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ELECTROCHEMILUMINESCENCE AS A DETECTION TECHNIQUE FOR REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

III*. A HIGH-PERFORMANCE FLOW CELL

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SUMMARY

A new flow cell has been designed and tested for continuous, medium-term use in an electrochemiluminescence detector. It is found that the cell efficiency may be maintained over periods of weeks by careful control of the excitation potential and by incorporating a cleaning agent within the eluent. A number of example chromatograms obtained in the presence of tetrabutyl ammonium chloride as a cleaning agent are reported.

INTRODUCTION

Electrochemiluminescence (ECL) is light emitted during the electrolysis of solutions of (usually) organic compounds. ECL was first observed[1] in 1927, although was not investigated in any detail[2,3] until 1964. There was much interest in the phenomenon throughout the late sixties, with particular emphasis on studies related to the mechanisms involved. During the 1970's the mechanisms were elucidated as the species involved became identified with the aid of electron spin resonance and magnetic field effects. Progress during this period has been thoroughly reviewed and the mechanistic processes summarised[4–6]. In recent years attempts have been made to enhance the ECL intensity observed from dilute solutions, generally to investigate the potential of ECL measurement as an analytical technique. The approaches adopted invariably reflect the requirements of the annihilation mechanism in that the emphasis has been on encouraging the interaction of the anion and cation formed during electrolysis, and on rigorously excluding species such as oxygen and water which are known to quench ECL emissions. ECL has been used as a detection technique for normal-phase liquid chromatography (LC), using a thin cell with tin oxide

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* For Part II, see ref. 10.

transparent electrodes[7,8]. However, the lifetime of the electrodes was said to be limited, and became very short in the presence of water.

We have been investigating the use of ECL as a detection technique for reversed-phase LC using relatively simple electrode assemblies which both avoid the complexity of moving electrodes and have much greater durability than tin oxide electrodes. We reported earlier[9] that a modest sensitivity could be achieved with a relatively simple two-electrode cell arrangement and non-deoxygenated eluents involving 10–20% water. Our initial experiments were carried out with a d.c. electrolysis current and involved flowing liquids through cells in which the electrodes were up to 2 cm apart. The major proportion of the emitted light was found to originate from the vicinity of the anode, even when the anode was upstream of the cathode. The light emission intensity from a given sample was found to be proportional to the total cell current, even at applied voltages up to 100 V d.c. We subsequently reported[10] on the improvement in sensitivity obtained through the use of an a.c. electrolysis potential, and more recently have detailed results obtained from lumograph studies[11], *i.e.* the variations of emission intensity as a function of electrolysis potential and frequency.

Our earlier experiments demonstrated that ECL offered some attractions as a detection technique for reversed phase high-performance liquid chromatography (HPLC), in that many classes of compounds appeared to emit light during electrolysis and the sensitivities for detection in a relatively crude cell were reasonable. However, potential problem areas were observed once the apparatus had been in use for any length of time. The two main problems we have observed are: (1) lack of reproducibility; on some occasions highly reproducible chromatograms could be obtained daily for several days, whereas at other times successive chromatograms differed both in terms of absolute peak height and even in relative peak heights for a multicomponent test sample. (2) Fouling of the flow cell; the first flow cells were glass tubes with pairs of platinum wires passed through the wall. The wires were small (requiring a magnifier for examination) and the electrolysis currents used routinely were small (typically < 1 mA). When we fabricated more substantial cells and operated with higher electrolysis currents and potentials, we found that the electrodes became covered with a brown deposit which, quite apart from lowering the light output, eventually blocked the cell.

Furthermore both problems became progressively worse over a period of about a year. It became clear that the more care we exercised in cleaning electrodes and purifying the components of the eluent, the more rapidly the problems returned. The present work was undertaken in an attempt to overcome these difficulties. We report below on a new flow cell design which allows operation at substantially higher currents than previously, and on new electronic and chemical approaches we have adopted to permit an ECL detector to operate reproducibly for long periods.

