Journal of Chromatography, 315 (1984) 135–143
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,186

ON-COLUMN UV ABSORPTION DETECTOR FOR OPEN TUBULAR CAPILLARY ZONE ELECTROPHORESIS

YVONNE WALBROEHL and JAMES W. JORGENSON*
Department of Chemistry, University of North Carolina, Chapel Hill, NC 27514 (U.S.A.)
(Received August 29th, 1984)

SUMMARY

A fixed-wavelength on-column UV absorption detector has been developed for open tubular capillary electrophoresis. The detection limits are 15 pg for isoquinoline and 250 pg for lysozyme. Detector response is linear over three to four orders of magnitude for each solute tested. This approach to detector design solves many problems encountered in the use of modified commercial high-performance liquid chromatography detectors. Examples of separations obtained by capillary zone electrophoresis with UV detection are given, including separations in non-aqueous and mixed media.

INTRODUCTION

High-voltage zone electrophoresis performed in open tubular capillaries offers several advantages over more conventional approaches to electrophoresis[1]. Efficient cooling of the separation compartment allows the use of very high voltages, resulting in high-resolution separations in a short time period. Since no stabilizers are used, zone broadening effects such as "eddy migration" and adsorptive interactions between solutes and stabilizers are eliminated. Under ideal circumstances, molecular diffusion is the primary source of zone broadening. On-line detection devices permit accurate measurement of migration times and zone concentration profiles in a format similar to a chromatogram.

Detection is a critical factor in the development of capillary zone electrophoresis. Separation efficiencies of up to one million theoretical plates have been reported for small ions with this technique[2]. Efficiencies of up to several million theoretical plates may be realized for proteins and other large solutes, if adsorption to the inside surface of the capillary can be eliminated[2]. The high separation efficiency of capillary zone electrophoresis makes small volume a requirement for a detection device. The low sample capacity of the capillary necessitates a highly sensitive detector. In our laboratory, these problems have been approached through the use of on-column optical detectors. Initial work involved the use of a home-made fluorescence detector[3]. This detector is quite sensitive, but limits detection to fluorescent solutes. Recent improvements on this detector have made it applicable to a wider range of solutes[4,5],

0021-9673/84/$03.00

but it remains that solutes must fluoresce in order to be detected. Preparation of fluorescent derivatives is sometimes a solution to this problem, but derivatization is not always feasible, so more general detection schemes are warranted.

UV absorption is a more general mode of detection which is useful for many solutes of interest. Previous work with on-column UV detection in capillary separation techniques has involved the use of modified commercial HPLC detectors[6–9] or detectors designed for capillary isotachophoresis[10]. None of these detectors has a sufficiently small illumination slit to accommodate capillaries of 80 μm I.D. or less.

Our experience with a modified Jasco UVIDEC 100-II HPLC detector revealed several problems. First, the source, a deuterium lamp, offers modest intensity over a broad spectrum, but relatively low intensity over narrow bandwidths. Secondly, our modification[9] makes no provision for securing the capillary firmly in the optical path. This presents two problems. There is no way to secure the capillary in an optimum position in the optical path, for greatest signal-to-noise ratio. There is also no way to ensure that the capillary will occupy the same spot in the optical path during subsequent experiments, so reproducibility is poor. Also, the capillary can vibrate in the optical path upon application of very high voltages, resulting in a noisy baseline. Finally, the slit width is much greater than the inner diameter of the capillary, so much of the light reaching the photodetector has not passed through the capillary. This has two effects. First, the linear dynamic range of the detector is reduced. Secondly, the maximum absorption signal that can be produced is relatively small, and signal-to-noise ratio is reduced.

In our laboratory, a UV absorption detector has been designed and built specifically for open tubular capillary zone electrophoresis. This detector has a "slit" width of only 100 μm, and thus spatial resolution is very good. It uses interchangeable line sources which offer high intensity over narrow bandwidths. The detector is linear over four orders of magnitude with detection limits as low as 15 pg for isoquinoline.

