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## CARBON-POLYMER CHIPS AS SENSITIVE ELECTROCHEMICAL DETECTORS FOR MICRO-LIQUID CHROMATOGRAPHY

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### SUMMARY

The construction and characterization of an on-line electrochemical detector especially suited for flow-rates in the order of a few microlitres per minute is described. The working electrode is tubular, of length 15 mm and I.D. 90  $\mu\text{m}$  (effective volume 100 nl). It is made of a small carbon-polymer chip, giving an inexpensive, practical and disposable construction. When used with packed fused-silica liquid chromatographic columns of 0.3 mm I.D., sub-picogram detection limits are obtained. The detector is linear for amounts up to 1 ng. An exceptional feature of this detector is that it stabilizes in a few seconds after potential onset (no baseline drift). The noise level is very constant and predictable. Some examples are given of the oxidative determination of phenolic compounds and catecholamines. Coulometric efficiencies, response *versus* flow-rate behaviour and peak dispersion characteristics are given.

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### INTRODUCTION

Chromatographic methods are rapidly expanding towards the use of small-diameter columns (less than 1 mm I.D.). The benefits are a lower cost of stationary-phase material and eluents and applicability to very small sample volumes. In our work, a method was needed for the determination of catecholamines in small biological samples. The use of sub-1-mm I.D. packed capillary liquid chromatographic (micro-LC) columns with electrochemical detection (ED) was appropriate for the problem, but a detector that fits these requirements could not be found.

Flow-through electrochemical detectors have been the subject of intensive research, especially for the determination of organic molecules in the oxidative mode<sup>1</sup>. Their use and characteristics are very well documented for conventional high-performance liquid chromatographic (HPLC) methods (column diameter larger than 1 mm)<sup>2,3</sup>.

Small column diameters and low flow-rates pose new problems in the construction of detectors. A few electrochemical detectors have been described for microbore LC systems<sup>4-6</sup>, for open-tubular capillary LC<sup>7</sup> and for micro-LC columns<sup>8-13</sup>. In the last instance, detection limits of 10 pg<sup>10</sup> were mentioned for detectors based on planar electrode systems. Therefore, our first experiments were directed towards laboratory-made wall-jet detectors, which have been used successfully in our laboratories for conventional HPLC systems (detection limits in the low-picogram range for 4.6 mm I.D. columns). With such detectors, good results were obtained for micro-LC also, but the baseline noise and drift were not reproducible. This phenomenon was ascribed to hydrodynamic conditions that are difficult to control at low flow-rates when planar glassy carbon electrodes are used. Miniaturization of the working electrode diameter from 3 mm to 1.5 and 0.8 mm and reduction of the working electrode-counter electrode distance from 50  $\mu\text{m}$  to a few micrometres did not improve this situation. For these reasons, a tubular electrode was designed in which carbon composite materials were used.

Such materials have already been used in planar<sup>14</sup> and in tubular<sup>15-17</sup> flow-through detectors for use with conventional HPLC columns (large internal volumes). For the latter type of detectors, a hole must be drilled into the material, which restricts the diameter and results in rough internal surfaces. For these reasons, and because reliable and sensitive planar electrode constructions are available for conventional HPLC systems (column I.D. larger than 1 mm), tubular detectors have never been competitive in this area. For the drastically reduced flow-rates used in micro-LC systems, planar working electrodes may well prove unsatisfactory. It was felt that narrow-bore tubular electrodes could provide good flow characteristics even at low flow-rates. Very narrow channels can be more easily produced in this material than in hard materials, such as glassy carbon. Further, carbon-polymer electrodes exhibit large accessible potential ranges, small and constant residual currents and reproducible surface activities<sup>18-20</sup>. On the other hand, their kinetic parameters are less favourable than those of freshly polished glassy carbon. We selected an ethylene-vinyl acetate copolymer containing 9% of vinyl acetate because of its high chemical inertness and its good electrochemical behaviour<sup>14,21</sup>. This paper describes the use of such a copolymer in a disposable tubular electrochemical sensor for application at low flow-rates.

