

Note

Electrochemiluminescence as a detection technique for reversed-phase high-performance liquid chromatography

II. Low-frequency a.c. electrochemiluminescence

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In a previous paper¹ we reported on the use of d.c. electrochemiluminescence (ECL) as a detection technique for a variety of materials separated by reversed-phase high-performance liquid chromatography (HPLC). In that work the ECL was excited by the passage of a steady d.c. current of approximately 4 mA between a pair of platinum wire electrodes in a small glass flow cell through which the chromatographic eluent passed. The eluent consisted of acetonitrile–water mixtures containing various amounts of a salt, such as tetrabutylammonium perchlorate. We reported that ECL emission was detected from a range of separated components passing through the cell and that the ECL signal rose as the current through the cell was increased, and pointed out that the limitation on the technique appeared to be the formation of gas bubbles within the cell caused by the electrolysis of the eluent.

We have been experimenting with various techniques for the suppression of bubble formation within the flow cell in order to achieve higher sensitivities than those reported. One of the effective techniques for reducing bubble formation appears to be reversing the direction of current flow before sufficient gas has been evolved at either electrode to give rise to a bubble. Additional suppression may be available by pressurising the cell, and we are continuing our experiments with cells capable of withstanding higher pressures. However, it is already clear that the use of a low-frequency, square-wave electrolysis current does dramatically improve the sensitivity of ECL in aqueous systems, and in this paper some of the details of this effect are described.

EXPERIMENTAL

The chromatographic apparatus and flow cell were as described in our earlier paper¹. However, the electronic equipment was modified to incorporate a variable-frequency square-wave generator and a device to enable the electrolysis current to be recorded. The arrangement is shown in Fig. 1. While for the purposes of this paper it is sufficient to treat the current measuring device as a milliammeter, it is perhaps

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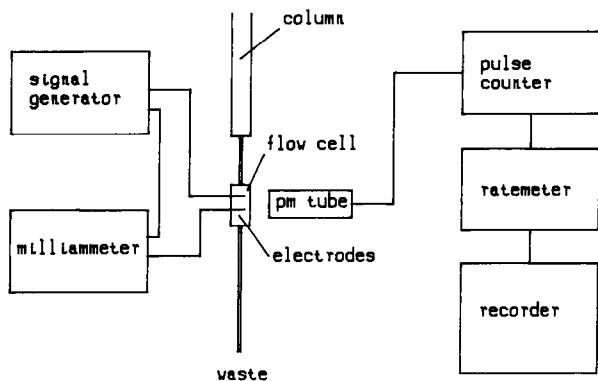


Fig. 1. Block diagram of the components used to record ECL chromatograms using low-frequency excitation.

worth pointing out that most digital multimeters are not suitable for the measurement of square-wave currents at the lower frequencies discussed below unless care is taken to synchronise the reading cycle with the waveform. A signal generator (Hewlett-Packard Model 8116A) was used to generate the applied waveform, and the electrolysis current was measured with a digital multimeter (Kiethley Model 175). Light intensity was measured by a photomultiplier (Thorn.EMI, Type 9804QB) and a digital pulse counter which provided an output voltage signal proportional to the number of photons per second being detected. The pulse preamplifier and counting system have been described².

The eluents and solvents used consisted of HPLC grade acetonitrile (Rathburn Chemicals) and solutions of tetrabutylammonium salts which were made by neutralisation of tetrabutylammonium hydroxide (Aldrich) with the appropriate acid (perchloric, nitric or phosphoric). Water was distilled and purified using a Water 1 unit (Gelman Sciences). For the chromatographic results given below the eluent consisted of acetonitrile-0.005 *M* tetrabutylammonium perchlorate in water (90:10). It was found that the purity of the salt had a pronounced effect on the intensity of ECL observed in subsequent experiments, and recrystallisation of the salt is essential to obtain useful emissions. The sample materials were all standard SLR grade and were used without further purification for chromatography purposes.

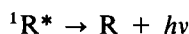
The chromatographic results presented below were obtained using one of our standard test mixtures. The mixture contained the following quantities in a standard 20- μ l sample: naphthalene (82 ng), acenaphthene (83 ng), fluoranthene (80 ng), pyrene (83 ng) and perylene (4.2 ng), and the sample components eluted in that order from our Waters RCSS 8- μ m C₁₈ column system.

