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Note

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

I. Preliminary experiments

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When a potential is applied between two electrodes separated by a conducting solution containing aromatic compounds, light is sometimes emitted. This phenomenon was first reported in 1964 by several groups¹⁻⁴, and has since been called electrochemiluminescence (ECL). ECL has developed into an important photochemical technique, providing much information about redox mechanisms of organic systems.

A recent review by Park and Tryk⁵ discusses the probable mechanisms for ECL, with particular emphasis on excited-state intermediates. The normal ECL process is thought to involve the production of radical cations at the anode and radical anions at the cathode, and these subsequently are annihilated in an electron transfer reaction. This annihilation can produce either singlet or triplet excited states, which can relax by fluorescence or phosphorescence, respectively, or which can be involved in further reactions. Processes 1-4 below provide the fluorescence mechanism of ECL with direct emission from the singlet excited state.



The methods that have been developed to investigate ECL have been reviewed by Faulkner and Bard⁶. There are two main experimental techniques for studying ECL. The first involves the application of an alternating potential at the working electrode so that the radical cations and anions are produced at the same electrode in alternate cycles. The annihilation process then takes place in the vicinity of the working electrode and is dependent on the stability of the redox products and their rates of diffusion. An analysis of the emission intensity requires a complicated mathematical treatment of the diffusion process and quantitative data are difficult to resolve.

The other method for studying ECL is based on two permanently polarized electrodes. In this instance the limiting step is the mixing of the oppositely charged radicals so that the annihilation process can proceed. There are two ways in which this mixing can be assisted. A rotating ring disc electrode may be used, in which event the rotation of the electrode causes a flow of solvent towards the face of the disc and then outwards, radially past the ring electrode. The light emission is dependent on the electrode configuration and the speed of rotation. The second approach is to construct a very thin cell so that diffusion of the radical ions produces a sufficient concentration of both species in the centre of the cell for the annihilation process to proceed.

The thin cell design with permanently polarized electrodes was adopted by Schaper and Schnedler⁷⁻⁹ for studying the ECL of compounds separated by liquid chromatography using non-aqueous eluents. They showed that the technique is fairly sensitive to some aromatic compounds when separated on a column with 1,2-dimethoxyethane as a solvent. They found that the solvent had to be electrolyte free and carefully dried, to avoid rapid and permanent degradation of the electrodes used, and that even then electrode lifetime was very limited. The solvent also had to be deoxygenated thoroughly to prevent quenching of the excited states and a consequent loss of sensitivity.

In this paper, we report the results of preliminary experiments with an ECL flow cell that can be used as a detector for a wide range of compounds in reversed-phase liquid chromatography or flow injection analysis (FIA) using eluents containing both water and an electrolyte. Spectroscopic examination of the light emitted during ECL has also been attempted with the aim of determining whether the emission is characteristic of the excited state of the test molecule rather than an excimer, exciplex or impurity.

EXPERIMENTAL

The chromatographic apparatus consisted of an ACS 300 series pump, a Rheodyne injection valve fitted with a 20- μ l loop and a Waters Assoc. 8 mm diameter ODS column contained in a Waters Z-Module cartridge assembly. All connecting tubing was 0.012 in. I.D. stainless steel. The solvent flow-rate was monitored with a Phase Separation flow meter. For these early investigations a relatively simple flow cell design was adopted, as illustrated in Fig. 1. Two platinum wire electrodes were set about 1 mm apart in a glass tube of *ca.* 0.062 in. I.D. The cell inlet was a short piece of 0.012 in. I.D. PTFE tubing, which was fixed inside the glass tubing with slow-setting epoxy resin. The outlet was 0.032 in. I.D. PTFE tubing and this was also fixed into position with epoxy resin the epoxy resin-covered area was then covered with black masking tape to minimize the effects of the long-lived phosphorescence of the epoxy resin. The total volume of the cell was about 100 μ l, but as we shall show later, most of the light originates from the vicinity of the anode and therefore the effective cell volume was much smaller.