EXPERIMENTAL

Fig. 1 shows a block diagram of the elements of the ECL detector used. The major elements are the flow cell, the excitation supply, and the light detection system, and these are described in detail below. The other equipment used consisted of an HPLC pump (Biotech Instruments), a sample loading system (a Rheodyne valve,
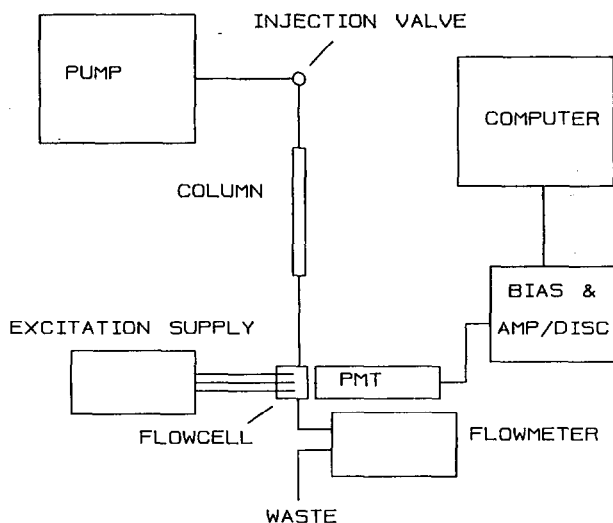
Fig. 1. Block diagram of ECL chromatography system.

Model 7125, fitted with a 20-μl loop), a 25-cm Spherisorb ODS (10 μm) column (Phase Separations), and a digital flow meter (Phase Separations) which monitored the flow-rate post cells. The chromatography was performed using the eluents described for each of the sample chromatograms reported below.

The flow cell design adopted is shown in Fig. 2, and was chosen to provide a large electrode surface area and a constant path length for current flow. The cell body was made from Kel-F, and tapped to accept standard Altex fittings to carry the eluent through 1/16 in. PTFE tubing. The electrodes were prepared from a disk of platinum 1 mm thick and approximately 10 mm in diameter. The disk was cut into two Ds and platinum wire conductors spot welded to each. The electrodes fitted into a circular recess in the cell body, and the conductors passed through narrow holes to the rear of the cell. The face of the cell (*i.e.* the part protruding beyond the recess holding the electrodes) was polished to accept a quartz window, held in position by a securing ring bolted to the cell body. The result was a narrow corridor between the two Ds of platinum, sealed at the rear by the Kel-F body, and at the front by the quartz window. Eluent entered this corridor via a hole drilled at one end of the corridor through to the rear of the cell, although to minimise dead volume we allowed the inlet PTFE tubing to pass through this hole and reach the bottom of the corridor. Eluent left the corridor via a (larger) hole drilled at the other end of the corridor. The dimensions of the corridor were approximately 10 × 1 × 1 mm, giving a cell volume of approximately 10 μl.

