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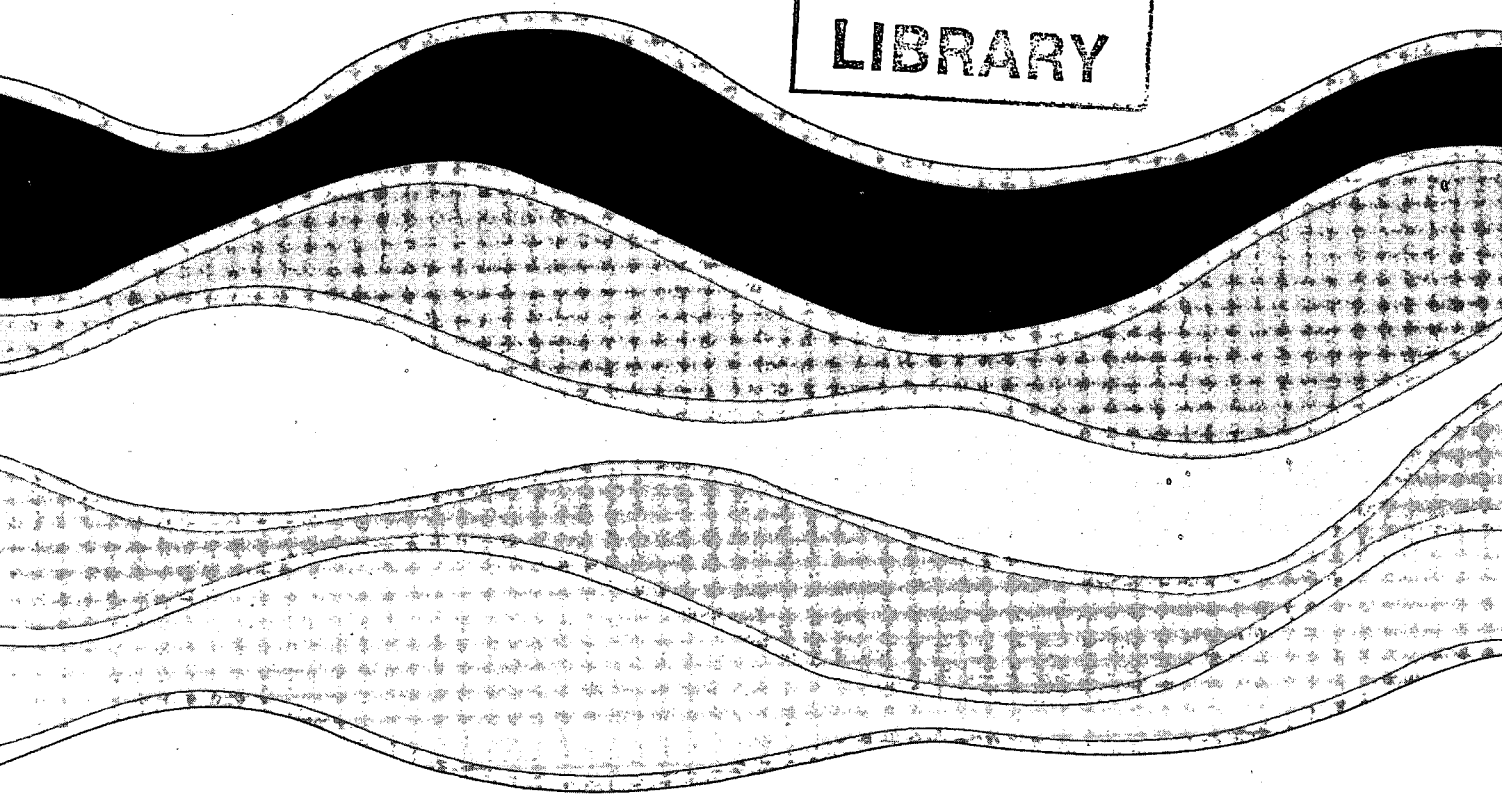

# MICELLAR ELECTROKINETIC CHROMATOGRAPHY: UV LONGITUDINAL FLOW CELL DESIGN WITH ENHANCED SENSITIVITY SUITABLE FOR ENVIRONMENTAL ANALYSES

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## MANAGEMENT PERSPECTIVE

Capillary electrophoresis in increasingly recognized as a potentially important new analytical separation technique because it brings speed, quantitation, reproducibility and automation to the very high resolving power of electrophoresis. Micellar electrokinetic capillary chromatography is based on solute partitioning between micelle phase and buffer phase. We feel that this technique would be suitable for environmental trace analysis of polar organic and inorganic pollutants.

This report describes the construction and evaluation of a unique, miniature UV-detection cell having a longitudinal flow-through design. The variable path length of the cell enhances sensitivity and thus extends the applicability of capillary zone electrophoresis to the environmental field. The variable path length flow cell was designed having a U-shaped configuration, a nanolitre volume and an optical path length of up to 10 millimetres. The optimal path length of the cell is between 3 to 4 millimetres. The design eliminates extracolumn contribution variance because there are no connectors required between the column and the cell.

An inherent feature of this design is that it can be applied to various separation techniques including HPLC and capillary gas chromatography. It can be employed as a selective detector by monitoring a UV-wavelength that is specific for selected contaminants.