## EXPERIMENTAL

### *Chromatographic equipment*

The chromatographic setup consisted of a high-pressure pump (Model M6000A; Waters Assoc., Milford, MA, U.S.A.), a 60-nl internal volume injector (Valco CV6U; VICI, Houston, TX, U.S.A.) and a 25 cm  $\times$  0.32 mm I.D. micro-LC column of the fused-silica type, filled with 5- $\mu\text{m}$  C<sub>18</sub> (RSL-Alltech, Eke, Belgium). All eluents were continuously degassed with helium. A conventional HPLC column (25

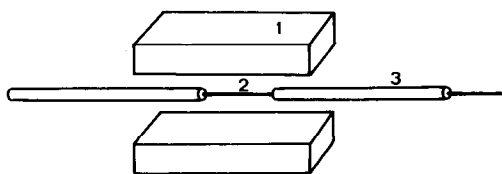


Fig. 1. Construction of a carbon-polymer tubular electrode. 1 = Piece of copolymer; 2 = stainless-steel wire of 90  $\mu\text{m}$  diameter; 3 = polyimide-coated fused-silica quartz capillary of 100  $\mu\text{m}$  I.D.

cm  $\times$  4.6 mm I.D.) served as a solvent by-pass. A splitter was placed between the pump and the conventional + micro-LC systems. Using this system, a flow-rate of 1–2 ml  $\text{min}^{-1}$  could be maintained with the HPLC pump.

#### *Electrochemical detector*

The working electrode was made of an ethylene-vinyl acetate copolymer containing 9% of vinyl acetate, mixed with 20% (w/w) of carbon. This material is obtained as sheets of thickness 1.3 mm, as described in refs. 20 and 21. For the fabrication of a working electrode chip, two rectangular pieces (5  $\times$  25 mm) were cut out of these sheets (see Fig. 1). A metal wire of 90  $\mu\text{m}$  thickness (stainless-steel syringe-cleaning wires from Hamilton, Reno, NV, U.S.A.) was inserted into two fused-silica capillaries of 100  $\mu\text{m}$  I.D., which coated with a polyimide layer, are readily available (RSL-Alltech). The inlet and outlet capillaries were 80 and 35 mm long, respectively, and the distance between them was 15 mm. The two capillaries must be inspected under a light microscope; they should not contain dust particles in the interior and the capillary ends contacting the plastic must be squarely cut. After cutting the capillaries with a glass cutter, the ends were polished with silicon carbide grinding paper (grit 600, Carbitet; Buehler, Lake Bluff, U.S.A.). Residual silicon carbide particles were removed from the capillaries by flushing with water. The capillaries and steel wire were placed between the two copolymer pieces and this construction was placed on an aluminium foil, and then on a hot-plate (200°C). A second hot-plate (200°C) was pressed manually against the upper piece of copolymer (also covered with aluminium foil to avoid contact of the plastic with the hot-plate). Application of pressure for a few seconds was sufficient to compress a chip to 1 mm thick.

The moulded assembly was removed from the hot-plates and allowed to cool in air for *ca.* 30 s. The steel wire was then pulled out and the tubular working electrode could be used as shown in Fig. 2. The short capillary dipped into a conducting solution (3.5 M potassium chloride was found to be appropriate for our experiments). A combined calomel reference electrode and platinum counter electrode was used to complete the detector (PT62 type; Schott Geräte, Hofheim a.Ts., F.R.G.). The contact points between the fused-silica capillaries and the copolymer were shock-protected by a drop of silicone rubber.

## RESULTS AND DISCUSSION

#### *Potential response behaviour: hydrodynamic voltammograms*

Electrochemical detectors are highly selective when compared with, *e.g.*, UV detectors<sup>22</sup>. Especially steep, well resolved voltammograms give added possibilities

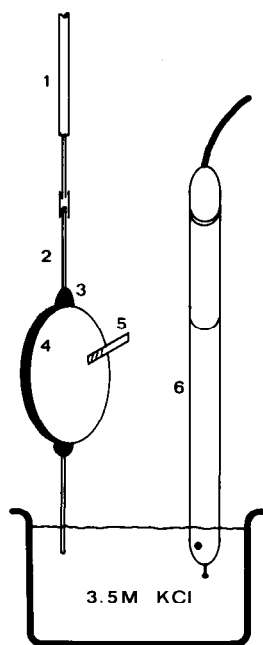


Fig. 2. Construction of electrochemical detector. 1 = Micro-LC column; 2 = quartz capillary; 3 = silicone rubber; 4 = carbon-composite working electrode; 5 = electrical contact; 6 = combined calomel-platinum counter electrode.

for differentiating between electroactive species. The shape of the voltammograms depends on the reaction kinetics, the  $iR$  drop ( $i$  = current;  $R$  = resistance) and the homogeneity of the electric field between the working and counter electrodes. Whereas the reaction kinetics depend on the electrode material used, the last two phenomena are strongly influenced by cell geometry.

