Journal of Chromatography, 462 (1989) 147–154
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 001

ADJUSTABLE APERTURE-WIDTH DETECTOR CELL FOR ON-COLUMN DETECTION IN CAPILLARY ZONE ELECTROPHORESIS

TIANSONG WANG and RICHARD A. HARTWICK*
Department of Chemistry, Rutgers University, Piscataway, NJ 08854 (U.S.A.)
and
PAUL B. CHAMPLIN
Applied Analytical Technologies, Inc., P.O. Box 668, Narragansett, RI 02882 (U.S.A.)
(First received August 19th, 1988; revised manuscript received September 27th, 1988)

SUMMARY

An on-column UV–VIS detector cell with an adjustable aperture width (coaxial to the capillary) for capillary zone electrophoresis was made and evaluated. The cell increased signal-to-noise ratio almost 6 fold and expanded the linear range of detection about one order of magnitude as compared with a 1.0 mm diameter aperture cell. Capillaries can be readily installed, removed and repositioned with the new design. The relationship between aperture width and observed column efficiency was established by computer simulation and varified by experiment. Both theoretical and experimental results conformed that an aperture width $\leq 1\sigma$ of the peak width will contribute an efficiency loss of no more than 10%. The cell was applied to the separation of monophosphate nucleotides.

INTRODUCTION

Capillary zone electrophoresis (CZE) or high-performance capillary electrophoresis (HPCE) is a very attractive separation technique. Because capillaries with 50–75 μm I.D. allow efficient dissipation of heat, suppress convection and permit faster exchange of molecules between the wall and the center of capillary, CZE can generate 10^5–10^6 theoretical plates within 30 min[1,2]. Although CZE is still in the early stage of development, it has successfully been applied to analysis of varied samples, such as proteins, amino acids, nucleosides, inorganic ions and neutral molecules.

The miniaturization of electrophoresis into capillary format brings numerous advantages, but also creates some difficulties, one of which is detection. For example, the standard deviation of a peak with a retention time 500 s and $2.5 \cdot 10^5$ theoretical plates is 1 s. In a capillary with 50 μm I.D. and a linear velocity of 1 mm/s, one second standard deviation corresponds to only 2 nl in volume. The detector cell volume must be small enough and the detector sensitivity high enough to meet these detection requirements without significant zone dispersion.

In order to cope with the nanoliter volume detection of capillary separations, on-column or in-column[3] detectors have been almost universally used, employing

0021-9673/89/$03.50

fluorescence[4,5], electrochemistry[6,7] and ultraviolet (UV) absorption[8,9] detection methods. UV detectors, though less sensitive than fluorescence detectors, are still the most widely used, because of their relative versatility. Yang[10] constructed an on-column UV detector by stripping the polymer coating of a capillary and placing the capillary into the light path of a detector. Terabe *et al.*[9] used a UV detector with 0.05 × 0.75 mm slit. Walbroehl and Jorgenson[8] used a 100 μm pinhole as the aperture of the detector cell. Spino *et al.*[11] fabricated a detector cell by glueing a capillary and two razor blades onto a cell block, producing an aperture about 6 mm × the capillary inner diameter. Kientz and Verweij[12] made a cell aperture by drilling a 0.4 mm diameter hole into the outer holder of the capillary. Foret *et al.*[13] fabricated an on-column UV detector based on optical fibers. In these designs, the aperture of the detector cell seems to have been selected arbitrarily, even though some authors[4,8,11] have discussed qualitatively the role of aperture width on detector performance.

The design of on-column UV detector cell for CZE should consider three important aspects: (1) Light should pass only through the inner diameter of capillary. When a large amount of light passes through the rim of the capillary, one result is that the signal will be very sensitive to the refractive index changes of solution and the distance between the capillary and photodetector[14]. Another result is that the signal-to-noise ratio (S/N) and the linear range of detection will be reduced[8]. (2) Aperture should have a width (along the axis of capillary) that keeps the efficiency loss within a predefined limit, or ideally the width should be adjustable to meet different requirements. (3) Installation and removal of capillaries should be convenient and accurate.

In this paper, a design of an adjustable aperture-width detector cell for CZE is presented which can meet these requirements. The performance of the new cell were evaluated experimentally. The relationship between the aperture width and apparent column efficiency was established by computer simulation, then verified by experiment.

EXPERIMENTAL

Detector cell construction

Fig. 1 is a schematic diagram of an adjustable aperture-width cell. The aperture body is constructed by sandwiching a shim between two pieces of metal (either stainless steel or brass), then the body is glued on the base. The washer with slits is rotatable. The aperture depends on both the thickness of the shim (25–75 μm) and the dimension of the slit (0.2–2 mm) on the washer. The aperture width along the axis of capillary can be changed by rotation of the washer. On the aperture body, there is a fine groove which retains the capillary in the correct position in the light path. Installation of a capillary is readily accomplished by loosening the capillary retainer, placing the capillary into the groove and tightening the retainer.

