Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1479–1483, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Rapid-scan hydrodynamic voltammetry and cyclic voltammetry of pharmaceuticals in flow injection analysis conditions*

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Abstract: An on-line rapid-scan electrochemical detector is described for HPLC and FIA systems. Its practical use in qualitative analysis is demonstrated for 19 drug substances. The detector can be operated to record convection/diffusion-controlled (S-shaped), or diffusion-controlled (peak-shaped) voltammograms. In the latter mode, on-line cyclic voltammetry measurements are possible. The cell can also be used as an amperometric detector for conventional, microbore and micro-LC methods. Detection limits are of the order of 10 pg (conventional and microbore HPLC), or at the sub-picogram level (micro-LC). For scanning work, drugs can be analysed at mg l^{-1} levels in an FIA setup.

Keywords: *Electrochemical; detectors; rapid-scan; HPLC; FIA.*

Introduction

Staircase rapid-scan voltammetry experiments in flow injection analysis (FIA) or high-performance liquid chromatography (HPLC) have been carried out in the 1980s by several laboratories [1–12]. The required digital electronic equipment is well-described in the literature, and can be made at reasonable cost. Research efforts are now directed towards the construction of optimal flow-cells for use in conventional, microbore and micro-LC systems. Constructed cells must have small RC time constants to be compatible with high scan-rates, and small internal volumes especially for micro-LC applications. Also a very important characteristic is the shape of the hydrodynamic boundary layer which is obtained at the electrode surface in flowing streams. Three main types of cell construction are used in the literature, i.e. carbon fibre electrodes [11, 12], thin-layer cells and wall-jet cells [8]. Tubular and porous electrode types seem rather non-compatible with rapid-scan experiments [10, 14].

We have shown in an earlier study [13] that a home-made large-volume wall-jet cell could be used as an amperometric detector in a broad range of eluent flow-rates, from conventional HPLC (1 ml min^{-1}) to micro-LC (5 μl min^{-1}). For rapid-scan voltammetry measurements in conventional HPLC, the conditions were given to obtain either convection/diffusion-controlled (S-shaped) or diffusion-controlled (peak-shaped)

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
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voltammograms. The time constant of the cell allowed scan-rates up to 10 V s^{-1}. In the present article, it is shown that cyclic voltammograms can be recorded in flowing streams when the cell is in "diffusion control" mode, and that the cell can be placed in a "convection/diffusion control" mode to record S-shaped voltammograms. The method is demonstrated for 19 drug substances.

Experimental

The flow-cell, potentiostat, computer and interfacing equipment were home-made (see also ref. 13). A three-electrode potentiostat was computer controlled to apply staircase waveforms: see Fig. 1A. The time-constant of the current follower was adjusted to 0.2 ms ($R1$ = 200 kΩ, $C1$ = 1 nF). Computer interfacing was done via 12 bit D/A and A/D converters using a Schneider CPC 6128 microcomputer programmed in PASCAL. The stepwidth of the staircase was 12 ms, unless otherwise specified. The stepheight was adjusted to obtain the required scan-rate. The current was sampled at the end of the stepwidth. Background voltammograms were subtracted electronically (Fig. 1A). The flow-cell was of the large-volume wall-jet type as shown schematically in Fig. 1B. Carbon paste (1 mm dia.; EA 267C, Metrohm, Herisau, Switzerland) working electrodes were used in cylindrical Delrin plastics of 10 mm dia. A 100-μm, i.d., polyimide coated fused silica capillary (RSL Alltech, EKE, Belgium) was connected to an HPLC injector (7125, Rheodyne, Cotati, USA). The other end was conducted in the electrochemical (EC) detector through SS chromatográphic tubing which also served as a counter electrode.

