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Amperostatic–potentiometric detection for micro high-performance liquid chromatography

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Abstract

Constant current is applied to a microelectrode which is "bathed" in the effluent from a micro-LC column. A micro indicator electrode senses the potential as electroactive solutes undergo electron transfer at the working electrode surface. In the absence of analyte, mobile phase components (principally water) are reduced or oxidized, generating a significant working electrode potential. Because the measured signal (potential or charge density) is significant near the detection limit, detection sensitivity may, under optimum conditions, be higher than for conventional amperometric detection. We report on the design, fabrication and evaluation of amperostatic–potentiometric (ASPEN) detection for micro-LC. The determination of phenol, *o*-chlorophenol, 2,4-dimethylphenol and *p*-benzylphenol, each at the femtomole level (injected amount), has been performed with a packed fused-silica capillary (250 μm I.D.), operating in the reversed-phase mode. The real and apparent limitations to this technique will be presented.

1. Introduction

1.1. Micro-LC with electrochemical detection

Microcolumn high-performance liquid chromatography (micro-LC), using capillaries with less than 500 μm I.D., is characterized by several fundamental measurement and practical analytical advantages. With dramatically reduced column dimensions the quantities of sample, mobile phase and packing material are reduced [1]. Micro-LC is ideal for the analysis of such unique samples as exotic physiological fluids and single cells [2,3]. Good kinetic performance is also characteristic of micro-LC [4]. Significant gains in detection limits can be realized by the enhanced mass sensitivity of coupled concentration-sensitive detectors [5]. Detector development has been problematic, however, due to stringent volumetric requirements.

Amperometry

One of the most suitable detection techniques for such small columns is microelectrochemical detection in the amperometric mode [6]. Amperometric detection currently provides the best detectability among the various electrochemical detection techniques [6–14]. The primary advantage of electrochemical detection for micro-LC lies in the fact that electrochemical processes take place at the surface of a microelectrode, resulting in very high detection efficiency, "virtually coulometric in the case where electroactive analytes are contained in the very thin annular

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flow region between the electrode and capillary wall" [11]. The unique compatibility between micro-LC and microelectrochemical detection is largely due to the fundamental features of microelectrodes. A relatively large current *density* at the microelectrode is achievable at low applied current and enhanced mass transport to and from the electrode surface is seen (both due to the small size and surface area). The use of carbon fiber working electrodes for microvoltammetric micro-LC detection was first demonstrated by Knecht et al. [10] in 1984 and has been steadily refined [6].

In the normal amperometric mode a constant potential is applied to the auxiliary reference electrode pair and the working electrode remains at virtual ground [15]. Current flows along the working electrode signal pathway to "earth" ground through a current-to-voltage converter and is amplified in the process. The magnitude of this current is proportional to the mass flux of electroactive species to the working electrode surface plus background contributions. Double layer capacitance and reaction (adsorption/desorption) of the electrolyte/mobile phase impurities at the working electrode surface contribute to the background current. Fluctuations in these, stimulated by surges in fluid flow, generally give small current and noise levels [16]. However, as one approaches the limit of detection (i.e less and less analyte mass flux), vanishingly small analyte current becomes indistinguishable from the background. In other words, at the detection limit analyte detection *sensitivity* is low with amperometric sensing.

The disadvantages of fixed potential detection are poor selectivity and generation of rather limited electrochemical information. Selective detection using a differential pulse amperometric technique has been demonstrated [11]. Though improved detection limits were also shown the technique does not give general information about the electroactive species in the sample.

Voltammetry

Potential scanning methods (voltammetry) give increased information content but they are usually less sensitive than amperometric techniques, largely due to the charging current associated with the rapid scan rates necessary for real-time chromatographic detection. By discriminating against background current, square-wave voltammetric detection [17,18] has been used to improve sensitivity and yet maintain the inherent selectivity of the potential scanning technique. Square-wave voltammetry is well suited to mercury because the relaxation of the charged double layer, induced by the pulses, is reproducible. Staircase voltammetry has demonstrated reasonable results at solid electrodes in chromatography at around 1 V/s sweep rate [7]. The coulostatic electrochemical technique discriminates against double layer charging current and is largely immune from solution resistance effects [19,20]. In this technique pulses are used to charge the double layer capacitor, changing the electrode-solution potential. White et al. [7] used a scanning microvoltammetric detector for on-column detection in open tubular liquid chromatography. It was noted that an advantage of microvoltammetric electrodes is the reduced double layer capacitance that permits rapid scanning of the electrode potential.

Potentiometry

Potentiometric electrochemical detection has been performed with capillary liquid chromatography. A micro ion-selective electrode has been used for potentiometric detection with micro-LC–electrochemical detection [21,22]. The utility of potentiometric detection with microanalytical separations has recently been addressed. For both direct and differential potentiometric detection, the ideal voltage measurement is necessary, i.e. no induced current flow. No change in electrical charge during transduction and amplification can be tolerated. Though this ideal is not perfectly attainable, modern electronic devices can allow very small absolute and differential voltage measurements to be made.

