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MODIFICATIONS OF THE WALL-JET ELECTROCHEMICAL DETECTOR FOR LIQUID CHROMATOGRAPHY AND FLOW ANALYSIS

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SUMMARY

Two modifications are suggested for the construction of electrochemical detectors for liquid chromatography and flow analysis. A convenient, large-volume wall-jet cell has a low-dead volume and performs like cells with microlitre volumes. A new electrode material, silicone rubber-impregnated carbon, is also conveniently prepared and used, and behaves much like carbon paste or glassy carbon.

INTRODUCTION

Electrochemical detectors have become very popular for solving some problems in liquid chromatography, *e.g.*, the low-level detection of biogenic amines¹⁻⁵. Electrochemical detectors respond only to those species which can be oxidized or reduced at the applied potential on the particular electrode material used in the detector and the selectivities are usually very different from those obtained with non-electrochemical detectors.

The name "electrochemical detector" has been mainly used in connection with amperometric or coulometric detectors. The working electrode of these detectors is kept at a constant potential against a suitable reference electrode, and the current flowing across the working electrode is measured. The current depends not only on the concentration of the determinand in the eluent but to a great extent also on the flow pattern of the eluent in the vicinity of the electrode. This flow pattern, on the other hand, determines the effective volume of the detector, and thus the band spreading caused by the detector. For these reasons the design of the flow geometry is particularly important in electrochemical detectors.

Three geometries have been most often used for electrochemical detection. In the thin-layer cell, the eluent passes through a thin, sandwich-type cell, with the working electrode mounted flush with one side wall of the flow channel. In the second variant, the working electrode is made from a porous material and the eluent flows

across the pores of the electrode. The third alternative is the wall-jet cell. Here the eluent streams through a small orifice into a liquid-filled space and forms a jet that impinges on the electrode surface, which is placed normal to the axis of the jet. Although wall-jet cells have been used for some years⁶, they have always been constructed in such a way that the volume of the liquid surrounding the jet was minimized, apparently in an attempt to keep the band spreading low. It has recently been shown⁷, however, that band spreading is very low, even if the geometrical cell volume is virtually infinite. Results obtained with a large-volume wall-jet cell are presented in this paper.

In addition to the need for an efficient cell geometry, electrochemical detection depends to a great extent on the material of the working electrode. For the oxidation of organic substances, various forms of carbon-based electrodes have found widest acceptance. Graphite or carbon materials cannot, in general, be used without pretreatment, however, because of the high background current. Glassy carbon, pyrolytic graphite and carbon preparations impregnated with paraffins have been found more useful. These materials can be judged from different properties such as signal-to-noise ratio, detection limit, ease of preparation, ease of handling and sensitivity to fouling. Apparently each of them has its own merits. We have attempted to add a further useful carbon preparation to this list. Our experience with a silicone rubber-impregnated carbon composition, similar to that used earlier^{8,9} for flow injection and medium-pressure chromatography, is reported here.

EXPERIMENTAL

Flow-injection experiments were carried out to demonstrate the capabilities of the large-volume wall-jet cell for this type of application. They also served for the assessment of the properties of the silicone rubber-impregnated carbon electrode. High-performance (HPLC) experiments showed the chromatographic performance of the large-volume cell, both in conjunction with a carbon-paste electrode and the new electrode composition.

Apparatus

The large-volume detector cell, shown in Fig. 1, was machined from Plexiglass. The orifice producing the liquid jet was a 0.2 mm I.D. stainless-steel capillary. The cell was connected to a custom-built potentiostat. The reference electrode was a saturated calomel electrode (SCE) and the auxiliary electrode was Pt foil.

In the flow-injection experiments (Fig. 2), the background electrolyte was pumped by an LKB Multiperpex 2115 peristaltic pump. Samples were injected from a two-loop Pierce MTS 24144 injector. The 40- and 120- μ l loops were used alternately. Signals were recorded with a Houston Instruments OmniScribe recorder.

