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COMPARISON OF THE DESIGN AND PERFORMANCE CHARACTERISTICS OF THE WALL-JET TYPE AND THIN-LAYER TYPE ELECTROCHEMICAL DETECTORS

SEPARATION OF CATECHOLAMINES AND PHENOTHIAZINES

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SUMMARY

A comparison of the design and performance characteristics of two commercially available high-performance liquid chromatography systems with electrochemical detection, the wall-jet electrode detector and the thin-layer electrode detector (marketed by EDT Research, Great Britain, and Bioanalytical Systems, U.S.A., respectively), is discussed. Performance characteristics such as detection limits, noise and sensitivities, linear dynamic ranges and design characteristics affecting column performance (band spreading) are reported. Catecholamines and phenothiazines served as model systems in the experiments.

INTRODUCTION

Work on solid-surface electrode materials so far seems to indicate that carbon paste and glassy carbon are likely to be the most useful as working electrodes in high-performance liquid chromatography systems with electrochemical detection. The three electrodes in the wall-jet-cell design[1] (equipped with a glassy carbon working electrode) and the thin-layer-cell design[2] (with a carbon paste electrode in it) are arranged in cell configurations of different geometry, resulting in differences, among others, in the flow patterns, mass transfer and uncompensated electric resistance between the electrodes.

For a given set of chromatographic conditions, detector design and working-electrode dimensions, the detector response (I) depends only on the concentration (c_0) and the fluid velocity (assuming that v, the kinematic viscosity of the mobile phase and D, the diffusion coefficient of the electroactive component in the eluent, are constant), as is expressed by eqn. 1 (for thin-layer type detectors) and eqn. 2 (for wall-jet detectors):

$$I = 0.68nFD^{2/3}bl^{1/2}v^{-1/6}u^{1/2}c_0 \quad (1)$$

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(derived by Levich[3] for the limiting current at a planar electrode immersed in a medium with laminar flow and

$$I = 1.60\, knFD^{2/3}a^{-1/2}r^{3/4}\nu^{-5/12}V^{3/4}c_0 \qquad (2)$$

(derived by Matsuda[4,5] for the limiting current at wall-jet electrodes), where n = the number of electrons exchanged, F = the Faraday constant; b and l are the width and length, respectively, of the electrode surface, u = linear fluid velocity in the cell, V = the volume flow-rate of the solution issuing from the jet, a = diameter of the jet, r = the radius of the working electrode, k = an empirical constant.

In this work the relationships between I and $\sqrt{u}$ or $V^{3/4}$, as well as I and c_0 were examined. Some performance characteristics of the two detectors, such as detection limits, noise and sensitivities, linear dynamic ranges and design characteristics affecting column performance (band spreading) were also compared.

EXPERIMENTAL

Apparatus

The liquid chromatograph was constructed from individual components. A Varian 8500 high-pressure pump was used. Samples were injected either by a Valco Model CV-6UHPa injector equipped with a 10-μl loop (when a LiChrosorb RP-8 column was used) or by a home-modified, syringe-loaded Altex AX 201-56 (Tefzel) valve with a 100-μl loop operated in the partial loop mode (when a glass column packed with Vydac CX, a pellicular cation exchanger, was employed).

The amperometric detector was composed of a Bioanalytical Systems potentiostat (LC-2A) and a flow cell of wall-jet type or thin-layer type (hereafter called the EDT and BS cells, respectively). The carbon paste for the working electrode in the BS cell was prepared from 65% (w/w) of graphite powder (Spektralkohle RW-A, Ringsdorff-Werke, Bonn, G.F.R.) and 35% (w/w) of Nujol. A Radelkis (Budapest, Hungary) type OH-814/1 strip-chart recorder was used.

Chemicals

Aqueous solutions were prepared from glass-distilled water redistilled from alkaline permanganate. A citrate–acetate aqueous buffer (2.6 g citric acid monohydrate, 3.0 g sodium acetate trihydrate and 0.50 ml glacial acetic acid, dissolved and diluted to 1 l by distilled water and adjusted to pH 5.3 with pellets of sodium hydroxide) was used as the eluent for ion-exchange separations. The eluent composition for most reversed phase (RP) separations was: acetonitrile–0.05 *M* phosphate buffer adjusted to pH 4.4 (75:25). Before use eluents were degassed *in vacuo*. Cation-exchange columns were dry-packed and the RP columns were slurry-packed in our laboratory. Acetonitrile (Uvasol) and methanol (Selectipur) were purchased from E. Merck (Darmstadt, G.F.R.).