1.2. The amperostatic–potentiometric (ASPEN) measurement

If an appropriate constant current is applied between a working electrode and a counter

electrode, a potential gradient is generated by the mass flux of electroactive species, to the working electrode. If this working electrode is a microelectrode it is reasonable to expect that the potential gradient would be significant in the same way that current density is significant for such an electrode in amperometry. This potential can be measured with an "indicating" electrode, using a high-impedance electrometer. Negligible charge is expected to flow if a suitable microelectrode like Ag/AgCl is used. In the constant-current mode, unlike the constant potential mode, solvent components react at the micro working electrode generating a background potential gradient. When a more easily electrolyzed species passes over the microelectrode it will preferentially react at the electrode surface, changing the potential such that this signal (potential gradient) would be related to the amount of analyte reacting at the electrode surface. At vanishingly small analyte concentration, there would be a comparatively large background signal level. However, the noise component will be present as a very small portion of this large signal. Our ability to detect smaller and smaller amounts of analyte becomes limited only by our ability to distinguish between smaller and smaller *differences* in the large signals. Thus, ASPEN detection may offer enhanced detectability at the lower limit of detection. ASPEN detection can, thus, be viewed as a simple modification of these familiar measurements. In its basic configuration, a two-electrode galvanostat would be coupled with a high-impedance electrometer (in the voltage mode) through a suitable indicator electrode. Alternatively, an indicator–reference electrode pair could be used for differential measurement of working electrode charge.

2. Experimental

2.1. Galvanostat/potentiostat

The instrumental set-up for ASPEN detection is shown in Fig. 1. An EG & G Princeton Applied Research (Princeton, NJ, USA) Model 273 potentiostat/galvanostat was used to apply constant current to the cell. The electrochemically generated potential was measured with a Keithley Instruments (Cleveland, OH, USA) Model 610C solid-state electrometer. Data acquisition was accomplished with a IBM PC computer system. The cell was shielded in a laboratory-made Faraday cage. The offset circuit was made in the departmental electronic shop. The lowest current that the galvanostat could apply was 25 pA. A current divider could not be used to reach lower currents because a constant-current condition could not be maintained.

2.2. Micro-LC

A Model 8500 syringe pump (Varian, Walnut Creek, CA, USA) and a Model CI4W injection valve (Valco, Houston, TX, USA) were used for all micro-LC experiments. The volume of injection valve was 80 nl. Slurry-packed capillary columns, 75 cm × 0.25 mm I.D., were prepared using published procedures [23]. Micro-LC was performed with microcolumns which contained Spherisorb ODS2 reversed-phase packing (Alltech, Deerfield, IL, USA) and an acetonitrile–phosphate buffer (65:35) mobile phase. The aqueous portion had a pH of 4.8 and was 0.02 *M* in total phosphate and $1 \cdot 10^{-4}$ *M* in EDTA. This mobile phase was degassed by vacuum ultrasonication and used for all transport and chromatographic studies, unless otherwise stated. All chromatograms were analyzed using DADiSP (DSP Development, Cambridge, MA, USA), digital signal processing software. All noise analysis was done using this system.

2.3. Reagents and chemicals

Acetonitrile (Optima grade), potassium phosphate (monobasic and dibasic) and hydroquinone were obtained from Fisher Chemical (Pittsburgh, PA, USA). EDTA was obtained from J.T. Baker (Phillipsburg, NJ, USA). Phenol, *o*-chlorophenol, 2,4-dimethylphenol and *p*-benzylphenol were obtained from Aldrich (Milwaukee, WI, USA). All the above chemicals were used without further purification.