Chromatographic work was carried out on three different systems, using the same electrochemical detector as described above:

(1) a Varian 5000 pump, a 100- μ l Valco sample loop, a 300 \times 4 mm I.D. column containing Micro-Pak MCH 10 C₁₈ (10 μ m), a Varian 9176 linear recorder and a CDS III integrator;

(2) a JASCO FAMILIC 300-S semimicro.pump with a pulse damper, a Labor MIM OE 320 sampler with a 20- μ l loop, a Nucleosil 10 C₁₈ (10 μ m) reversed-phase column (100 \times 4.6 mm I.D.) (laboratory-packed) and a Servogor linear line recorder;

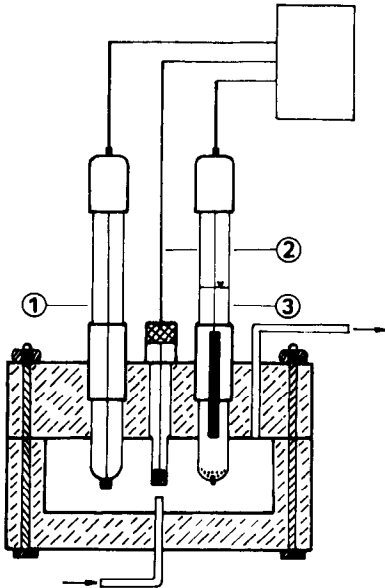


Fig. 1. Large-volume wall-jet amperometric detector cell. 1, Auxiliary electrode; 2, working electrode; 3, reference electrode.

(3) a Labor MIM OE 312 pump with a laboratory-built pulse-damper, a Rheodyne 7010 sampler with a 100- μ l loop, a 250 \times 4.6 mm I.D. column containing LiChrosorb RP-18 (10 μ m) (laboratory-packed) and a Radelkis OH 814/1 linear line recorder.

Scanning electron microscopic (SEM) investigations were carried out on a JEOL JSM 50/A instrument with an accelerating voltage of 20 kV. The investigated surfaces had been treated with Au vapour.

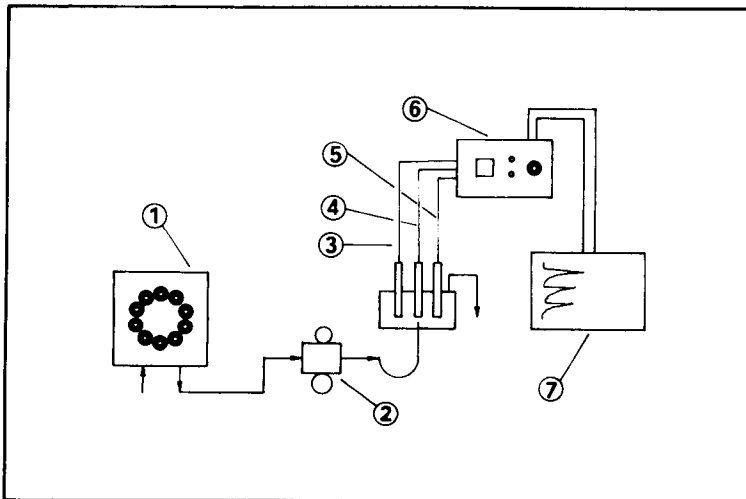


Fig. 2. Schematic diagram of the flow-injection setup. 1, Peristaltic pump; 2, injector; 3, auxiliary electrode; 4, working electrode; 5, reference electrode; 6, potentiostat; 7, recorder.

Chemicals

To prepare the eluents, doubly distilled water, acetonitrile (Merck, LiChrosolv), methanol (Merck, LiChrosolv; Reanal, p.a.) potassium dihydrogen phosphate (Reanal), Na_2EDTA , octanesulphonic acid (Fluka), heptanesulphonic acid (Fluka) and sodium lauryl sulphate (Reanal) were used. The other chemicals used were of analytical-reagent and chromatographic grade.

The background electrolyte for all flow-injection experiments was a sodium acetate-citric acid buffer, adjusted to pH 5.2, and containing 1 mM Na_2EDTA .

Electrode preparation

Glassy carbon from Carbon Lorraine was glued into the Plexiglass electrode body and polished well. Spectrographic carbon powder was ground and mixed with pharmaceutical paraffin oil to obtain the carbon paste electrodes, the proportions being chosen so as to obtain an almost brittle product. Carbon paste electrodes were also prepared by substituting polysiloxane for the paraffin oil. Silicone rubber-impregnated membranes were made from the silicone oil-based carbon paste by addition of a cross-linking agent and catalyst. The pastes were applied into a well at the lower end of the Plexiglass electrode body. The silicone rubber-impregnated graphite was cured in sheets of about 2 mm thickness. Circular membranes of 4 mm diameter were cut from the sheet and glued on the lower end of the electrode body.