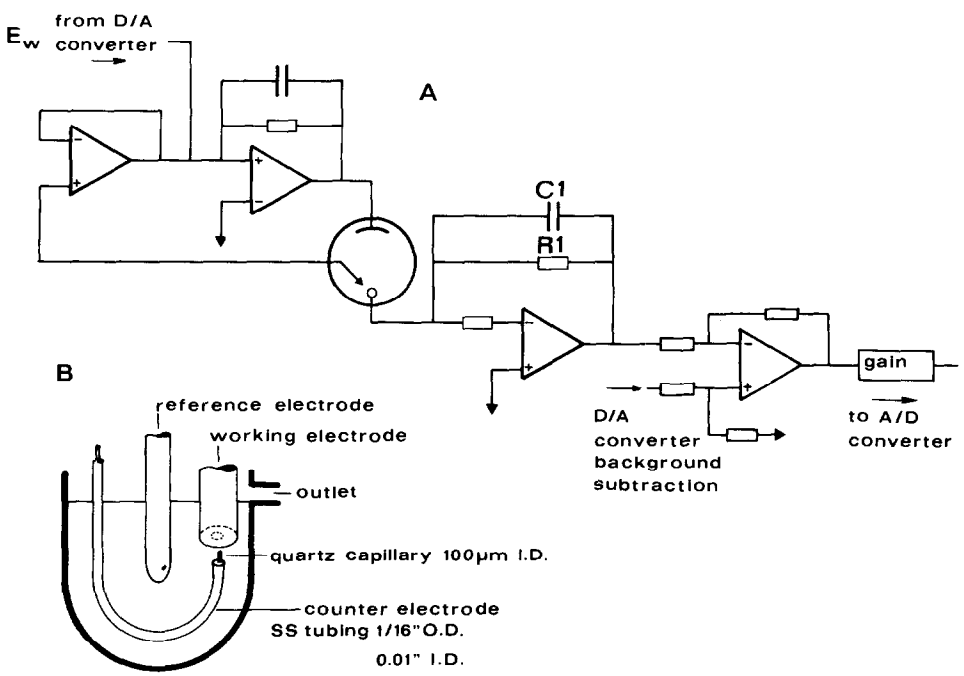

Figure 1
A: Schematic representation of the potentiostat, D/A and A/D interfacing and computer control. B: Schematic representation of the large-volume wall-jet flow cell.

The jet nozzle extended 0.5 mm out of this tubing, and was placed at a distance of 0.5 mm from the working electrode. The reference electrode was a standard calomel electrode (B 2910 Schott, Hoffheim a.Ts., FRG). Phosphoric acid (0.01 M)–methanol (85:15, v/v) was used as an eluent in the FIA setup comprising an HPLC pump (SP 8810, Spectra Physics, Santa Clara, USA) plus the injector and detector (see above). Injection volumes were 0.5 ml. All drug substances were of pharmacopoeial purity.

Results and Discussion

With the large-volume wall-jet detector configuration shown in Fig. 1, it is possible to choose the experimental conditions (working electrode diameter, flow-rate, scan-rate) to obtain convection/diffusion-controlled (S-shaped) voltammograms, diffusion-controlled (peak-shaped) voltammograms, or a combination of both [13]. If the scan-rate (a) is small with respect to the flow-rate (v), S-shaped voltammograms will be obtained. If v is large as compared with a, the voltammograms are of the peak-shaped type. This is so because v determines the shape of the hydrodynamic boundary layer, for a certain electrode diameter. A great advantage of the large-volume wall-jet detector, is that its hydrodynamic characteristics are very reproducible. Using a 1 mm dia. electrode, and a scan-rate of 0.83 V s^{-1}, convection/diffusion-controlled voltammograms are obtained at a 1.5 ml min^{-1} flow-rate. This is shown for the o-dihydroxy phenolic compound caffeic acid in Fig. 2A. When a cyclic voltammogram is recorded in these conditions, the reverse scan (curve b) will yield no useful information as oxidized products are swept away from the electrode before they could eventually be reduced. The forward scan (curve a) however corresponds to a "hydrodynamic voltammogram". Such hydrodynamic voltammograms are normally obtained with non-scanning detectors by repetitively injecting the compound and measuring the response at different electrode potentials (see ref. 15). This method is however very time consuming. Curve (a) in Fig. 2A still deviates a little from the typical S-shape, as a small peak component is present at the onset of the limiting current plateau. This peak component could be further decreased by increasing flow-rate, or decreasing scan-rate. Hydrodynamic voltammograms provide the necessary information to choose a selective potential for use in amperometric detection [15] in HPLC or FIA. A more diffusion-controlled voltammogram is obtained in Fig. 2B, by lowering the flow-rate to 0.5 ml min^{-1}. Lowering the flow-rate results in an increase of the diffusion layer thickness. The oxidized compound will still be present in the diffusion layer at the electrode surface during the reverse scan, and the reversibly oxidized caffeic acid molecule clearly shows a reduction wave (curve b).