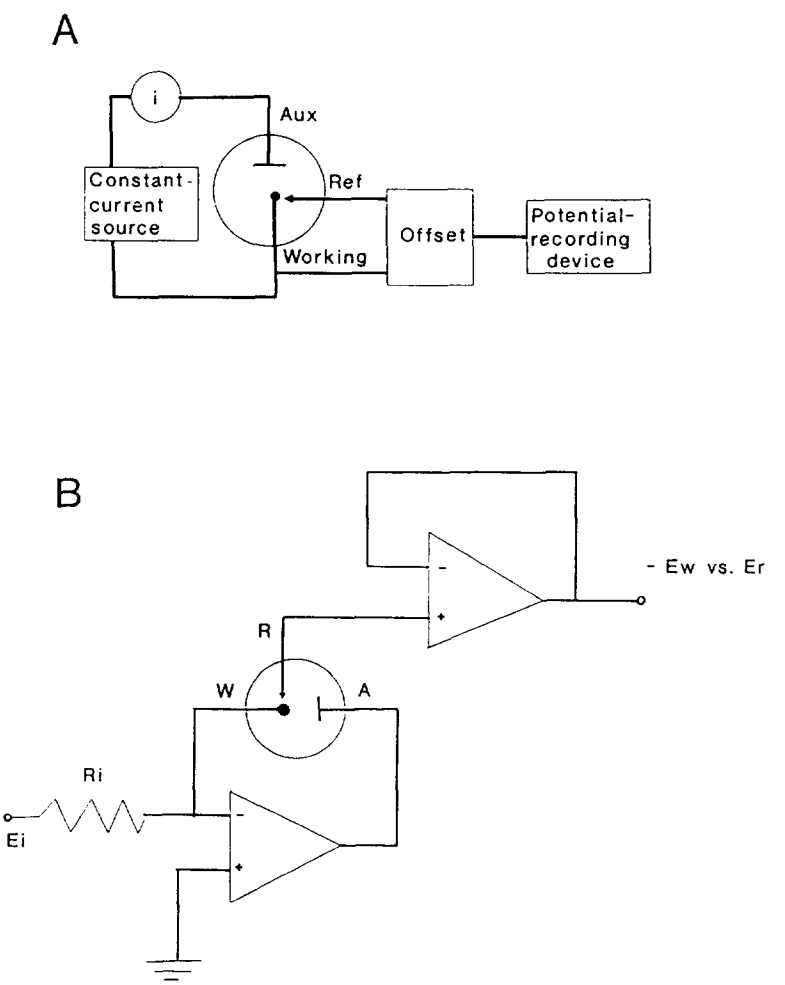

Fig. 1. (A) Simplified block diagram of ASPEN measurement apparatus; (B) simplified schematic of three-electrode cell in amperostatic (galvanostatic) mode. Aux (A), Ref (R) and Working (W) = Auxiliary, reference and working electrodes, respectively; i = current; E_i = input voltage; R_i = input resistance ($i = E_i/R_i$); E_w and E_r = working electrode and reference electrode potentials, respectively.

2.4. Construction of electrodes

In order to make a carbon fiber working electrode, 7 μm diameter Magnamite Type AS-4 carbon fiber, obtained from Hercules, was cleaned by soaking in hot (80–90°C) concentrated sulfuric acid for several hours. It was rinsed in distilled water, then methanol and finally dried in ambient air. A single carbon fiber was then inserted into a 530 μm I.D. capillary so that 2 to 3 mm was protruding outside the capillary and then it was glued with optical adhesive (No. 63) which was obtained from Norland Products (New Brunswick, NJ, USA). Mercury and copper or tin wire were used for electrical connection.

Ag/AgCl reference electrodes were made by soldering a 5 mm length of 0.215 mm diameter silver wire (Aldrich) to an aluminum or copper lead. The lead was then sealed in a 530 μm I.D. capillary, leaving about 3 mm of silver wire exposed. The silver surface was cleaned by placing only the protruding portion in a 3 *M* nitric acid solution, for 1 h. It was then im-

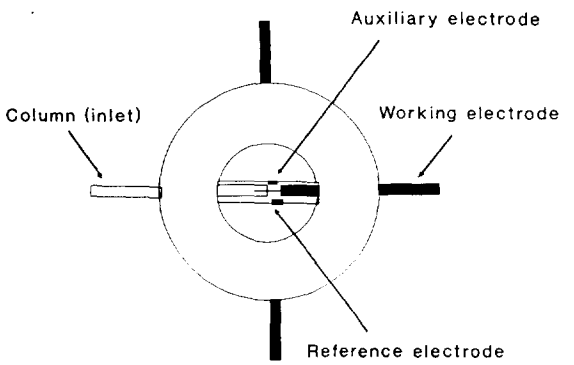

Fig. 2. Drawing of flow-through cell.

mersed in a 1 *M* potassium chloride (with few drops of HCl) solution and the potential was held at 1.0 V for about 24 h.

2.5. Construction of electrochemical cell

A polyether ether ketone (PEEK) "tee" connector from Alltech was used to fabricate the flow-through cell. Holes of 0.035 in. (1 in. = 2.54 cm) diameter were drilled through the long axis of 1/4 × 28 T.P.I. (threads per inch) nylon plugs; the electrodes were inserted through these holes and into the "tee", as shown in Fig. 2. For one pair of holes, oriented at 180°, a carbon fiber microelectrode was inserted into the lumen of a fused-silica capillary. Reference and auxiliary electrodes were inserted through the other holes. An observation hole had been bored in the exact center of the "tee", through the top surface, to enable viewing with a microscope. This hole also served as an outlet for mobile phase. The reference electrode was placed as close as possible to the working electrode surface.

