A High-Sensitivity Diode Array Detector for On-Column Detection in Capillary Electrophoresis

The small peak volumes in CE demand special optical design to maximize sensitivity. High light throughput, good stray light suppression, and precise alignment are necessary. The diode array detector design focused on good matching of the illumination system and the spectrometer, precise alignment of the capillary and optical elements, and mechanical and thermal stability.

by Patrick Kaltenbach

Ultraviolet (UV) and ultraviolet/visible (UV/Vis) absorbance detection currently is the most commonly used detection technique in capillary electrophoresis (CE). The universal nature of this detection principle, its widespread use in high-performance liquid chromatography (HPLC), and its relatively simple adaption for on-column detection are some of the reasons for its popularity.

Using a diode array detector, absorption at a large number of wavelengths can be measured simultaneously, giving spectral information. Thus the area of application can be further enlarged. Since the observed spectrum is characteristic for any analyte, this information can be used for identification of unknown compounds. Multiwavelength detection, peak identification, and peak purity determination are capabilities that make the diode array detector a powerful tool and an ideal detector for CE.

The small peak volumes in CE demand special optical design to maximize sensitivity. High light throughput, good stray light suppression, and precise alignment of the small capillary are necessary to get the best possible performance.

Optical System

Fig. 1 shows a schematic overview of the optical system of the HP CE diode array detector. A new type of prealigned deuterium lamp is used to increase the overall light throughput and thus improve the sensitivity of the system. This type of lamp has a much smaller emission spot than lamps previously used for HPLC, making it superior for illumination of small sample areas. The polychromatic light of the lamp is focused onto the detection window of the capillary by an achromatic lens system. These detection windows are usually made by removing a small section of
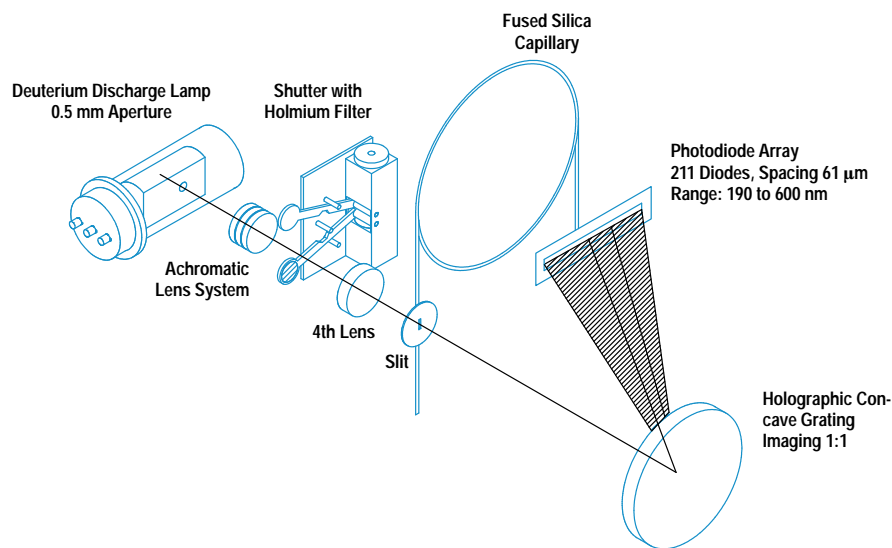
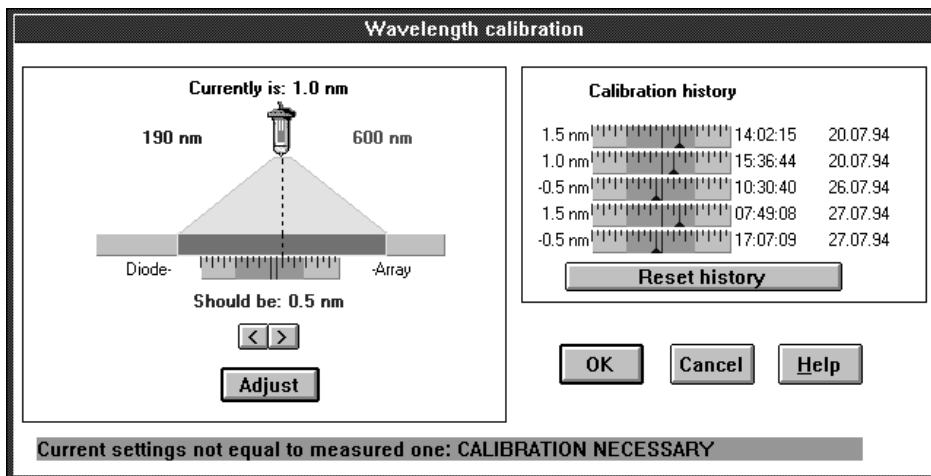**Fig. 1.** Schematic overview of the CE diode array detector optics.