The remaining components of the detection system are shown schematically in Fig. 2. A Shandon voltage supply (0-400 V d.c.) was used to provide a d.c. current between the two platinum electrodes. Both the current through the cell and the voltage across it were monitored. The intensity of the ECL emission and background

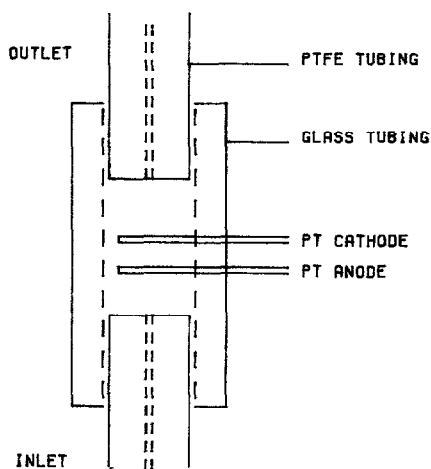


Fig. 1. Diagram of flow cell construction.

signal were found to be more closely related to the cell current than the potential difference between the two electrodes, and for this reason the system was operated in constant-current mode, the power supply adjusting the applied potential in order to maintain the current at a pre-determined level. At high cell currents there was considerable evolution of gas, predominantly from the cathode. In an attempt to minimize the disturbance that these bubbles caused to current flow, the cell was held in a vertical position with the cathode above the anode and the solvent flowed upwards so that the gas bubbles were rapidly flushed from the cell.

The light output from the cell was monitored using a Thorn-EMI 9804QB photomultiplier tube operated at approximately 900 V, and the output from the

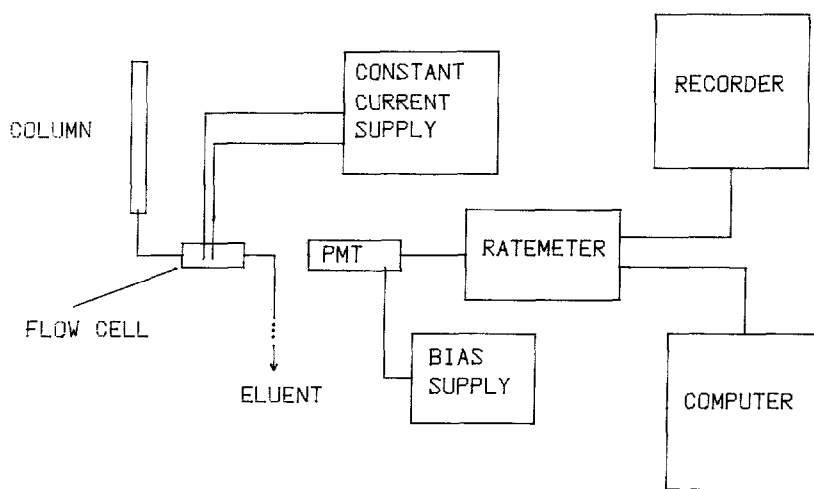


Fig. 2. Principal components of the ECL detector system. PMT = Photomultiplier tube.

anode was amplified and converted into logic pulses, which were passed to a photon-counting ratemeter¹⁰. The ratemeter output was digitized using a Hewlett-Packard 3478A digital multimeter, and the signal level passed via the GPIB to a CBM PET computer for analysis, graphical output and storage on disk.

For ECL spectroscopy the sample was pumped through the flow cell at approximately 2 ml min^{-1} by a single-piston Eldex pump. The cell was positioned at the entrance slits of a single photon-counting spectrometer¹¹, which corrected for spectral efficiency and background.

Water was distilled, purified in a Water-1 purifier (Gelman Sciences) and subsequently filtered through a $0.45\text{-}\mu\text{m}$ Millipore filter. HPLC-grade acetonitrile was filtered but otherwise used as supplied (Rathburn Chemicals). Tetrabutylammonium salts were prepared freshly by neutralizing an aqueous solution of tetrabutylammonium hydroxide with the acid of the appropriate anion. Tetrabutylammonium hydroxide was used as supplied (Aldrich). Samples were weighed and dissolved in the appropriate solvent for liquid chromatography or spectroscopy. Most samples were of Standard Laboratory Reagent (SLR) grade and were used without purification.

RESULTS

A range of aromatic compounds was tested for ECL and Figs. 3 and 4 show ECL chromatograms recorded from mixtures of a number of active compounds. For these examples the eluent was acetonitrile-water (9:1) made 0.005 M in tetrabutylammonium perchlorate, flowing at approximately 2 ml min^{-1} . The chromatograms shown were recorded with cell currents of approximately 4 mA , which was about the maximum that could be passed before gas bubble formation became a nuisance (the cell was not designed to withstand the high pressure needed to suppress bubble formation). In general, the compounds that produced the greatest ECL emission intensity were those which exhibit conventional fluorescence with a high quantum efficiency. Although at this stage we were not concentrating on achieving high sensitivity (and the column system used was selected for its durability rather than its efficiency), the limit of detection for most compounds under the electrical conditions used for Figs. 3 and 4 was in the region of 100 ng (injection volume $20 \mu\text{l}$), although for some compounds, such as perylene and diphenylanthracene, the limit of detection was about 10 ng .