3. Results and discussion

3.1. Background signal and noise characteristics

One of the first studies was directed to examining the importance of inlet capillary inner diameter on detector performance. Fig. 3 shows a plot of background potential as a function of applied current for inlet fused-silica capillary

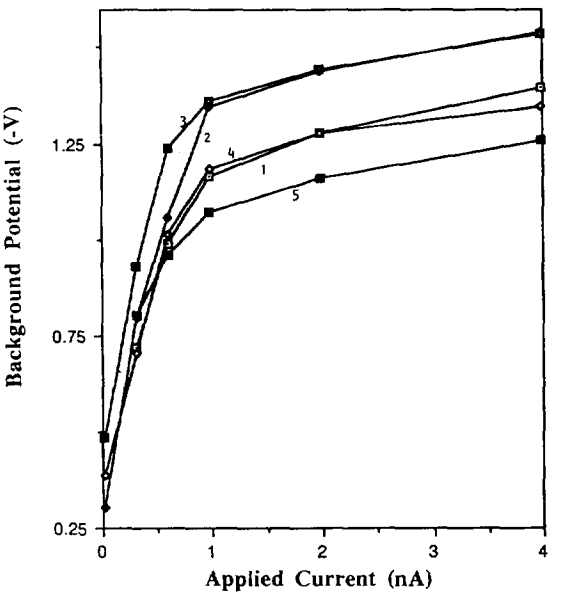

Fig. 3. Plot of background potential (−V) versus applied current (nA) for capillaries of various internal diameters. Capillary I.D.: 1 = 15 μm; 2 = 19 μm; 3 = 50 μm; 4 = 73 μm; 5 = 98 μm.

I.D.s of 15, 19, 50, 73 and 98 μm. In each case, the carbon fiber working electrode was inserted to a depth of 2 mm into the capillary and the mobile phase solution flowed at approximately 5 μl/min across the electrode. The measured background potential becomes more negative with increasing applied current. A "limiting potential" condition is attained in this controlled-current mode just as a limiting current is achieved in controlled-potential experiments. Application of an anodic current between the working and auxiliary electrodes implies that the working electrode potential should be positive. The measured background potential is equal to $-E_w$ versus E_r (where E_w and E_r are the working electrode and reference electrode potentials, respectively) because these potentials were measured relative to earth ground. Therefore, a negative value of background potential corresponds to a positive working electrode potential. There does not appear to be a trend to the limiting background potentials, in terms of inlet capillary inner diameter.

With an applied current, the working elec-

trode potential increases in order to pass this current. Electroactive mobile phase components will undergo electron transfer and the working electrode will take on a potential, dependent on the magnitude of the applied current and the nature and concentration of the available electroactive species. With an anodic bias and an aqueous mobile phase, water oxidation:

$$2H_2O \rightarrow O_{2(g)} + 4H^+ + 4e^-$$

will be the predominant electrochemical reaction at the working electrode. This probably explains the plateau of 1.4 V (versus ground potential) at >1 nA applied current.

When the working electrode potential exceeds the standard potential for water, application of higher currents does not increase the working electrode potential significantly, since solution resistance probably accounts for the remaining current dissipation. The potential (electrode surface charge) depends on the current density at the working electrode and the cell resistance. Due to its very small surface area, the current density for the microelectrode is high, hence there tends to be a rather high background potential for a very small applied current. For example, the surface area of the carbon fiber working electrode ranges from about $4 \cdot 10^{-4}$ cm^2 to $7 \cdot 10^{-4}$ cm^2. At an applied current of 250 pA the current density would be 0.62 to 0.28 μA/cm^2. Since the carbon fiber was of the same dimensions in each of our experiments, we would not expect the current density to be affected by inlet capillary inner diameter. On the other hand, the resistance of the flow-through cell is very high and would likely cause the cell resistance to be higher for capillaries of smaller diameters. Therefore, a higher background potential would be expected with the 15 and 19 μm I.D. capillaries, irrespective of the applied current. This is not seen in Fig. 3. One possible explanation for this is variation in fiber placement inside the inlet capillaries, which would be more problematic with the smaller-I.D. capillaries This would cause rather drastic differences in cell resistance. Another explanation lies in the differences between linear velocity and volumetric flow. Since each of the cells was operated at constant volumetric flow-rate, a much higher mobile phase linear velocity would accompany the smaller-I.D. inlet capillary. The measured noise did not appear to signal such changes in linear velocity as the noise ($3 \cdot 10^{-4}$ V) remained within a factor of two at the different applied currents used in this study. Drift in the background signal was observed, especially at 600 pA. Drift *decreases* tremendously when currents exceeding 600 pA were applied. The sloping baseline showed some resemblance to a potential–time profile of a charging capacitor. A possible source of drift is the gradual dissolution of the thick AgCl film on the reference electrode.

3.2. Relationship of applied current and potential in ASPEN detection

Hydroquinone (HQ) was used as an oxidizable analyte for evaluation of the applied current–potential relationship. Here, a constant anodic current was applied and the peak height of an injected solute was measured. In the absence of analyte, water would give the background potential, as discussed above. Since HQ is more easily oxidized than water, the potential at the working electrode decreases during HQ elution and reaction at the working electrode. In order to record the small changes in potential accompanying the presence of electroactive analytes, an offset circuit was used. This device applied a reverse polarity signal to the electrometer output in order to maintain a "0" response voltage. This response signal is plotted against applied current for several concentrations of HQ in Fig. 4. As shown, the response increases with applied current for nearly all the HQ concentrations up to a maximum applied current level of about 0.600 nA. At higher applied current dramatic decreases in response are seen. Even though each concentration represents different injected masses of HQ, the point at which no more HQ results in higher response occurs at nearly the same applied current. This may be attributable to oxygen evolution at the working electrode. Molecular oxygen would hinder transport of electroactive species to the electrode and may also