Dopamine–HCl (DA), noradrenaline–HCl (NA) and adrenaline–HCl (A) were purchased from Chrompack (Middelburg, The Netherlands). The phenothiazines fluphenazine (Fl), T-412 and GYKI-22441 as well as α-methyldopamine-HBr (α-MDA) were synthesized in our institute. All other chemicals were of analytical-reagent grade.

Phenothiazine stock solutions were prepared in the eluent. Catecholamine stock solutions were made up in 0.1 *M* trichloroacetic acid containing 4.2 mg sodium hydrogen sulphite per litre. Dilutions were prepared daily in the respective solvent.

RESULTS AND DISCUSSION

The detectors were operated in the oxidation mode. The diameter of the carbon paste electrode was 4.8 mm (the size of the electrode-well was modified in our laboratories) and that of the glassy carbon electrode was 3 mm. Instead of a theoretically meaningful comparison of the two cells in a flow injection analysis system[6,7], the BS cell was tested mainly with an ion-exchange column, and the EDT cell was tested with ion exchange and RP columns.

Response dependence on the flow rate

BS cell. In this cell, the three electrodes are placed relatively far apart, resulting in a larger uncompensated resistance through the cell and a relatively big cell space.

Eqn. 1 indicates that a linear relationship exists between $\sqrt{u}$ and the detector response when a thin-layer type cell is used. Linear velocity in the BS cell can be changed by changing the flow-rate through the column or by changing the spacer thickness. In the usual flow-rate range for electrochemical detectors (0.3–1.0 ml/min) a linear relationship was found between I and $\sqrt{u}$ in the range $0.3 < u < 0.6$ ml/min. At flow-rates higher than 0.6 ml/min, however, the relationship was no longer linear (the decrease in response was probably due to the fact that the effective plate number of the ion-exchange column decreased with increased flow-rate). The noise level did not change between 0.3 and 0.5 ml/min, but showed a gradual increase when the flow-rate was higher than 0.5 ml/min. Thus, a flow-rate of 0.6 ml/min was accepted as the best compromise.

When u was changed by changing the spacer thickness (at a flow rate of 0.6 ml/min), a 51-μm spacer was used instead of the usual 127-μm one. The increase in $\sqrt{u}$ was 1.58-fold; the response increase, however, was only a factor of 1.25, roughly the same as that reported previously[8], and owing to a commensurable increase in the noise level, no change in the signal-to-noise ratio (S/N) was found.

Fig. 1A shows a typical response for picomole amounts of catecholamines and the usual noise level of the BS cell. The typical detection limits (S/N 2:1, injected amount) for NA, A and DA were 10, 9 and 18 pg, respectively.

In the routine determination of rat-brain catecholamines we found variations in S/N whenever a new working electrode was used. Therefore, we adopted the practice of testing each newly packed electrode with DA, in the system shown in Fig. 1A, after an equilibration of 15–20 h. Only electrodes exhibiting an S/N value of at least 20 for 250 pg of DA standard were considered as acceptable for use. The electrode-to-electrode variation was relatively wide and ranged over an S/N value of 5–35, however carefully the walls of the flow cell were polished and the paste and working electrode prepared.

EDT cell

In this cell the electrodes are screwed into the cell body close together in order