RESULTS AND DISCUSSION

The chromatographic performance of the wall-jet cell depends on several variables: the distance of the electrode from the orifice, the diameters of the orifice and

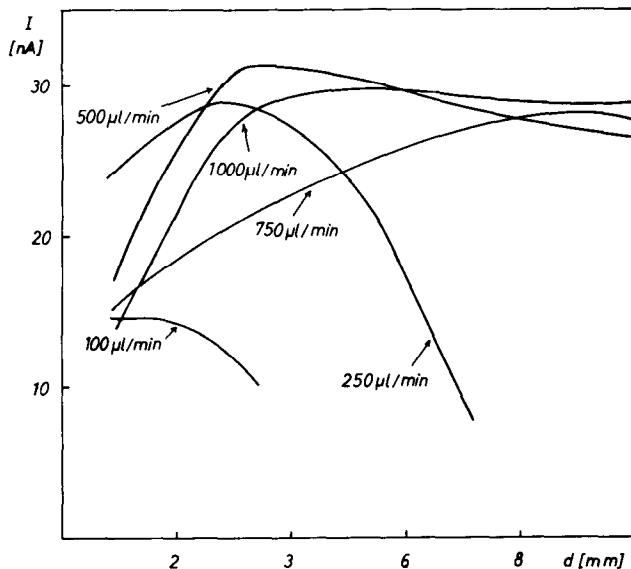


Fig. 3. Effect of orifice-electrode distance and of flow-rate on chromatographic peak height in the large-volume wall-jet cell. Column: 100×4.6 mm I.D., Nucleosil 10 C_{18} , 10 μm . Mobile phase: 0.05 M KH_2PO_4 -0.75 mM octanesulphonic acid, sodium salt (OSS)-5% (v/v) methanol (pH = 4.1). Test compound: VMA. Electrode: paraffin oil-based carbon paste. Applied potential: 0.75 V vs. SCE.

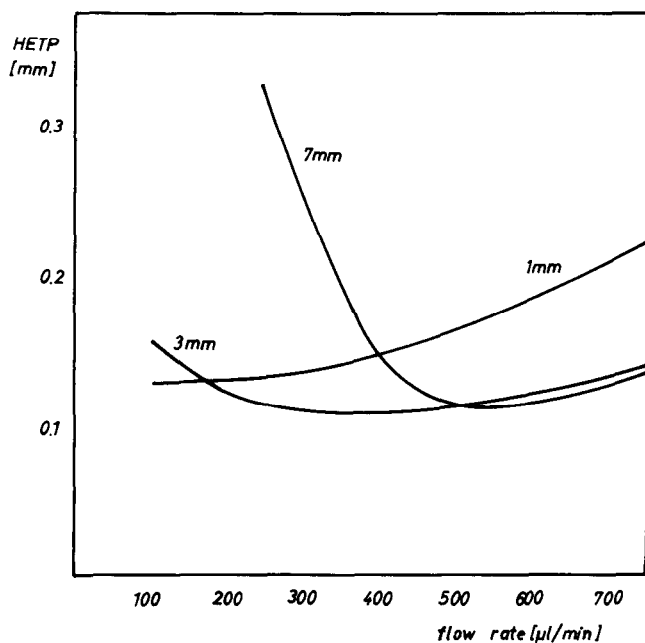


Fig. 4. Effect of flow-rate and orifice-electrode distance μ on the HETP of the chromatographic system. Conditions as in Fig. 3.