INSTRUMENTAL

Optical layout

A schematic diagram of the optical layout for the on-column UV detector is given in Fig. 1. The UV source is a 7-W cadmium "pen-ray" lamp (CD-480, Ultra-Violet Products, San Gabriel, CA, U.S.A.). This lamp emits radiation from both sides, so emission from one side of the lamp is used as the reference beam and the other is used as the sample beam. For added flexibility, it may be interchanged with zinc and mercury lamps of a similar design. The reference and sample beams pass through 100-μm pinholes (1546, Oriel, Stratford, CT, U.S.A.). The positions of the pinholes are adjusted through the use of pinhole positioning mounts (1538, Oriel). The pinhole mounts were slightly modified so that the capillary can be mounted flush to the pinhole. The capillary is clamped in place by two nylon set screws on either side of the pinhole. The use of a "reference capillary" is optional and was omitted in this work because it was of no advantage. The beams are then passed through interference filters (229 nm, No. 2290/200, Barr Assoc., Concord, MA, U.S.A. or 326 nm, No. P10-326-A, Corion, Holliston, MA, U.S.A.) to select the wavelength of interest. The transmitted light is then detected by two photomultiplier tubes (R212, Hamamatsu, Japan).

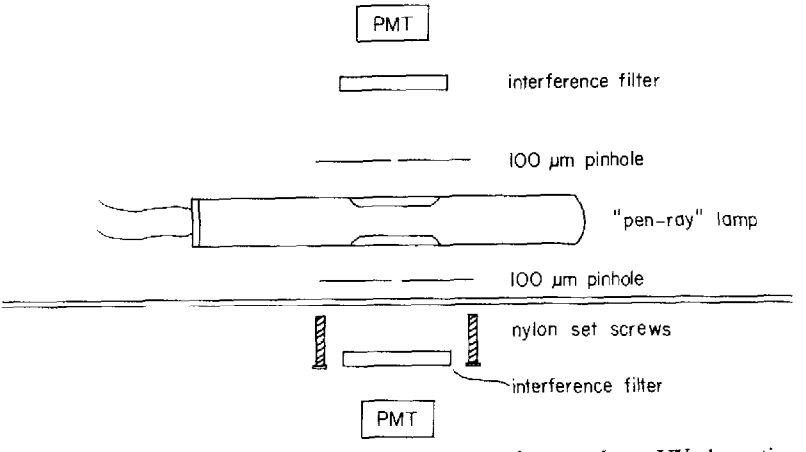

Fig. 1. Exploded schematic diagram of optical layout for on-column UV absorption detector.

The optical system is held together by a detector block, which was machined from aluminum, 15.2 × 12.7 × 5.1 cm. The lamp is held within the block by a large set screw. The lamp position may be optimized by loosening the set screw and moving the lamp until the desired position is attained. The pinhole positioners, filter mounts, and housings of the photomultiplier tubes (PMT) are attached to one another and to the detector block. The position of the optical path with respect to the capillary is optimized via the moveable pinhole. This design solves most of the problems which were encountered with the modified commercial high-performance liquid chromatography (HPLC) detector.

Signal processor

In preliminary work, signal processing was achieved by a home-made analog electrometer. This consisted of current-to-voltage transducers with variable gain, for both signal and reference channels, followed by an analog subtractor and a low-pass filter. This method of signal processing was abandoned in favor of a microcomputer controlled signal processor. It consists of two current-to-voltage transducers with variable gain. The voltage outputs of the two channels are read simultaneously by two 16-bit integrating analog-to-digital converters (IADC), which are controlled by an Atari 800 microcomputer (Atari, Sunnyvale, CA, U.S.A.). The computer is equipped with a total of four 16-bit IADC's, two 16-bit digital-to-analog converters, two disk drives, a printer, and a plotter. The raw data is stored in the computer's RAM and may also be stored on disk. Actual signal processing occurs after the electropherogram is collected. It is accomplished by a BASIC program and involves either subtraction of the reference channel data from the sample channel data, similar to the analog circuit, or calculation of absorbance. The latter is preferable, because of increased linear dynamic range. However, it is more time consuming than a simple baseline subtraction. In this work, the absorbance calculation method was used.

Electrophoresis set-up

Electrophoresis was performed in fused-silica capillaries which varied in length

from 75 to 100 cm and were 75 μm I.D. (Scientific Glass Engineering, Austin, TX, U.S.A.). A regulated high-voltage d.c. power supply (Bertan Assoc., Syosset, NY, U.S.A.) delivering from 0 to 30 kV was used to drive the electrophoresis. A small section of the polyimide coating was removed from the fused-silica capillary to permit transmission of UV light through the capillary. The fused silica is transparent to about 190 nm. This set-up has been described in detail elsewhere[1,2,11–13].

