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VITREOUS CARBON-BASED COMPOSITE ELECTRODE AS AN ELECTRO-CHEMICAL DETECTOR FOR LIQUID CHROMATOGRAPHY

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SUMMARY

The characteristics of an electrochemical detector for liquid chromatography with an array of vitreous carbon electrodes in a thin-layer channel are described. This array is fabricated by filling the pores of a reticulated vitreous carbon disk with non-conductive epoxy. The resulting flow-by electrode exhibits a flow-rate independent response due to its nonlinear mass-transport properties. For the same reason, the detector shows 3–5 improvements in signal-to-noise characteristics over commercial detectors with continuous glassy carbon electrode, operated under otherwise identical conditions. Epinephrine, dopamine, acetaminophen and potassium ferrocyanide were used as test systems to give detection limits at the pg level. Repetitive injections yield peak heights with a relative standard deviation of 0.3%. The cell is easy to construct from inexpensive materials.

INTRODUCTION

The recent progress in high-performance liquid chromatography (HPLC) has stimulated the development of many detectors. Among them, an important role is played by electrochemical detectors that exhibit high sensitivity and selectivity. The principal requirements for such detectors have recently been reviewed[1,2]. A number of electrode materials have been used for electrochemical detection, including many forms of carbon. The most popular electrode material appears to be glassy (vitreous) carbon. Recent studies in several laboratories[3–6] suggest that improved signal-to-noise characteristics at carbon electrodes are obtained when the working electrode is comprised of graphite particles immobilized in an inert binder. The resulting electrode surface is composed of an array of carbon microelectrodes surrounded by inactive insulating regions. Tallman and co-workers[3–5] described the construction of Kel-F-graphite (Kelgraf) composite electrodes and evaluated their performance in HPLC. Caudill *et al.*[6] used a flow detector with an electrode consisting of an array of carbon fibers. Shah and Honigsberg[7] described the use of the PTFE-graphite electrode as a detector for HPLC. For such composite electrodes non-linear diffusion is the predominant mode of mass-transport (at moderate time scales). In this case, a steady-state limiting current is achieved when the diffusion layer thickness exceeds the di-

ameter of the carbon active site. For a microdisk (of radius r) this current is given by:

$$i_1 = 4rnFCD \tag{1}$$

where n is the number of electrons per mole, F is Faraday's constant and C and D are the bulk concentration and diffusion coefficient, respectively, of the analyte. Due to their unique mass-transport behavior, composite electrodes exhibit signal-to-noise improvement compared to continuous electrodes. This improvement appears to be the result of enhancing the signal due to radial diffusion of the analyte to the individual microelectrodes (*i.e.*, larger response than that obtained at continuous electrodes of the same *active* area) and reducing the flow-dependent noise.

In this report we describe a new electrochemical flow detector with an array of vitreous carbon electrodes in a thin-layer channel. Such carbon composite electrode is obtained by filling the pores of reticulated vitreous carbon (RVC) with non-conducting epoxy and polishing to give a smooth surface. RVC is a three-dimensional open-pore "foam" material possessing many electrochemical, hydrodynamic and mechanical properties[8]. The three-dimensional RVC has been exploited as an effective working electrode in a variety of flowing systems[9-12]. However, a composite (two-dimensional) RVC flow detector has not been reported. A recent study describing a batch composite electrode based on RVC has been published during the preparation of this manuscript[13]. The results obtained in this study by cyclic voltammetry and rotating disk voltammetry show that the behavior of the composite (two-dimensional) RVC electrode can be described by simple theories of nonlinear diffusion. Herein we show that the electrochemical flow detector with a RVC-based composite electrode possesses the advantages of improved signal-to-noise characteristics and flow-rate independent as compared to solid (continuous) glassy carbon flow cells. In addition, the detector is easy to construct compared to composite electrodes made of arrays of carbon fibers.