Fig. 2. Wavelength calibration window.

the polyimide coating of the fused silica capillaries. A slit behind the capillary blocks stray light and defines the entrance slit of the spectrometer. The shutter is used to cut off radiation for dark current compensation or to drive a holmium oxide filter into the optical path for wavelength calibration purposes. The spectrometer consists of a holographic concave grating which disperses the light onto a photodiode array. 205 diodes are used for a wavelength range from 190 to 600 nm, which gives a resolution of 2 nm per diode. Grating, array, and signal electronics are very much the same as for the HP G1306A diode array detector and the HP 79854A multiwavelength detector for HPLC.[1]

To optimize the overall performance of the system, the design focused on good matching of the illumination system and the spectrometer, precise alignment of the capillary and optical elements, and mechanical and thermal stability. The optics bench is precision machined and exchangeable parts such as the lamp or the alignment interface (slit) are self-aligning. To maintain optical precision, the system recognizes when the user accesses these exchangeable parts. A wavelength calibration is performed every time the instrument senses a possible change and if necessary the user is asked to confirm new calibration settings (Fig. 2).

Since the spectrometer f-number of 4 represents the basic limit on the light throughput of the system, the lamp and lenses are designed to match this limitation to optimize the overall light throughput.

Ray tracing software was used to calculate aberrations, to determine theoretical optimums for positioning of the optical elements, and to maximize light throughput. Fig. 3 shows a ray tracing plot of the optical system, and Fig. 4 shows in detail a ray trace through a capillary with 150-μm inside diameter—for example, a 50-μm capillary with an extended lightpath with BF = 3 (BF = bubble factor, the increase in the diameter of the capillary at the detection window of the detector, as explained in the article on page 62).

Theory

On-column detection approaches often use setups similar to that shown in Fig. 5. In a small area near the outlet the capillary is illuminated with light from a UV/Vis light source, and a slit of width o is placed behind the capillary to block stray light through the capillary wall. The light transmitted through the slit is detected. Using the Lambert-Beer law, the detector signal for an ideal cell is determined by:

$$A(\lambda) = e(\lambda)CD = \log \frac{I_0(\lambda)}{I(\lambda)},$$

where $A(\lambda)$ = absorbance in absorbance units (AU) as a function of wavelength, $e(\lambda)$ = extinction or molar absorption
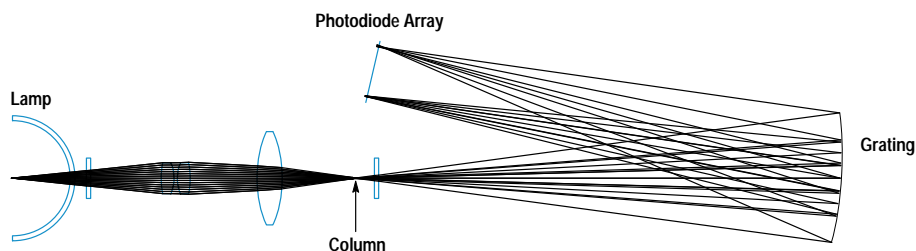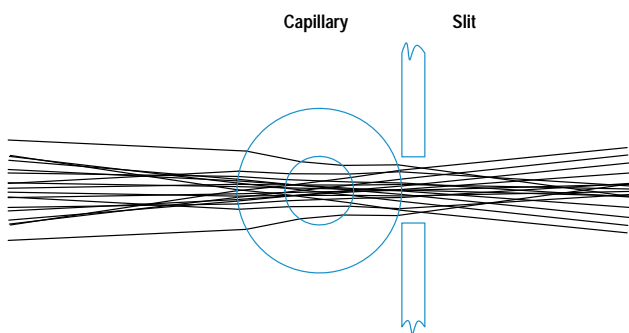

Fig. 3. Raytrace of wavelengths 190 and 600 nm through the CE diode array detector optics.

Fig. 4. Raytrace of 190, 254, and 600 nm through a water-filled capillary.

coefficient as a function of wavelength, C = molar solute concentration, D = path length, I_0 = incident photon flux, and I = transmitted photon flux.

If stray light passing through the column wall can reach the detector, the equation has to be rewritten:

$$A = \log \frac{I_0 + I_S}{I + I_S},$$

where I_S = stray light through the capillary wall.