Several electrolytes were tested in addition to tetrabutylammonium perchlorate, including the nitrate, sulphate and phosphate counter ions of tetrabutylammonium and lithium perchlorate. In each instance currents of up to 5 mA were achieved with similar ECL chromatograms and background light levels. These results demonstrate that, although different chemical reactions may have occurred at the electrodes under these drastic electrochemical conditions, the supporting electrolyte was not responsible for the light emission and did not substantially affect the ECL process. The detailed results of these studies and the effect of varying the solvent composition will be the subject of a later paper.

The chromatographic efficiency of the Z-Module cartridges was not as high as that of many columns now commercially available, so some tests were carried out using flow injection analysis (FIA) in order to determine the limit of detection (LOD) of the detector in the absence of column broadening. As expected, the LOD for most

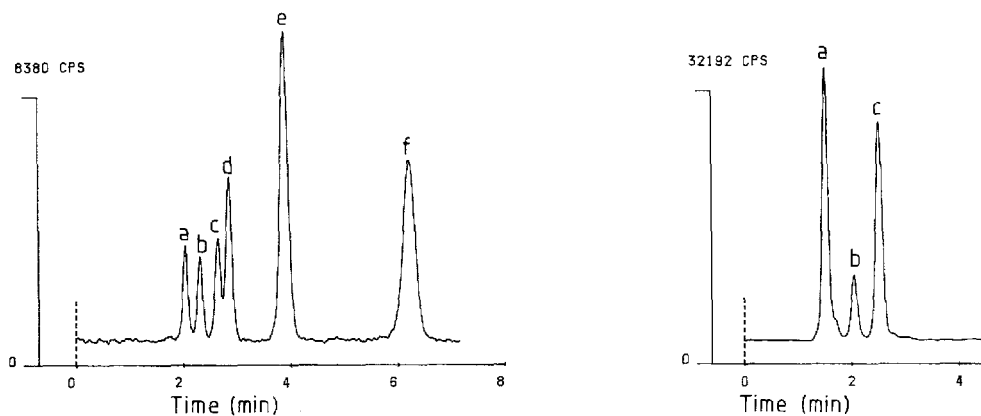


Fig. 3. ECL chromatogram of (a) naphthalene (1.4 μg), (b) acenaphthene (1.3 μg), (c) fluoranthene (1.6 μg), (d) pyrene (1.3 μg), (e) perylene (0.29 μg) and (f) anthanthrene (0.35 μg) injected in a 20- μl sample, after separation on a Waters Assoc. Radial-Pak ODS cartridge in acetonitrile-water (9:1) containing 0.005 *M* tetrabutylammonium perchlorate. Cell current = 4 mA.

Fig. 4. ECL chromatogram of (a) carbazole (2.0 μg), (b) chrysene (1.8 μg) and (c) *m*-terphenyl (1.9 μg) injected in a 20- μl sample, after separation on a Waters Assoc. Radial-Pak ODS cartridge in acetonitrile-water (9:1) containing 0.005 *M* tetrabutylammonium perchlorate. Cell current = 4 mA.

compounds under FIA conditions was considerably better, about five times lower than with the column in the circuit. However, it is clear that the relatively large dead volume of the cell described here has a significant and detrimental effect, and we hope for better LODs as the cell design is improved.

In order to ascertain the nature of the light-emitting species, emission spectra of a number of ECL systems were recorded. Figs. 5 and 6 show the ECL spectra of perylene and diphenylanthracene, respectively. These were recorded using a higher cell current than that used to obtain the chromatograms discussed above (hence the high noise level), and for recording the spectra a constant concentration of the test compound was pumped through the cell. Allowing for the fact that the resolution was low (slit widths of 2 mm were used), the spectra are essentially the same as photon fluorescence spectra for the parent species, confirming that the emission is due to the relaxation of the singlet excited state of the test molecule. The count rate under these conditions was very low, requiring counting times of 50 or 100 sec at each wavelength. At lower cell currents the emission was too weak to be able to record a spectrum. It is important to note that no other emissions from the test solutions within the wavelength range 200–600 nm were observed.

