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# Axial-beam absorbance detection for capillary electrophoresis

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## ABSTRACT

A novel approach to absorbance detection has been demonstrated for capillary electrophoresis utilizing an incident light beam directed along the capillary axis. The resulting absorption path length is roughly equal to the width of the sample bands present. This represents a 60-fold improvement in analytical path length over conventional on-column absorbance detectors without any loss of separation efficiency. The method can be used with completely aqueous buffer systems or those containing organic modifiers, and is applicable to both fused-silica and PTFE capillaries.

## INTRODUCTION

The spread of capillary electrophoresis (CE) applications in recent years has led to the development of many detection schemes, each with its own degree of selectivity and sensitivity to fit a specific purpose. Two characteristics have allowed absorbance detection to earn its place among the most popular of these techniques. First, if the proper incident wavelengths are chosen (*e.g.*, the ultraviolet region), this one detector can be used for almost every class of organic compound. Second, this technique usually does not require sample derivatization or special buffer considerations.

Ultra-high separation efficiencies and low mass detection limits, which have become the main attraction of CE, require that detector volumes be small, thus favoring on-column detectors. By convention, on-column absorbance detection in CE has been carried out with the incident light beam directed perpendicular to the capillary walls [1–3]. While some of these designs have demonstrated reasonable detection limits owing to high light source stability, they are seriously hindered by short absorption path lengths. Diffusion processes along the axis of a CE column keep the minimum band width attainable to about 3 mm [4,5]. If, instead of the standard configuration, one directs the light beam along the capillary, then the entire 3-mm band width becomes the absorption path length. In a 50-$\mu$m capillary, given the same source stability, a 60-fold improvement in detection limits could be obtained with no loss of separation efficiency.

Increasing analytical path lengths by directing the incident light beam along the axis of a capillary cell is not a new idea. Fuwa and co-workers [6–8] obtained analytical path lengths in excess of 1 m in glass and polymer capillaries. Axial absorbance

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detection has also been demonstrated for smaller capillaries incorporating open-tubular liquid chromatography [9]. In that application, a laser beam was focused into one end of a 10-$\mu$m fused-silica capillary using a single-element lens. With the proper choice of solvents (*i.e.*, solvents having a greater refractive index than that of fused silica), light entering the capillary is transmitted by total internal reflection at the interface between the solution and the capillary wall. At the capillary exit, light is collected with a microscope objective and imaged onto either a photomultiplier tube or a photodiode.

This research involves the application of the axial-beam technique to detection in CE. Two strategies have been studied. The first of these takes advantage of the partial internal reflectance at the water/fused-silica interface to propagate light along the capillary axis. The second utilizes polymers of low refractive index and buffer systems with organic modifiers to bring about total internal reflectance.

## EXPERIMENTAL

### *Axial detection system*

Fig. 1 shows a schematic diagram for axial detection. The light source was a 10-mW, 632-nm He–Ne laser (GLG5261, NEC, Mountain Valley, CA, USA). The beam was directed through two mirrors on pivoting mounts (2 × 2 in.; Newport, Fountain Valley, CA, USA) and focused using a 2.5-cm focal-length lens (KPX076; Newport) mounted on a three-dimensional translational stage. Both the front and rear cells were constructed from Plexiglas blocks with channels bored in a three-dimensional "T-shape" to allow introduction of the capillary, indicent light beam, electrode wire and liquid buffer. These cells contained windows fabricated from mi-

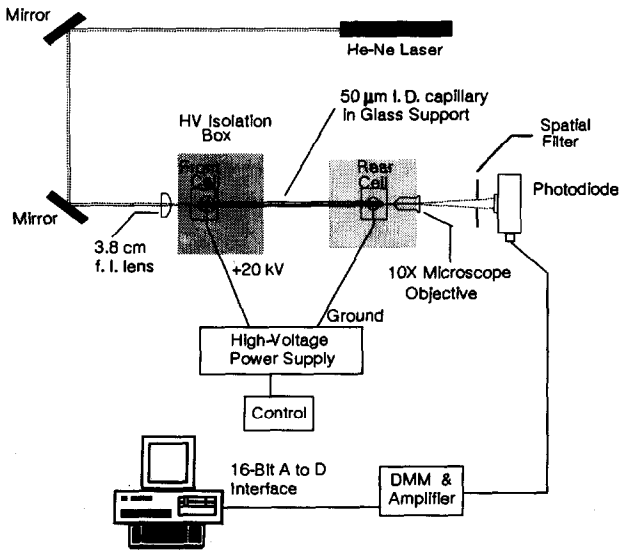


Fig. 1. Schematic diagram of axial absorbance detection in CE. The dotted lines indicate the path of the laser beam. A to D = Analog-to-digital; DMM = digital multimeter; f.l. = focal length; HV = high-voltage.