A typical detector response is shown in Fig. 6. The lower limit of detection is determined by the baseline noise of the detector and its sensitivity, which can be described by the slope dA/dC of the detector response. The upper limit of detection is determined by the nonlinearity of the detector response at higher concentration levels. Unwanted stray light causes a deviation of the response from the theoretical linear slope according to the Lambert-Beer law. The steepness of the slope, in contrast to typical HPLC detection cells, is dependent on the effective path length (related to the inside diameter of the capillary) and the stray light through the

capillary wall. As a result, there is a linear range in which the detector can be operated to get reliable results.

Sensitivity, Noise, and Linear Dynamic Range

This section discusses the parameters that influence the performance of the diode array detector and the trade-offs that have to be made to optimize the system.

A big disadvantage of the on-column detection approach is the possibility of light passing through the transparent capillary wall without interacting with the sample. Figs. 4 and 5 show clearly that not all rays propagate through the center of the circular cross section of the capillary. This decreases the linearity of the detector and reduces the effective path length, which determines the sensitivity of the detector. To maximize the response (effective path length) and linearity of the detector, the width o of the slit must be as small as possible.

Since the slit defines the entrance aperture of the spectrometer, the slit width o has an impact on the optical bandwidth. A 60-µm slit width corresponds to a 2-nm optical bandwidth of the CE diode array detector.

At the lower limit of detection, baseline noise becomes important. As long as the baseline noise of the signal is limited to Schottky noise (noise associated with the photocurrent generated by the photodiodes), the baseline noise is dependent on the total light throughput reaching the detector and is inversely proportional to the square root of the light throughput (photocurrent):

$$I_{S\ p\text{-}p} = \frac{6\sqrt{2qI_{ph}BW}}{2.3I_{ph}},$$

where $I_{S\ p\text{-}p}$ = peak-to-peak value of the Schottky noise current, q = charge of the electron, I_{ph} = photometric signal (current), and BW = electrical bandwidth.

Cross Section View
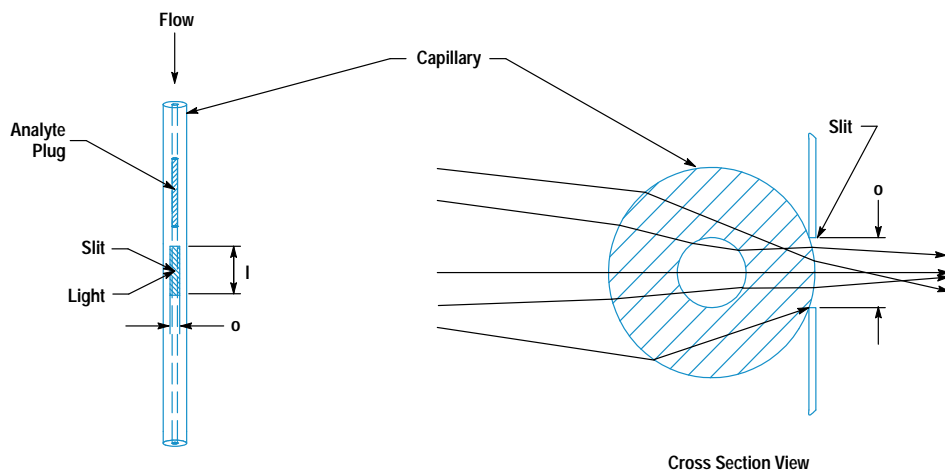
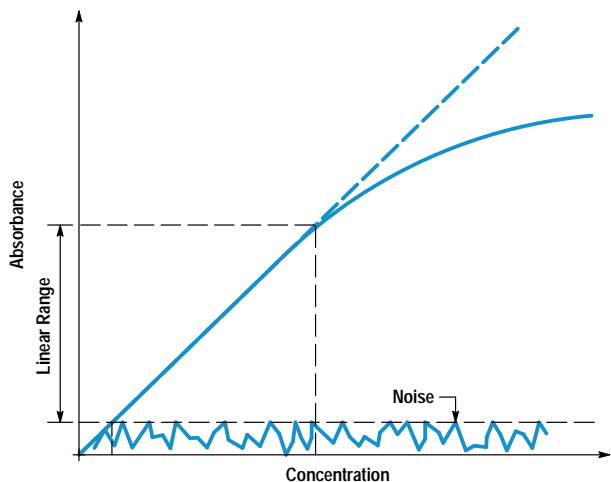**Fig. 5.** Slit and capillary in an on-column detection approach.