Nineteen drugs belonging to 10 different pharmaceutical classes (see ref. 15) were injected in the FIA setup, and rapid scans were recorded in convection/diffusion conditions (1 mm electrode dia., 1 ml min^{-1} flow-rate and 0.83 V s^{-1} scan-rate), see Fig. 3. In these conditions, the ratio $(i_p - i_l)/i_l$ (cf. Fig. 2A curve a) will be very small. Reactions are convection/diffusion controlled, and the voltammograms are identical to hydrodynamic voltammograms obtained by repetitive injections and manual change of the working electrode potential. The shapes of the voltammograms of Fig. 3 compare very well with those of the published hydrodynamic voltammograms obtained on glassy carbon using a manual procedure [15]. An easily measurable parameter for comparison is the potential at the start of the wave, E_s ($E_{1/2}$ values are often difficult to determine because the limiting current plateaus are not well-defined). A plot of E_s values of the data published on glassy carbon [15] versus E_s values measured from Fig. 3, showed good

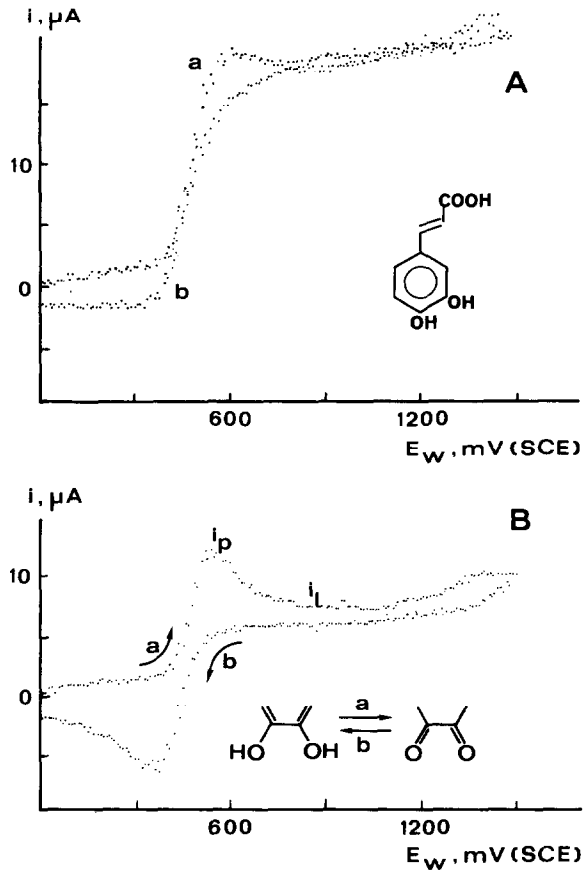

Figure 2
Cyclic voltammetry of caffeic acid (77 mg l^{-1}) at 0.83 V s^{-1}, at 1 ml min^{-1} flow-rate (A) and at 0.5 ml min^{-1} flow-rate (B).

linearity. The correlation coefficients between both data sets was 0.97 (17 products). However, E_s values were 25% higher as a mean, on carbon paste electrodes. This means that carbon paste shows higher overpotentials. As the background currents obtained at carbon paste are smaller than those obtained at glassy carbon (at the same potential), this will have no negative effects on signal/noise ratios. When the described cell was used as an amperometric detector (i.e. at a fixed potential), detection limits were better for glassy carbon based electrodes, as compared to carbon paste based electrodes. For conventional columns (4.6 mm dia.) these were typically around 10 and 500 pg, respectively (10 μl injections). The limits of the rapid-scan technique were found to be in the mg l^{-1} concentration range in FIA mode (no dilution by a chromatographic column). This is less sensitive than amperometric detection which is used for trace analysis. The application of rapid-scan techniques is therefore most interesting for qualitative analysis of microgram amounts of substances.