Evaluation of the new cell

The new cell aperture (60 μm × 0.95 mm) was examined on a Kratos SF770 UV detector (Applied Biosystems, Ramsey, NJ, U.S.A.) by static method (filling the capillary with test solution with no flow and applied voltage). Fused-silica capillary was 75 μm I.D. × 195 μm O.D. (Polymicro Technology, Phoenix, AZ, U.S.A.).

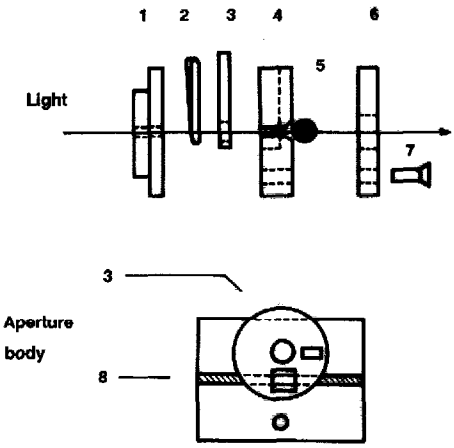

Fig. 1. The schematic diagram of the new cell. (1) Base (Plexiglass); (2) spring; (3) washer with slits; (4) aperture body (stainless steel or brass); (5) capillary; (6) capillary retainer (Plexiglass); (7) screw; (8) shim.

Acetophenone in acetonitrile was used to determine signal-to-noise ratio and linear range of detection. The wavelength of detection was 240 nm ($\varepsilon = 1.3 \cdot 10^4$). "Gradient elution" was performed by alternatively filling the capillary with 100% methanol or methanol–water (60:40), its absorbance being measured at 330 nm.

Since no commercial cells for CZE or capillary HPLC currently are available with which to compare the new cell's performance, an SF770 standard flow cell was used. The capillary was taped on the surface of the SF770 cell and across the aperture (1.0 mm diameter), after which the signal-to-noise ratio and linear range of detection were measured.

In order to demonstrate the performance of the new cell, a mixture of nucleotides (AMP, CMP, GMP and UMP, 1 mg/ml each) was analysed (the instrumentation is described below).

Aperture width and observed column efficiency

Computer simulation of the effect of aperture width on observed column efficiency was performed on an IBM compatible personal computer, using EUREKA software (Borland International, Scotts Valley, CA, U.S.A.).

A CZE apparatus similar to that described by Jorgenson and Lukacs[2] was constructed and used to verify the simulation results. The same detector and cell mentioned above were used. The power supply (0–30 kV) was a model PS/MJ30P0400-11 (Glassman High Voltage, Whitehouse Station, NJ, U.S.A.). Buffer solution was 0.05 *M* sodium dodecyl sulfate (SDS) in borate–phosphate solution (pH = 7.0)[9]. Fused-silica capillary was 50 μm I.D. × 355 μm O.D. The total capillary length was 84 cm, while the length from the injection end to the detector was 60 cm. The capillary was rinsed with 0.1 *M* potassium hydroxide (20 min, about 100 μl), water and the buffer solution respectively, then was conditioned under high voltage for 24 h. The test sample was 0.6 mg/ml thymidine in same buffer solution. Injection: 2.0 kV and 10 s. Analysis: 20 kV and 32 μA. The wavelength of detection was 267 nm, time constant was 0.05 s. All data were collected with MAXIMA™ chromatography software

(Dynamic Solutions, Venture, CA, U.S.A.) at a sampling rate of 20 points/s. The data were then downloaded to Lotus 123™ (rev. 2.01) software (Lotus Development, Cambridge, MA, U.S.A.) where second moments of the peaks were calculated.

RESULTS AND DISCUSSION

Performance of the new cell

Higher signal-to-noise ratio is especially important to the CZE detector, because the amount of sample introduced into the capillary is very limited. In addition to low electronic noise, a well designed cell is critical to enhance the signal-to-noise ratio. Table I presents the data of signal and noise measured with the new cell and with the SF770 cell. From this table, it is seen that although the noise measured with the new cell (60 μm × 0.95 mm aperture) is 2.5 times higher than that with the SF770 cell (1.0 mm diameter aperture), the signal obtained from the new cell is 14.7 times higher than that from the 1.0 mm circular aperture cell, resulting in an overall improvement in signal-to-noise ratio of 5.9-fold.