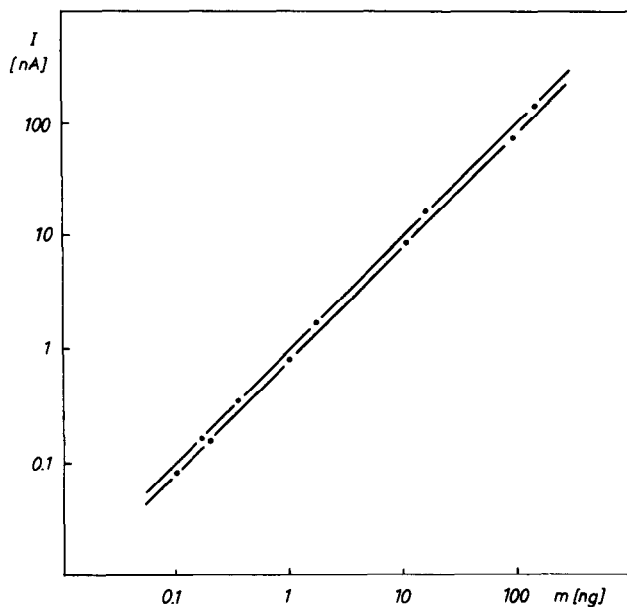


Fig. 5. Linearity test with the wall-jet cell as chromatographic detector. Column: 300×4.6 mm I.D. Micro-Pak MCH 10 C_{18} , $10 \mu\text{m}$. Mobile phase: $0.05 \text{ M KH}_2\text{PO}_4$ adjusted to pH 4.1-1 mM OSS -10% (v/v) acetonitrile. Flow-rate: $1 \text{ cm}^3/\text{min}$. electrode: paraffin oil-based carbon paste. Applied potential: 0.75 V vs. SCE. (O) Adrenaline ($r^2 = 0.9999$); (●) dopamine ($r^2 = 0.9993$).

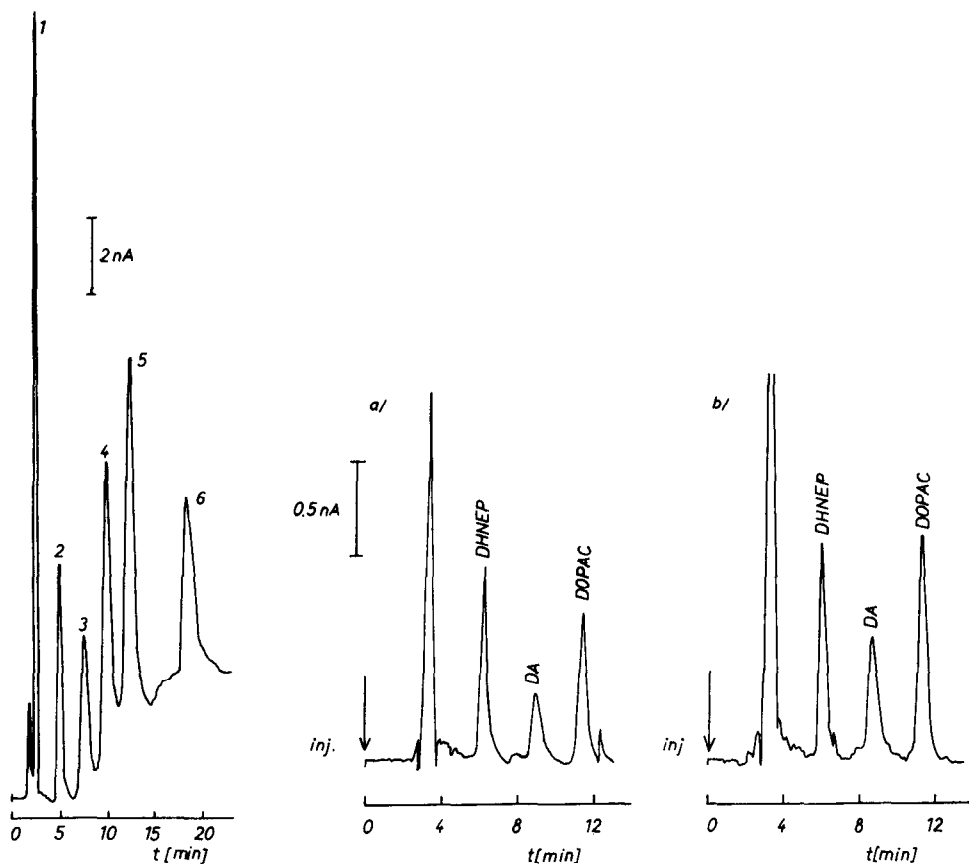


Fig. 6. Compatibility test of the detector with gradient elution. Column: 300×4.0 mm I.D. Micro-Pak MCH 10 C₁₈, $10 \mu\text{m}$. Mobile phase components: (A) $0.05 \text{ M KH}_2\text{PO}_4$ – 100 mg/dm^3 OSS; (B) acetonitrile. Gradient profile: 0–2 min: 2% (v/v) B in A; 2–5 min: 1.0% B/min; 5–10 min: 1.5% B/min. Flow-rate: $1 \text{ cm}^3/\text{min}$. Electrode: paraffin oil-based carbon paste. Applied potential: 0.75 V vs. SCE . Test compounds: 1, vanillylmandelic acid 18.8 ng , 3,4-dihydroxyphenylalanine (dopa) 14.3 ng ; 2, 5-hydroxytryptophan 9.4 ng ; 3, norepinephrine (noradrenaline) 13.2 ng ; 4, 5-hydroxyindoleacetic acid 23.1 ng ; 5, epinephrine (adrenaline) 16.9 ng , homovanillic acid 24 ng ; 6, dopamine 9.8 ng .