The cell was subsequently modified to allow a third electrode to be introduced as close to the corridor region as possible. In this work the third electrode has been a fine platinum wire passed, via an additional Altex connector, into the eluent outlet hole. In practice we position the end of this wire as close to the cell's corridor as possible. This electrode is used to provide a reference level for the control of the working electrode level. While a platinum wire is not normally considered to be
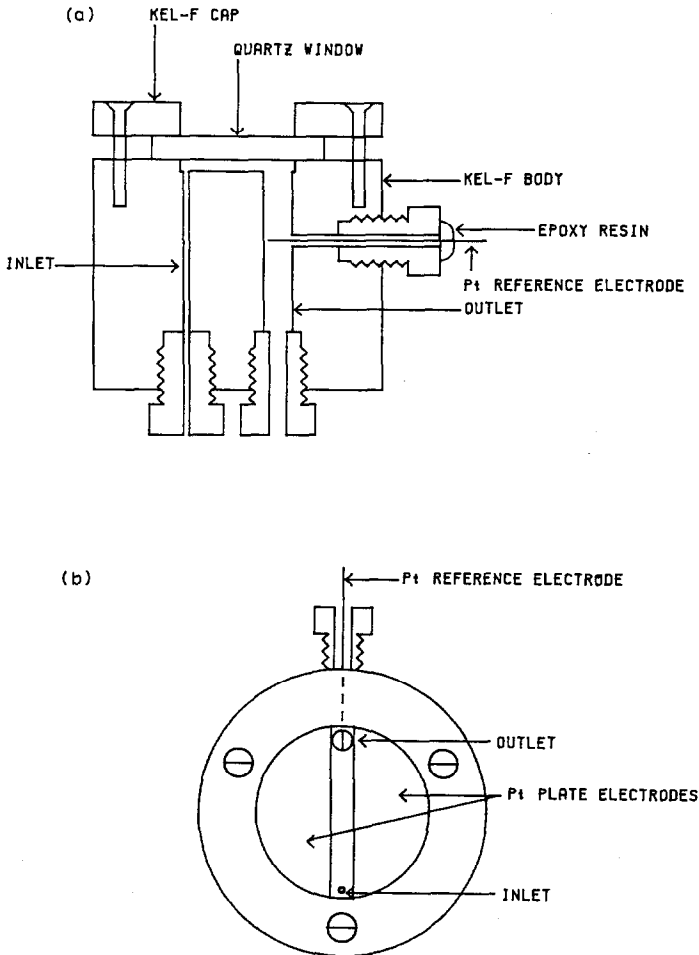
Fig. 2. Diagram of the ECL flow cell used, showing the cautela view (a) and the photomultiplier's view of the electrodes (b). The active volume of the cell is the channel between the electrodes, which in this work had a volume of approximately 10 μl.

adequate as a reference electrode for electrochemical measurements, it does offer the advantage of simplicity and small size, and was found to be useful under the conditions of the present experiments.

   The excitation supply system is shown schematically in Fig. 3. The unit maintains one of the electrolysis electrodes at ground potential and sets the potential of the other to a level which causes the potential of the reference electrode to equal a predetermined level. The circuit compares the buffered potential difference between the reference electrode and ground with a level input from a potential divider (adjustable between 0 and 5 V), using the difference to drive one of the two 759 power amplifiers. The amplifier driven, and so the electrode which operates as a cathode, is selected by a 4066 CMOS switch which operates at a frequency determined by an
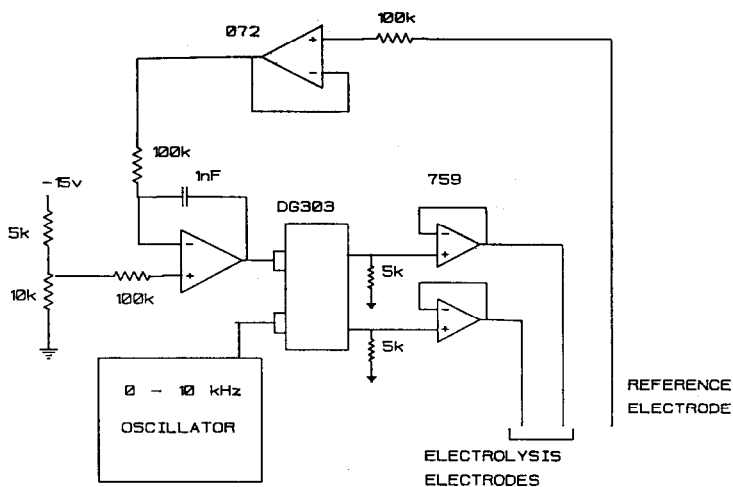
Fig. 3. The electrolysis supply circuit which maintains a constant amplitude for the potential between the electrolysis anode and the reference electrode. The electrolysis electrode acting as anode changes every half cycle. (k = kΩ).

external TTL oscillator, the two switched channels opening and closing alternately. The amplifier which is not driven through the switch has its input pulled to ground through the 10 kΩ resistor, thus ensuring that the appropriate electrolysis electrode (the anode) is operating at ground potential.

The light measurement system is shown in Fig. 4. It consists of a photomultiplier tube (Thorn EMI type 9789QB) together with a low-power bias supply (operated at approximately 1000 V), a preamplifier/discriminator which converts those anode pulses exceeding a preset threshold into TTL compatible logic pulses, and a low-cost microcomputer (Commodore 64) which counts the logic pulses and calculates the pulse rate. Details of the operation of this simple photon counting system have been reported[12].