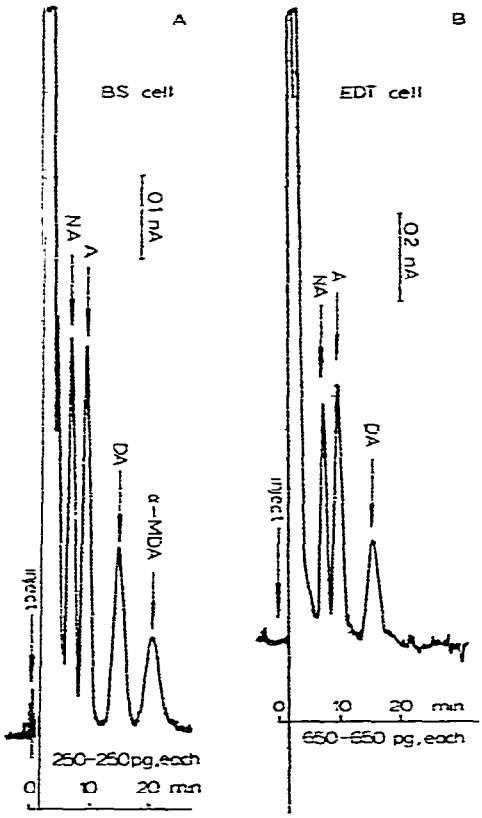

Fig. 1. Cation-exchange separation of catecholamines NA, A and DA, using α-MDA as internal standard. Column, Vydac CX (35 μm) 500 × 2 mm I.D.; eluent, citrate–acetate buffer; flow-rate, 0.6 ml/min; chart speed, 10 cm/h; temperature, ambient. Detection: (A) BS cell, oxidation potential + 550 mV (carbon paste *vs.* Ag/AgCl); spacer thickness, 127 μm; effective cell volume *ca.* 3 μl; sample size, 250 pg each. (B) EDT cell, oxidation potential + 900 mV (glassy carbon *vs.* Ag/AgCl); effective cell volume, 0.25 μl; sample size, 650 pg each.

to minimize the uncompensated resistance through the cell. By turning the working electrode it is possible to change the cell volume between 0 and 100 μl.

Eqn. 2 predicts that I changes linearly with $V^{3/4}$ if a flow-cell working on the wall-jet principle is employed. Increased peak heights were experienced with increased volume flow-rate in high efficiency RP systems (strongly non-aqueous) when the flow-rate was increased from *e.g.* 0.50 to 0.83 ml/min, but the increase in the response factor was between 1.04–1.22-fold, instead of the expected 1.46-fold increase, and there was no response increase with low efficiency (theoretical plate height, $H \geq 1$ mm) ion-exchange systems.

Theoretically, there is no linear-velocity dependence with the wall-jet design if V is constant. However, this assumption is no longer valid if the effective cell volume is smaller than 5 μl. The analysis of catecholamines and phenothiazines served as model systems in support of this statement. The separation of phenothiazines examined is shown in Fig. 2.

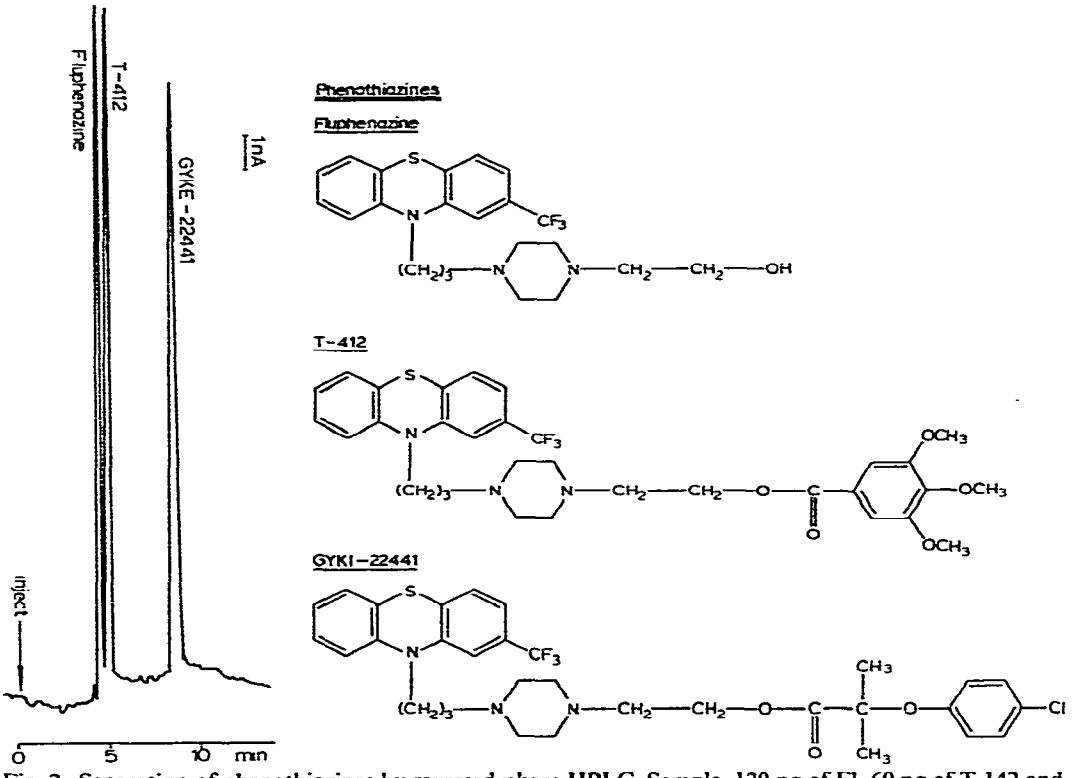

Fig. 2. Separation of phenothiazines by reversed-phase HPLC. Sample, 120 ng of Fl, 60 ng of T-142 and 150 ng of GYKI-22441 (injected amount); column, LiChrosorb RP-8 (10 μm) 250 × 4.6 mm I.D.; eluent, 0.05 *M* phosphate buffer (pH 4.4)–acetonitrile (25:75); flow-rate, 0.83 ml/min; chart speed, 30 cm/h; temperature, ambient. Detection: EDT cell, oxidation potential + 1.2 V (glassy carbon *vs.* Ag/AgCl); effective cell volume, 0.50 μl.