The detection cell was evaluated for the determination of polar phenolic analytes directly in aqueous solutions. The flow through cell increased sensitivity for the analytes under study by up to 300 times that of conventional on-column detection.

## SOMMAIRE À L'INTENTION DE LA DIRECTION

L'électrophorèse sur colonne capillaire est de plus en plus reconnue comme nouvelle technique de séparation analytique importante parce qu'au pouvoir de résolution très élevé de l'électrophorèse s'ajoutent la rapidité, la possibilité de quantification, la reproductibilité et l'automatisation. La chromatographie sur colonne capillaire par effets électrocinétiques des colloïdes micellaires est fondée sur le fractionnement du soluté entre la phase micellaire et la phase tampon. Nous estimons que cette technique conviendrait bien à l'analyse de polluants organiques et inorganiques polaires présents à l'état de trace dans l'environnement.

Le présent rapport décrit la construction et l'évaluation d'un nouveau type de cuve miniature, longitudinale à circulation pour la détection dans l'ultraviolet. La longueur variable de la cuve augmente la sensibilité et permet donc d'appliquer l'électrophorèse de zone sur colonne capillaire à l'analyse d'échantillons prélevés dans l'environnement. La cuve à circulation de longueur variable est en forme de U, possède un volume de l'ordre du nanolitre et un trajet optique pouvant atteindre 10 millimètres. Le trajet optique optimal de la cuve se situe entre 3 et 4 millimètres. Cette conception élimine la variance attribuable à des facteurs autres que ceux relatifs à la colonne grâce à la suppression des raccords entre le tube capillaire et le système de détection.

Une caractéristique inhérente de ce modèle est qu'il peut être appliqué à différentes techniques de séparation, notamment à la chromatographie liquide à haute performance et à la chromatographie en phase gazeuse sur colonne capillaire. Il peut être utilisé comme appareil de détection sélectif par l'utilisation d'une longueur d'onde UV caractéristique des contaminants choisis. La cuve de détection a été évaluée pour le dosage direct dans des solutions aqueuses de substances polaires cibles comme les phénols. La sensibilité de cette méthode pour les substances cibles à l'étude est jusqu'à 300 fois supérieure à la détection classique par lecture directe sur la colonne.

## ABSTRACT

Greatly improved sensitivity is reported for micellar electrokinetic capillary column separations by using a novel variable pathlength flow cell of nanolitre volume instead of conventional on-column detection. The extracolumn variance has been overcome through the elimination of connections between the capillary and the detection system. Enhanced efficiency of approximately 180,000 plates/meter and the improved separation for a mixture of phenols using a 50 $\mu$m diameter capillary column demonstrate the merits of the design. The 10 nL, 6 mm long flow cell exhibited an approximate 300 fold increase in sensitivity. Construction details are provided.

# RÉSUMÉ

Comparativement à la détection classique par lecture directe sur la colonne, les séparations sur colonne capillaire par effets électrocinétiques des colloïdes micellaires sont beaucoup plus sensibles grâce à une nouvelle cuve à circulation de longueur variable dont le volume est de l'ordre du nanolitre. La variance due à des facteurs autres que ceux relatifs à la colonne a été éliminée grâce à la suppression des raccords entre le tube capillaire et le système de détection. Les avantages de cette méthode sont une plus grande efficacité, soit environ 180 000 plateaux théoriques par mètre et une meilleure séparation d'un mélange de phénols sur une colonne capillaire de 50 $\mu$m de diamètre. La cuve à circulation de 10 nL et de 6 mm de longueur est environ 300 fois plus sensible. Le présent rapport renferme les détails de construction du système de détection.

## INTRODUCTION

Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MECC) have become rapidly advancing analytical techniques. They employ high potential fields resulting in highly efficient separations of ionic and nonionic micellar solutes. High efficiency combined with the ability to handle ultrasmall volumes of sample make these techniques very promising for many environmental separations.

The major drawback with micellar electrokinetic chromatography in environmental analysis is the lack of highly sensitive and selective detectors. This stems from the fact that the physical properties of the analytes and the electrophoretic buffers often overlap. While a suitable compromise can be found in selective detection, the physical similarities between the solutes and separation media make universal detection very difficult. With these limitations, detectors based on the principles of the Beer-Lambert law, have been dominant in this area. Recently, ultrasensitive UV-absorption detector cells have been described (1,2) for capillary and microbore liquid chromatography and this type of cell could potentially be utilized in MECC. Small column dimensions and narrow solute bands demand detectors with minimal dead volume and fast responses in order to maintain the efficiency inherent in these techniques. In addition, detection methods must not affect the potential field across the capillary column, and therefore, detectors that are capable of detection within the capillary itself have been predominant. At present, UV absorption detection, although very versatile, has been shown to be less sensitive than amperometric detectors (3,4).

In this paper, we explore the potential of a longitudinal UV absorption cell in capillary zone electrophoresis with normal and micellar solutions. First, we report on the use of a UV-absorption cell that is a part of the capillary tubing itself and demonstrate the advantage of removing a variance caused by the extracolumn contribution of connectors. Second, the UV behaviour of several phenols of environmental concern in

micellar solutions is presented. Finally, from this data, we assess the strength and limitations of UV-detection with the longitudinal flow cell arrangement.

