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Note

Construction of a simple wall-jet electrochemical flow-cell for high-performance liquid chromatography

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Electrochemical detection in high-performance liquid chromatography (HPLC) offers considerable improvements in sensitivity and selectivity over the ultraviolet (UV) absorption detectors currently used for most applications. A wide variety of flow-cell designs have been proposed for electrochemical detection[1–9] and these have recently been reviewed[10–12]. The two most popular designs for amperometric detection are based on wall-jet and thin-layer constructions and both types of flow-cell are commercially available along with appropriate electronic control units.

Electrochemical flow-cells can be constructed with small internal volumes relative to UV detector flow-cells. This presents interesting possibilities for applications involving narrow-bore columns or high-speed separations using short columns packed with small (< 5 μm) particles where extra-column band broadening must be reduced to a minimum. The construction of a low-volume flow-cell is complicated by the need to position three electrodes (working, auxiliary and reference) and the eluate inlet in close proximity. This has led to relatively complex constructions often involving intricate drilling and machining of stainless steel, Kel-F or PTFE. Furthermore, current commercial flow-cells have not been designed for applications where extra-column band broadening is critical. Connecting tubing between the column and the detector may be a major drawback in this respect and consequently there is considerable scope for improving the eluate inlet connections.

The present paper describes the simple construction of an amperometric flow-cell of the wall-jet type which uses a glassy carbon working electrode, a stainless-steel auxiliary electrode and a silver/silver chloride reference electrode. The construction uses only commercial parts which require minimum modification and the design allows for a variable flow-cell volume (down to *ca.* 0.3 μl). Particular attention has been given to providing a low-volume connection between the HPLC column and the flow-cell.

EXPERIMENTAL

Materials and construction

The detector flow-cell (Fig. 1) was constructed from commercially available parts made from 316 stainless steel, Kel-F or PTFE. The cell body, which also acts

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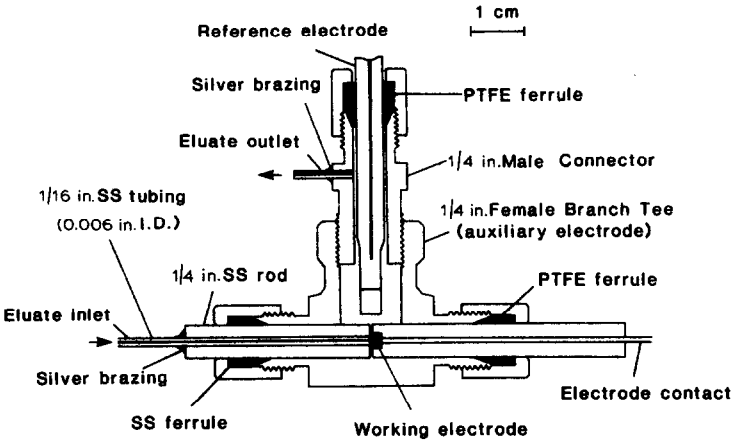

Fig. 1. A vertical section through the wall-jet electrochemical flow-cell. The details of the construction are given in the text. SS = Stainless steel.

as the auxiliary (or counter) electrode, was a 1/4-in. Female Branch Tee (Swagelok, part No. SS-400-3-4TTF, South London Valve and Fitting Co., London, U.K.) which was drilled out to 1/4 in. I.D. along the main axis to accept the working electrode and the eluate inlet tube, both of which were 1/4 in. in diameter.

A 1/4-in. Male connector (Swagelok, part No. SS-400-1-4) was modified by drilling through one of the hex-flats to take a 1/16-in. stainless-steel tube (0.020 in. I.D.) which was brazed into place to act as the eluate exit. In addition the Male connector was also drilled out to 1/4 in. I.D. along its axis to accept the reference electrode.