Figs. 3–7 demonstrate a strong response dependence on cell volume (at constant V) when the cell volume is reduced below 3 μl, indicating a "linear-flow-rate"-like dependence under such conditions. This phenomenon may be attributed to the fact that, instead of a radial free flow of the eluate on the surface of the electrode (as assumed by eqn. 2), there is a directed flow from the working space towards the outlet. When the cell volume was changed between 0.25 and 3 μl, the response showed power functions of u approaching $u^{1/2}$ and $u^{3/4}$, with neat aqueous systems (aqueous buffers) resulting in a response dependence on $u^{3/4}$, while with strongly non-aqueous systems a response dependence on $u^{1/2}$ was found (see Figs. 3–7).

With glassy carbon electrodes the noise level strongly depends on the quality of the electrode surface polishing[9]. When polished well, the electrode in the EDT cell exhibited a regular noise level that was not affected by an increase in V (in the range 0.3–1.0 ml/min) or a decrease in the cell volume (so S/N was in direct proportion to the response throughout this range).

Fig. 1B shows a typical response for picomole amounts of catecholamines and the usual noise level of the EDT cell. The typical detection limits for NA, A and DA in the cation exchange system were 40, 36 and 90 pg, respectively. In efficient RP

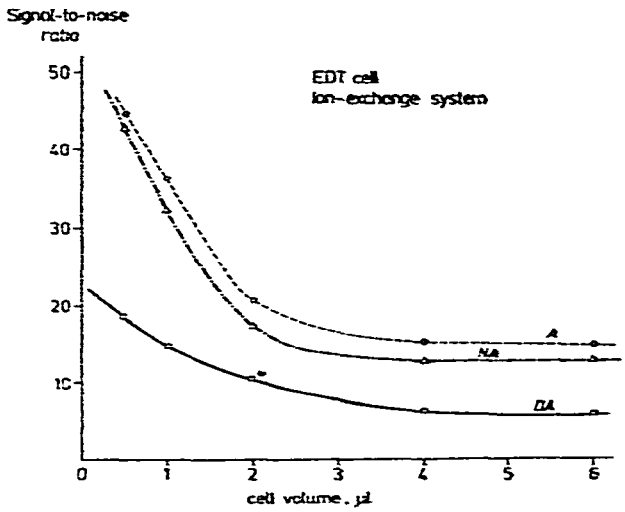

Fig. 3. Influence of cell volume on detector response (S/N). Conditions as in Fig. 1B. Sample, 1 ng of NA, A and DA.

systems ($H \leq 0.02$ mm) the detection limits were about half of these values (see Fig. 5).

A comparison of Figs. 1A and B suggests the conclusion that better detection limits can be expected in neat aqueous systems from the BS cell, if pre-tested working electrodes are used, than from the EDT cell, in spite of a possibly inferior mass