In Fig. 3, hydrodynamic voltammograms (HDVs) obtained on a chip-type electrode are compared with those measured with a classical wall-jet type of detector. Curves a and b are the HDVs of caffeic acid (a reversibly and rapidly oxidized *o*-

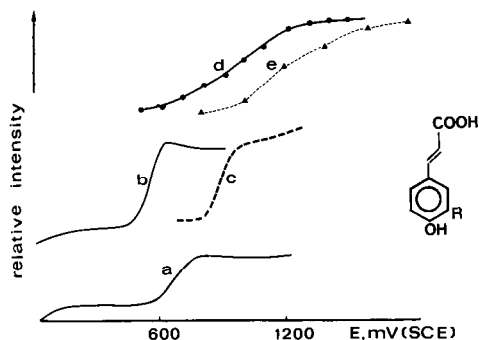


Fig. 3. Hydrodynamic voltammograms of caffeic acid ( $R = OH$ ) and *p*-coumaric acid ( $R = H$ ) in micro-LC systems.

dihydroxyphenolic acid) on a planar copolymer electrode (3 mm I.D., 20% carbon) and on a planar glassy carbon electrode (3 mm I.D.), respectively. Both electrodes were mounted in the same wall-jet cell so that the observed differences are due only to the electrode material, and not to the cell geometry. This detector had the working and counter electrodes in a parallel opposed configuration; the stainless-steel inlet line was used as the counter electrode. The reaction kinetics are faster on the planar glassy carbon (curve b) than on the planar copolymer electrode (curve a). Curves a and b were recorded by on-line rapid-scanning ( $1 \text{ V s}^{-1}$ ) staircase voltammetry. This technique (see also ref. 23), which is expected to become increasingly used in routine HPLC detection, is very sensitive to the  $iR$  drop and cell geometry. The same HDVs were obtained with a more cumbersome method in which repetitive injections and manual changes of electrode potential were used. The latter method was used to record the voltammograms in Fig. 3d and e for caffeic acid and *p*-coumaric acid respectively, with the tubular copolymer detector shown in Fig. 2. When this cell geometry is used, the HDVs are considerably shifted towards apparently higher applied potentials. However, these "overpotentials" can only partly be ascribed to sluggish electrode kinetics, as the copolymer electrode showed good kinetic behaviour when used in a planar form (curve a). They are ascribed to the special geometric configuration of the working and counter electrodes. This results in a reduced resolving power; the caffeic acid-*p*-coumaric acid pair is better resolved with the glassy carbon wall-jet detector (curves b and c, respectively) than with the plastic chip detector (curves d and e).

### Linearity

When used with micro-LC columns, the detector shows a linear response behaviour for injected amounts from 5 to 1000 pg (Fig. 4a). Above 1 ng, deviations from linearity begin to occur. The above concentration range (three decades) can be considered as the working range for organic trace analysis with micro-LC methods. For use with conventional HPLC methods (4.6 mm I.D. columns,  $1 \text{ ml min}^{-1}$  flow-rate), a

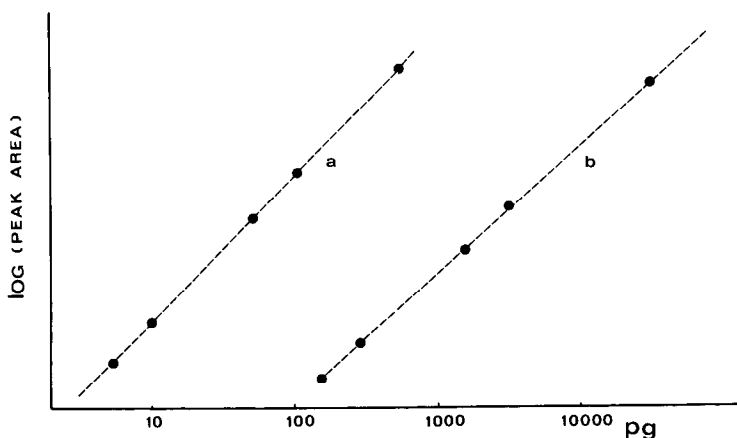


Fig. 4. Calibration graphs for the tubular electrode in (a) micro-LC and (b) conventional HPLC ( $C_{18}$  column,  $25 \text{ cm} \times 4.6 \text{ mm}$  I.D.). 3,4-Dihydroxybenzoic acid was used as a test substance. The eluent composition was methanol- $0.01 \text{ M}$  orthophosphoric acid (30:70). Flow-rate: (a)  $0.005$  and (b)  $1 \text{ ml min}^{-1}$ .

linear response behaviour was obtained from 0.1 to 50 ng (Fig. 4b, and other measurements). On a non-logarithmic (linear) scale, the calibration graphs show a zero intercept.