The 19 drug substances were subjected to cyclic voltammetry under diffusion-controlled conditions. Such conditions were obtained for a 1 ml min^{-1} flow-rate (1 mm dia. electrodes) at a scan-rate of 8.3 V s^{-1} (6 ms stepwidth, 50 mV stepheight). All compounds, except salicylamide, showed irreversible redox properties (no reduction in

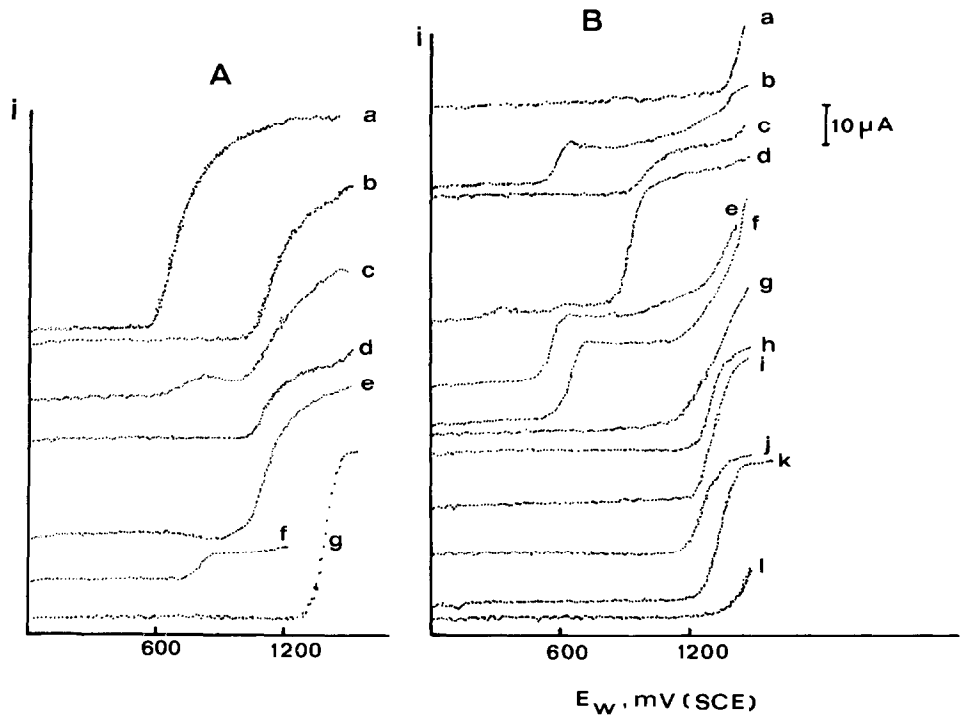

Figure 3
A: Hydrodynamic voltammetry in convection/diffusion-controlled conditions of paracetamol (a), procaine (b), salicylamide (c), sulphadiazine (d), sulphanilamide (e), diethylstilbestrol (f) and oxprenolol (g); and B: oxacilline (a), thiaridazine (b), narcotine (c), desipramine (d), levomepromazine (e), chloropromazine (f), carbenicilline (g), amilaride (h), triamtereen (i), codeine (j), papaverine (k) and amitryptiline (l). All compounds were injected in 50–80 mg l^{-1} concentrations.

the background scan). This means that series dual electrode systems can be used to obtain a higher selectivity for salicylamide only.

Acknowledgement — L. J. Nagels thanks the NFWO for a grant.

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[*Received for review 16 May 1989; revised manuscript received 29 May 1989*]