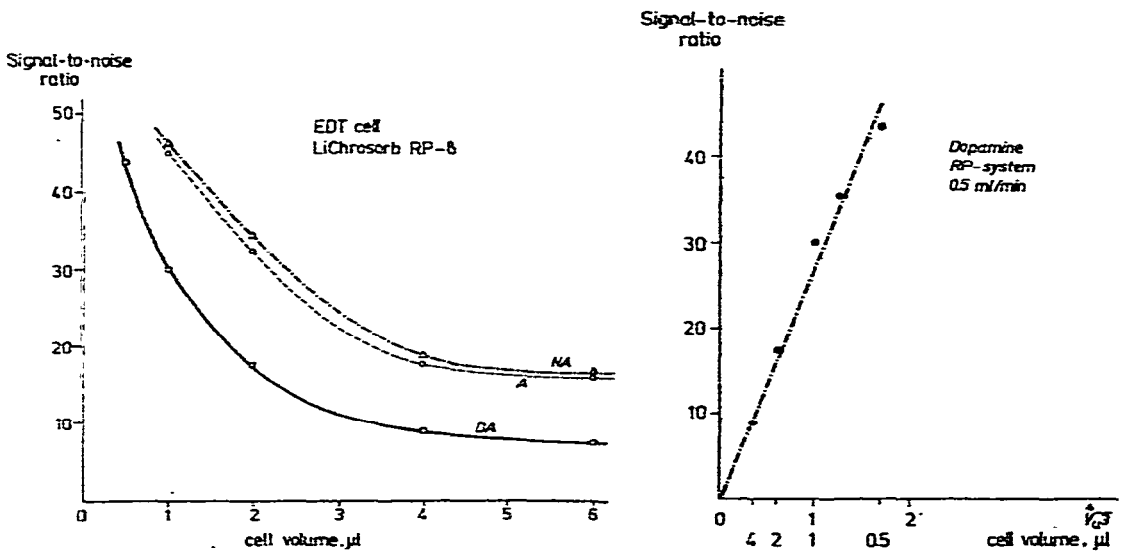

Fig. 4. Influence of cell volume on detector response (S/N). Sample, 1 ng of NA, A and DA; column, LiChrosorb RP-8 (10 µm) 250 × 4.6 mm I.D.; eluent, 0.1 *M* phosphate buffer (pH 5.3)–methanol (98:2); flow-rate, 0.83 ml/min. Detection: EDT cell, oxidation potential + 1 *V* (Ag/AgCl).

Fig. 5. S/N *vs.* $u^{3/4}$ graph of the DA curve in Fig. 4, depicting the relationship between the response and "linear flow-rate" with the wall-jet cell, in aqueous systems.

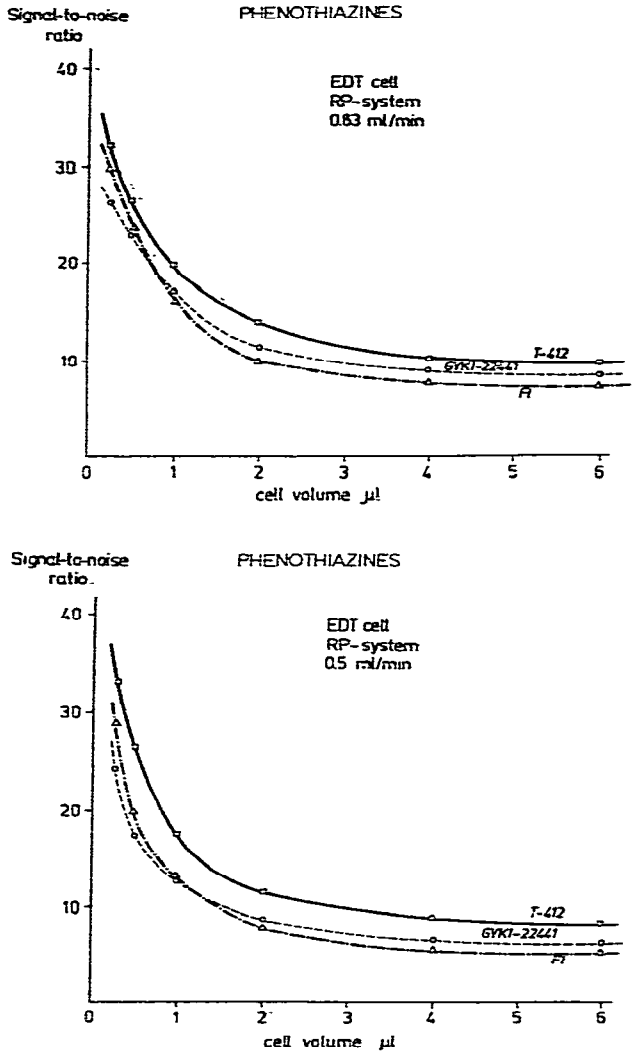

Fig. 6. Influence of cell volume on detector response (S/N). Chromatographic and detection conditions as in Fig. 2 (except for sample size and cell volume). Sample, 15 ng of Fl, 10 ng of T-142, 25 ng of GYKI-22441.

transfer and a higher uncompensated electric resistance with flow cells of the former design.

