µl with a standard deviation of 1.8 µl. The droplet volume, V_d, found from the period of dropping at a known flow--rate, was also constant within this range. The mean droplet volume was 58.8 µl (standard deviation, 0.7 µl) for 0.2 mm jet and 52 µl for 0.4 mm jet.

It follows from the equation of the exponential response that 99.5% of the steady-state amplitude can, under optimal conditions, be attained after disconnection of the 5.3 V_r/V_d droplets, i.e. after about three droplets. However, a simple consideration shows that full deflection can be reached at the latest after two droplets on a step change in the concentration. The sample zone that just passed through the jet tip leads to a drop of electrolyte in which the zone is diluted, but the following drop is composed only of the sample zone and the detector should indicate the full deflection. The slower response observed can be explained by an assumption that the drops are imperfectly disconnected from the jet tip. It must be borne in mind that a spherical drop 60 µl in volume would have a diameter of almost 5 mm and the translational velocity of the liquid is more than half metre per second with a 0.2mm jet and a flow-rate of 1 ml/min. Thus the detector operates probably with elongated electrolyte drops, deposited along the external circumference of the jet, from which a part is always imperfectly separated.

Calibration Curve

When the 0.2 mm jet was used, a linear calibration curve was obtained from 5 x 10^{-6} to 10^{-4}M concentrations, at a flow-rate of 0.5 ml/min. The slope of the calibration straight line with a regression coefficient of 0.9994 was 2.26 x 10^5 uA.l.mol^{-1}. The response non-linearity at higher current densities is most probably caused by a high potential gradient inside the electrolyte drop that cannot be compensated, even if a positive feedback is introduced. This is supported by the fact that when using an electrolyte with a higher conductance (0.2M acetate buffer containing 5% methanol, in which the measured resistance between the mercury and the auxiliary electrode was 65 kΩ) the linear section of the calibration curve is doubled with the positive feedback.

On the other hand, a linear calibration curve (with a regression coefficient of 0.9987) was obtained in a concentration range of 5×10^{-6} to 2.5×10^{-4}M even in a medium of 50% methanol, when using the 0.4mm jet. The upper limit of this interval was given by the operational range of the potentiostat that attained the saturation voltage at a current of ca. 55 µA. The detection with the larger jet was virtually equally sensitive as that with the narrower jet, 2.23×10^{5} µA.l.mol^{-1}.

The Dependence of the Signal Amplitude on the Flow-Rate

The conditions of the transport of the electroactive substance toward the surface of a hollow drop electrode are very complicated. Convection caused by the growth of the drop and that caused by the flow of the electrolyte are combined and the situation is further complicated by the above mentioned inhomogeneity of the potential distribution over the working electrode surface. Therefore it will only be stated that the current signal increases linearly with increasing volume flow-rate (Fig. 5) and that the dependence deviates from linearity when using the smaller jet in the region of higher current densities, similar to the calibration curve.

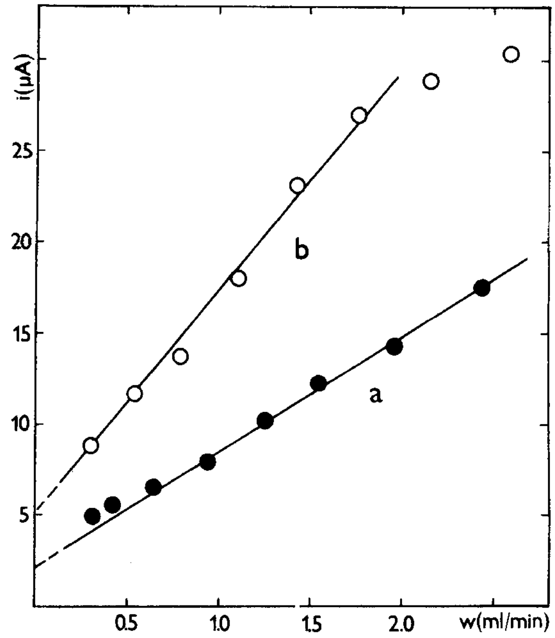

FIGURE 5. Dependence of the signal amplitude on the flow-rate. Vertical jet detector, jet diameter 0.2 mm. 400 µl of a) 2x10^{-5} M ONPh and b) 5x10^{-5} M ONPh injected.

Response Reproducibility

The detector response reproducibility **is comparable to that of a classical dropping mercury** electrode. Twenty measurements of samples at a concentration of 5 x 10^{-5}M at a flow-rate of 0.5 ml/min. yielded a mean value of the current of 11.46 µA with a standard deviation of 0.08 µA.