Measurements of the linear range of detection indicate that the upper limit of concentration is about $3 \cdot 10^{-3}$ *M* for both cells mentioned in Table I. However, the lower limit is different, being $3 \cdot 10^{-5}$ *M* for the new cell (S/N = 2.3, measured), $2 \cdot 10^{-4}$ *M* for the SF770 cell (S/N = 2.5, extrapolated), because the new cell produces a higher signal-to-noise ratio. Therefore, the linear range of detection with the new cell is expanded about one order of magnitude. When using detectors with reduced noise, the linear range and detection limit could be improved further.

As an on-column detector, the capillary as well as the solution in it acts as a lens in the light path. When the photodetector is positioned far from the capillary, the baseline response is sensitive not only to the refractive index of solution, but also to the position of the capillary. Vindevogel *et al.*[14] recommended that gradient elution was a quick method for cell quality determination. The new cell has been examined for refractive index sensitivity by examining baseline shifts using 100% methanol and methanol–water (60:40) solution. The results are summarized in Table II. The data in Table II show that the baseline shift is about 0.02 a.u. This relatively large shift is due largely to the long distance (about 4 cm) between the cell and the photodetector on SF770 detector, which has been shown to cause refractive index sensitivity in the literature[14]. Table II shows that the repeatability of absorbance measurement is good, the maximum difference is $6.2 \cdot 10^{-3}$ a.u. This result indicates that the groove on the new cell can reliably position the capillary.

TABLE I

COMPARISON OF SIGNAL AND NOISE MEASURED WITH TWO CELLS*

Cell	*Aperture dimension*	*Signal (a.u.)*	*Noise (a.u.)*	*S/N*
New cell	60 μm × 0.95 mm	$2.2 \cdot 10^{-2}$	$1 \cdot 10^{-3}$	22
SF770 cell	1.0 mm diameter	$1.5 \cdot 10^{-3}$	$0.4 \cdot 10^{-3}$	3.7

* $3 \cdot 10^{-4}$ *M* acetophenone, 75 μm I.D. × 195 μm O.D. capillary.

TABLE II

BASELINE SHIFT MEASURED WITH NEW CELL (60 μm × 0.95 mm APERTURE)

Solutions	*Absorbance (10^{-3} a.u.)*				
	*1**	*2*	*3*	*4*	$\bar{x} \pm$ *S.D.*
Methanol–water (60:40)	24.4	19.4	22.6	25.6	23.0 ± 2.7
Methanol	1.6	3.1	1.3	1.4	1.9 ± 0.8
Δa.u.	22.8	16.3	21.3	24.2	21.2 ± 3.4

* 1, 2, 3 and 4 means the absorbance values are measured after reinstallation of capillary.

Fig. 2 is an electropherogram obtained using the new cell. The separation is quite good. The column efficiency calculated from the UMP peak is $6.5 \cdot 10^4$ theoretical plates (10% peak height).

Aperture width and observed column efficiency

Required detector cell volumes are often estimated using the model of a mixing chamber[15]. However, this consideration is not applicable for an on-column detector, since the separation is still in progress in an on-column detector cell. In this case, the apparent column efficiency or resolution, as Guthrie and Jorgenson[4] have pointed out, depends on the aperture width along the axis of capillary. However, no quantitative discussion was presented in their paper.

The quantitative relationship can be readily derived as follows. Imagine a gaussian function, g(x), which represents the concentration profile generated by a separated compound, an aperture with a width W is the mathematical equivalent of a rectangle placed over this function, the signal obtained from the aperture at any instant being given by

$$\text{Signal} = \int_{x-W/2}^{x+W/2} g(x)\mathrm{d}x/W \qquad (1)$$

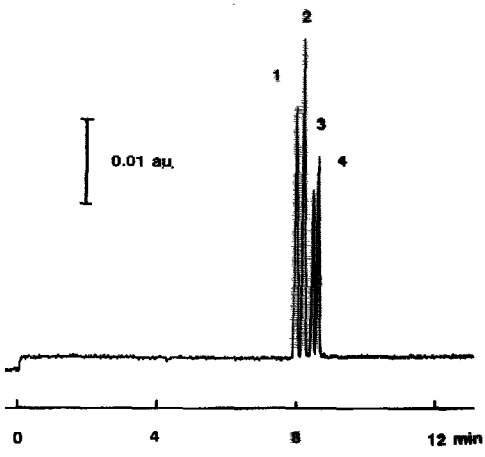

Fig. 2. Separation of nucleotides. 1 = GMP; 2 = AMP; 3 = CMP; 4 = UMP (about 1 mg/ml). 50 μm × 355 μm capillary, 60 cm long to detector. Detector cell aperture, 60 μm × 1.4 mm. 0.05 M SDS in borate–phosphate buffer (pH = 7.0). Injection, 2.0 kV and 15 s. Analysis, 25 kV and 50 μA.