EXPERIMENTAL

"Static" linearity test

The American Society for Testing and Materials has recently published a static linearity test for fixed-wavelength UV absorption HPLC detectors[14]. This method evaluates the linearity of the detector independent of chromatographic factors such as injection and column effects. A similar method was used to evaluate the capillary electrophoresis detector, without introducing complication from the electrophoretic injection technique. To test for linearity at 229 nm, lysozyme and isoquinoline were used. At 326 nm, acridine and basic fuchsin were the test solutes. Stock solutions were made for each compound and series of standards were made by serial dilution of the stock solutions. Lysozyme was dissolved in an aqueous buffer, while the other compounds were dissolved in acetonitrile with an acidic modifier.

The "static" linearity test was carried out as follows. The detector output was zeroed with the sample capillary filled with solvent. The capillary was then flushed via suction with a standard solution until a constant detector reading was observed on the computer monitor. At this point, the capillary was flushed with solvent until the baseline returned. Three readings were made for each concentration of standard.

Electrophoretic technique

This technique has been published in greater detail elsewhere[1,2,11–13]. Capillaries were filled with the electrophoretic medium of choice. This was done by dipping one end of the capillary into the solution and applying suction. Once the capillary was filled, both ends of the capillary were dipped into beakers containing the electrophoretic medium. The positive end of the capillary, where samples were introduced, was attached to the high-voltage power supply with a graphite electrode. The negative end of the capillary was connected to ground in a similar fashion. The detector was located on-column about 15 cm from the negative (ground) end of the capillary. Samples were injected by electromigration[1].

RESULTS AND DISCUSSION

Linearity

Scott[15] has stated that the function

$$y = Ac^r \tag{1}$$

where y is the detector response, c is the sample concentration, A is a constant, and r is the response index, can be used to describe the output of all detectors which are approximately linear. Thus the equation

$$\log y = \log A + r \log c \tag{2}$$

TABLE I

LINEARITY DATA: RESPONSE INDEX AND CORRELATION COEFFICIENT

Compound	*Wavelength (nm)*	*Response index (r)*	*Corr. coeff.*
Isoquinoline	229	0.963	0.9997
Lysozyme	229	0.999	0.9991
Acridine	326	0.960	0.9994
Basic fuchsin	326	0.983	0.9970

arises, which should be linear with a slope of r. Scott arbitrarily stated that for the response of a particular detector to be linear, r should lie between 0.98 and 1.02. The closer r is to unity, the more linear the response. It should be pointed out that "non-linear" detectors can still be utilized, as long as r is known with sufficient accuracy. The standard curves for the four test solutes were computed in log–log format using a linear least squares fit. The r values as well as the correlation coefficients for the curves are given in Table I. The detector shows good linearity at both 229 and 326 nm for the solutes tested. Isoquinoline and acridine have r values slightly less than 0.98, which may be a result of a dilution error during sample preparation or decomposition of the solute over time. In all cases, the correlation coefficients are very close to unity, so a calibration curve can be used with good accuracy.

The linear dynamic range was four orders of magnitude for lysozyme and basic fuchsin and greater than three orders of magnitude for isoquinoline and acridine. At higher concentrations, there is a severe problem with non-linearity. This could be caused by two problems. One is that at very high solute concentrations, there are deviations from Beer's law, due to changes in the refractive index of the sample solution. The other is the fact that the "slit" or pinhole size is larger than the inside diameter of the capillary. Thus a certain amount of light reaches the photodetector without first passing through the sample. This was meassured by drawing a totally opaque sample into the capillary and measuring the percentage transmittance (%T). Isoquinoline–acetonitrile (1:1) was used as the sample. The %T was measured as 7.24%, which corresponds to an absorbance of 1.14. As sample absorbances approach this value, significant non-linearity will be observed. For the low absorbances normally encountered in open tubular capillary electrophoresis, the detector possesses adequate linearity. A solution to this problem would be to use a smaller pinhole. The next smaller commercially available pinhole is 50 μm in diameter. This is

TABLE II

DETECTION LIMITS

Compound	*Wavelength (nm)*	*MDC (mol/l)*	*MDA*	
			pg	*fmol*
Isoquinoline	229	$8.6\ 10^{-7}$	15	120
Lysozyme	229	$2.8\ 10^{-7}$	250	18
Acridine	229	$1.4\ 10^{-6}$	150	820
Acridine	326	$6.9\ 10^{-6}$	710	3900

smaller than the capillary, but reduces light throughout to one-fourth of its previous value, and therefore increasing detector noise. Since the 100-μm pinhole offers good linearity at absorbances commonly encountered in capillary electrophoresis, and is less costly than smaller pinholes, it has been retained in the design.