#### Detection limits and baseline stability

Chromatographic detection is easily and reproducibly obtained in the picogram range, as shown in Fig. 5 for phenolic compounds (A) and catecholamines (B). On the basis of the gallic acid peak in Fig. 5A (peak a), the detection limit was calculated to be 500 fg. This is far below the detection limits reported by Goto *et al.*<sup>10</sup> (10 pg) for planar glassy carbon electrodes used in micro-LC systems. The response of the catecholamines as a function of the applied working electrode potential declines for potentials above 1200 mV. This behaviour was also observed by Yoshiura *et al.* with glassy carbon electrodes (ref. 24, Fig. 3).

Fig. 5C shows that the tubular copolymer detectors can also be used successfully with conventional HPLC systems. The three phenolic compounds were injected in the 100–200-pg range. However, at these flow-rates, the glassy carbon-based wall-jet detector had even better signal-to-noise characteristics (results not shown). Unfortunately, a great disadvantage of the latter detectors is the long stabilization time required to measure these low amounts (baseline drift).

For the small currents measured in Fig. 5A and B, the noise level was unaffected by external events, such as touching the glass beaker (Fig. 2) or the reference electrode. Such effects, which are due to high cell impedances, are frequently observed for many classical cell designs. The “chip” electrode described here allows the use of a reference electrode with a platinum fibre junction. This has distinct advantages over ceramic membrane types, as the latter occasionally tend to give high impedances because of deposition of solid potassium chloride.

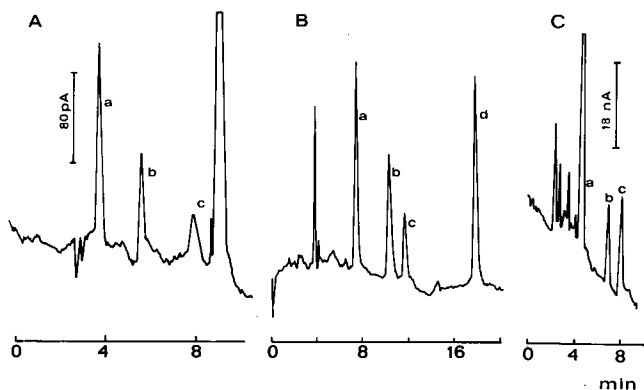


Fig. 5. Application of carbon-copolymer tubular electrodes in (A and B) micro-LC and (C) conventional HPLC. (A) Separation of (a) 8.2 pg of gallic acid, (b) 6.6 pg of 3,4-dihydroxybenzoic acid and (c) 6 pg of 3,4-dihydroxybenzaldehyde in the micro-LC system. Eluent, methanol–0.01 M phosphoric acid (30:70). Detector potential, +1600 mV [vs. a saturated calomel electrode (SCE)]. (B) Separation of 10–20-pg amounts of (a) noradrenalin, (b) adrenalin, (c) 3,4-dihydroxybenzylamine and (d) dopamine. Eluent, 3 mM heptanesulphonate + 100 mg/l EDTA + disodium hydrogenphosphate-citric acid buffer pH 3.5,  $10^{-2}$  M + 5% acetonitrile. Column, see text; detector potential, +900 mV (vs. SCE). (C) Separation of (a) 240 pg of gallic acid, (b) 190 pg of 3,4-dihydroxybenzoic acid and (c) 210 pg of 3,4-dihydroxybenzaldehyde on a conventional  $C_{18}$  column (25 cm  $\times$  4.6 mm I.D.). Eluent, methanol–0.01 M orthophosphoric acid (30:70); detector potential, +1600 mV (vs. SCE).

Faraday-cage shielding of the detector components was not required. The measured currents are still considerably higher than those which are measured with ED in open-tubular capillary systems, where shielding is necessary<sup>7</sup>. In our study of the tubular "chip" electrode, external noise pick-up did occur occasionally when the working electrode was fouled or clogged, or when the calomel electrode junction was disrupted. Noise was also due to a non-grounded pneumatic pump; grounding immediately eliminated the noise. Adhesion of gases to the electrode surface occasionally reduced the coulometric efficiency. This was quickly remedied by placing the chip plus inlet and outlet capillaries in a test-tube filled with methanol and applying a vacuum for a few minutes.