EXPERIMENTAL

Apparatus

The liquid chromatographic system (Bioanalytical Systems LC-303) consisted of a dual piston pump (PM-30A), a Rheodyne Model 7125 injector (20-μl loop), a Biophase ODS-5 μm reversed-phase column (25 cm × 4.6 mm I.D.) and an amperometric detector (LC-3A) connected to a Houston Omniscribe strip-chart recorder. The flow injection system was described earlier[14], except that a 20-μl sample loop was used. Cyclic voltammetry was performed in a beaker using a Princeton Applied Research Model 264A polarographic analyzer.

Electrode construction

The thin-layer cell design is shown schematically in Fig. 1. The "homemade" lower block was made of plexiglas to combine with a commercial upper block (Model T1-5, Bioanalytical Systems), containing the sample solution inlet and outlet (not shown). A hole (7 mm diameter narrowed to 1 mm) was drilled through the center of the plexiglas body to contain the composite electrode (a) and the copper wire
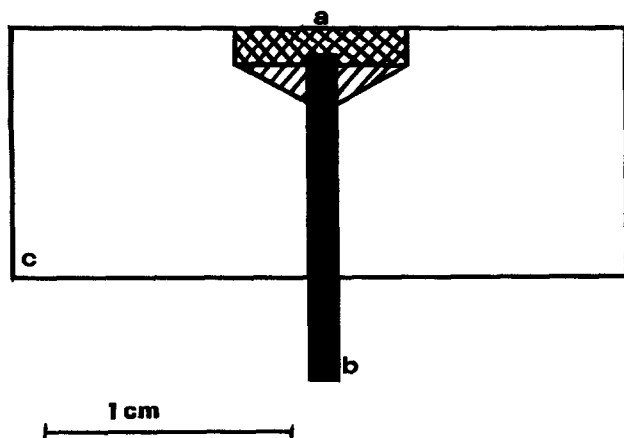
Fig. 1. Thin-layer detector with RVC-based composite electrode. (a) composite electrode; (b) electrode contact; (c) plexiglas body. (The upper block is not shown.)

electrical contact (b). A 4 × 1, 60-pores per inch (ppi) RVC disk (7 mm diameter, 2 mm thickness) was placed in the resulting hole, pressed into the edge of the copper wire. Non-conducting epoxy (Epotek 349, Epoxy Technology, Watertown, MA, U.S.A.) was used to fill the pores of the RVC. The electrode was cured at 60°C for 24 h. The resulting two-dimensional surface was then polished with 600-grit SiC sandpaper, followed by 3000-mesh diamond dust and 1.0- and 0.05-μm alumina slurries. After the absence of voids on the final surface was assured, the two detector blocks were bolted together, exerting pressure on the Teflon spacer that separated them. The reference and auxiliary electrodes were located in a downstream compartment (Model RC-2A, Bioanalytical Systems). All potentials are reported *versus* Ag/AgCl reference electrode (Model RE-1, Bioanalytical Systems). A commercial glassy carbon thin-layer detector with similar geometry (T1-5, Bioanalytical Systems) was used as the continuous electrode in comparison studies.

Reagents

All aqueous solutions were prepared in double distilled water. Stock solutions of epinephrine, acetaminophen, dopamine hydrochloride (Sigma) and potassium ferrocyanide (Baker) were made up fresh each day. The dopamine and epinephrine solutions were prepared in 0.1 M perchloric acid while the acetaminophen was dissolved in the mobile phase. For flow injection analysis dopamine was dissolved in deionized water while 0.05 M phosphate buffer served as supporting electrolyte and carrier solution. The mobile phases were: (1) 0.2 M potassium dihydrogen phosphate and (2) 0.15 M chloroacetic acid sodium salt, 25 mg/l sodium octyl sulfate adjusted to pH 3 with perchloric acid. Both mobile phases contained 1 g/l disodium EDTA. A 1 M potassium chloride solution (adjusted to pH 3 with hydrochloric acid) was used in the cyclic voltammetry experiment.