Errors

The instruments used in recording potentials, current and light levels were calibrated and unlikely to provide significant sources of error for either d.c. elec-
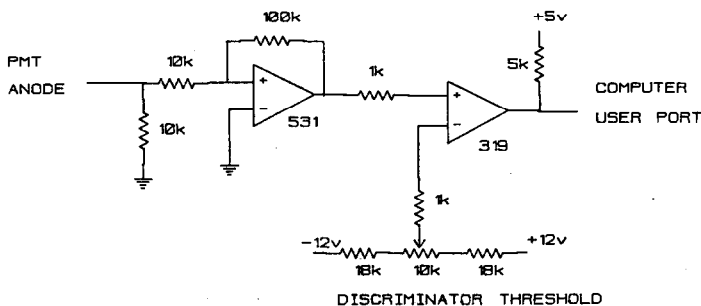

Fig. 4. The ECL intensity measuring system.

trolysis or for square wave electrolysis above 10 Hz. For low-frequency electrolysis the current measurements do fluctuate as the sampling frequency of the multimeter is of the same order as the electrolysis frequency. However, we have taken the maximum values recorded for the purposes of this work. (An alternative technique has been developed for recording currents during lumograph studies, and these will be reported in due course.) For the present we assign error limits of 10% to current reading below 10 Hz. The chromatograms recorded below were reproducible to within 5% from a given set of samples and eluent. Over a period of time and using different batches of eluent and samples, the reproducibility was found to be within 10%.

Materials

The sample materials, purity and manufacturer used in this work are: anthracene, naphthalene, Analar (Fisons); acenaphthylene, chrysene 95% (Aldrich); fluoranthene, 98% (Aldrich); pyrene, perylene, >99% (Aldrich); di-butylphthalate, di-hexylphthalate, di-octylphthalate, >98% (Phase Separations Qual-kit). Pesticide samples (courtesy of the Laboratory of the Government Chemist; no detailed purity information available): 1-naphthyl methylcarbamate (carbaryl), 4-chloro-2-butynyl-3-chlorophenylcarbamate (Barban), 1,1,1-trichloro,2,2-bis(4-methoxyphenyl)ethane (methoxychlor), 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE), 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT).

All test compounds were used without further purification. Tetrabutylammonium perchlorate (TBAP) was prepared by neutralising a concentrated solution of tetrabutylammonium hydroxide (Aldrich) with AR perchloric acid (Fisons) and was purified by two recrystallisations from ethylacetate–pentane. HPLC-grade acetonitrile (Rathburn Chemicals) was found to have a water content of about 700 ppm. Dry acetonitrile was prepared from HPLC-grade acetonitrile, either by distillation from phosphorus pentoxide or by standing over 3 Å molecular sieve (BDH Chemicals) as discussed in an earlier paper[11]. Distilled water was further purified using a Water-1 purification unit (Gelman Sciences) and filtered through a 0.45-μm, Millipore filter.