Sensitivity

The detector sensitivity was evaluated for the four compounds in terms of minimum detectable concentration (MDC). MDC is defined as the sample concentration producing a signal-to-noise ratio of two. The MDC was determined during the "static" linearity test and is thus independent of the electrophoretic process. It is also useful to know the detection limit of the entire system in terms of minimum detectable amount (MDA) of solute.

In order to consider the detection limit of the electrophoretic technique with UV detection, it is necessary to evaluate the effect of applying high voltage to the capillary on detector noise level. As high voltage is applied to the capillary, the detector noise level increases. The increase in noise is proportional to voltage and at 30 kV, the detector noise is twice as large as it is with no voltage applied. So if 30 kV is applied, the MDC is twice its value with no voltage applied.

To determine the minimum detectable amount (MDA) of solute in capillary electrophoresis with on-column UV detection, samples of three of the four test solutes were injected onto the electrophoresis set-up described previously. The minimum detectable amounts are given in Table II in terms of grams and moles of solute. These values reflect the sensitivity of the electrophoretic system with UV detection. The noise level is $1 \cdot 10^{-4}$ a.u. at 229 nm and $6 \cdot 10^{-5}$ a.u. at 326 nm. The greatest source

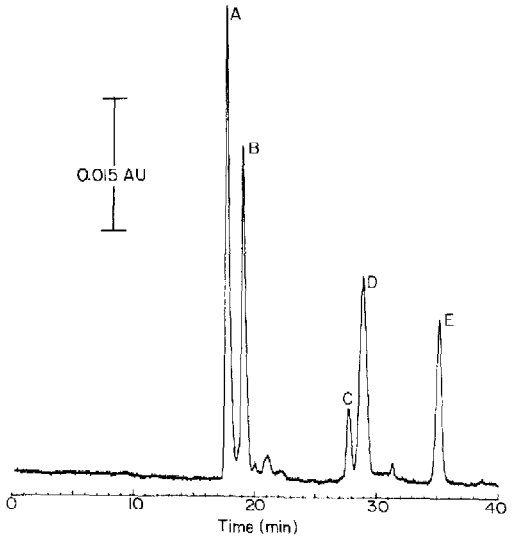

Fig. 2. Electropherogram of protein standards, the concentration is 0.2% (w/v) in each protein. Surface modified fused-silica capillary[2]; 75 cm × 75 μm I.D., filled with 0.025 *M* phosphate buffer at pH 6.86; applied voltage, 15 kV. A, egg white lysozyme; B, horse heart cytochrome *c*; C, bovine pancreatic ribonuclease *a*; D, bovine pancreatic α-chymotrypsinogen; E, equine myoglobin.

of noise is probably due to a mismatch in the intensity fluctuations of the channels of light. This could perhaps be improved by using the output of only one side of the pen-ray lamp with a beam splitter. The detection limits for all compounds are good, and that for isoquinoline is especially so. Since the absorption maximum for isoquinoline lies near 229 nm, the detection limit is much lower than for the other test solutes. Better results could be obtained for the other test solutes by operating nearer to their absorption maxima. This would require the use of other line sources, or a variable-wavelength source.

Applications

To show the utility of the UV detector in capillary zone electrophoresis, some representative separations are included. Fig. 2 shows a separation of five proteins in a surface modified fused-silica capillary with detection at 229 nm. This separation was reported previously[2] using a modified HPLC detector. The new detector shows a reduction in drift and low-frequency noise. Since the peptide bond absorbs at 229 nm, the detector is generally used for proteins. Fig. 3 shows a separation of five quinoline-type compounds in a non-aqueous medium with detection at 229 nm. This is the first reported separation by capillary electrophoresis in a totally non-aqueous medium, and it shows that non-aqueous capillary electrophoresis deserves further investigation. It is particularly interesting to note that a baseline separation was obtained for quinoline and isoquinoline, which are geometric isomers. Since the two