## EXPERIMENTAL

### Reagents

All standards were purchased from Aldrich Chemical Co., Milwaukee, Wisc., U.S.A., and dissolved in water at concentrations shown in Table 2. These solutions were kept at 10°C until use. Monobasic and dibasic sodium phosphate were purchased from Mallinkrodt Co., Paris, Kentucky, U.S.A. Doubly distilled water was boiled for 10 minute for degassing. A buffer solution of pH 7 was prepared by mixing 210 mL of 0.025 M $Na_2B_4O_7$ and 250 mL 0.05 M $NaH_2PO_4$. To this buffer 6.62 g of sodium dodecyl sulfate (SDS) was added to make a 0.05 M SDS solution. This solution was then passed through a 0.45 $\mu$m filter. The concentrations of analytes in the test solutions are given in Table 1.

### Separation System

A component based system from SpectroVision Inc. (5) consisting of a DA-30 power supply and a DS-4 sample delivery system was employed. The capillary electrophoresis system was equipped with an open capillary tube (20 to 200 $\mu$m i.d.) that ran between two aqueous buffer solution reservoirs. The DA-30 high voltage power supply (0-30 kV) could be used in either a constant current or a constant voltage mode of operation. Operating parameters were controlled through membrane keys and selected values displayed on LED control panel. The DS-4 sample delivery accessory consisted of platinum cathode and anode electrodes positioned in small plastic reservoirs containing buffer solution.

## Injection

Electrokinetic injection was employed to introduce samples into the capillary column (7). The reservoir at the positive side of the high voltage end of the capillary was removed and replaced with a reservoir containing the sample. Voltage was applied for short time intervals of 3 and 5 sec, generally using a separation voltage of 18 kV. The buffer reservoir was then replaced and the separation was allowed to proceed. All calibration standards were prepared in the buffer solution without SDS. The injected amount was calculated using the equation given by Burton et al.(8). The injected quantities (Q) are given in Table 3 and injected amounts were calculated as follows:

$$Q = V_c.C_{std}.t_i/t_r$$

where Q is the injected quantity, $V_c$ is the column volume (nL), $t_i$ is the injection time (sec) and $t_r$ is the retention time (sec) of the analyte and $C_{std}$ is the concentration of the analyte in ng/$\mu$L. For this equation to be valid, the analyte must be dissolved in the electrophoretic buffer and the injection voltage must be the same as the voltage used for the analysis. The response of the UV-detector to analytes passing through the flow cell was monitored.

## Construction of Capillary Flow Cells

An 80 cm length of 50 $\mu$m i.d. fused silica tubing (250 $\mu$m o.d.) from Polymicro Technologies, Phoenix AZ, was used to construct the cells. Two windows were made employing the technique described by Lux et al. (6). These windows were approx. 1.5 mm in length and were 1.7 to 6 mm apart, depending on the insert used, and were located 40cm from the beginning of the capillary tubing. The tubing was pressurized to 10 psi (0.7 atp) with helium and a 90 degree bend was made at the 40 cm mark of the first window. This L-shaped fused silica capillary was then passed through the hole in the insert and a very fine oxygen rich flame was used to make another 90

degree elbow on the detector side of the insert, exactly where the second window was located. This U-shaped capillary tubing was mounted in the cell holder depicted in Figure 1 and cemented into place using silicone rubber sealant.

The resulting flow cell had an optical path that could be varied between 1.7 to 6 mm and a volume of 11 nL or less. Since the cell and electrophoretic capillary column are integrated into one piece of tubing, dead volume as a result of connectors is completely eliminated. Also measured values of absorbence are significantly greater than those obtained using a 50 $\mu$m i.d. on-column capillary flow cell due to the longer optical path.

## RESULTS AND DISCUSSION

In capillary zone electrophoresis special requirements are imposed on the cell volume of a detector. The total volume of the system represents the sum of the individual volumes of the column, the injector, the detector and the unions. These can be expressed as statistical variances and contribute to the deterioration of the separation process. It is generally accepted that the detector volume should be at most 1:10 of the peak volume to avoid introducing extracolumn broadening effects (9). This means, volumes must include all connections to the capillary column as expressed by the contribution of the column variances:

$$\sigma^2_{tot} = \sigma^2_{col} + \sigma^2_{inj} + \sigma^2_{det} + \sigma^2_{union} + \sigma^2_{elec}$$

Thus, the total variance of the CZE system equals the sum of each of its individual sources of dispersion caused by the capillary, the injector volume, the detector volume, the connecting union dead volumes and the response time of the electronics.

Standard detectors for micro liquid chromatography columns of 1 mm i.d. cannot be adopted for MECC. This means cell volumes must be less than 100 nL. The specifications of different path length flow cells constructed from 50 $\mu$m i.d. capillaries are given in Table 2.

The shape of the detector cell and its design are also important. It turns out that the physical lengths of the eluted zones are essentially the same, if the detector's inner diameter is identical to that of the corresponding capillary tubing. U-shaped flow cells designed as described earlier, provide higher sensitivities than those described in (1). The gain in sensitivity obtained, using a properly designed U-cell under CZE conditions is directly related to the increase in path length, as shown in Table 2. The internal diameter of all cells is 0.05 mm. The optical transmittance of the flow cells showed very good linearity. The resulting capillary column efficiencies for various phenols and different cell path lengths are illustrated in Figure 2.

