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Absorbance detector for capillary electrophoresis based on light-emitting diodes and photodiodes for the deep-ultraviolet range

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ABSTRACT

A new absorbance detector for capillary electrophoresis featuring relatively high intensity light-emitting diodes as radiation sources and photodiodes for the deep-UV range was developed. The direct relationship of absorbance values and concentrations was obtained by emulating Lambert-Beer's law with the application of a beam splitter to obtain a reference signal and a log-ratio amplifier circuitry. The performance of the cell was investigated at 255 nm with the detection of sulfanilic, 4-nitrobenzoic, 4-hydroxybenzoic and 4-aminobenzoic acid and the indirect detection of acetate, propionate, butyrate and caproate using benzoate as the displacement dye molecule. Vanillic acid, L-tyrosine and DL-tryptophan as well as the sulfonamides sulfamerazine, sulfathiazole and sulfamethazine were determined at 280 nm. Good linearities over 3 orders of magnitude were obtained. The noise level recorded was as low as 50 μAU and the drift typically <200 μAU/5 min.

1. Introduction

Capillary electrophoresis is in principle very simple in that for separation essentially only an inexpensive capillary and a high voltage supply are required. For commercial instruments the standard method of detection is via molecular absorption in the UV and visible ranges. The source of radiation for these detectors is broadband emittors in the form of deuterium and tungsten lamps, monochromators are used for wavelength selection and photomultipliers for intensity measurement. Such a detector is then perhaps the most complex part of a CE-instrument.

Light-emitting diodes (LEDs) have been employed as alternative radiation sources in instrumentation for the analytical sciences since the first report on such a use by Flaschka et al. in 1973 [1]. As the emission bands of LEDs are relatively narrow (typically about 30 nm) monochromators or optical filters are not generally required when carrying out molecular absorption measurements as these spectral widths are well matched to the absorbance bands of molecules. Other advantages are compact size and robustness, low power consumption and low heat production. A further analytically important benefit is the high stability of the output intensity of LEDs. The original work by Flaschka et al. [1] was based on a then available red LED, but since that time LEDs with progressively shorter emission wavelengths have become available and their analytical applications have been extended to many different fields. Two recent general reviews are available [2,3].

Tong and Yeung in 1995 were the first to describe a purpose built absorption detector for CE based on a green LED as radiation source [4]. The indirect detection of inorganic anions via the displacement of permanganate in the background electrolyte was demonstrated. Macka et al. in 1996 reported the direct detection of alkali and alkaline earth metals as their Arsenazo complexes using green and yellow LEDs fitted into a commercial detector in place of the conventional light source [5]. Butler et al. in 1997 demonstrated the use of a green LED for the direct detection of the complexes of transition metals with 4-(2-pyridylazo) resorcinol and the indirect detection of inorganic cations and anions using organic displacement dyes [6]. Macka and coworkers in 2002 also introduced the use of an LED emitting at 380 nm in the near UV-range, which was demonstrated for the indirect determination of inorganic anions via the displacement of chromate as a probe dye [7]. This is a common method for these ions when determined with conventional commercial CE instruments as otherwise they are not accessible by optical detection. A number of further reports on the use of LEDs in detectors for capillary electrophoresis have appeared over the years and the developments up to 2009 have been reviewed by Xiao et al. [8,9].

Abbreviations: CE, capillary electrophoresis; UV, ultraviolet; LED, light-emitting diode; HPLC, high performance liquid chromatography; AU, absorbance unit.

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A limitation to the employment of LEDs for analytical purposes, including their use in capillary electrophoresis, has been the restriction to visible light and the near-UV range. Most organic molecules absorb in the deep-UV range below 300 nm, but not in the visible range, and for this reason absorption detectors for the separation methods of column chromatography and capillary electrophoresis employ wavelengths typically of 280, 255 or 210 nm. However, in recent years deep UV-LEDs down to wavelengths of about 250 nm have become commercially available. Schmid et al. [10] in 2008 and Bomastyk et al. [11] in 2011 reported detectors for standard HPLC instruments based on UV-LEDs of 280 and 255 nm. This was followed by a report on a detector for narrow bore HPLC [12]. Kraiczek et al. [13] reported a HPLC detector employing an array of different UV-LEDs which features wavelength flexibility through simple electronic switching. Very recently Sharma et al. [14] reported an absorption cell for capillary liquid chromatography employing a 260 nm UV-LED. With all of these detectors, low noise, high stability and detection limits were achieved which were comparable with that of more complex and expensive commercial detectors. The use of deep-UV LEDs has also been reported for the quantification of O_3 [15,16] directly in the gas phase.