A second flow cell was constructed, similar to that shown in Fig. 1, but with a 3-cm gap between the electrodes. This enabled either electrode to be covered so that the light emission from the individual electrodes could be measured separately. The ECL emissions from a range of chromatographically separated compounds were recorded under similar conditions to the chromatograms recorded above. When the first electrode in the liquid flow was the anode, the emission intensity detected from the anode was approximately ten times greater than that detected from the cathode for all of the compounds tested. As the cathode was 3 cm downstream, it seems highly unlikely that significant concentrations of aromatic anion radical would reach

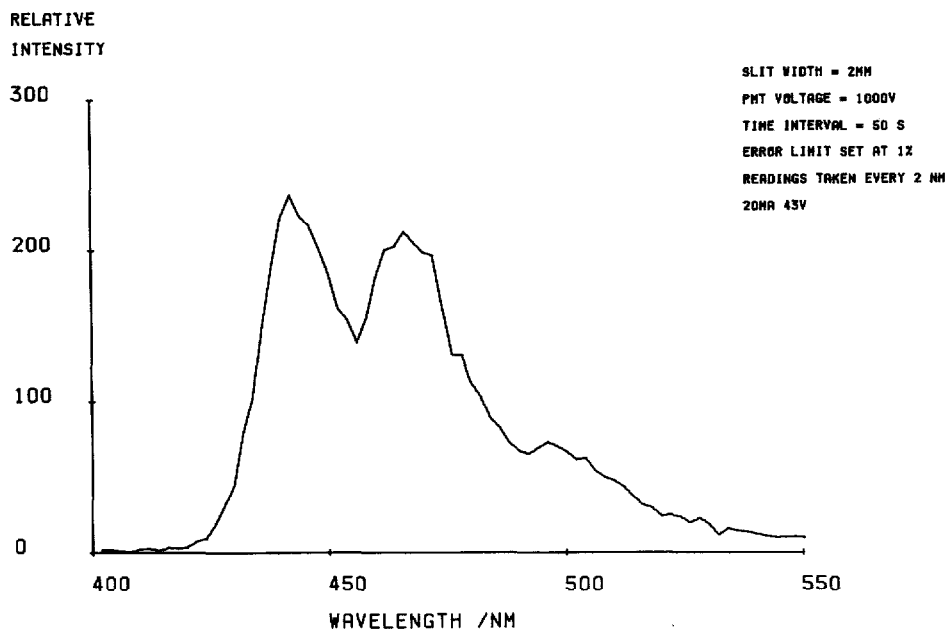


Fig. 5. ECL spectrum of perylene (0.0001 *M*) in acetonitrile-water (9:1) containing 0.025 *M* tetrabutylammonium perchlorate. Cell current = 20 mA.

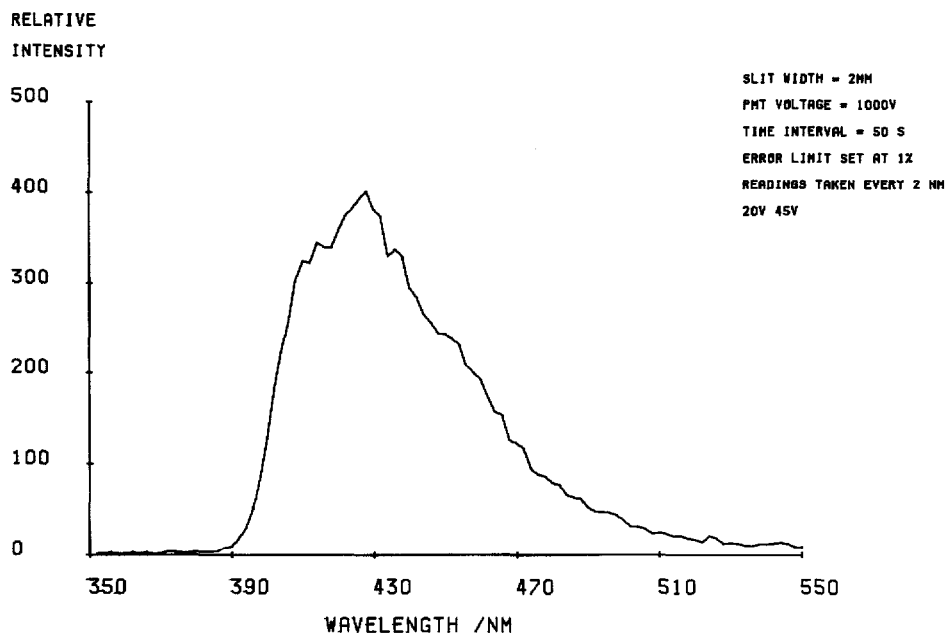


Fig. 6. ECL spectrum of diphenylanthracene (0.0005 *M*) in acetonitrile-water (9:1) containing 0.025 *M* tetrabutylammonium perchlorate. Cell current = 20 mA.

the vicinity of the anode, as would be necessary if the emission was to be brought about by the normal ECL annihilation process. When the cathode was made the first electrode in the liquid flow there was little change in the emission at the anode or cathode.