croscope slides and were sealed on all sides except the top with epoxy (Devcon, 5-min). The capillary was mounted in a glass support (borosilicate glass tubing, 25 cm × 5 mm O.D., × 1 mm I.D.) by gluing at both ends and through three evenly spaced holes drilled perpendicular to the length of the tube. This support was used to maintain rigidity and straightness.

Light emitted from the capillary exit was collected with a 10× microscope objective (BM2888, 16 mm, Bausch and Lomb, Rochester, NY, USA) and focused onto a spatial filter (size ranging from 1 to 10 mm depending on the distance between the objective and the filter). The resulting beam intensity was converted to an electrical signal via a photomultiplier tube (R928; Hamamatsu, Middlesex, NJ, USA) or a photodiode (1 $cm^2$ area, S179004N; Hamamatsu) and amplified through a digital multimeter (160B; Keithley Instruments, Cleveland, OH, USA). Data collection and analysis were accomplished using an IBM AT computer with a 16-bit analog-to-digital conversion board (2827; Data Translation, Marlboro, MA, USA). A smoothing algorithm [10] was applied to sequential data files, providing an effective time constant compatible with the injection peak widths.

*Beam profile measurements*

Intensity profiles at the exit of the capillary were measured by focusing the exiting light through the 10× microscope objective onto a white background at the location of the spatial aperture. Images were then recorded using a CCD camera (Series 200; Photometrics, Tucson, AZ, USA) through a 90-mm macro lens (Tamron, Tokyo, Japan) and converted to 50 × 50 matrices of pixel intensities.

*Electrophoresis*

The separation of bromocresol green and bromothymol blue was carried out in a fused-silica capillary (75 μm I.D., 150 μm O.D.; Polymicro Technologies, Phoenix, AZ, USA) and in a PTFE capillary (50 μm I.D., 150 μm O.D.; Zeus Industrial Products, Raritan, NJ, USA). The power supply, PS/MJ30PO400-11 (Glassman, Whitehouse Station, NJ, USA) was operated at +20 kV applied across two chromel electrodes positioned at the ends of the capillary (capillary exit at ground). Electrokinetic injection was accomplished by placing a sample solution in the front cell, activating the power supply momentarily, then flushing the front cell with running buffer prior to the separation.

*Buffers and reagents*

For electrophoresis in the fused-silica capillary, the running buffer consisted of 10 m*M* disodium phosphate (certified ACS grade; Fisher, Fair Lawn, NJ, USA), adjusted to pH 7.5 using phosphoric acid. When the PTFE capillary was used, the buffer was 10 m*M* disodium phosphate and 50 m*M* sodium dodecyl sulfate (Sigma, St. Louis, MO, USA) in 50% (v/v) ethylene glycol (Fisher, purified grade), adjusted to pH 7.6. All buffers were degassed by applying vacuum while the buffer bottle was agitated in an ultrasonic bath. Bromocresol green and bromothymol blue were obtained from J. T. Baker (Phillipsburg, NJ, USA).

## RESULTS AND DISCUSSION

### *Partial internal reflectance*

An advantage of this mode of axial absorbance detection for CE is that it can be carried out using the same aqueous buffer solutions and fused-silica capillary as in standard absorbance detection. According to Lei *et al.* [7], the total intensity of light transmitted through a long glass capillary cell containing water consists of light rays taking many paths through the capillary center or through the walls. The secret of obtaining a useful analytical signal from the light emitted at the exit of a very small capillary (less than 100 $\mu$m I.D.) is to separate wall-propagated light from center-propagated light. This was accomplished by imaging the exiting light beam on a spatial filter using a microscope objective. An important difference between the present work and the published results for liquid chromatographic detection [9] is evident. In the latter instance, much smaller diameter capillaries (approaching 5 $\mu$m) are needed to provide highly efficient separations. The smaller tubes are not suitable for transmitting light by partical internal reflectance because of the ratio of the cross-sectional areas of the liquid *versus* the walls is too small to allow proper discrimination. On the other hand, CE works well in capillaries up to 75 $\mu$m, and partial internal reflectance can be implemented.