**Column Dispersion Variance**

As previously shown (1,2), the effect of extracolumn variance can be simply evaluated by calculating the decrease in plate number for the different path lengths of flow cells. Since we were able to eliminate the contribution of the unions to the variance, we assume that the major contribution to the variance is due to the detector path length. Comparing the on-column cell variance of longitudinal flow cells having different path lengths illustrates an acceptable simplified means of estimating the maximum allowable pathway for achieving maximum sensitivity of the UV-detection system with minimum loss of theoretical plates due to band broadening.

As shown in Fig. 3, loss in column efficiency as measured by a decrease in the theoretical number of plates results from increasing the cell path length because the analyte although injected as a narrow band, remains in the cell for a longer length of time.

**Sensitivity**

The detector sensitivity was evaluated for the four analytes in terms of minimum detectable amount (MDA) of solute (10). Two test solutions containing four solutes were injected onto the electrophoretic capillary as described previously. The minimum detectable amounts using a S/N ratio of 5:1 are given in Table 4 in terms of nanograms of solute that reached the UV cell. These values reflect the sensitivity of the electrophoretic system using UV-detection. A noise level of $4.2.10^{-4}$ a.u. at 260 nm was observed and the detection limits achieved for all analytes were excellent relative to those obtained employing conventional on-column detection.

The injected quantities of different analytes in nanograms are shown in Table 4. The loss of efficiency (L.E.) of a capillary column can be expressed as a ratio of second moments (standard deviations, $\sigma$) of the two zones. It can be calculated from the theoretical plate number, N of these two peaks as:

$$L.E. = N_{theo}/N_{obs}$$

where $N_{theo}$ is the theoretical plate number for the 0.05 mm on-column flow cell and $N_{obs}$ is the observed value for a different path length of the longitudinal U-shaped cell (11). We assume that peaks represent concentration profiles having gaussian shapes. Thus, $\sigma$ is proportional to the width of the peak along a given length of the cell and the $\sigma$ of the zone can be calculated as:

$$\sigma = L/\sqrt{N}$$

where L is the cell path length and N is the theoretical plate number of an analyte. Data obtained for phenol are tabulated in Table 5.

Electropherograms depicted in Figure 4 show that even a 4.02 mm path length cell exhibits only about a 30% loss in efficiency, which makes the cell an acceptable alternative for very diluted samples. A 197 times increase in integration units was observed in comparison to the 0.05 mm on-column cell, which is very significant for the UV-detector and makes it an attractive detector for environmental analysis in micellar electrokinetic separations.

## CONCLUSION

It is well recognized that the MECC and capillary electrophoresis are in the development stages and their ultimate utility in the environmental field remains to be evaluated. There are many applications from different fields for which the micellar electrokinetic separation technique is much more efficient than high performance liquid chromatography. We have found that the MECC technique is simple to use. However, the limited elution range and detector sensitivity in aqueous buffered solutions are presently identified as serious constrains of the technique. On the other hand, benefits may overweigh current limitations. In this paper we have reported our contributions to improve the detection limits obtained using UV-spectrophotometry by means of a U-shaped flow-through cell. The results presented were obtained using a simple test mixture and equipment. This cell design utilizes variable path length inserts that are easily interchangeable and substantially eliminate variances caused by connecting tubings, and provide significantly greater sensitivities than conventional on-column detection.

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**Table 1. Specification of Testing Solutions**

| Analyte | R.T. (min) | Standard 1 (ng/μL) | Standard 2 (ng/μL) |
|---|---|---|---|
| **Phenol** | 6.07 | 26.3 | 263.0 |
| **1-Phenyl ethanol** | 7.82 | 72.5 | 725.0 |
| **o-Cresol** | 8.40 | 29.5 | 295.0 |
| **2,6-Dimethyl phenol** | 10.94 | 25.0 | 250.0 |

**Table 2. Specification of the Flow Cells**

| Parameter | On-Column | Capillary Flow Cells | | | |
|---|---|---|---|---|---|
| Volume (nL) | 2.00 | 3.60 | 6.80 | 7.90 | 10.50 |
| Path length (mm) | 0.05 | 1.85 | 3.45 | 4.02 | 5.37 |
| Configuration | Perpendicular | Longitudinal | | | |

**Table 3. Responses of Various Analytes Using Different Pathway Cells**

| Analyte/Path length, mm | 0.05 | 1.85 | 3.60 | 4.02 | 5.37 |
|---|---|---|---|---|---|
| Phenol | 6420 | 504 700 | 940 620 | 1,045 133 | 1,526 210 |
| 1-Phenyl ethanol | 2200 | 308 040 | 573 210 | 641 770 | 767 060 |
| o- Cresol | 2150 | 270 030 | 501 865 | 579 210 | 745 430 |
| 2,6-Dimethyl phenol | ND | 68 700 | 149 470 | 182 230 | 241 240 |

Note : Responses are given in integration area counts.

