

CASE STUDY:

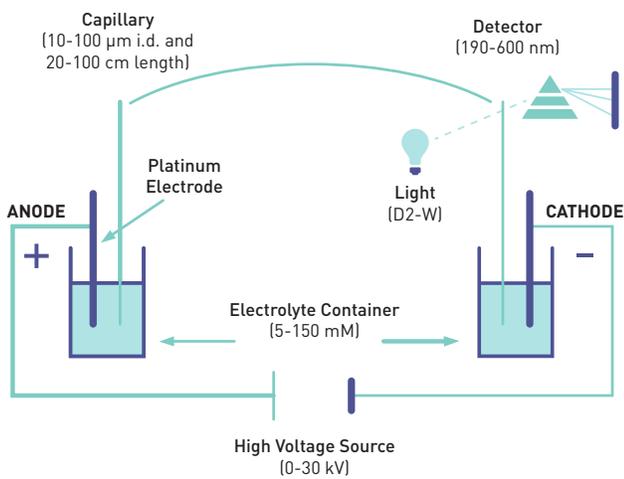
University of Basel

University of Basel is a pioneer in the research of innovative detection methods in liquid chromatography, capillary electrophoresis and other separation techniques.

CHALLENGE

Capillary electrophoresis (CE) is a separation technique for mixtures of ions in a capillary tube by the application of a high electrical voltage. Samples are introduced at one end of the system, and then an electric field is applied which causes analytes to migrate toward the opposite end, passing through a detector along the way. These detectors predominately use UV-Vis spectroscopy and laser induced fluorescence (LIF) methods.

FIGURE 1



Schematic description of capillary electrophoresis

HIGH LIGHT OUTPUT UVC LEDs
ENABLE THE USE OF INEXPENSIVE
SILICON PHOTODIODES FOR
DETECTION, THUS, SIGNIFICANTLY
REDUCING SYSTEM COST.

CE is primarily used for the analysis of charged molecules and has a niche in labs focused on genomics and proteomics. Because of the small diameter of the typical CE capillary (25 μm - 100 μm), CE sample volumes are some of the smallest of any modern separation method—as small as 1 nL. Because CE requires very small volumes of samples, it is advantageous for analyzing rare or expensive substances and used for protein and nucleic acid characterization, and in drug development to separate both basic and chiral pharmaceuticals. In addition, CE is highly amenable to automation which is advantageous in genetic sequencing and human identification by DNA fingerprinting.

Traditional UV-Vis detection methods in CE typically use broadband emitters, such as deuterium lamps, as their primary light source, monochromators for wavelength selection and photomultipliers (PMTs) for signal measurement. The detectors are therefore the most complex part of a CE instrument. CE manufacturers select deuterium lamps due to their relatively high light output at deep UV wavelengths. However, these lamps require a very stable (and expensive) power supply to maintain their performance and a warm-up period of up to 30 minutes to allow the lamp to reach thermal equilibrium. For this reason, most lamps are left on while not in use so that the instrument is ready as needed—wasting much of the lamp's useful life. Deuterium lamps are also bulky and have a large electrical power requirement (20-30 W). The resulting CE detectors are complex and have high total system costs.

The potential benefits of UVC LED-based CE detectors have been known for some time as the monochromaticity of LEDs eliminates the need for costly filters or monochromators. In addition, they can be powered with relatively inexpensive constant current drivers, have a smaller footprint and can use inexpensive silicon photodiodes for detection, replacing costly PMTs. However, the earliest commercialized UVC LEDs based on sapphire substrates had low light output, causing PMTs to still be required for detection—detracting from the basic premise of CE being a simple, low cost technique with potential for portability.

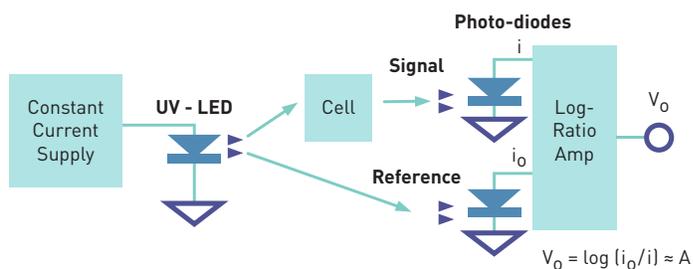
SOLUTION

The availability of high light output, long lifetime UVC LEDs from Crystal IS has enabled designers to replace deuterium lamps with UVC LEDs for detection in CE systems. The high intensity enables replacement of the PMT with simple silicon photodiodes for detection. Furthermore, they have a higher stability of light output which enables lower detection limits.