RESULTS AND DISCUSSION

We have determined that the formation of the deposit on the platinum electrodes could be prevented by maintaining a concentration of chloride ions in the carrier electrolyte. (A similar effect is produced by bromide ions.) Fig. 5 shows the variation of the signal-to-noise ratio (*i.e.* peak height to r.m.s. noise ratio) for the chromatographic peak produced from 100 ng of naphthalene eluted at 2 ml min^{-1} using an eluent of acetonitrile–water (95:5), with the concentration of added chloride, in the form of tetrabutylammonium chloride. (Electrolysis electrode–reference electrode potential difference 7 V and electrolysis frequency 5 Hz.) The ratio was determined from the average of six successive sample injections over a fixed period of 1 h. Thus variations in peak height (and we have found that reductions in the peak heights of successive peaks are a reliable indication that deposit is forming) show up as a reduction in the signal-to-noise ratio. The results indicate that a concentration of tetrabutyl ammonium chloride of less than $5 \cdot 10^{-4}$ M is insufficient to prevent
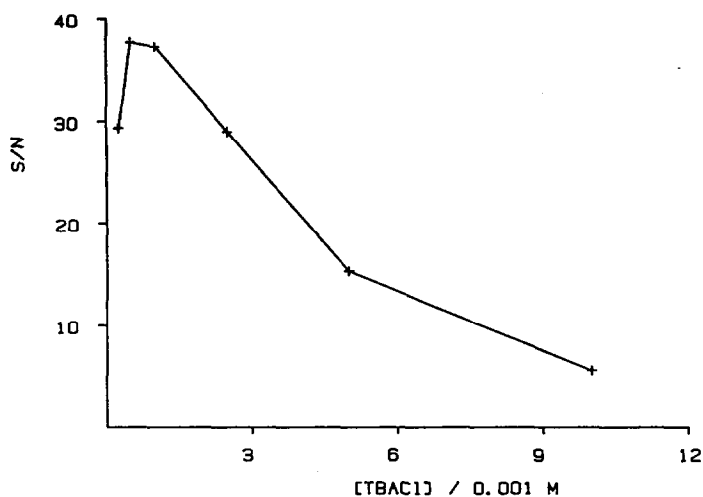
Fig. 5. Variation of the signal-to-noise ratio (S/N) recorded from 100 ng samples of naphthalene as a function of tetrabutylammonium chloride (TBACl) concentration in an acetonitrile–water (95:5) eluent. See text for chromatographic and ECL details.

the formation of deposit, and that much higher concentrations, while preventing the formation of deposit, also reduce the intensity of ECL emission from the sample. Similar results have been obtained using other sample materials. From these results we selected $5 \cdot 10^{-4}$ M as the optimum concentration of tetrabutylammonium chloride for this particular eluent.
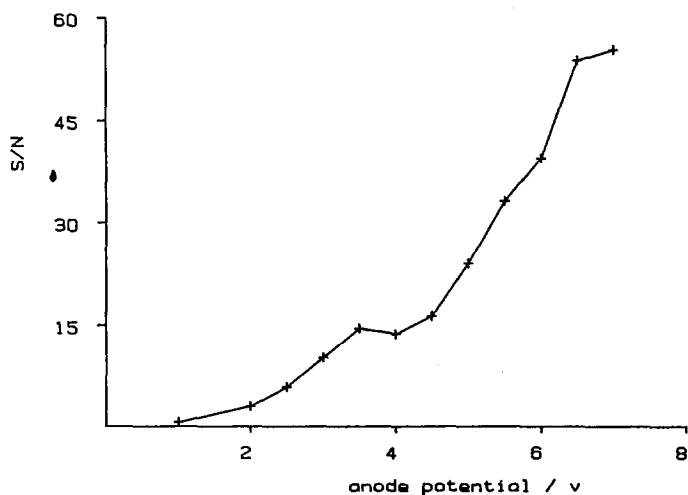

Fig. 6. Variation of the signal-to-noise (S/N) ratio from 100 ng samples of naphthalene as as function of the electrolysis anode–reference electrode potential difference at an electrolysis frequency of 5 Hz. See text for eluent details.