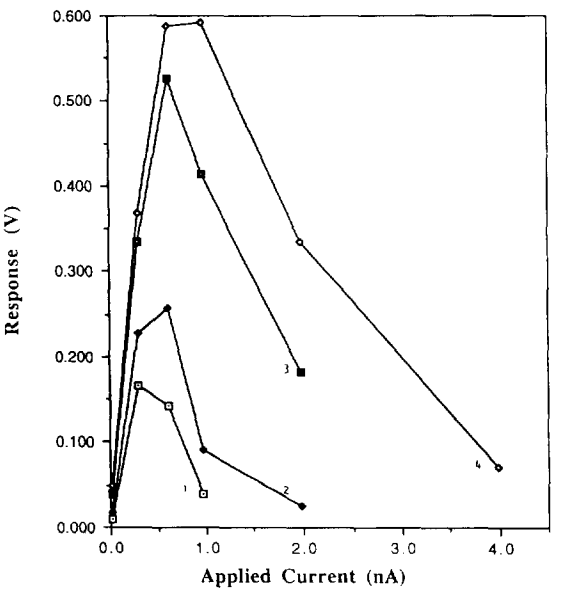

Fig. 4. Plot of ASPEN detector response (V) versus applied current (nA) at various hydroquinone concentrations. HQ concentrations: $1 = 5.10 \cdot 10^{-6}$ *M*; $2 = 1.02 \cdot 10^{-5}$ *M*; $3 = 2.60 \cdot 10^{-5}$ *M*; $4 = 5.10 \cdot 10^{-5}$ *M*.

contribute to preoxidation of HQ. The 0.600 nA threshold is not distinct, however. Note that $1.02 \cdot 10^{-6}$ *M* HQ reaches a maximum response at an applied current of 0.300 nA and there is a gradual shift in the current threshold to almost 1.0 nA at $5.10 \cdot 10^{-5}$ *M*. This shows that a somewhat greater response can be obtained for low analyte concentrations by applying lower currents. Another interesting conclusion stems from the slopes of these curves at currents < 0.5 nA. Since the HQ concentrations are different (by virtue of the different injected masses) yet the slopes in these regions are almost the same, it is reasonable to conclude that the response in this region is not transport-dependent, but rather kinetically limited. To our knowledge, this a totally unique situation for microelectrochemical detection.

3.3. Response and sensitivity

Classically speaking, the concentration response function is linear for amperometric detectors and logarithmic for potentiometric detectors. Table 1 is a compilation of response versus log of HQ concentration at the various applied currents and inlet capillary diameters tested. The linear regression slopes and correlation coefficients are also shown. Note the high correlation coefficient (R^2) values. With the lowest value of 0.92, these results suggest that most of the available data are accurately represented by the model. Note also that the slopes of the response curves increase with applied current up to 1.975 nA. Compare these results to those of Table 2. Table 2 is a list of linear regression data for response versus HQ concentration, again at various applied currents and inlet capillary I.D.s. Apart from the much higher slope values, there is generally much poorer correlation of data with this model, as evidenced by the lower R^2 values.

Further evidence of "anomalous" detector response is seen in actual plots of these data.

Table 1
Linear regression data summary from ASPEN detector response versus log HQ concentration for capillaries of different I.D.

Capillary diameter (μm)	Current applied (pA)	Slope	R^2
15	300	0.1422	1.00
	600	0.2268	0.99
	975	0.351	0.98
	1975	0.677	0.97
19	300	0.1053	0.99
	600	0.2725	0.97
	975	0.3056	0.92
	1975	0.2283	0.97
50	300	0.1937	0.99
	600	0.3542	0.96
	975	0.5882	0.98
	1975	0.4364	1.00
73	300	0.1127	0.99
	600	0.2547	1.00
	975	0.3447	0.98
	1975	0.6747	0.97
98	300	0.1299	1.00
	600	0.2362	0.99
	975	0.3234	0.98
	1975	0.5787	0.97

Table 2
Linear regression data summary from ASPEN detector response versus HQ concentration for capillaries of different I.D.

Capillary diameter (μm)	Current applied (pA)	Slope	R^2
15	300	3994.65	0.87
	600	6221.61	0.85
	975	$1.007 \cdot 10^4$	0.87
	1975	$1.367 \cdot 10^4$	0.92
19	300	3055.46	0.89
	600	8295.79	0.92
	975	$1.020 \cdot 10^4$	0.96
	1975	5005.97	0.99
50	300	5572.17	0.89
	600	$1.100 \cdot 10^4$	0.93
	975	$1.246 \cdot 10^4$	0.97
	1975	7398.96	0.99
73	300	3381.47	0.92
	600	6344.45	0.83
	975	9815.82	0.87
	1975	$1.339 \cdot 10^4$	0.90
98	300	3653.39	0.87
	600	6522.45	0.84
	975	9077.44	0.86
	1975	$1.141 \cdot 10^4$	0.90

Fig. 5 shows plots of detector response versus HQ concentration at several different applied currents for a 50 μm I.D. inlet capillary. Note that the greatest response level is achieved at 0.600 nA applied current throughout the concentration range. The greatest sensitivity (slope) is found at 0.975 nA applied current, as confirmed by the results of Table 1. The range of concentrations included in Fig. 5 is very narrow and is meant to show calibration plots rather than the entire response curves.