Linear dynamic range, stability and reproducibility

Both detectors have a wide linear dynamic range (D_L) in neat aqueous systems. The upper limit is virtually determined by the properties of the circuitry in the potentiostat, with the BS cell, *e.g.* $D_{L(NA)} \geq 1.05 \cdot 10^{-11}$–$4.0 \cdot 10^{-7}$ g, a range of more than four orders of magnitude. The graphs obtained fulfil the linearity criterion suggested by Fowlis and Scott[10]. In strongly non-aqueous systems, however, the range was only

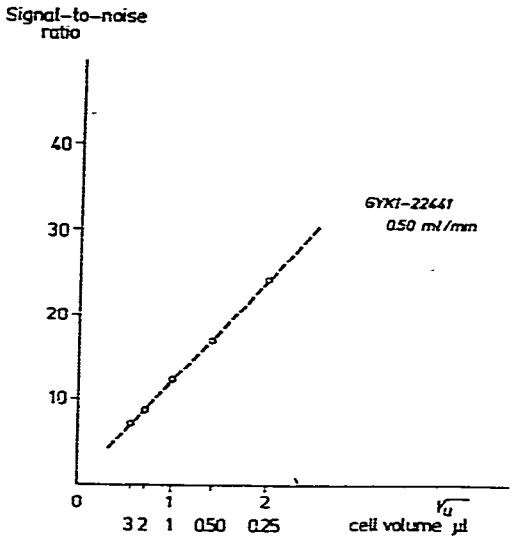

Fig. 7. S/N *vs.* $\sqrt{u}$ graph of the GYKI-22441 curve in Fig. 6B, showing the relationship between the response and "linear flow-rate" with the wall-jet cell, in strongly non-aqueous systems.

2–3 orders of magnitude with the EDT cell (owing to a much higher residual current and an increased impedance between the electrodes), and the BS cell could not be used at all.

The stability of the carbon paste electrode in neat aqueous systems was reasonably good. In routine determinations of rat-brain catecholamines S/N slowly decreased over the test limit of 20 for 2–6 months and no electrochemical passivation problems were found within this period. The glassy carbon electrode in the EDT cell, however, needed frequent repolishing (every day in mixed aqueous–organic systems) and daily refilling of the reference electrode was necessary.

Reproducibility in neat aqueous systems was good with both cells. Catecholamine standards were measured with a relative standard deviation of ± 3–6% (without an internal standard) in the subpicomole range. Reproducibility of determinations with the EDT cell in mixed systems was somewhat poorer.

Band spreading

Band spreading caused by the BS or EDT flow cell was studied in a direct injection mode as suggested by Poppe[6] and in a high efficiency RP system (see the legend for Fig. 4), using NA and A as test compounds and a UV detector (Variscan, Varian) with a 8-μl flow cell, for comparison. A short 0.3-mm PTFE capillary was used for connecting a home-made septum injector or the RP column to the BS or EDT cell, respectively. The flow-rate was 0.6 ml/min. Peak width was measured at 0.607 of the peak height (2σ).

Compared with the UV flow cell, no extra band spreading was found with the BS cell. The EDT cell did not cause any extra band spreading, either, as long as the cell volume was in the volume range of practical importance (below 5 μl) and showed some adverse effect only when the cell volume was larger than 20 μl.

Based on the comparison described above, the BS cell has been chosen in our laboratory for the routine determination of catecholamines in rat-brain regions of 1–2 mg. After a simple extraction with 0.1 *M* trichloroacetic acid (which eliminates the need for the usual purification step of selectively adsorbing the catecholamines on alumina[11] or solvent extraction[12]) the centrifuged brain extract was directly injected into the chromatographic system shown in the legend of Fig. 1A (the retention of NA was insufficient even in RP systems containing an ion-pairing agent). By using α-MDA as internal standard (instead of the widely used 3,4-dihydroxybenzylamine) all three catecholamines were determined simultaneously in the picomole range with a relative standard deviation of ± 5–7%.

CONCLUSION

The response of both commercial detectors exhibited a remarkably smaller flow-rate dependence than predicted by theory. On the other hand, a strong "linear-flow-rate"-like dependence was found with the EDT cell when the volume of the working space was smaller than 3 μl. Theory predicted a volume-flow-rate dependence only.

With the problem of the reproducibility of the preparation of the working electrode being unsolved, a test procedure for newly packed carbon-paste electrodes has been proposed. With pre-tested working electrodes, the BS cell exhibits 4–5 times better detection limits for catecholamines than the EDT cell. The latter, however, can also be used in strongly non-aqueous systems at a comparable sensitivity to UV detectors (see Fig. 2).

There was no significant difference between the two cells in linear dynamic ranges, reproducibility and band spreading effects, but, in our hands, the long-term stability of pre-tested carbon-paste electrodes was remarkably better in neat aqueous systems than that of glassy carbon electrodes.

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