RESULTS AND DISCUSSION

Slezynski *et al.*[13] have illustrated that a batch composite electrode based on
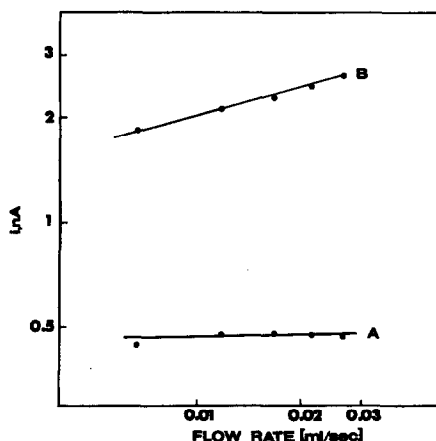
Fig. 2. Logarithmic dependence of peak current upon volume flow-rate. (A) RVC-based composite electrode; (B) continuous glassy carbon electrode. Injections of 10 ng acetaminophen; applied potential, $+0.7$ V; mobile phase, 0.2 M KH$_2$PO$_4$ (with 1 g/l disodium EDTA); channel thickness, 0.25 mm.

RVC possesses many of the characteristics of an array of microelectrodes. Although the individual carbon active sites have non-uniform geometry, they are well separated from one another (by hundreds of micrometers) and have large boundary density. As a result of these properties the behavior of such composite electrode can be described by the theory of non-linear diffusion. Therefore, forced-convection processes show minimum effect on the resulting response (at moderate time scales). For monitoring flowing streams, such as chromatographic effluents, this property of the RVC-based composite electrode offers various advantages since the detector response becomes flow-rate independent and the flow-dependent noise is reduced. To examine this characteristic of the RVC-based composite flow-by electrode, chromatographic peaks for acetaminophen were recorded at different flow-rates ranging from 0.4 to 1.6 ml/min (Fig. 2A). Also shown in Fig. 2 is the corresponding peak current *versus* flow-rate dependence obtained using a commercial detector with a continuous (solid) glassy carbon electrode (B). The peak current at the RVC-based composite electrode is essentially the same over the entire range of flow-rates employed. In contrast (and as expected), an increase in peak current is obtained at the continuous electrode upon increasing the flow-rate. Least-squares treatment of the log-log dependences shown in Fig. 2 yields straight lines with slopes of 0.04 $\pm$ 0.02 (A) and 0.23 $\pm$ 0.02 (B). It should be emphasized that the flow-rate independent response shown in Fig. 2A was obtained without compromising the dead volume of the detector. A 0.25-mm spacer thickness was used to obtain these data. In contrast, a spacer thickness of 1.2 mm was required to exhibit similar flow-rate independence using the carbon-fibers based flow-by composite electrode[6]. This improvement in the flow-rate independent behavior may be attributed to larger separation between the individual active sites. Since high sensitivity can be achieved even at low flow-rates (with no additional extra-column band broadening) the RVC-based composite electrode may be attractive for modern chromatographic systems (*e.g.*, microbore HPLC) characterized by low flow-rates and low peak volumes. Other approaches to minimize the flow-rate dependence, based on the use of supplementary forced convection (*e.g.*, rotation[15])
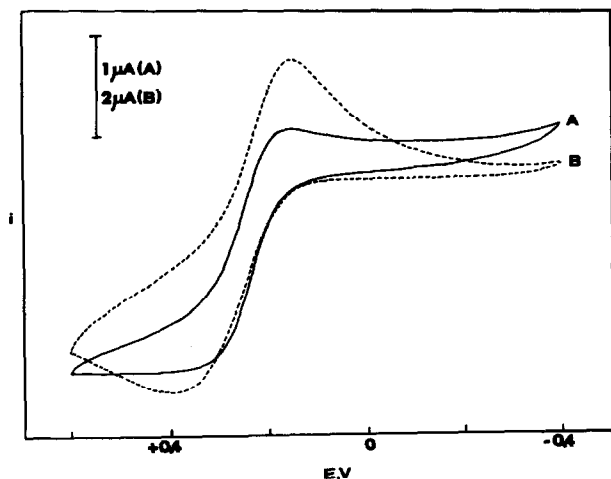
Fig. 3. Cyclic voltammograms of 1 mM K$_4$Fe(CN)$_6$ in KCl (1 M, pH 3). (A) RVC-based composite electrode; (B) continuous glassy carbon electrode. Scan-rate, 20 mV/sec.