We look again at the aspect of inlet capillary I.D. Table 1 shows that inlet capillary has no clear influence on response sensitivity. An examination of the noise data from these measurements (data not shown) indicates, at most, a two-fold variation over the range 15–98 μm I.D. This is quite different from typical behavior for amperometric detection [24]. If the response

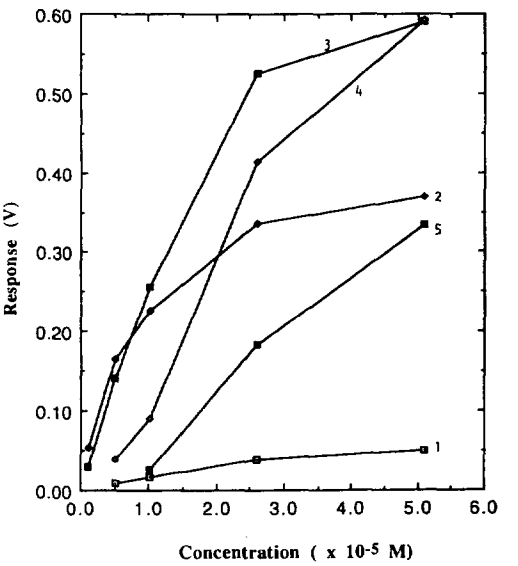

Fig. 5. Plot of ASPEN detector response (V) versus HQ concentration at various applied currents. Applied current: 1 = 0.025 nA; 2 = 0.30 nA; 3 = 0.60 nA; 4 = 0.975 nA; 5 = 1.975 nA.

were mass-transport limited there would have been an optimum or, at least, some effect of inlet capillary diameter. Our data show no influence of inlet capillary diameter on response sensitivity, detection limits or background noise. It is quite possible that without a careful noise analysis [25] such effects have been overlooked. Admittedly, we have not proven superior detection sensitivity near the detection limit, compared to microamperometric detection. However, we have not pushed the instrumentation or measurement to its limits in this preliminary study. Therefore, we can conclude from these results that ASPEN detection is not characterized by a mass-transport limited analytical signal as is microamperometric detection. Obviously, design flexibility and versatility are inherent in this conclusion and positive attributes of ASPEN detection.

3.4. ASPEN detection of phenols

A four-component mixture of phenols (phenol, *o*-chlorophenol, 2,4-dimethylphenol and *p*-benzylphenol) was analyzed by micro-LC.