Detector with Horizontal Jet Axis

In the detector with the horizontal jet axis the current signal has no oscillations, because the solution does not drop, but forms a thin layer between the external surface of the jet and the mercury. The effective

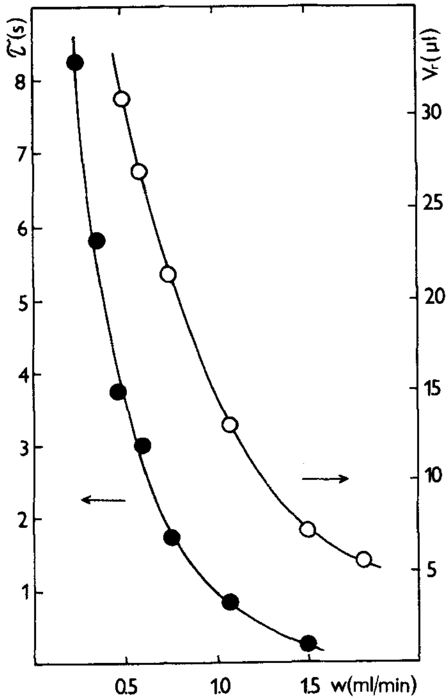

FIGURE 6. Dependence of the response volume and the time constant on the flow-rate. Horizontal jet detector, jet diameter 0.4 mm. 400 μl of $2x10^{-5}$ M ONPh injected.

surface area of the working electrode (on which the electrochemical reaction can occur) is not exactly defined, which leads to certain peculiarities in the detector behaviour, as described below.

Response Rate

As can be seen from the time constants given in Fig. 6, the detector with the 0.4 mm jet exhibits a dynamic behaviour similar to that of the detector with the vertical jet. However, the response volume does not remain constant when the flow-rate is changed and relatively rapidly decreases with increasing flow-rate.

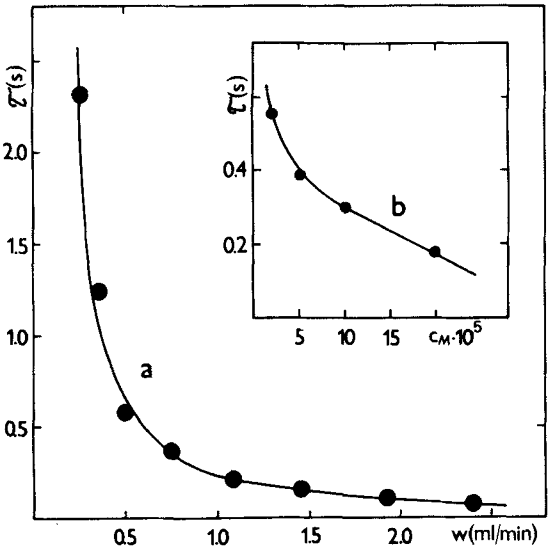

FIGURE 7. Horizontal jet detector: time constant vs. the flow-rate (a) and the concentration of o-nitrophenol (b). Jet diameter 0.2 mm, 400 µl injected. a) constant concentration $2x10^{-5}$ M, b) constant flow-rate 0.5 ml/min.

When the 0.2 mm jet is used, the response rate is substantially increased and the time constant becomes to a certain extent dependent on the electroactive substance concentration (Fig. 7). This somewhat unexpected dependence can be understood when realizing that with increasing concentration, i.e. increasing electrolytic current, the inhomogeneity of the potential distribution over the working electrode surface also increases. Thus the electrode effective surface area decreases, leading to a decrease in the volume in which the detection takes place.

Calibration Curve

The deterioration of the geometry between the reference electrode tip and the working electrode sur-

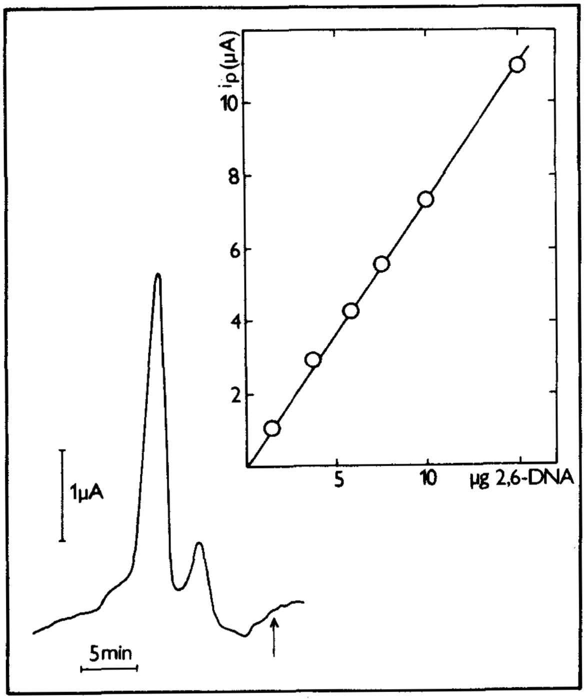

FIGURE 8. Horizontal jet detector: chromatogram of 5 µg of 2,6-dinitroaniline and the corresponding calibration curve. Jet diameter 0.2 mm, flow-rate 1.5 ml/min.

face (compared with the previous detector) causes a narrowing of the linear dynamic range. Even if the 0.4 mm jet was used, the linear calibration curve was only obtained from 5 x 10^{-6} to 10^{-4}M concentration at a flow-rate of 0.5 ml/min. and this interval could be extended to 2 x 10^{-4}M by introducing positive feedback. The slope of the calibration curve with a regression coefficient of 0.9986 was 2.13 x 10^{5} µA.l.mol^{-1}. Therefore the detection sensitivity was virtually the same as that for the detector with vertical jet.