Macka and coworkers in 2009 in a short communication reported the first design of a detector for CE based on a 255 nm UV-LED as a light source and showed limited preliminary results for the detection of 4 nucleotides [17]. Due to the utilization of an early low intensity UV-LED a photomultiplier tube was employed to measure the light intensity. Rudaz and coworkers in 2009 also reported a UV-LED based detector for CE, but no details on the design of the detector nor its performance parameters were given [18,19]. The detector reported herein was fitted with newer LEDs of higher intensity than previously available and is based on photodiodes. Its performance in CE was evaluated for the commonly used wavelengths of 280 and 255 nm.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade. Sulfanilic acid was purchased from Merck (Zug, Switzerland). Sodium butyrate and sodium propionate were obtained from Lancaster Synthesis (White Lund, Morecambe, England) and Riedel-de Haën (Seelze, Germany), respectively. The other chemicals were products of Sigma-Aldrich (Buchs, Switzerland) or Fluka (Buchs, Switzerland). Deionized water from a NANO-Pure water purification system (Barnstead, IA, USA) was used throughout the experiments. Standard solutions were prepared in water, except for sulfamerazine, sulfathiazole and sulfamethazine, which were dissolved in methanol. All solutions were degassed in an ultrasonic bath and filtered through 0.2 μm nylon filters purchased from BGB Analytic (Boeckten, Switzerland). The capillaries were preconditioned with 1 M NaOH for 10 min, rinsed with deionized water for 10 min and finally flushed with the electrolyte solutions for 30 min. After each separation, they were reconditioned with the electrolyte solutions for 5 min.

2.2. Instrumentation

The high intensity UV-LEDs emitting at 255 nm (model 7YS, $P_{100\,mA}$ = 1.8 mW) and 280 nm (model 74P, $P_{100\,mA}$ = 1.5 mW) were obtained from Crystal IS (Green Island, NY, USA). The UV-photodiodes (SG01L-C, SG01L-B18) were sourced from Sglux Solgel Technologies (Berlin, Germany). A polyimide coated fused-silica capillary (50 μm ID, 360 μm OD) from Polymicro Technologies (Phoenix, AZ, USA) and a fused-silica extended light path capillary (G1600-62232, 50 μm ID, 360 μm OD, bubble factor = 3) from Agilent Technologies (Agilent, Waldbronn, Germany) were employed for separations. The beam splitter (G344312000) was sourced from Qioptiq Photonics (Munich, Germany). The 4 mm diameter fused-silica ball lens (No. 67385), and the 50 μm and 100 μm wide optical slits of 3 mm length (air slits Nos. 38559 and 38560, respectively) were products of Edmund Optics Germany (Karlsruhe, Germany). The mechanical parts (holders and adjustable positioning stages) were made in our workshop from black poly(methyl methacrylate) (PMMA) or from aluminium. The log-ratio amplifier (LOG102) was obtained from Texas Instruments (Austin, TX, USA). The separations of target ions were carried out by using a purpose-made portable capillary electrophoresis instrument, which is a refinement of the design first developed by Kubáň [20]. It consists of a case made from PMMA with dimensions of 310 × 220 × 260 mm. This was fitted with a microswitch to interrupt the high voltage for safety when opened for manipulations inside. It includes a dual polarity high voltage power supply (CZE2000, Spellman, Pulborough, UK), which has maximum output voltage of ±30 kV at 300 μA, and associated control electronics. The signals were recorded and digitized with the use of an e-corder data acquisition system (Model ED401) and the Chart software package (both from EDAQ, Denistone East, NSW, Australia).

3. Results and discussion

3.1. Design of detector

The physical arrangement of the detector was a modification of the previously reported design for narrow bore chromatography [12] and the circuitry used was an adaptation of the one reported elsewhere [11]. The overall set-up of the cell is shown in Fig. 1A. The UV-LED was driven with a constant current source at 100 mA. The light beam was divided into signal and reference paths. This allows a compensation for the temperature dependence of the output

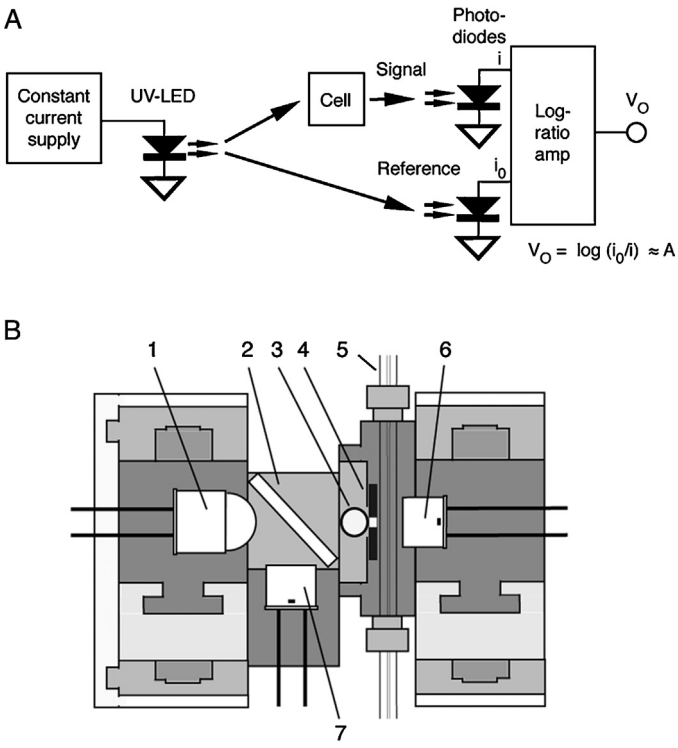

Fig. 1. Design of the detector. (A) Overview. (B) Assembly: 1) UV-LED in positioning stage, (2) beam splitter, (3) ball lens, (4) optical slit, (5) capillary, (6) signal photodiode in positioning stage, (7) reference photodiode.