Fig. 7. Determination of catecholamines in rat brain perfusates, (a) in resting state and (b) after stimulation with 20 mmol/dm^3 KCl. Column: 250×4.6 mm I.D. Chromsil RP-18, $10 \mu\text{m}$. Mobile phase: 0.14 M monochloroacetic acid buffer (pH 3.0)–6% (v/v) acetonitrile–1% (v/v) tetrahydrofuran– 50 mg/dm^3 OSS– $0.05 \text{ mM Na}_2\text{EDTA}$. Flow-rate: $0.75 \text{ cm}^3/\text{min}$. Electrode: paraffin oil-based carbon paste. Applied potential: 0.75 V vs. SCE . Internal standard: 1.6 ng of dihydroxynorepinephrine (DHNEP), added before alumina clean-up. DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid.

of the electrode and the flow-rate. The orifice and the electrode diameters were usually kept at convenient sizes of 0.2 and 4 mm, respectively, and only the orifice–electrode distance and the flow-rate were varied. The effect of these variables on the peak height and on the HETP is shown in Figs. 3 and 4. The general conclusion is that one should avoid very low flow-rates and very small orifice–electrode distances in this type of cell. There are fairly wide limits of near-optimum performance and the remainder of the results were measured in this range. The linearity of the detector

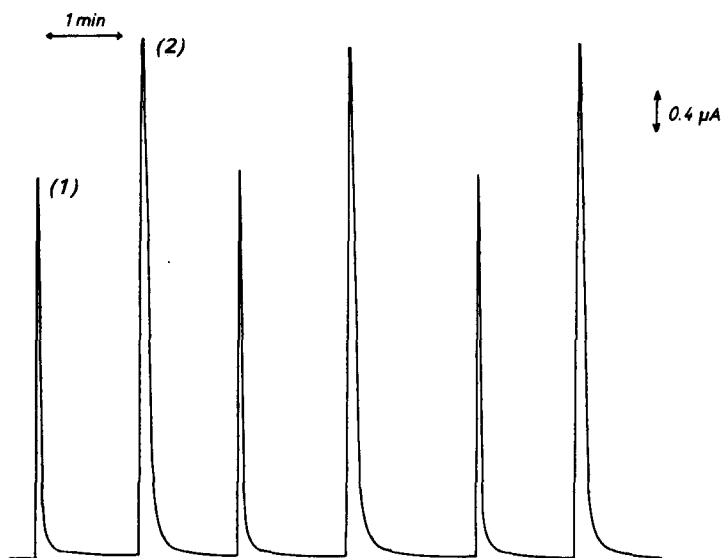


Fig. 8. Flow-injection peaks obtained with the large-volume wall-jet cell. Flow-rate: $1 \text{ cm}^3/\text{min}$. Electrode: silicone rubber-impregnated carbon. Applied potential: 0.65 V vs. SCE. Sample injected: (1) 600 ng of dopamine; (2) $1.8 \text{ }\mu\text{g}$ of dopamine.

was checked for epinephrine (adrenaline) and dopamine chromatographic peaks (Fig. 5). The detection limit, defined as $3s/k$, where k is the slope of the linear calibration line and s is the standard deviation, calculated from the baseline noise, was found to be *ca.* 10 pg for both compounds. The detector behaves fairly well in gradient elution (Fig. 6). It also works well with biological samples (Fig. 7).

Flow-injection peaks of dopamine, obtained with the large-volume detector and the silicone rubber-impregnated carbon electrode, are shown in Fig. 8. The cell follows the abrupt concentration change on the front side of the peak quickly. An earlier study¹⁰ showed the effective cell volume to be *ca.* $7 \text{ }\mu\text{l}$ in the optimal operating range. The flow-injection peak height was found to depend linearly on the injected

TABLE I

PERFORMANCE OF DIFFERENT ELECTRODE MATERIALS IN THE FLOW INJECTION SYSTEM

Injected substance: dopamine. Orifice-electrode distance: 3.5 mm .