University of Basel evaluated performance in CE with Optan UVC LEDs for the commonly used wavelengths of 255 nm and 280 nm¹. In their design, the light from the LED is split into two paths, with a split ratio of 80:20. This allows a compensation for change in the intensity of the UV LED. Two silicon photodiodes are used: one photodiode monitors the primary UV light passing through the capillary (signal) and the second photodiode directly monitors the secondary light from the LED to act as the reference. The logarithm of the ratio of the signal and reference intensities is used to determine the UV absorbance of the analytes and thus, their concentration.



FIGURE 2

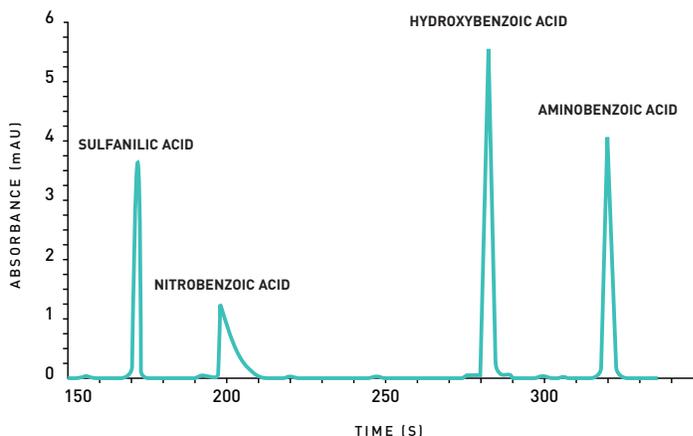


Schematic design of the CE detector

The baseline noise of the detector, a measure of the limit of detection, was evaluated. The peak-to-peak noise value over a period of 60 seconds was determined to be 50 μ AU. This is much better than the 100 μ AU noise level for the CE detector using deep UV LEDs on sapphire. In addition, the sapphire-based UV LED detector uses a photomultiplier tube to compensate for the relatively low light output of the light source. The baseline noise performance also matches that of significantly more complex and expensive commercial UV-detectors for current capillary electrophoresis instruments (<50 μ AU for the Agilent 7100 Capillary Electrophoresis system, <30 μ AU for the PrinCE-C 700 system).

The linearity performance of this detector was then investigated by filling it with standard solutions in a wide range of concentrations. Hydroxybenzoic acid and L-tyrosine, which have strong absorption bands at 255 and 280 nm, respectively, were used to carry out this evaluation. The calibration curves were found to be linear up to the highest concentrations tested. The correlation coefficients (r) were determined as 0.9996 and 0.9995 for 4-hydroxybenzoic acid (255 nm) and L-tyrosine (280 nm), respectively. The good linearity confirms the adherence of the detector response to Lambert-Beer's law as well as negligible levels of stray light and dark current on the photodiodes for the relevant absorbance range.

FIGURE 3



Electropherograms for aromatic acids with detection at 255 nm

The separation performance was evaluated with the four aromatic compounds, sulfanilic acid, 4-nitrobenzoic acid, 4-hydroxybenzoic acid and 4-aminobenzoic acid by zone electrophoresis in a capillary with 50 µm ID and direct detection at 255 nm (Figure 3). The results show that at the optimum detection wavelength, the system is able to provide an acceptably high sensitivity. In addition, the baseline noise under separation conditions was found to be similar to the values obtained without application of the separation voltage.

1. Duy Anh Bui, Peter C. Hauser, "Absorbance detector for capillary electrophoresis based on light-emitting diodes and photodiodes for the deep-ultraviolet range", *Journal of Chromatography A* 1421 (2015), 203-208

"WITH NEW HIGH INTENSITY DEEP UV LEDS IT WAS POSSIBLE TO CONSTRUCT A DETECTOR FOR CE BASED ON PHOTODIODES, RATHER THAN THE PHOTOMULTIPLIER TUBE USED PREVIOUSLY. THE BASELINE NOISE MATCHES THAT OF MORE COMPLEX AND EXPENSIVE COMMERCIAL UV-DETECTORS FOR CE INSTRUMENTS. IN ADDITION, THE LOW CURRENT CONSUMPTION AND COMPACTNESS MAKES THE LED BASED DETECTOR ALSO SUITABLE FOR USE IN PORTABLE, BATTERY OPERATED CE INSTRUMENTS."

Professor Peter Hauser, Department of Chemistry, University of Basel

Crystal IS **ADVANTAGE**

LEDs respond instantaneously, consume relatively little power and offer design freedom over traditional light sources. In addition, Crystal IS UVC LEDs deliver:

- >Excellent stability of light for lower detection limits
- >Superior spectral quality for measurement linearity over a wide concentration range
- >Little or no radiated heat, which is ideal for heat sensitive samples



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