or differential pulse detection[16], require some compromise in the resolution or detectability, respectively. For other on-line analytical applications, the virtual independence of response upon flow-rate permits uncritical control of the flow-rate, *i.e.*, high precision even in systems with poorly controlled flow-rates. The unique mass-transport characteristics of the present electrode are evidenced also from a cyclic voltammetry experiment. Fig. 3 compares cyclic voltammograms obtained at the 60
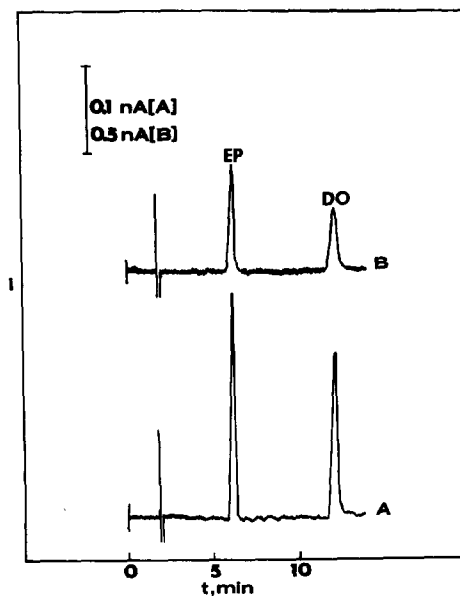

Fig. 4. Chromatograms for an injection of 0.9 ng epinephrine and dopamine obtained at the RVC-based composite electrode (A) and continuous glassy carbon electrode (B). Flow-rate, 1.2 ml/min; applied potential, +0.7 V; mobile phase, 0.15 M chloroacetic acid (pH 3, with 1 g/l disodium EDTA); channel thickness, 0.25 mm.
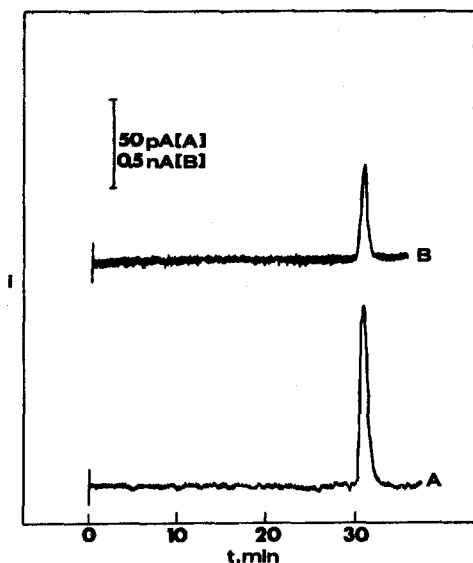
Fig. 5. Chromatograms for an injection of 2 ng acetominophen obtained at the RVC-based composite electrode (A) and continuous glassy carbon electrode (B). Flow-rate, 1.5 ml/min; applied potential, mobile phase and channel thickness, as in Fig. 2.

ppi RVC-based composite electrode (A) and the continuous glassy carbon electrode (B). In contrast to the peak-shaped response observed at the continuous electrode, an S-shaped response is obtained at the composite electrode due to the non-linear diffusion. A similar S-shaped response was obtained with an array of carbon fibers electrodes[6] and with 80- and 100-ppi RVC-based composite electrodes[13].