An intensity profile at the exit end of the capillary, as seen at the spatial filter, is shown in Fig. 2. The peak of intensity at the middle of the image represents light propagated through the capillary center while the ring of intensity around the outside represents wall-propagated light. Interference modes within the small bore of the tube are probably responsible for the nodes seen in the center peak. This mode structure changes with heat build-up along the capillary or fluctuations in the refractive index of the buffer. Evidence for the complete spatial separation of wall- and center-propagated light can be seen when the capillary is filled with an opaque dye solution. In this instance, only the outer ring of intensity remained in the image. An aperture size for the spatial filter was chosen so as to allow only the center peak to pass on to the photomultiplier or photodiode, thus excluding wall-propagated light from the signal.

Fig. 3 is a sample electropherogram incorporating partial internal reflectance axial absorption detection. On injection of the sample, the transmitted intensity drops to a low level owing to the combined absorption of both sample components. This level is maintained as the samples migrate within the column, even though they are physically being separated by electrophoresis. The separation of bromocresol green and bromothymol blue is indicated by two sharp rises in intensity, each representing a loss in absorbance due to the elution of the individual compounds. Bromothymol

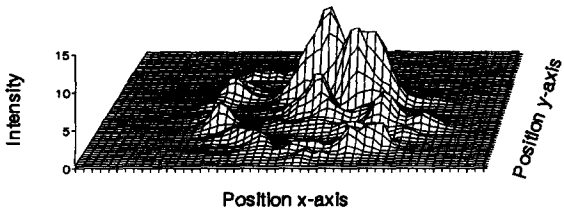


Fig. 2. Intensity profile at the exit end of a fused-silica capillary. Capillary I.D. 75 $\mu$m, O.D. 150 $\mu$m.

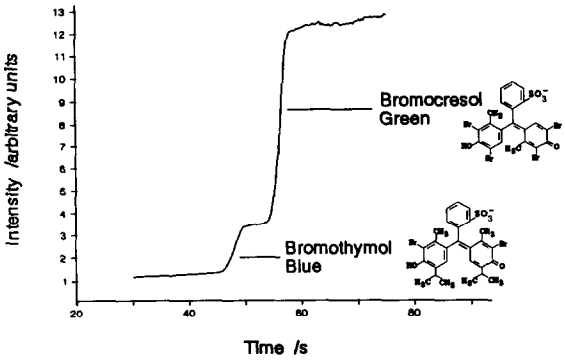


Fig. 3. Separation of bromothymol blue and bromocresol green. The separation took place in a fused-silica capillary (75 μm I.D.). Buffer, 10 m*M* phosphate adjusted to pH 7.5; analyte concentration, $2 \cdot 10^{-5}$ *M* each; applied potential +20 kV; electrokinetic injection time, 2.5 s. A data smoothing routine was applied with an effective time constant of 2.5 s.

blue elutes first, owing to its higher p$K_a$ value and consequently lower degree of ionization and lower affinity for the positive electrode. The "integrating" nature of this electropherogram results in lower detection limits because the absorbance of the entire length of each analyte band is measured continuously from the beginning of the run until elution [11]. As has been shown previously, this type of integral electropherogram (chromatogram) preserves all the information in a normal electopherogram, such as elution time, peak area and peak width. In fact, the first-derivative plot of these data produces the normal electropherogram [9].

*Total internal reflectance*

Total internal reflectance allows efficient transmission of light along the liquid-filled core of the capillary, analogous to an optical fiber. When this phenomenon is applied to axial detection, two distinct advantages are seen. The first advantage arises from the fact that all the light rays impinging on the capillary, within a certain cone of acceptance, are completely internally reflected. The results are less noise associated with small capillary vibrations in the electric field and greater reproducibility of measured absorbances. A second advantage is that as very little loss of light occurs, longer capillaries (in excess of 1 m) in conjunction with higher applied potential can be used for greater electrophoretic resolution.