Fig. 6. Diode array detector response characteristic.

Expanding the slit, therefore, will decrease the baseline noise. Thus the optimum slit width o is a trade-off between light throughput (noise), linearity, sensitivity, and spectral resolution. An important attribute for detector performance is the linear dynamic range, calculated as:

$$\text{Linear Dynamic Range} = \frac{\text{Upper Limit of Detection (AU)}}{3 \times \text{Noise (AU)}},$$

where the upper limit of detection is given by the point where the detector response deviates 1% from the expected linear response (see Fig. 6).

Different capillary inside diameters are used in CE (typically 25 µm to 75 µm). For optimum performance the slit size must be well-matched to the inside diameter. Using the right slits, a high linear dynamic range can be achieved for standard capillaries and even further improved using extended lightpath capillaries (Fig. 7).
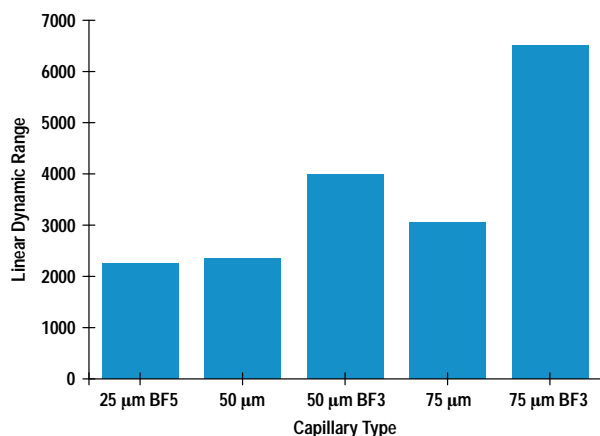

Fig. 7. Typical values for the linear dynamic range using different capillary inside diameters. BF = bubble factor, the increase in the diameter of the capillary at the detection window of the detector.

The slit height l along the separation axis has an influence on the resolution (peak width) and peak height of a detected peak. It also has an impact on the light throughput and influences the baseline noise as already mentioned. To minimize the impact of the detector on the efficiency of the separation the slit height should be as small as possible. Fig. 8 shows the theoretical distortion of a Gaussian peak detected through a rectangular slit. For simplification, the mathematical model neglects the separation (mobility) of the analyte (peak) in the detection window. The deformation is given by:

$$\text{Signal Deformation} = \frac{\text{Observed Signal}}{\text{True Signal}},$$

where the observed signal is the detector output using a slit with height l, and the true signal is the detector output using an infinitely small slit.

Longer slits result in a loss of resolution (broader peaks) and decreased peak height of the recorded signal. However, as long as the slit is small enough compared to the peak width, slightly longer slits do not significantly degrade resolution and they improve the signal-to-noise ratio by allowing more light to reach the photodiodes. As a rule of thumb, the slit height should be smaller than the parameter σ of the detected peak, where the Gaussian peak shape can be described by the formula:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{(x-\mu)^2}{\sigma^2}}$$

$$\text{Peak Width} = \sqrt{5.54}\,\sigma \text{ (full width at half maximum).}$$