The ability to detect minute levels of electroactive analytes in chromatographic effluents depends on the signal-to-noise characteristics of the detector. The non-linear diffusion that characterizes flow-by composite electrodes can lead to enhancement of the signal-to-noise ratio[4]. Such enhancement is attributed to the enhanced signal (via the enhanced flux of the analyte to the surface) and reduced noise (especially if flow-noise is the dominant type of noise). In spite of the high signal-to-noise ratios obtained with other types of composite electrodes, no direct comparison to a continuous electrode has been made. Fig. 4 compares chromatograms for a mixture of 0.9 ng epinephrine and dopamine obtained with the flow-by RVC-based composite electrode (A) and a commercial detector with a continuous glassy carbon electrode (B), under otherwise the same conditions. Signal-to-noise ratios of 66 and 48 are observed for epinephrine and dopamine using the composite electrode. Based on a signal-to-noise ratio of 2, these data correspond to detection limits of 27 pg (0.15 pmol) epinephrine and 37 pg (0.19 pmol) dopamine. In comparison, the continuous glassy carbon electrode yields signal-to-noise ratios of 17 (epinephrine) and 10 (dopamine) that correspond to detection limits of 100 pg and 170 pg, respectively. Overall, 3.7- and 4.6-fold improvements in the detectability are obtained with the RVC-based composite electrode. A similar behavior is shown in Fig. 5 that compares chromatograms, obtained with the two cells, for injections of 2 ng acetaminophen. In this case, signal-to-noise ratios of 17.3 and 6.6 are observed at the composite and continuous
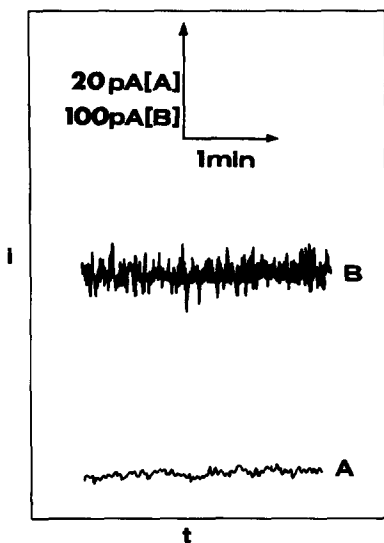
Fig. 6. Comparison of background noise level. (A) RVC-based composite electrode; (B) continuous glassy carbon electrode. Flow-rate, 0.8 ml/min; applied potential, +0.2 V; mobile phase and channel thickness, as in Fig. 2.

electrodes, respectively, yielding to a 2.7-fold improvement in the detectability. Notice also the different current scales employed when using the two electrodes. Although the composite electrode has a larger geometric area than the continuous electrode (1.54 cm^2 vs. 0.283 cm^2), significantly smaller signal and noise levels are observed. For the 4 × 1 60-ppi RVC specimen used in the present study the active area fraction is about 7%[13], i.e., the active area of the present electrode is about 0.11 cm^2. Thus, the area of the continuous glassy carbon electrode is 2.62 times larger than the active area of the RVC-based composite electrode. However, this difference in the active area of the two electrodes is not accompanied by a similar change in their signal and noise levels. Due to the non-linear diffusion of the composite electrode, the signal does not decrease as much as the noise, resulting in improved detectability.

Careful examination of the baselines of Figs. 4A and 5A reveals some baseline fluctuations that are attributed to flow irregularities. A similar phenomenon has been observed with other composite electrodes[4,6] and has been attributed to surfaces which are not microscopically smooth. As the present electrode was fabricated without prior degassing, the tiny voids on the surface can lead to detrimental effects (especially when they are in contact with active carbon sites). The flow irregularities may be ascribed also to the deviation from planarity that occurs when polishing a surface composed of components with different hardnesses. Such deviation has been confirmed by the topographical analysis of Slezynski et al.[13]. Fig. 6 shows amplified recorder tracings of the background currents and noise levels for the composite (A) and continuous (B) flow-by electrodes at a flow-rate of 0.8 ml/min and potential of +0.2 V. The composite electrode exhibits a peak-to-peak noise of about 2 pA. Similar baseline fluctuations were observed at flow-rates of 0 and 1.6 ml/min and at a potential of +0.7 V (not shown). Therefore, the noise observed at both electrodes
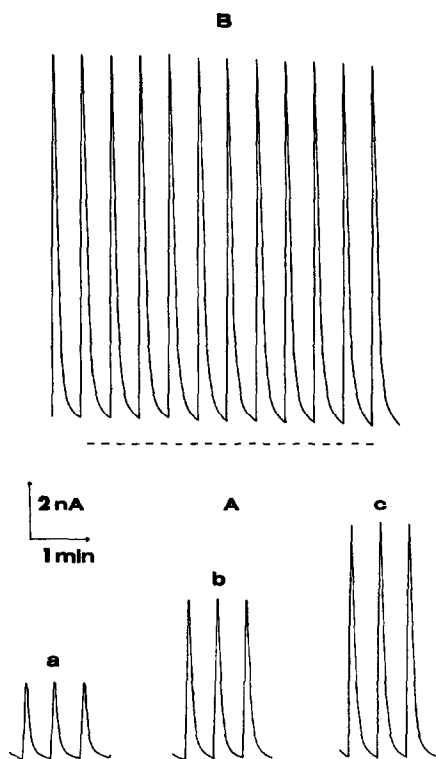
Fig. 7. Sensitivity and precision obtained for flow injection measurements of dopamine. (A) Injections of 1.9 (a), 3.8 (b) and 5.7 (c) ng dopamine. (B) Twelve successive injections of 7.6 ng dopamine. Flow-rate, 1.0 ml/min; applied potential, +0.7 V; supporting electrolyte, 0.05 M phosphate buffer; channel thickness, 0.125 mm.