Electrode material	Residual current (nA)	Resistance (Ω)	Physical surface area (mm^2)	Sensitivity	
				nA/ng	nA/ng $\cdot \text{cm}^2$
Glassy carbon	~ 1	2	19.6	~ 3	~ 15
Carbon paste (silicone)	~ 3	5	12.6	~ 2	~ 16
SR-carbon	~ 1	4	12.6	~ 10	~ 80

sample concentration between 10^{-8} and 10^{-4} M. The slope of this straight line can be used to characterize the sensitivity of the cell with respect to dopamine at the potential applied. When electrodes made from other materials were used, the linear relationship with the sample concentration remained, but the sensitivity was different. Table I shows the observed sensitivities, together with the residual current and electrode resistance for three electrode materials. The silicone rubber composition is found to have a higher sensitivity than the glassy carbon or the silicone-based carbon paste. The paraffin oil-based carbon paste had a similar sensitivity to that of the silicone rubber composition, as shown by two chromatograms obtained under identical conditions (Fig. 9).

An attempt has been made to explain the observed differences in sensitivity at least partly by differences in the active electrode surface. Electron micrographs show that the glassy carbon electrode has a smooth surface, whereas in the surface of the silicone rubber composition grains of size 5–50 μm are discernible at the same magnification. The carbon paste electrodes are difficult to prepare with reproducible sensitivity. Considerable differences between the actual and the geometrical electrode surfaces might also play a role.

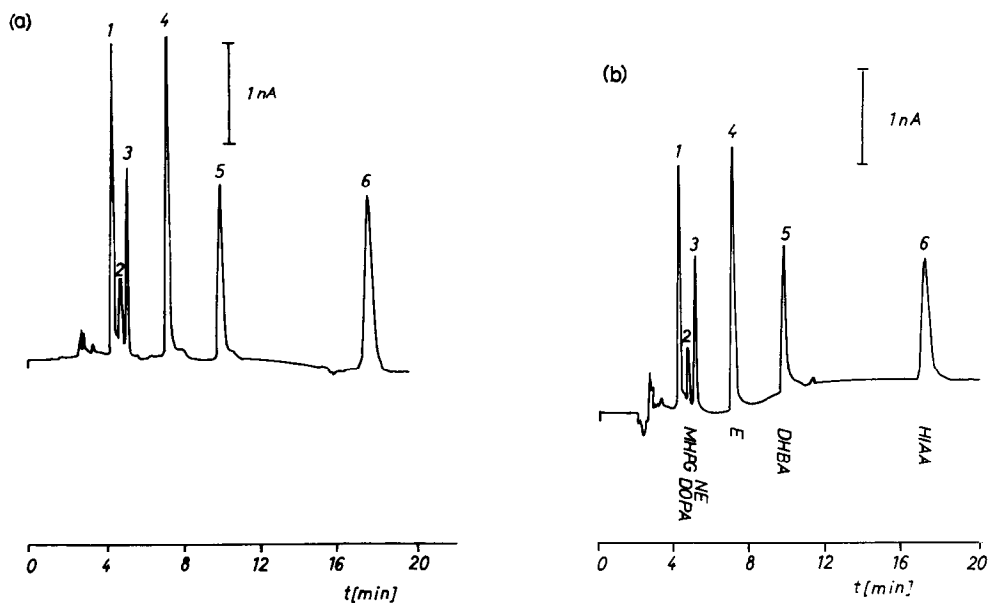


Fig. 9. Comparison of the silicone rubber-impregnated carbon electrode with the paraffin oil-based carbon paste electrode in the wall-jet cell. Column: 250×2 mm I.D. Ultrasphere ODS, 5 μm . Mobile phase: 0.1 M KH_2PO_4 –0.75 mM OSS–1.6 mg/dm³ Na_2EDTA –5.2% (v/v) methanol, pH adjusted to 3.5. Flow-rate: 0.4 cm³/min. Applied potential: 0.75 V vs. SCE. Electrode: (a) silicone rubber-impregnated carbon; (b) paraffin oil-based carbon paste. Test compounds: 1, dopa 2 ng; 2, 3-methoxy-4-hydroxyphenylglycol 540 pg; 3, norepinephrine (nondieneline) 2 ng; 4, epinephrine (adrenaline) 3 ng; 5, dihydroxybenzylamine 1.2 ng; 6, 5-hydroxyindoleacetic acid 2 ng.

CONCLUSION

Both the large-volume wall-jet cell and the silicone rubber-impregnated carbon electrode are viable alternatives to the existing systems for electrochemical detection. In addition, they have certain specific advantages. The large-volume cell is very easy to build and to handle, and it can accommodate virtually any commercial working, reference and auxiliary electrodes. The silicone rubber electrode composition is more robust than the pastes, and it can be made in the laboratory in any size and shape from inexpensive materials

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