The eluate inlet jet was constructed from a 35-mm length of stainless-steel rod (1/4 in. diameter), which was drilled out along its length to take a 50-mm piece of 1/16-in. (0.006 in. I.D.) stainless-steel tube. The 1/16-in. tube was inserted into the drilled out rod and then brazed into position such that one of its ends was flush with the end of the 1/4-in. rod. The inlet jet was then inserted into the main body and held in place by a stainless-steel ferrule and a standard 1/4-in. nut. The end of the jet was positioned centrally as shown in Fig. 1. The column outlet was connected to the protruding end of the 1/16-in. tube by a zero dead volume 1/16-in. connector.

The working electrode (part No. MF 2012) and the reference electrode (part No. MF 2020) assemblies were manufactured by Bioanalytical Systems (West Lafayette, U.S.A.) and were obtained from Anachem (Luton, U.K.). Both parts were used as supplied requiring no modifications. The working electrode was constructed from a Kel-F rod (55 × 6.5 mm diameter) with a glassy carbon disc (3.1 mm diameter) inserted into one end and a metal rod passing down the centre to provide an electrical contact. The reference electrode was of the silver/silver chloride type and consisted of a glass body (ca. 70 × 6 mm diameter) with a porous vycor salt bridge.

The working electrode and reference electrode were held in position by 1/4-in. PTFE ferrules and stainless-steel nuts as shown in Fig. 1. By removing the Male connector and the reference electrode, a feeler gauge could be inserted into the body such that the gap between the working electrode and the eluate inlet jet could be

adjusted. A gap of 0.0015 in. (*ca.* 40 μm) has been used in the present study giving a volume of *ca.* 0.3 μl adjacent to the working electrode surface.

The flow-cell has been tested using a commercial electronic control unit (Bioanalytical Systems, Model LC4) obtained from Anachem. A commercial thin-layer flow-cell (Model TL8A) from the same manufacturer was used with the same electronic control unit for comparative studies.

Chromatography

The flow-cell has been tested with a variety of HPLC eluents but the present work was carried out with a single system involving a silica column (250 × 5 mm I.D.) packed with Spherisorb S5W (Phase Separations, Queensferry, U.K.). The eluent was methanol–aqueous buffer (9:1, v/v); the buffer was prepared by mixing ammonia (35% w/w, 94 ml), nitric acid (70% w/w, 21.5 ml) and water (884 ml) and adjusting the pH to 10.1 with ammonia.

HPLC was carried out using a Waters 6000A pump and a Rheodyne 7125 injection valve fitted with a 10-μl sample loop. An eluent flow-rate of 2 ml/min was used unless otherwise stated. For the analysis of morphine the working electrode was set to a potential of +0.6 V relative to the reference electrode making the chromatographic conditions similar to those described by White[6]. Chromatograms were recorded using a Linseis (Model 480L) or a Servoscribe (Model RE 541.20) potentiometric recorder.

RESULTS AND DISCUSSION

The flow-cell described in this paper is based on the wall-jet principle first introduced by Yamada and Matsuda[1] but represents a considerable simplification of existing designs. The construction uses commercially available parts requiring minimum modification which can be completed in a few hours. The reference electrode is placed downstream of the working electrode and this ensures that any leakage of the reference electrolyte will not contaminate the glassy carbon surface. The electroactive flow-cell volume can be adjusted as required but for routine use a gap of 0.0015 in. (*ca.* 40 μm), giving a volume of *ca.* 0.3 μl, has given satisfactory results. No attempt to optimise this spacing has been made in the present study. The volume of the eluate inlet tube is also low (< 1 μl) and this means that the flow-cell is particularly suitable for applications where reductions of extra-column band broadening are important. Preliminary experiments using the flow-cell with narrow-bore HPLC columns (1 mm I.D.) have demonstrated good plate counts.