When samples were injected in amounts corresponding to about one third of the stationary current signal (18.6 µl), a linear calibration curve was obtained in a concentration range of 10^{-6} to 3×10^{-4}M. The straight line with a regression coefficient of 0.9970 had a slope of 5.7×10^{4} µA.l.mol^{-1} for amounts of 25.8 to 775.5 ng of o-nitrophenol. For the detector with the 0.2 mm jet, a substantially narrower linear dynamic range was obtained with the same flow-rate, namely, from 5×10^{-6} to 2×10^{-5}M. The calibration curve obtained from four experimental points had a regression coefficient of 0.9983 and a slope of 4.3×10^{5} µA.l.mol^{-1}. In spite of the discouragingly narrow linear dynamic range, this detector was tested in combination with a liquid chromatograph, as it could be expected that its rapid response would be favourable. Fig. 8 depicts the response in the detection of 2,6-dinitroaniline. The calibration curve, obtained intentionally for large amounts injected (1.5 to 15 ug) at -1.0V is reliably linear because of low concentrations of the electroactive substance in the eluted zone. It can be simply derived (5) that the concentration at the peak top was 1.2×10^{-5}M for 10 µg of 2,6-dinitroaniline injected.

Dependence of the Signal Amplitude on the Flow-Rate

This dependence was again followed for the steady-state current signal at concentrations of 2×10^{-5} and 5×10^{-5}M, from 0.25 to 2.4 ml/min.

With the 0.4 mm jet, the plot in the logarithmic coordinates yielded straight lines with slopes of 0.47 and 0.52 and the respective regression coefficients, 0.9929 and 0.9983. The current signal amplitude thus increases with the square root of the flow-rate, similar to thin-layer detectors with solid electrodes. The dependence is complicated by the high potential gradient when the small jet is used, and the dependence exhibits a negative deviation from the square-root dependence for high flow-rates.

Response Reproducibility

As mentioned above, the effective surface area of the working electrode is not exactly defined in this detector type. Therefore, certain rules must be observed to obtain reproducible results, in contrast to the detector with the vertical jet.

The detector exhibits optimal performance when the mercury surface is virtually dry, which can be attained by modifying the liquid outlet from the detector. The outlet to the waste that is lower than the detector is through a stainless-steel tube brought through the detector wall to a distance of about 1.5 cm above the jet base. After filling the compartment with mercury, the mercury is forced to the waste by the liquid; utilizing the siphon effect (for short-time opening of the upper closing), all excess mercury and electrolyte are removed. When the upper closing is again sealed, the absence of the electrolyte on the surface of the mercury and a constant mercury level are ensured. The detector prepared in this way yields well reproducible response after conditioning under flow conditions for several minutes. Twenty measurements on samples with a concentration of 2×10^{-5}M, using the 0.2 mm jet and a flow-rate of 0.5 ml/min., gave the mean current value of 8.83 µA with a standard deviation of 0.17 µA.

The results obtained in the testing of the detectors, some of which are given in the table, indicate that detectors of good properties can be constructed using this principle. The vertical jet detector is suitable e.g. for continuous analyzers, because of its slower response and a broad linear dynamic range. The construction with the closed mercury also ensures the

TABLE 1

Important Properties of Tested Detectors

Detector	Jet diameter (mm)	Time constant at 1ml/min. (s)	Response volume (µl)	Linear dynamic range (mol.l^{-1})
Vertical jet	0.2	1.8	31.7	5×10^{-6}-10^{-4}
	0.4	-	-	[a]5×10^{-6}-2.5×10^{-4}
Horizontal jet	0.2	[b]0.22	4.0	5×10^{-6}-2×10^{-5}
	0.4	1.0	15.0	5×10^{-6}-10^{-4}

[a] Limited by the operational range of the potentiostat

[b] Dependent on the electroactive substance concentration

safety of the operator during the measurement. The detector with a horizontal jet with a small diameter, which has some properties similar to those of solid--electrode thin-layer detectors, seems to be suitable for detection in liquid chromatography where small amounts are involved and a fast response is required. In a further development of detectors of this type, the attention should be directed to broadening of their linear dynamic range, which is directly connected with the geometry of the electrodes.

REFERENCES

1. Štulík, K. and Pacáková, V., Electrochemical Detection Techniques in High - Performance Liquid Chromatography. J. Electroanal. Chem. 129, 1, 1981.

2. Trojánek, A., Elektrochemické detektory v analytických průtokových zařízeních, Chem. Listy 76, 695, 1982.

3. Trojánek, A. and Holub, I., Current Sampling in Polarography without Synchronization with the Drop Time. Anal. Chim. Acta 110, 161, 1979.

4. Hanekamp, H.B., Bos, P., Brinkman, U.A.Th. and Frei, R.W., A Comparison of Different HPLC Detector Designs Based on the Dropping Mercury Electrode Principle. Fresenius Z. Anal. Chem. 297, 404, 1979.

5. Scott, R.P.W., Liquid Chromatography Detectors, Elsevier, Amsterdam, Oxford, New York, 1977, p. 11.