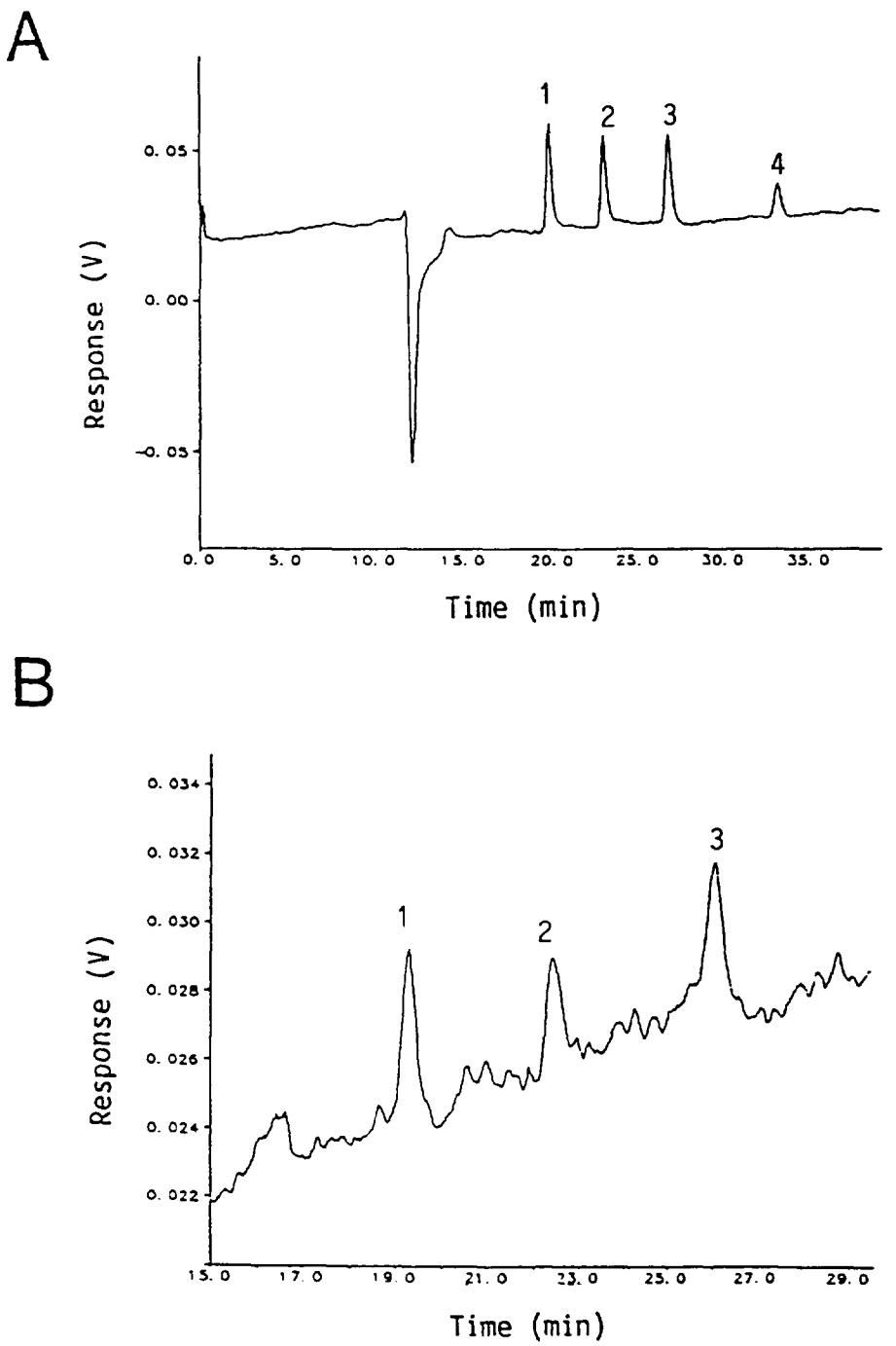

Fig. 6. (A) Chromatogram of phenol mixture: 1 = 0.714 pmol phenol; 2 = 0.80 pmol *o*-chlorophenol; 3 = 0.80 pmol 2,4-dimethylphenol; 4 = 0.756 pmol *p*-benzylphenol. (B) Chromatogram of a ten-fold dilution of mixture as in (A).

Fig. 6 shows two chromatograms of this mixture, each obtained at 0.600 nA applied current and a 50 μm I.D. inlet capillary. Fig. 6A represents an injection of 0.714 pmol phenol, 0.80 pmol *o*-chlorophenol, 0.80 pmol 2,4-dimethylphenol and 0.756 pmol of *p*-benzylphenol. Fig. 6B is an injection of a ten-fold dilution of that shown in Fig. 6A. *p*-Benzylphenol was not seen in the signal for Fig. 6B. Fig. 7 shows the peak heights of each component plotted against the applied current, now in the range of 0.1 to 4 nA. Note that for each analyte except 2,4-dimethylphenol a limiting response occurs between 1 and 2 nA. The response for 2,4-dimethylphenol is maximum at 0.300 nA and it steadily decreases with applied current. This can be attributed to the almost 200 mV lower half wave potential for 2,4-dimethylphenol [26], compared to phenol and *o*-chlorophenol, as shown in Table 3. Mass detectabilities, as limits of detection, for each phenol are also shown in Table 3. It is reasonable to conclude that an applied current of 0.600 nA for the chromatograms of Fig. 6 represents nearly optimum detection response for *all* these analytes. Had microamperometric detection been employed, detection selectivity, due to constant applied potential, may have excluded 2,4-dimethylphenol from detection. ASPEN detection appears, then, to be a more universal type of microelectrochemical detection technique than conventional amperometry, voltammetry or coulometry. Much more evaluation work remains before this can be confirmed.

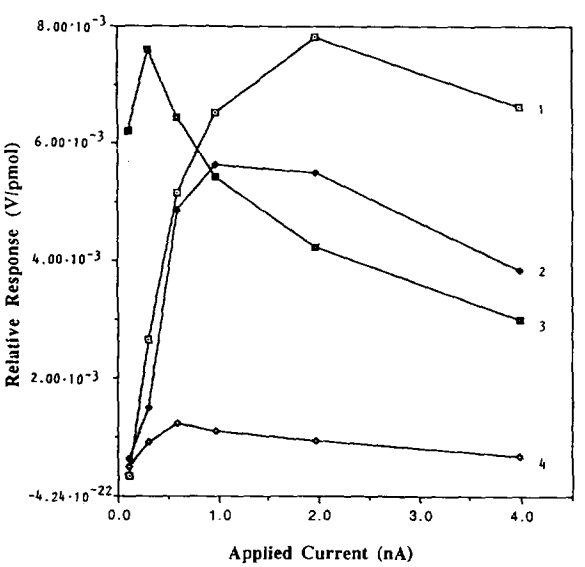

Fig. 7. Plot of relative response (V/pmol) versus applied current (nA) for the phenols. 1 = Phenol; 2 = *o*-chlorophenol; 3 = 2,4-dimethylphenol; 4 = *p*-benzylphenol.