The variation of the signal-to-noise ratio recorded from the same sample and an eluent of acetonitrile–water (95:5), made $5 \cdot 10^{-3}$ M in tetrabutylammonium perchlorate and $5 \cdot 10^{-4}$ M in tetrabutylammonium chloride, as a function of the amplitude of the electrolysis electrode–reference electrode potential difference is shown in Fig. 6. Again the eluent flow-rate was 2.0 ml min^{-1} and the electrolysis frequency was 5 Hz. The maximum potential difference which can be generated by our present a.c. potentiostat is 7 V (corresponding to an electrolysis voltage of about 10 V), and the results of Fig. 6 indicate that it may be desirable to go to somewhat
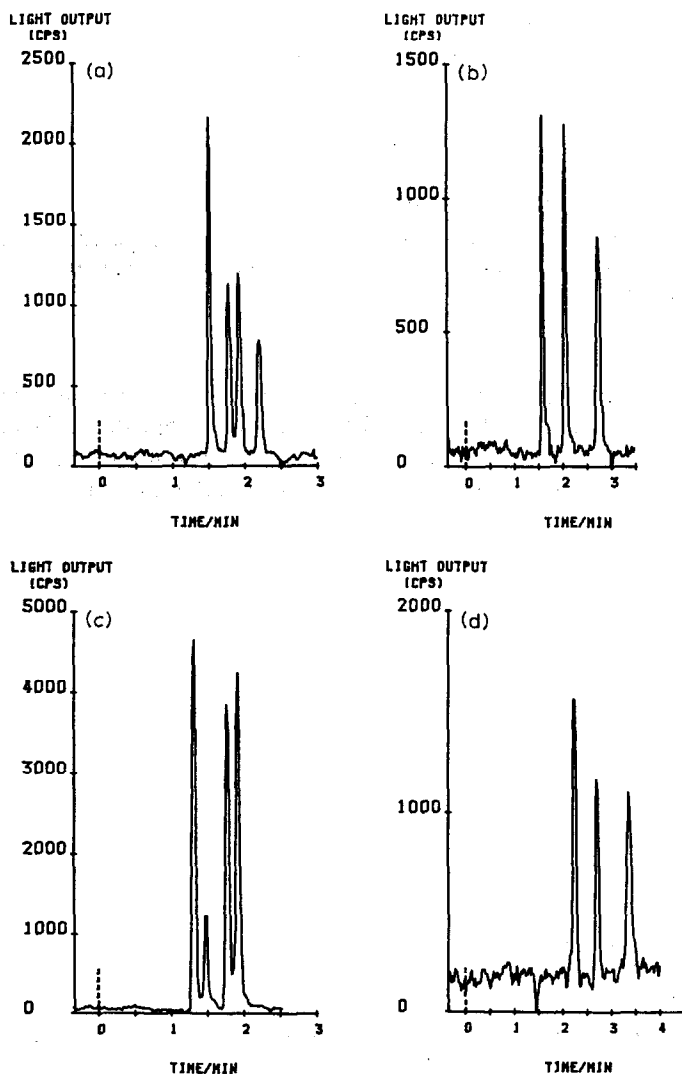

Fig. 7. Typical chromatograms recorded using the ECL flow cell of Fig. 2 in the presence of chloride. (a) *ca.* 27 ng each of naphthalene, anthracene, fluoranthene and chrysene; (b) *ca.* 14 ng each of acenaphthylene, pyrene and perylene; (c) carbaryl (96 ng), methoxychlor (150 ng), DDT (295 ng) and DDE (54 ng); (d) *ca.* 500 ng each of di-butyl, di-hexyl and di-octyl phthalates.

higher potentials. The chromatograms reported below were obtained with an electrolysis electrode–reference electrode potential difference of 7 V.

Some typical ECL chromatograms obtained using the eluent and potential described above are shown in Fig. 7, and serve to illustrate a selection of the materials which may be detected by ECL. Fig. 7a shows a chromatogram obtained from a sample of polynuclear aromatic hydrocarbons, consisting of (in order of elution), naphthalene (26.9 ng), anthracene (29.5 ng), fluoranthene (26.8 ng) and chrysene (28.4 ng). Fig. 7b shows the separation of acenaphthylene (13.6 ng), pyrene (14.8 ng) and perylene (13.4 ng). Fig. 7c shows a chromatograms from a sample of pesticides, consisting of carbaryl (96 ng), methoxychlor (150 ng), p,p'-DDT (295 ng) and p,p'-DDE (54 ng), eluted using the same conditions. Fig. 7d shows the separation of a series of phthalate esters, di-butyl (507 ng), di-hexyl (542 ng) and di-octyl (720 ng), recorded using an eluent of acetonitrile made $5 \cdot 10^{-3}$ M in tetrabutylammonium perchlorate and $5 \cdot 10^{-4}$ M in tetrabutylammonium chloride, with an electrolysis electrode–reference electrode potential difference of 7 V and an electrolysis frequency of 60 Hz.