**Table 4. The Injected Quantities of Selected Analytes Using Electrokinetic Injection at Nanogram Level**

| Analyte | M.W. | Electrokinetic Injection at Two Concentration Levels | | | |
|---|---|---|---|---|---|
| | | S-1 3 sec | S-1 5 sec | S-2 3 sec | S-2 5 sec |
| Phenol | 94 | 0.274 | 0.457 | 2.74 | 4.57 |
| 1-Phenyl ethanol | 122 | 0.556 | 0.933 | 5.56 | 9.33 |
| o-Cresol | 108 | 0.224 | 0.373 | 2.24 | 3.73 |
| 2,6-Dimethyl Phenol | 122 | 0.146 | 0.243 | 1.46 | 2.43 |

S-1 and S-2 refer to Standard 1 and 2.

**Table 5. Observed Losses in Efficiency of the Capillary Column for Different Path Length Cells**

| **Path length, mm** | | **0.05** | **1.85** | **3.60** | **4.02** | **5.40** |
|---|---|---|---|---|---|---|
| **N** | [TP/m] | 158 800 | 138 200 | 126 800 | 121 700 | 75 600 |
| $\sigma^2$ | [$min^2$] | 0.000125 | 0.00498 | 0.01010 | 0.01150 | 0.01964 |
| **L.E.** | [ %/100] | 1.000 | 1.147 | 1.252 | 1.305 | 2.100 |

L.E.- loss in efficiency

**Table 6. The Injected Quantities of Different Analytes at Two Injection Times in Nanograms**

| Compound | M.W. | Time of injection in seconds | | | |
|---|---|---|---|---|---|
| | | Standard 1 | | Standard 2 | |
| | | 3 s | 5 s | 3 s | 5 s |
| Phenol | 94 | 0.274 | 0.457 | 2.74 | 4.57 |
| 1-Phenyl ethanol | 122 | 0.559 | 0.933 | 5.60 | 9.33 |
| o-Cresol | 108 | 0.224 | 0.373 | 2.24 | 3.73 |
| 2,6-Dimethyl phenol | 122 | 0.146 | 0.243 | 1.46 | 2.43 |

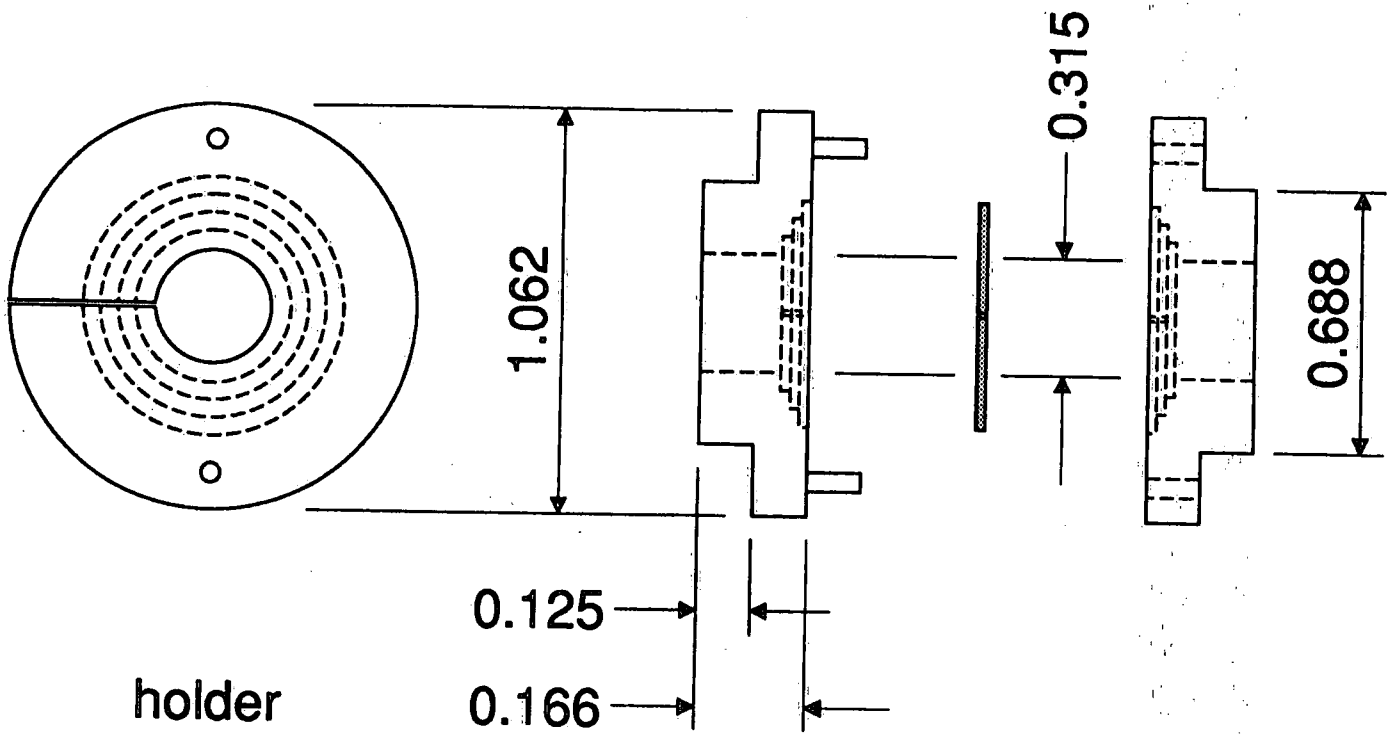
## FIGURE CAPTIONS

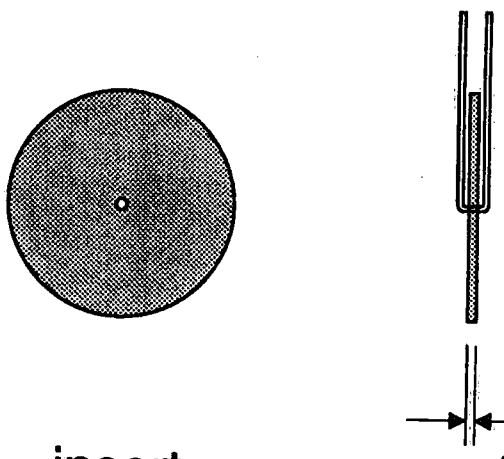
Figure 1. U-Flow cell construction details.