Table 3
Analytical data for phenols

Analyte	$E_{1/2}$ vs. SCE (V)	LOD (fmol)
Phenol	0.633	70
o-Chlorophenol	0.625	76
2,4-Dimethylphenol	0.459	76
p-Benzylphenol	–	76

$E_{1/2}$ = Half-wave potential; SCE = saturated calomel electrode; LOD = limit of detection. See Ref. [26] for $E_{1/2}$ data.

4. Conclusions

ASPEN detection appears to be a feasible microelectrochemical detection technique. With a constant applied current (in the 0.2–2 nA range) a surface charge is created on a carbon fiber working electrode in response to the concentration and identity of electroactive species flowing past the electrode surface. In the absence of more easily oxidized species, the surface charge depends on mobile phase (water oxidation) components. When an easily oxidized electroactive eluite flows by, the electrode's surface charge changes only enough to allow the applied current to pass. This modulation of surface charge or potential can be easily detected and has been shown to be proportional to analyte mass. Theoretically, this combination of effects should give ASPEN detection certain advantages for trace-level sensing. Unfortunately, these advantages were not seen in this preliminary study.

More work is needed before a thorough understanding of this technique is attained. Studies of response sensitivity at the limit of analyte detection are planned. A thorough noise analysis is needed. The influence of applied current upon detection efficiency is warranted, because slow

electrode kinetics may alter detection temporal performance at low applied current. Substantial reductions in limit of detection may result from further minimization of noise, as in a dual-channel–differential sensing mode.

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References

[1] M.V. Novotny and D. Ishii (Editors), *Microcolumn Separations —Columns, Instrumentation and Ancillary Techniques*, Elsevier, Amsterdam, 1985.
[2] T.M. Olefirowicz and A.G. Ewing, *Anal. Chem.*, 62 (1990) 1872.
[3] B.R. Cooper, J.A. Jankowski, D.J. Leszczyszyn, R.M. Wightman and J.W. Jorgenson, *Anal. Chem.*, 64 (1992) 691.
[4] G. Guichon and H. Colin, in P. Kucera (Editor), *Microcolumn High-Performance Liquid Chromatography*, Elsevier, Amsterdam, 1984, pp. 1–38.
[5] M.V. Novotny, *Anal. Chem.*, 53 (1981) 1294A.
[6] A.G. Ewing, J.M. Mesaros and P.F. Gavin, *Anal. Chem.*, 66 (1994) 527A.
[7] J.G. White, R.L. St. Claire III and J.W. Jorgenson, *Anal. Chem.*, 58 (1986) 293.
[8] M. Goto, E. Sakura and D. Ishii, *J. Chromatogr.*, 238 (1982) 357.
[9] K. Slais and D. Kourilova, *J Chromatogr.*, 258 (1983) 57.
[10] L.A. Knecht, E.J. Guthrie and J.W. Jorgenson, *Anal. Chem.*, 56 (1984) 479.
[11] R.L. St. Claire III and J.W. Jorgenson, *J. Chromatogr. Sci.*, 23 (1985) 186.
[12] K. Slais and M. Krejci, *J. Chromatogr.*, 235 (1982) 21.
[13] Y. Hirata, P.T. Lin, M V. Novotny and R.M. Wightman, *J. Chromatogr.*, 181 (1980) 287.
[14] M. Goto, Y. Koyangi and D. Ishii, *J. Chromatogr.*, 208 (1981) 261.
[15] P.T. Kissinger, *Laboratory Techniques in Electroanalytical Chemistry*, Marcel Dekker, New York, 1984, p. 163.
[16] D.M. Morgan and S.G. Weber, *Anal. Chem.*, 56 (1984) 2560.
[17] R. Samuelson, J. O'Dea and J. Osteryoung, *Anal. Chem.*, 52 (1980) 2215.
[18] J. Wang, E. Ouziel, C. Yarnitzky and M. Ariel, *Anal. Chim. Acta*, 102 (1978) 99.
[19] T.A. Last, *Anal. Chem.*, 55 (1983) 1509.
[20] A.C. Barnes and T.A. Nieman, *Anal. Chem.*, 55 (1983) 2309.
[21] A. Mant and W. Simon, *J. Chromatogr. Sci.*, 21 (1983) 326.
[22] S.R. Muller, W. Simon, H.M. Widmer, K. Grolimund, G. Schomburg and P. Kolla, *Anal. Chem.*, 61 (1989) 2747.
[23] D.C. Shelly, V.L. Antonucci, T.J. Edkins and T.J. Dalton, *J. Chromatogr.*, 458 (1989) 267.
[24] S.G. Weber and W.C. Purdy, *Anal. Chim. Acta*, 100 (1978) 531.
[25] S.G. Weber and J.T. Long, *Anal. Chem.*, 60 (1988) 223.
[26] L. Meites and P. Zuman (Editors), *Electrochemical Data*, Part 1, Vol. A, Wiley, New York, 1974.