RESULTS AND DISCUSSION

Using our standard test mixture, chromatograms were recorded as a function of the frequency of the applied square wave. The variation in the background emission level and the ECL peak heights recorded for pyrene and naphthalene are shown in Fig. 2. It is clear that alternating the electrolysis current makes very little difference to the peak heights until the frequency exceeds about 10 Hz, beyond which

the intensity of ECL emission drops rapidly for both compounds (and in fact for the others). For reasons which are not clear, the background light level falls noticeably below the d.c. level even for the lowest frequency used (1 Hz), and at 5 Hz a significant improvement in signal-to-noise ratio (the noise being the variance in the background) is achieved. However, it is important to note that the main reason for the improvement in the chromatographic signal-to-noise ratio lies in the reduction of background noise rather than background signal level, and this in turns arises from the much reduced formation of gas bubbles which, under d.c. conditions, generally cause the electrolysis current and ECL emission intensity to fluctuate.

The variation in light emission with the frequency of the current adds weight to our earlier suspicion that the ECL observed in these air-equilibrated, aqueous systems arises from a mechanism quite different from the classical annihilation process



However, we confirm that the spectra of the ECL of perylene and diphenylanthracene remain unchanged over the range 0–10 Hz, so that it is likely that the emission does arise from the relaxation of ${}^1R^*$ in both d.c. and low-frequency a.c. excited ECL.

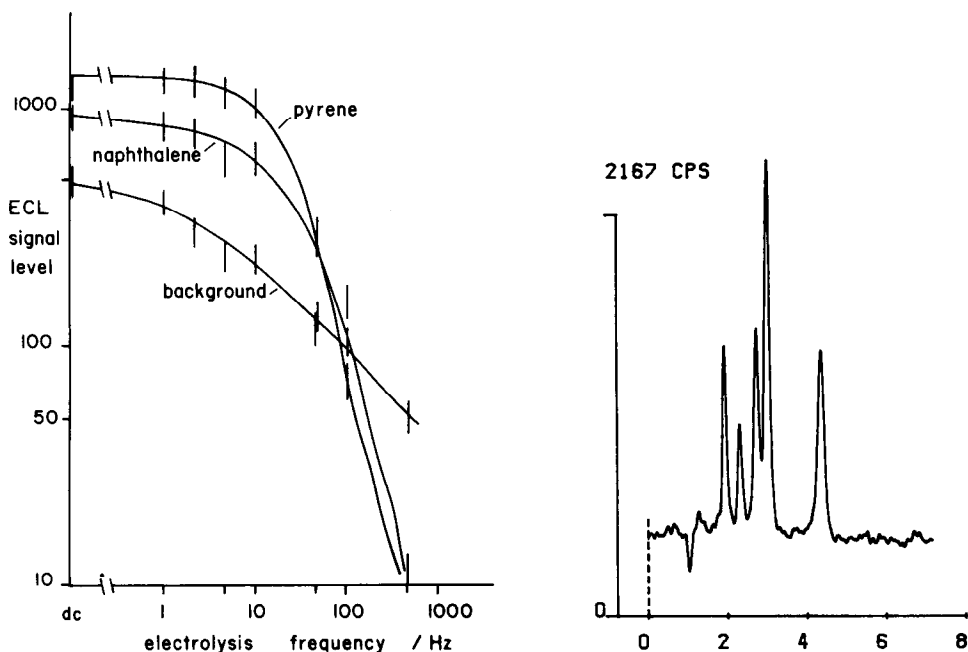


Fig. 2. Variation of ECL signal level with excitation frequency. The background signal levels shown are the levels of continuous background emission, while the levels for naphthalene and pyrene are the heights of chromatographic peaks.

Fig. 3. Example of an ECL chromatogram recorded using an excitation frequency of 5 Hz and an electrolysis current of 4 mA.

We did not attempt to quantify the variation in gas evolution during the electrolysis. However, we did observe that gas evolution was much reduced by the use of a.c. rather than d.c. current, and that the higher the current used, the greater was the frequency required to prevent the appearance of any gas bubbles in the flow from the cell. In practice we could operate at 5 Hz with a current of 4 mA without any detectable bubble formation, whereas previously 4 mA had been the limit of cell current and at this level extensive bubble formation had seriously contributed to the noise level of the recorded baseline.

A typical chromatogram of our test mixture is shown in Fig. 3, where the negative peak marks the solvent front (the sample solvent contained no electrolyte). When compared with Fig. 3 of our previous paper (in which the first four components were microgram quantities) it is clear that a substantial improvement in sensitivity has been achieved. The limits of detection have been reduced to between 5 and 20 ng, although perylene is still exceptional and has a lod of about 0.4 ng. While these limits are not remarkable it must be pointed out that the cell used to record these emissions consists of two platinum wires fused into a thick walled glass tube. We expect to have an improved design of flow cell available shortly and will report on the results achieved.

ACKNOWLEDGEMENTS

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