Figure 2. Linearity of responses for analytes under study for four selected path lengths.

Figure 3. Observed losses of number of theoretical plates due to an increased cell path length.

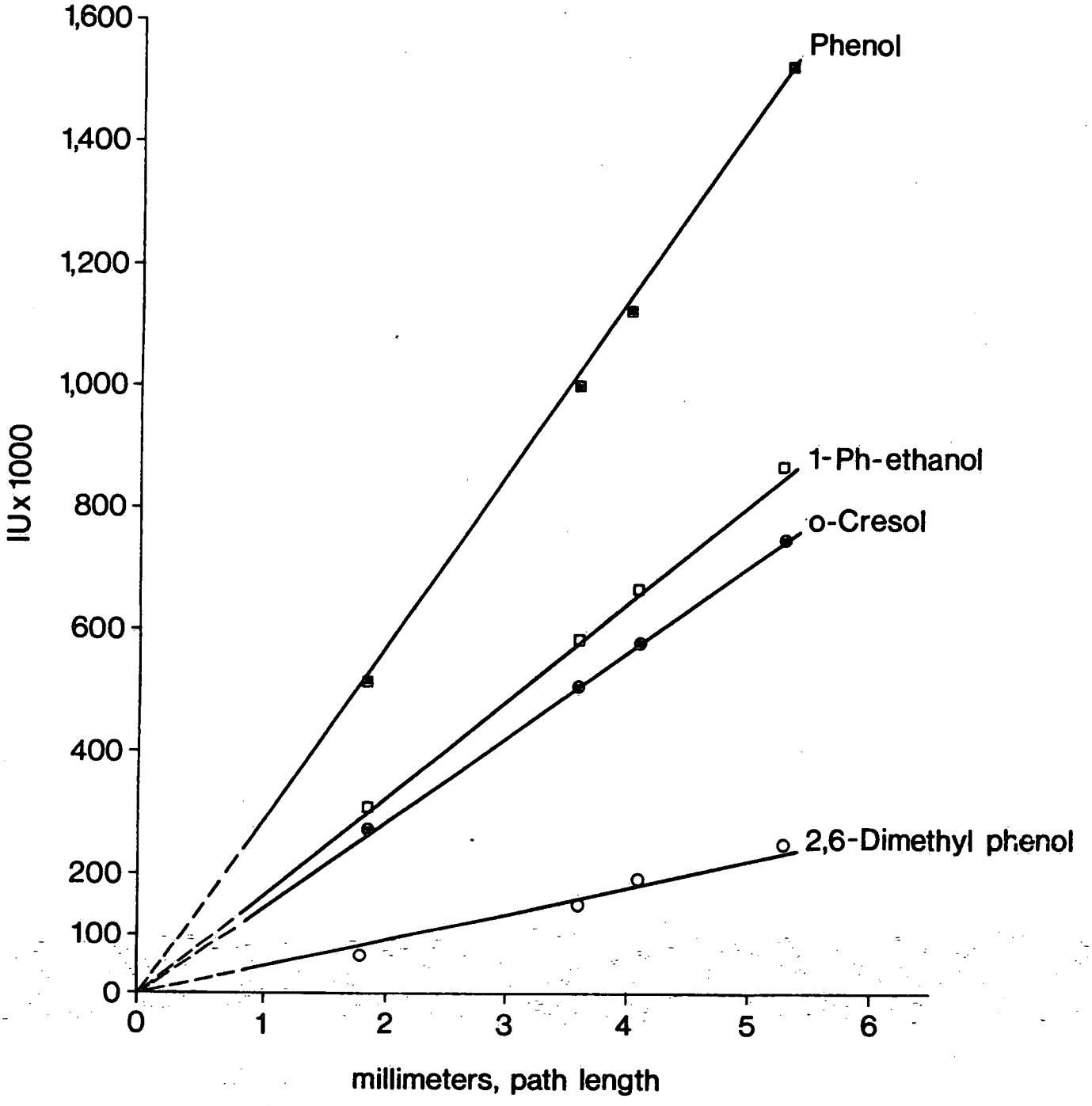
Figure 4. Electropherograms showing flow cell performances for separation of phenolics. 1 phenol; 2 1-phenyl ethanol; 3 o-cresol; 4 2,6-dimethyl phenol.