The condition for total internal reflectance is that the refractive index of the liquid filling the capillary must be greater than the refractive index of the capillary itself. PTFE has a refractive index in the range 1.35–1.38 [8] and has been well characterized in zone electrophoresis [12]. Although the refractive index of water is only 1.33 [13], much less than that PTFE, organic modifiers [14] can be added to the buffer to raise the refractive index above 1.38 and may actually enhance electrophoretic resolution. We chose ethylene glycol as an additive because of its high refractive index enhancement per unit concentration added. The liquid medium naturally must remain ionic for proper electrophoresis.

Fig. 4 shows that the same two test compounds can be separated in the ethylene

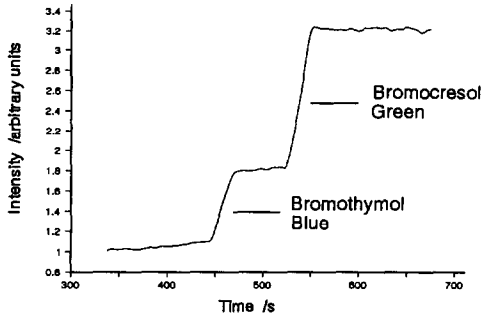


Fig. 4. Electropherogram of bromothymol blue and bromocresol green separated in a PTFE capillary (50 $\mu$m I.D.). Buffer, 50% (v/v) ethylene glycol–10 m$M$ phosphate–50 m$M$ SDS adjusted to pH 7.6; bromothymol blue concentration, $5 \cdot 10^{-5}$ $M$; bromocresol green concentration, $2 \cdot 10^{-5}$ $M$; applied potential, +20 kV; injection time, 20 s. The effective time constant was 20 s.

glycol–water-based buffer. The key ingredient in the separation buffer is 50 m$M$ sodium dodecyl sulfate (SDS) [15]. Our explanation of the role of SDS is as follows: the presence of the organic modifier favors ion-pair formation for the two test compounds. These ion pairs tend to interact with the PTFE capillary, causing band smearing. SDS micelles provide a moving organic phase which carries the analyte ion pairs along and gives each of the compounds a distinct mobility based on its affinity for the micellar phase. SDS ions also adsorb to the PTFE walls, producing a zeta potential and generating electroosmotic flow similar to that seen in silica capillaries. The evidence for enhanced electroosmotic flow is that, in the presence of SDS analyte flow is directed toward the cathode, whereas in its absence this flow is directed toward the anode.

*Detection limits*

The main source of noise limiting the signal-to-noise ratio of the transmitted light beam is capillary vibration. These effects of capillary vibration have been discussed earlier [9], and in the present instance vibration arises from electrostatic motion in the very large applied potential field. With the PTFE capillary glued onto a glass support and by applying a digital smoothing routine [10] with an effective time constant of about 5 s, the signal-to-noise ($S/N$) ratio measured over 20 s (one peak width) is 600 for the transmitted beam. This gives an absorbance limit of detection (ALOD at $S/N = 3$) of $2 \cdot 10^{-3}$.

In order to compare our results with the performance of the common cross-beam absorbance detector, we must use a criterion that is independent of the molar absorptivity of the analyte. This can be accomplished by using the ratio of the absorbance limit of detection to the absorbance path length (ALOD/$b$). For a typical cross-beam system [16], the ALOD was $5 \cdot 10^{-4}$, resulting in ALOD/$b$ = $1 \cdot 10^{-2}$/mm for a 50-$\mu$m capillary. Our system exhibits an ALOD/$b$ of $7 \cdot 10^{-4}$/mm, a 15-fold improvement over the cross-beam arrangement. It is also important to note that if the capillary diameter is decreased further, such as in the case when reduced Joule heating is needed to maintain a high separation efficiency, there will be a proportionate increase in this improvement factor. Note that we are comparing an integral electrophe-

rogram with the standard electropherogram. As pointed out earlier [11], integration of standard electropherograms is not always possible because of drifting baselines.

In summary, we have shown that an axial-beam absorption detection mode is feasible for CE. Even though a laser is used here for ease of coupling to the capillary tube, there is no reason why a conventional light source cannot be used. For example, the commercial absorption detectors for CE are already capable of collimating light from a xenon lamp to illuminate 25–75-$\mu$m capillaries. If the capillary can be held rigidly, then the higher stability of conventional light sources can even translate into better detectability. As discussed earlier [9], the present detection mode has a limited dynamic range at high concentrations because of depletion of transmitted light, but the lower concentration range is extended because of better detectability.

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