HP offers a variety of capillary lengths and inside diameters to cover the needs of a wide range of applications. Three standard inside diameters are available at the moment: 25, 50, and 75 µm. The 50-µm and 75-µm capillaries are offered with and without extended lightpaths (bubbles), whereas the 25-µm capillaries are only offered with a five times expanded inside diameter at the detection point (BF = 5).
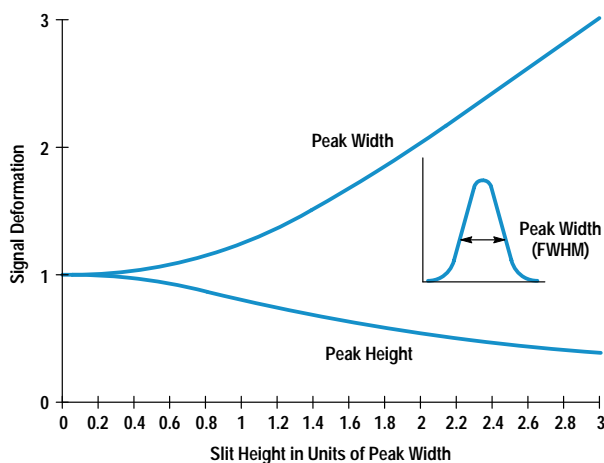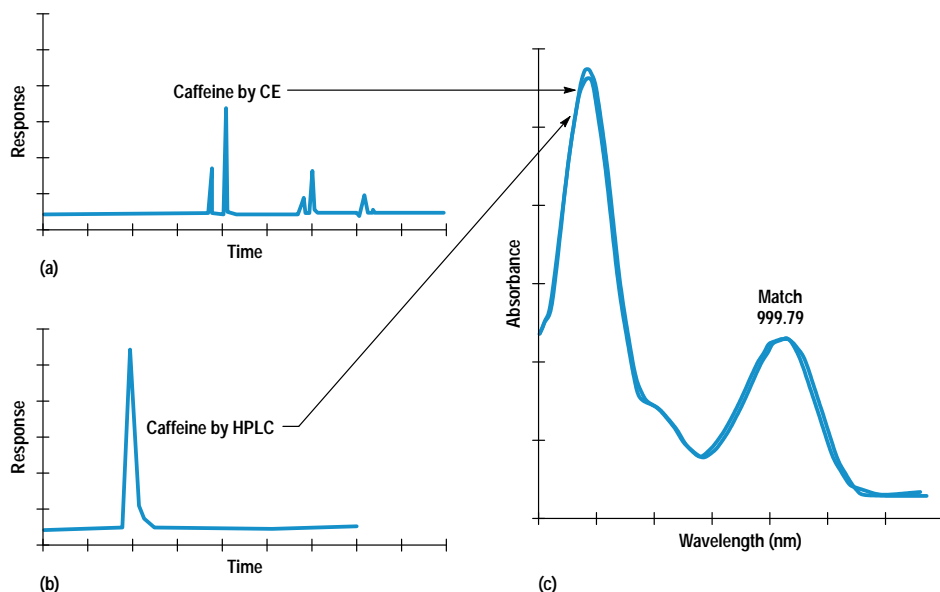

Fig. 8. Signal deformation by increasing the slit height.

Fig. 9. Comparison of caffeine spectra acquired by (a) CE and (b) HPLC. (c) Overlay of caffeine spectra from CE and HPLC runs. CE conditions: buffer = 11.7 mM borate, 8.3 mM phosphate, 50 mM SDS, pH 8.9; Sample = acetaminophen, caffeine, naproxen, guaifenesin, phenacetin, noscapine; Capillary i.d.= 50 μm, L_{eff}=56 cm; injection pressure/ time product = 50 mbar·s; E =370 V/cm; T = 37°C; detection wavelength/bandwidth = 200/16 nm. LC conditions: Hypersil ODS column, 100×2.1 mm, 5-μm particle size; T = 40°C; mobile phase = water/acetonitrile (85/15) at 0.4 ml/min; 10 ng caffeine on column; detection wavelength/bandwidth = 254/4 nm; slit width = 2 nm.

Table I shows the dimensions of the slits that are offered for each capillary type. The minimum useful signal bandwidth indicates the related optical bandwidth.

Table I
Capillary Inside Diameters and Slit Dimensions

Capillary Inside Diameter	Slit Dimensions (o×l)	Minimum Useful Signal Bandwidth
25 μm, BF = 5	120×80 μm	4 nm
50 μm	40×620 μm	2 nm
50 μm, BF = 3	145×145 μm	5 nm
75 μm	55×620 μm	2 nm
75 μm, BF = 3	200×150 μm	7 nm

BF = Bubble factor, the increase in the diameter of the capillary at the detection window of the detector.

Spectral Quality
Capillary electrophoresis is becoming more and more important as an orthogonal technique to HPLC. The use of spectral libraries for peak identification, using spectra taken in HPLC, can be very helpful.

However, the optical design of the CE diode array detector must ensure that no distortion occurs because of different effective path lengths for different wavelengths. The ray trace in Fig. 3 indicates that rays of different wavelengths may travel different paths through the cell resulting in different effective path lengths. Special care has to be taken to minimize distortion; otherwise, spectra will differ from those taken with an HPLC system.

As an example, Fig. 9 compares the spectrum of caffeine acquired with a 50-μm standard capillary on the HP CE diode array detector with that obtained from an HPLC system equipped with a 2-nm slit. The match is as good as typical matches obtained on the same instrument with repetitive analysis.[2]

Acknowledgments

References
1. A. Wiese, et al, "A New Generation of LC Absorbance Detectors," *Hewlett-Packard Journal*, Vol. 41, no. 2, April 1990.

2. D. Heiger, et al, "Diode Array Detection in Capillary Electrophoresis," *Electrophoresis*, November 1994.