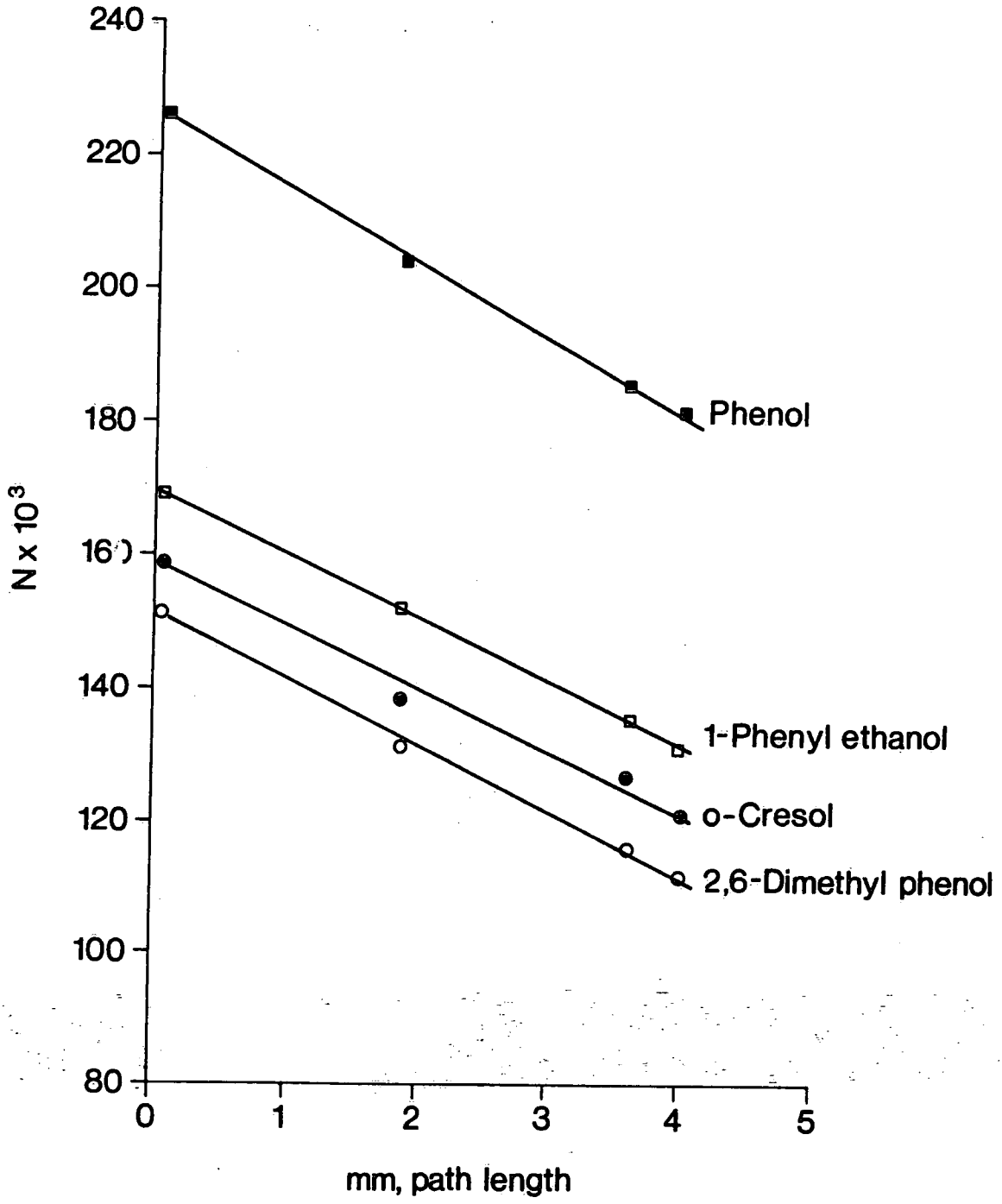

all dimensions in inches

FIG. 1




Linearity of responses for four analytes vs. cell path length

Fig. 2.




Observed loss of theoretical plates vs. path length

Fig 3.