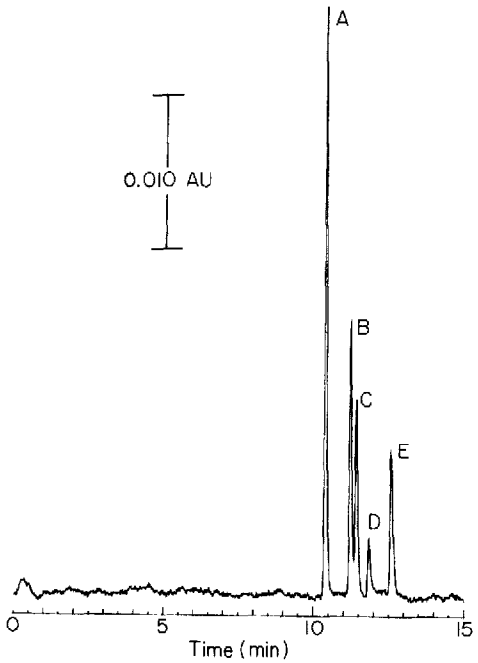

Fig. 3. Electropherogram of standard organic bases in non-aqueous medium. Untreated fused-silica capillary; 100 cm × 75 μm, filled with 0.05 *M* tetraethylammonium perchlorate, 0.01 *M* hydrochloric acid in acetonitrile. Applied voltage, 20 kV. A, $5 \cdot 10^{-4}$ *M* isoquinoline; B, $5 \cdot 10^{-4}$ *M* quinoline; C, $5 \cdot 10^{-4}$ *M* quinaldine; D, $1 \cdot 10^{-3}$ *M* 8-quinolol; E, $1 \cdot 10^{-3}$ *M* acridine.

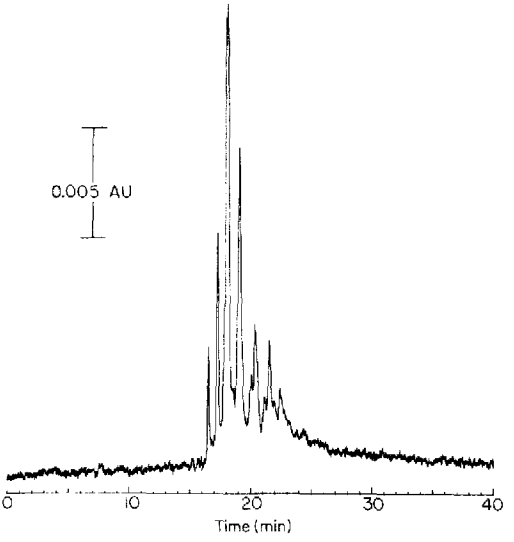

Fig. 4. Electropherogram of basic fraction of solvent refined coal in mixed solvent medium. Untreated fused-silica capillary, 100 cm × 75 μm I.D., filled with 0.05 *M* tetraethylammonium perchlorate, 0.01 *M* HCl in acetonitrile–water (75:25); applied voltage, 20 kV. Detection wavelength, 229 nm.

have identical molecular weights, and in the solvent used should be of the same charge, one might expect them to have identical migration times. One explanation for their separation may be differences in solvation geometry. Fig. 4 shows the use of capillary zone electrophoresis with UV detection applied to a complex sample. It is a separation of the basic fraction of solvent refined coal in a mixed water–organic medium. No attempt was made to identify the zones. These examples show the applicability of capillary zone electrophoresis with UV detection to a variety of samples and demonstrate that the technique has promise in the area of electrophoretic separations in non-aqueous media.

ACKNOWLEDGEMENTS

We thank W. S. Woodward for his assistance with electronic and computer aspects of this work. We also thank Ernest Dawes of Scientific Glass Engineering for the kind gift of fused silica. Support for this work was provided by E. I. duPont de Nemours and Company, by the National Science Foundation under grant CHE-8213771, and by the University Research Council of the University of North Carolina.

REFERENCES

1 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
2 J. W. Jorgenson and K. D. Lukacs, *Science*, 222 (1983) 266.
3 E. J. Guthrie and J. W. Jorgenson, *Anal. Chem.*, 56 (1984) 483.
4 E. J. Guthrie, J. W. Jorgenson and P. R. Dluzneski, *J. Chromatogr. Sci.*, 22 (1984) 171.
5 J. S. Green and J. W. Jorgenson, in preparation.
6 F. J. Yang, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 83.

7 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 113.
8 T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.*, 248 (1982) 241.
9 K. D. Lukacs, *Ph.D. Thesis*, University of North Carolina, Chapel Hill, NC, 1983.
10 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
11 J. W. Jorgenson and K. D. Lukacs, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 230.
12 J. W. Jorgenson and K. D. Lukacs, *Clin. Chem.*, 27 (1981) 1551.
13 J. W. Jorgenson and K. D. Lukacs, *J. Chromatogr.*, 218 (1981) 209.
14 *Standard Practice for Testing Fixed-Wavelength Photometric Detectors Used in Liquid Chromatography*, ANSI/ASTM E 685-79, American Society for Testing and Materials, Philadelphia, PA, 1979.
15 R. P. W. Scott, *Liquid Chromatography Detectors*, Part 1, Elsevier, Amsterdam, 1977, Ch. 3, p. 9.