appears to be random, presumably consisting of electrical and chemical contributions. The absence of flow fluctuations is expected from the dual piston pump and flow dampener employed. In contrast, using a reciprocating pump and a continuous electrode the flow-noise usually dominates the baseline fluctuations[6]. No special effort has been made in the present study to further reduce the noise levels. The important point to notice is that under the same environment the overall signal-to-noise ratio is improved at the composite electrode (even when the flow-noise does not dominate). A further improvement in the detectability can be expected for chromatographic systems equipped with single piston pumps, $i.e.$, when the flow-noise is the dominant type of noise. As was discussed earlier, significantly higher noise levels may be observed due to additional voids on the surface of the composite electrode (especially when such voids are in direct contact with the active carbon sites). Therefore, careful electrode preparation (preferentially under vacuum) is recommended.

The analytical utility of the flow-by RVC-based composite electrode is illustrated in Fig. 7. Fig. 7A is a reproduction of a chart-record for flow injection measurements of dopamine in the $5 \cdot 10^{-7}$–$1.5 \cdot 10^{-6}$ M concentration range (corresponding to 1.9–5.7 ng for the 20-μl injection). These three measurements are part of six concentration (C) increments ($5 \cdot 10^{-7}$–$3 \cdot 10^{-6}$ M range) for which linear

response was obtained. Least-squares treatment of these data yielded the equation: $i(nA) = (0.62 \pm 0.01) C(10^{-7} M) - (0.70 \pm 0.32)$, with $s_{yx} = 0.34$ nA and $r = 0.999$. A detection limit of about 40 pg dopamine would be expected based on the signal-to-noise characteristics (a signal-to-noise ratio of 2) of Fig. 7A(a). High linearity was obtained also for ten chromatographic injections of acetominophen over the 2–20-ng range. A least-squares treatment of these data yielded a slope of 46 nA/ng (correlation coefficient, 0.999; intercept, 4 nA; conditions, as in Fig. 5). Fig. 7B demonstrates the precision obtained for twelve successive injections of a $2 \cdot 10^{-6} M$ (7.6 ng) dopamine solution. An injection-rate of 120 samples per hour was employed. The mean peak found was 16.1 nA with a range of 16.0–16.2 nA and a relative standard deviation of 0.3%. The reproducibility of the results compares favorably with that observed at continuous carbon electrodes. Tallman and Weisshaar[4] have illustrated recently that composite electrodes tend to resist fouling, as a result of the unique geometry. Similar improvement in the reproducibility has been observed with graphite-PTFE composite electrodes[7].

In conclusion, the RVC-based composite electrode shows considerable promise for monitoring flowing streams, such as chromatographic effluents. The electrode exhibits improved signal-to-noise characteristics, flow-rate independence and high precision, all for a modest investment. Work in this laboratory is continuing to exploit the unique properties of RVC-based composite electrodes. For this purpose, different RVC specimens are being used to tailor a composite with optimized signal-to-noise characteristics.

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