An interesting advantage of the tubular micro-LC detector is the almost total lack of baseline drift (such drifts are a common phenomenon with glassy carbon working electrodes). The detector can be used a few seconds after application of the electrode potential. This potential can even be changed during analysis without excessively disturbing the chromatogram. The latter behaviour is very exceptional for an electrochemical detector. With the tubular "chip" electrodes, automatic disruption of the applied potential at very high current densities becomes very feasible. This would protect the electrode from accidental fouling by abnormally high concentrations of substances.

After a 14-day period of uninterrupted analysis (3,4-dihydroxybenzoic acid was injected 5–10 times daily in 600-pg amounts under the conditions stated for Fig. 5A), no significant change in the detector response could be observed. During this work, fifteen "chip"-type electrodes were assembled and tested, and all yielded results comparable to those shown in Fig. 5.

#### *Coulometric efficiency*

Coulometric efficiencies decrease when increasing concentrations of organic solvents are used in the mobile phase (Fig. 6A), and this also occurs with the glassy

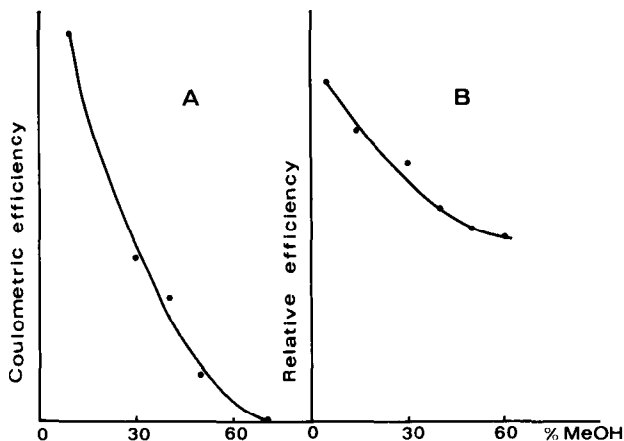


Fig. 6. Decrease of the response of (A) a tubular carbon-copolymer detector and (B) a wall-jet glassy carbon detector with increasing methanol concentrations in the eluent (methanol–0.01 M orthophosphoric acid). (A) Recorded under micro-LC conditions; (B) recorded under conventional HPLC conditions. Gallic acid was used in both systems. MeOH = Methanol.

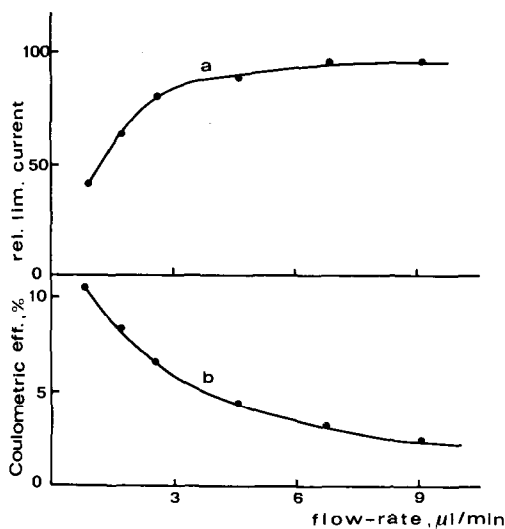


Fig. 7. Variation of (a) the limiting (lim.) current and (b) the coulometric efficiency (eff.) with flow-rate under micro-LC conditions. The curves were recorded with 3,4-dihydroxybenzoic acid as the test substance. Eluent, methanol-0.01 M orthophosphoric acid (30:70).

carbon-based wall-jet detector (Fig. 6B). In Fig. 6A, the real coulometric efficiency of the "chip"-type detector is shown. It is as high as 30% for organic solvents in 10% (v/v) concentration. For the measurements shown in Fig. 6A, the conductivities of all chromatographic eluents were adjusted to 2 mmho with potassium chloride to minimize differences in the  $iR$  drop). Such high efficiencies at low flow-rates were also noted by Goto *et al.*<sup>10</sup>. The coulometric efficiency of the detector also decreases with increasing flow-rate (see Fig. 7b); a decrease from 10% to 2% occurs when the flow-rate is increased from 1 to 12  $\mu\text{l}/\text{min}$  for an eluent containing 30% of organic solvent. Electrochemical detectors with high or low coulometric efficiencies are usually classified as coulometric or amperometric devices, respectively. The first are mass-sensitive, whereas the second are concentration-sensitive. With the micro-tubular detectors that were used in this study at low-flow rates, some intermediate behaviour is to be expected.

