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Deep UV-LED Based Absorbance Detectors for Narrow-Bore HPLC and Capillary Electrophoresis

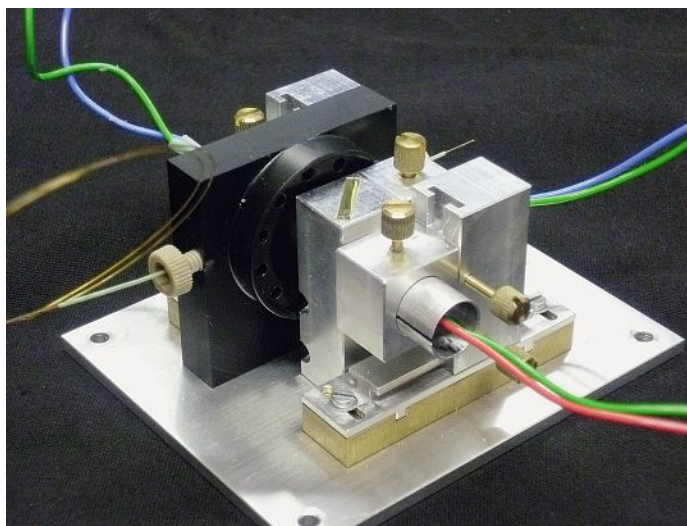
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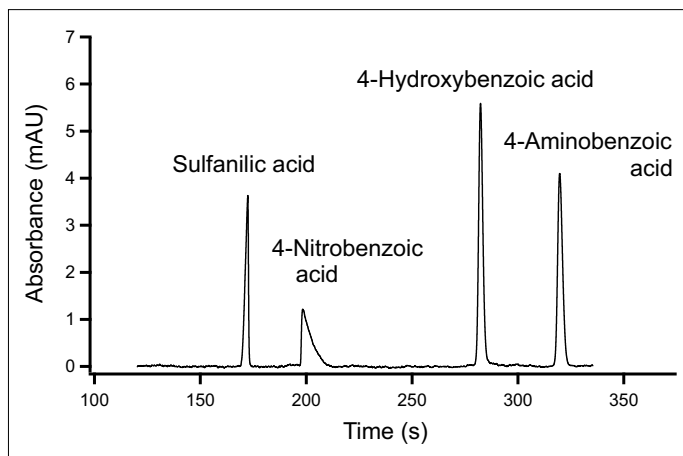
Keywords: Capillary detection · CE · Deep UV-LED · HPLC · UV photodiode

The most common detection method for the analytical separation techniques of HPLC and capillary electrophoresis (CE) is absorbance measurement in the deep-UV range (below 300 nm) as a large number of organic species absorb in this wavelength region. Conventional UV detectors are based on deuterium discharge lamps coupled to a monochromator for wavelength selection. Light-emitting diodes (LEDs) for this wavelength range have been produced in recent years. They have bandwidths of typically 30 nm, which makes them well suited for direct absorbance measurements of molecules without requiring a monochromator. Only UV-photodiodes and a log-ratio amplifier integrated circuit for emulating Lambert-Beer's law are required to complete the electronic circuitry.

Narrow-bore HPLC has primarily been developed for use with mass-spectrometric detection, for which only small amounts of analytes are sufficient. However, the savings in eluent consumption makes this approach also attractive for use with optical detection when ultimate sensitivity is not required. In CE narrow channels are essential to limit the Joule heating associated with the ionic current along the separation path.



The detector cell for capillary electrophoresis.



Detection of aromatic acids in capillary electrophoresis using a 50 μ m ID capillary with a 255 nm LED.

The design of LED-based detectors for these narrow gauge methods is more challenging than for standard HPLC. Due to the small available volumes, the construction of dedicated Z-shaped flow cells is not possible and the measurement has to be made transverse to the flow path. The narrowness of the necessary apertures requires careful attention to efficient light coupling and avoidance of stray light. High mechanical stability is also required in order to minimize noise due to mechanical fluctuations. Despite these hurdles excellent performance with regard to baseline noise (low μ AU range), reproducibility of peak areas ($\sim 1\%$), and linearity of calibration curves (correlation coefficients >0.999) could be obtained with LEDs of the commonly used wavelengths of 255 and 280 nm for both, narrow-bore HPLC (250 μ m ID) and CE (50 μ m ID).

The inexpensive LED-based devices display a capability comparable to standard commercial detectors. Their compact size and low power requirements make them also suitable for portable battery-powered instruments.

Received: September 30, 2015

References

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