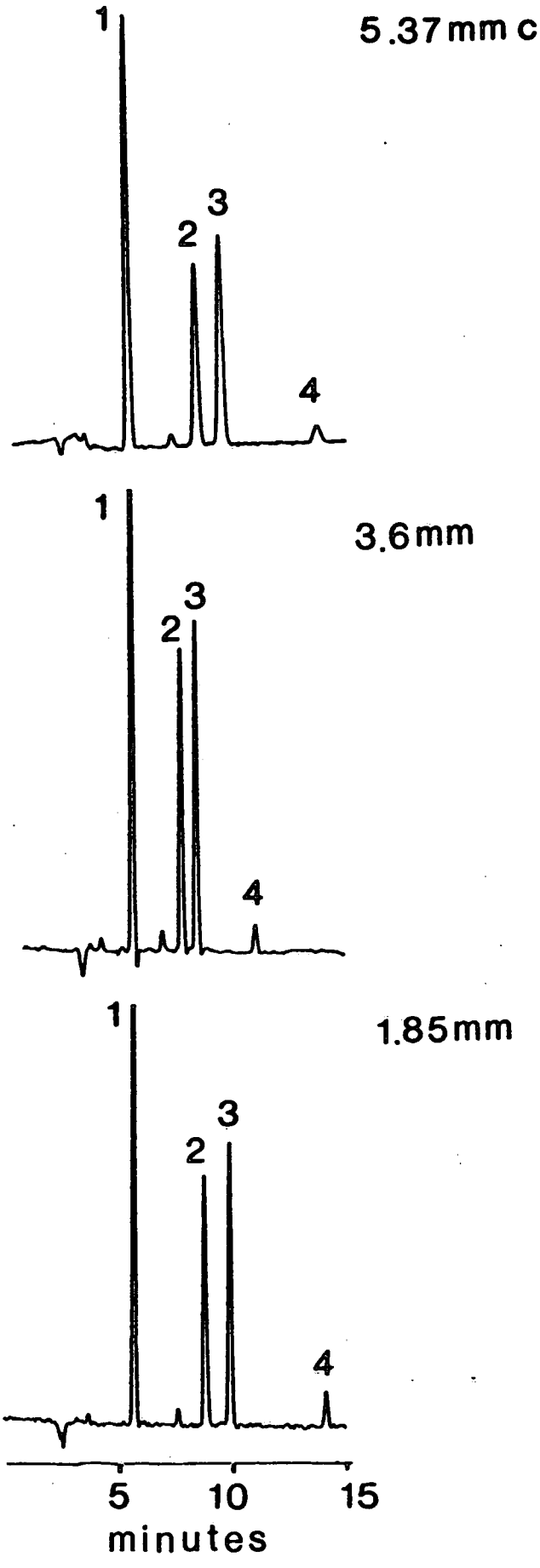
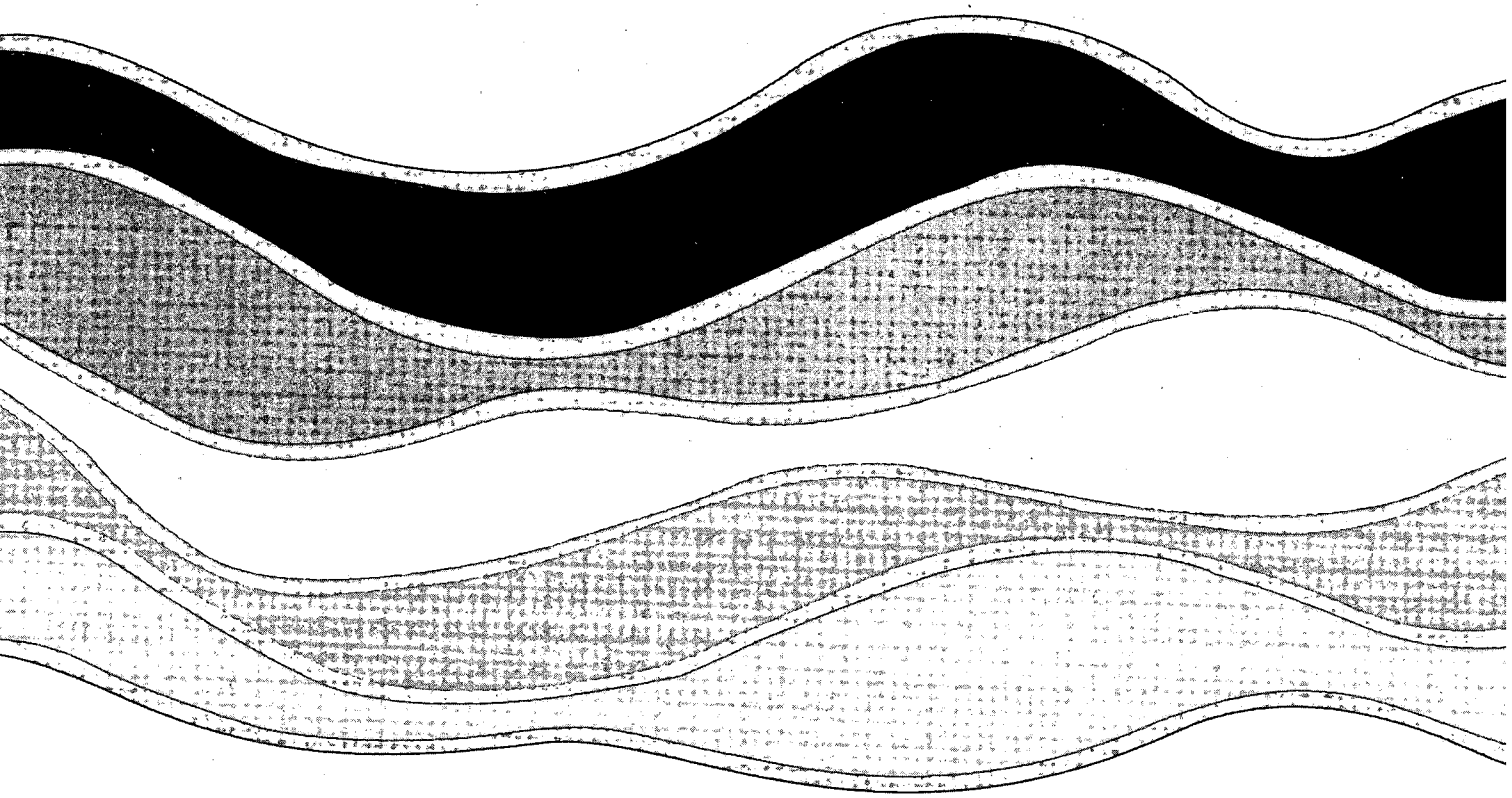



Fig. 4.



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