While the external masking of the electrodes in these experiments could not ensure that all unwanted photons were excluded from the photomultiplier (the body of the cell was Pyrex, and some unwanted emission could have reached the photomultiplier after multiple reflections), the intensities recorded from the cathode must contain large uncertainties. However, it is clear that the light emission was predominantly from the vicinity of the anode, and that the emission intensity was not significantly affected by the direction of liquid flow.

Several different electrode materials have been incorporated in ECL flow cells of the basic design discussed above. Cells have been constructed with platinum, stainless steel, copper, aluminium, lead and silver as the cathodic material, although we have only used platinum as the anode in each instance, because of the difficulties of anodic corrosion with most other metals. The cathodic material has shown little, if any, effect on the detected intensity of ECL emission from a range of test samples. The intensity of ECL emission increases in direct proportion to the cell current, independent of the cathode material. This observation provides further support for our view that the light emission is due to an anodic process and the cathode is required only to provide electrons to complete the cell reaction.

DISCUSSION

The normal ECL of aromatic molecules can be produced at a single electrode if an alternating current is used to generate anions and cations at the one electrode, or by two permanently polarized electrodes if there is a means by which the redox products can mix. The mechanism has been well studied⁵ and there appears to be no doubt that such ECL is due to the annihilation of radical anions and radical cations resulting in the production of an excited-state species. This species may emit light during a relaxation process (eqns. 1-4), or it may react again to produce a different excited state species which subsequently fluoresces.

In order to study ECL by the radical annihilation process, where diffusion is responsible for the movement of charged radicals, it should be necessary to apply potentials only sufficient to oxidize and reduce the test compound(s). However, two more recent studies^{7,12} have shown that by applying more extreme potentials substantially more light is produced. Schaper and Schnedler⁷⁻⁹ rationalized this in terms of a multiplicity factor, on the assumption that a molecule can be oxidized/reduced more than one time. They calculated that in their thin-layer cell (inter-electrode distance approximately 30 μm) rubrene could undergo the annihilation process up to eight times. They noted that the light emission was related to the cell current and concluded that the current was predominantly due to electrohydrodynamic (EHD) convection. The rate of movement of ions was thus linked directly to the applied voltage. On the other hand, Zhivnov and Rumyantsev¹² studied the ECL of several compounds in dimethylformamide, acetonitrile and acetone in a cell with two permanently polarized electrodes placed 7 mm apart. They noted that ECL was produced at the cathode after only 0.6-0.8 sec. They proposed a speculative mechanism based on electrons tunnelling through the cathode surface to produce excited-state species. This mechanism seems unlikely, as a high electric field would be produced very rapidly on switching on the applied voltage and one would therefore expect

almost instantaneous light emission. The pause of 0.6–0.8 sec suggests that this was the time required for the radical cations to diffuse the 7 mm (theirs was a static system) to the vicinity of the cathode, where light emission took place. However, it remains difficult to explain their observation that the light intensity was related to cell current and increased progressively at higher applied voltages. In their experimental arrangement EHD convection would not make a significant contribution to the cell current as the electrodes were a long way apart and they used a high concentration of supporting electrolyte.

In our experiments we observed that light emission is closely related to cell currents of up to 20 mA with a potential difference across the cell of up to 200 V. In our experimental arrangement there is a flow of solvent past the electrodes, which have only a limited active area of catchment in the cell, so it is unlikely that the aromatic compound could be oxidized/reduced more than once, and consequently could not be involved in the normal annihilation process more than once. Another ECL mechanism must be responsible for our results and it is possible that this may play an important role in the high-voltage ECL observed by others.

CONCLUSION

We have shown that d.c. electrochemiluminescence can be used for detecting components eluting in aqueous eluents. This technique has been applied to the detection of a range of aromatic molecules chromatographically separated in acetonitrile–water mixtures, and spectroscopic examination of the emissions indicates that they are similar to the conventional fluorescence emissions of the compounds. Detection limits for the system that we have described are not yet as low as those achieved using a normal phase ECL detector based on a thin cell design^{7–9}, but the system does operate long-term in the presence of non-deoxygenated and aqueous eluents. We are continuing our investigations with lower volume flow cells and experimenting with techniques for the suppression of gas bubbles, in the hope of producing significant improvements in the sensitivity of the system.

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