Although we are still seeking improvements in sensitivity, we are encouraged by the reproducibility of chromatograms. Fig. 8 shows a pair of chromatograms recorded from the same sample solution four weeks apart (the system was in virtually full time use in between). This result is typical of many others and indicates that the electrodes may be maintained in a relatively constant condition by the use of chloride ion in the eluent during electrolysis. An important consequence of the improvement in the consistency of the condition of the electrodes is that the linearity of response as a function of sample size has also improved considerably. Fig. 9 shows how the integrated peak area (*i.e.* the total number of photons detected, corrected for back-
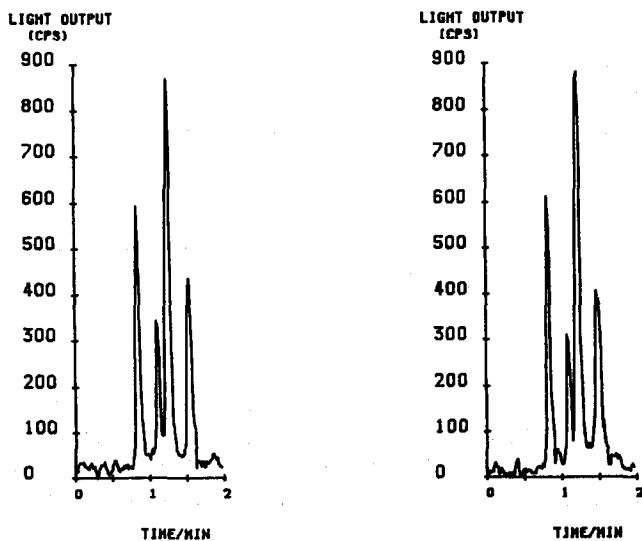

Fig. 8. Two ECL chromatograms recorded from a mixture of naphthalene, anthracene, fluoranthene and chrysene four weeks apart, illustrating the medium term reproducibility of the system when used with chloride containing electrolyte.
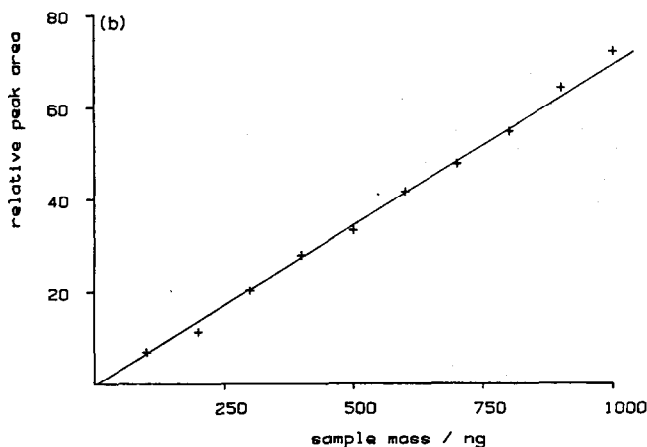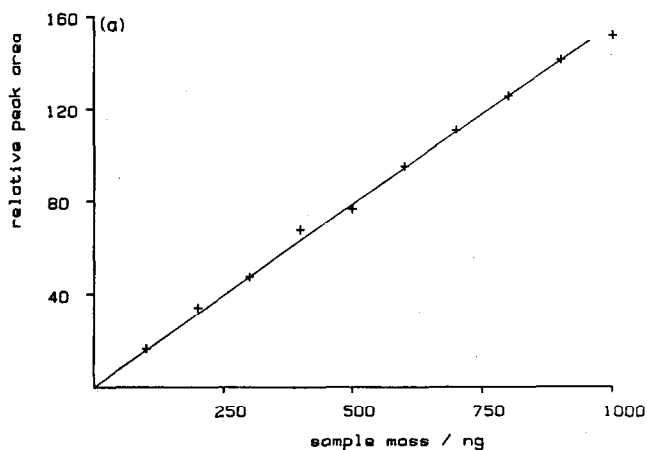
Fig. 9. Variation of peak area as as function of sample size for samples of (a) naphthalene and (b) Barban. Elution conditions as in Fig. 7.

ground) varies with the mass of sample injected for an aromatic hydrocarbon (naphthalene, Fig. 9a) and a pesticide (Barban, Fig. 9b), over the range 10–1000 ng.

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