The close proximity of the three electrodes (working, auxiliary and reference) ensures a low internal resistance and this facilitates a wide dynamic range. Tests involving injections of morphine have shown excellent linearity of response over at least four orders of magnitude (Fig. 2). The sensitivity is also good and 50 pg of morphine was detected with a signal-to-noise ratio higher than 5:1 (Fig. 3a). Further improvements in sensitivity were achieved by increasing the time constant (filter) on the control unit which reduced noise relative to the signal (Fig. 3b). These detection limits are comparable to results obtained with the commercial thin-layer flow-cell operated under identical conditions. The absolute noise level observed was very good (*ca.* 40 pA at a flow-rate of 1 ml/min) and identical to that observed with the com-

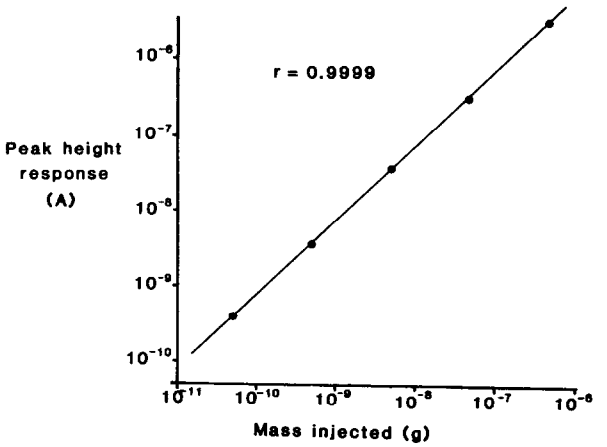

Fig. 2. Peak height response (A) for morphine injections demonstrating excellent linearity over four orders of magnitude. Column: Spherisorb S5W, 5 μm, 250 × 5 mm I.D. Eluent: methanol–aqueous buffer, pH 10.1 (9:1, v/v). Flow-rate: 2 ml/min. Potential: +0.6 V relative to the silver/silver chloride reference electrode. Injection volume: 10 μl.

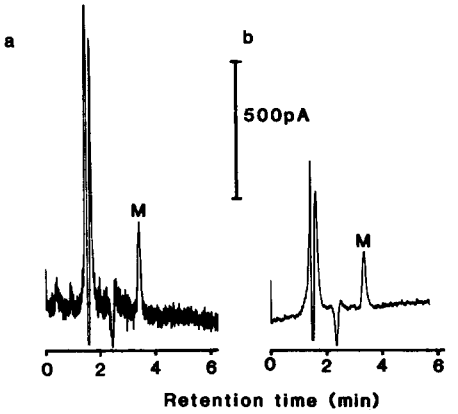

Fig. 3. Chromatograms for morphine (M, 50 pg injected) obtained at two filter (time constant) settings; (a) 0.5 sec, (b) 2.0 sec. Conditions as in Fig. 2.

mercial thin-layer flow-cell operated under the same conditions. These results are also comparable to those reported by White[6] (*ca.* 50 pA) for a home-made wall-jet/thin-layer hybrid flow-cell with the same eluent and flow-rate.

Peak height reproducibility on repeated injection of morphine (500 ng) was acceptable; for twelve consecutive injections made over a period of 40 min the coefficient of variation was lower than 5%. Similar results have been obtained using commercial flow-cells[13]. The literature data suggest that electrochemical detection is capable of better precision (*e.g.*, ref. 14). However, it is probable that the reproducibility is as much a function of the chromatographic system as it is of flow-cell design.

Over the course of a working day some loss in response was observed comparable to that seen with other commercial and home-made flow-cells[13]. The loss in response could be reversed by dismantling the flow-cell and polishing the surface of the working electrode. An equally successful approach involved the application of a negative potential to the working electrode for several minutes with the eluent flowing.

In conclusion, the wall-jet electrochemical flow-cell described in this paper is simple to build and at the present time can be constructed at approximately one fifth of the cost of a commercial flow-cell. It demonstrates comparable performance (noise, sensitivity and linear dynamic range) to commercial and home-made flow-cells reported in the literature and is equally reliable in routine use. Furthermore, the low internal volume allows detection with minimum peak broadening.

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