The observed function, f(x), will be given by the set of signals calculated from eqn. 1. The loss in efficiency can be expressed as the ratio of second moments:

$$\text{Distortion} = M_{2\text{obs}}/M_{2\text{tru}} = N_{\text{tru}}/N_{\text{obs}} \tag{2}$$

where M_2 is second moment of a peak and N is the theoretical plate number of the peak. The result of a computer simulation according to this consideration is shown in Fig. 3.

From Fig. 3, it can be seen that when the aperture width is larger than 1σ, the distortion becomes significant and increases non-linearly with increasing aperture width. For example, when the aperture width is 3.5 σ, the distortion is about 2, that is, the observed column efficiency is only half of the true efficiency. A regression equation is obtained from the data of Fig. 3:

$$\text{Distortion} = 0.993 + 0.0152W + 0.0771W^2 \tag{3}$$

where W is aperture width in σ units. The regression coefficient is 0.99999.

The theoretical and experimental results relating the effect of aperture width on observed column efficiency are compared in Table III. In general, good agreement is found between experiment and theory. Both experimental and theoretical results indicate that if the aperture width is $\leq 1\sigma$ of the peak width, the loss in column efficiency resulting from aperture width will be no more than 10%. Narrower the aperture width will reduce the loss in column efficiency, but conversely, increase noise. Therefore, it is recommended that when designing an on-column optical detector, aperture width should be about 1σ (in length unit) of the peak interested.

The σ of a peak can be calculated from eqn. 4,

$$\sigma = L/\sqrt{N} \tag{4}$$

where L is the column length from the injection end to the detector. Typically, L is 500 mm and N is $3 \cdot 10^5$, producing a σ value of 0.9 mm.

Another effect of aperture width is decreasing signal intensity. The computer

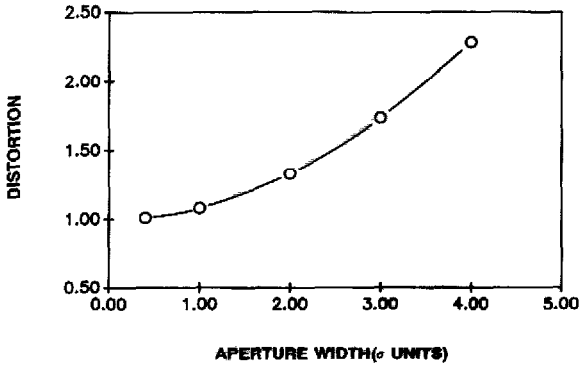

Fig. 3. The computer simulation results of the effect of aperture width on observed column efficiency.

TABLE III

EFFECT OF APERTURE WIDTH ON OBSERVED COLUMN EFFICIENCY

Parameters	*Aperture width*			
	0.22 mm (0.26σ)*	*0.41 mm (0.49σ)*	*0.95 mm (1.13σ)*	*1.4 mm (1.7σ)*
M_{2exp}**	0.33	0.35	0.36	0.39
Distortion (exp)***	1.00	1.06	1.09	1.18
Distortion (eqn. 2)	1.00	1.02	1.11	1.23

* From the peak of thymidine (retention time 6.81 min) $\sigma = M_2^{1/2} \times$ velocity of band $= 0.33^{1/2} \times 600/6.81 \times 60 = 0.84$ mm.

** Second moment (s^2), $n = 3$, S.D. $= 0.01$ s^2.

*** The M_2 value obtained from 0.22 mm aperture width was assigned to the true M_2 value.

simulation results indicate that observed peak height will decrease with increasing aperture width. For example, as the aperture width increases from 0.1 to 1.0σ, peak height decreases 4%. At present, the precision of peak height measurement in this laboratory is not good enough to verify this theoretical expection.

CONCLUSION

The new cell shown in Fig. 1 was made and evaluated. This cell increases signal-to-noise ratio almost six times and expands the linear range of detection about one order of magnitude over that observed using a simple 1-mm hole for an aperture. The capillaries can be easily installed, removed and repositioned into the new cell.

Computer simulation of the effect of aperture width on apparent column efficiency was performed. The relationship between aperture width and observed column efficiency has been established (eqn. 3) and verified experimentally. Both theoretical and experimental results indicate that when the aperture width $\leq 1\sigma$ of the peak width, the loss in column efficiency resulted from aperture width will be no more than 10%.

ACKNOWLEDGEMENTS

Supports for this research was provided in part by the Center for Advanced Food Technology (CAFT), and by a grant from the PQ Corporation.

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