Another important feature of electrochemical detectors is the dependence of limiting currents on flow-rates. For the tubular detector described here, this dependence is given in Fig. 7a. Limiting currents were obtained by measuring peak heights. As the chromatographic column was part of the system, a normalization was applied to this peak height to account for the variation in peak broadening (normalization to the same peak volume). After an initial increase in the limiting current with increasing flow-rate, this current becomes almost flow-insensitive. A 50% decrease in limiting current occurred when the flow-rate was decreased from 5 to 1  $\mu\text{l}/\text{min}$ . Such dependences have been the subject of several studies, and mathematical expressions have been formulated for hypothetical systems<sup>25</sup>. As was stated by Roosendaal and Poppe<sup>26</sup>, much remains to be done to cope with realistic situations, such as electrodes in flow-through detectors. The experimental conditions used in this study (small diameters, low flow-rates and high coulometric efficiencies) provide new material for such



TABLE I

PEAK VARIANCE (nl) AFTER INJECTING NORADRENALIN IN DIFFERENT VOLUMES IN A FLOW-INJECTION SETUP, CONSISTING OF A WALL-JET DETECTOR (EQUIPPED WITH 1.5 AND 3 mm DIAMETER GLASSY CARBON ELECTRODES) AND A CHIP-TYPE CARBON-POLYMER DETECTOR

Detector type	Injection volume (nl)		
	60	20	500
Wall-jet (1.5 mm)	285	372	593
Wall-jet (3 mm)	306	427	467
Chip	244	337	354

studies. A curve such as Fig. 7a also yields practical information on the flow-rates that are still useful with 90  $\mu\text{m}$  I.D. tubular electrodes from the point of view of detector sensitivity. The curves compare well with those obtained in a slightly different way by Goto *et al.* on planar glassy carbon working electrodes used under micro-LC conditions (ref. 10, Fig. 7).

#### *Other working electrode dimensions and materials*

Tubular working electrodes of I.D. 40 and 200  $\mu\text{m}$  and with lengths varying from 2 to 20 mm were also tested. The smaller inner diameter gave a considerably higher baseline noise but had little effect on peak height. The 200- $\mu\text{m}$  I.D. electrodes showed substantially the same behaviour as the 90- $\mu\text{m}$  I.D. electrodes (signal-to-noise ratio, linearity, voltammogram shape, baseline stability and response *versus* flow characteristics). The 90- $\mu\text{m}$  I.D. electrodes gave less peak broadening and were therefore found to be the most satisfactory. The use of copolymer material with higher carbon contents (30%) was not found to be satisfactory, as cracking of the electrode material and subsequent leaking of the cell occurred.

#### *Peak broadening*

Extra peak dispersion was determined by measuring the total variance (in nanolitres) after the injection of different volumes of catecholamine solutions in a flow-injection setup (injector + detector only). This variance was calculated by using moment analysis to account for peak shapes deviating from Gaussian forms. Table I compares the results obtained with a wall-jet detector (3 and 1.5 mm diameter working electrodes), and the results obtained with a tubular "chip" detector. In this respect also the tubular detector had better characteristics than the wall-jet detector. This is not surprising, as the working electrode has a smaller inner diameter than the quartz connector tubing.

#### CONCLUSIONS

Tubular detectors made of carbon-polymer material are very useful for micro-LC. Sub-picogram detection limits are obtained and the response is linear up to 1 ng. The discriminating power for different electroactive compounds is lower than with

planar glassy carbon-type detectors. Because they are easy to use and have reliable performance characteristics, these tubular detectors are very convenient for application at low flow-rates. They can also be used at higher flow-rates (up to 2 ml min<sup>-1</sup>), but in this instance the demands are not as great because classical glassy carbon detectors perform very well in this range. It is to be expected that the new detectors will be useful in applications other than HPLC. Biosensors seem likely candidates, especially when electrode-coupled enzymes are envisaged, because low flow-rates may well prove to be compatible with efficient enzymatic product conversion. Another area of application could be the analysis of small sample volumes in flow-injection methods at microlitres per minute flow-rates (possibly with micro-pump systems<sup>27</sup>).

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