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Multiple-channel ultra-violet absorbance detector for two-dimensional chromatographic separations

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ABSTRACT

In recent years, much research has gone into developing online comprehensive two-dimensional liquid chromatographic systems allowing for high peak capacities in comparable separation times to that of one-dimensional liquid chromatographic systems. However, the speed requirements in the second dimension (2nd-D) still remain one challenge for complex biological samples due to the current configuration of two column/two detector systems. Utilization of multiple 2nd-D columns can mitigate this challenge. To adapt this approach, we need a multiple channel detector. Here we develop a versatile multichannel ultraviolet (UV) light absorbance detector that is capable of simultaneously monitoring separations in 12 columns. The detector consists of a deuterium lighthouse, a flow cell assembly (a 13-channel flow cell fitted with a 13-photodiode-detection system), and a data acquisition and monitoring terminal. Through the use of a custom high optical quality furcated fiber to improve light transmission, precise machining of a flow cell to reduce background stray light through precision alignment, and sensitive electronic circuitry to reduce electronic noise through an active low pass filter, the background noise level is measured in the tens of μAU. We obtain a linear dynamic range of close to three orders of magnitude. Compared to a commercialized multichannel UV light absorbance detector like the Waters 2488 UV/Vis, our device provides an increase in channel detection while residing within the same noise region and linear range.

1. Introduction

In recent years, comprehensive two-dimensional (2D) high performance liquid chromatography (HPLC) has become an emerging research topic because it can achieve a high peak capacity within a comparable separation time to that of one-dimensional liquid chromatography [1]. Two-dimensional separations may be performed either online or offline [2] depending on the desired result and time allocated for the total separation. The first online 2D-HPLC system was designed by Erni and Frei in 1978 [3] and utilized gel permeation chromatography (GPC) and reverse phase liquid chromatography (RPLC) in the first and second dimensions respectively. They separated Senna-glycoside with GPC over a course of 10 h, collected and re-injected alternatively seven fractions using two sample loops in an eight-port valve for RPLC. This laid the groundwork for 2D-HPLC. If online 2D-HPLC is desired in a reduced total separation time, the 2nd-D must include both the separation and re-equilibration steps in the fraction-collection time of the 1st-D. In order to meet this requirement, setups have included two to four columns for use in the 2nd-D separation. Bushey and Jorgenson [4] developed the first online 2D-HPLC system in 1990 to implement this strategy. They employed cation exchange and size exclusion columns for their two separation dimensions to separate a protein sample. By doing so, they created the foundation for the next generation of 2D-LC systems to come.

A good 2D-HPLC system should have two orthogonal separation dimensions. One can obtain improved orthogonality [5] by combining a variety of chromatographic modes of LC including ion-exchange chromatography (IEX) [6], size exclusion chromatography (SEC) [7], normal phase liquid chromatography (NPLC) [8], reverse phase liquid chromatography [9], supercritical fluid chromatography (SFC) [10], hydrodynamic interaction liquid chromatography HILIC [11], etc. A first dimension (1st-D) of IEX has been combined with a second dimension (2nd-D) of RPLC in conventional 2D-HPLC schemes for peptide separation due to their relatively high orthogonality, fast re-equilibration time [12] and compatibility with mass spectrometry (MS) [13]. However, to this day, limited separation speed in the second separation dimension is still a major drawback for complex biological samples due to the current configuration of two-column/two-detector systems [14].

Abbreviations: UV, ultraviolet; μHPLC, micro-High Performance Liquid Chromatography; 2D-HPLC, two-dimensional high performance liquid chromatography; MS, mass spectrometry; IEX, ion exchange chromatography; RPLC, reverse phase liquid chromatography

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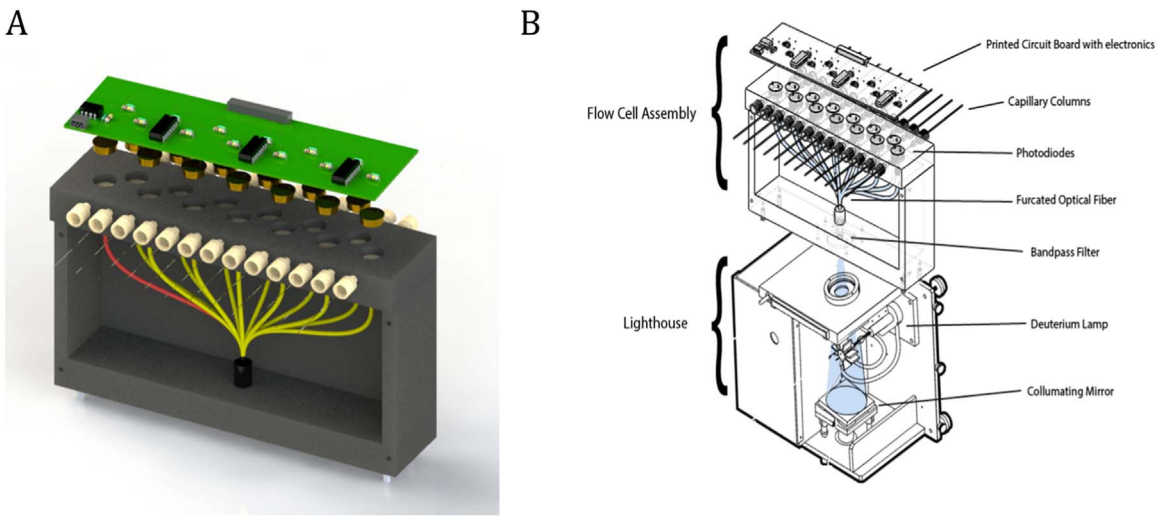

Fig. 1. 3D Rendering of completed optical fiber assembly housing. The machined housing consisted of an aluminum shell that was powder coated black to eliminate light interference. (A) It housed a 13-furcated fiber optic cable and had a top flow cell piece comprising of 12 individual flow cells and a reference input. On top attached a printed circuit board integrating the photodiodes, amplification circuitry, and connections for source power and output signals. (B) The light path is shown from the Newport Apex Monochrometer Illuminator through the furcated fiber optic cable into each of the flow cells.

Current commercial systems utilize a series of valves in order to collect and store samples for future separation while still configured with only one column [15,16]. This system, although robust and reproducible, does not address the issue of the limited separation speed in the 2nd-D separation since these systems still run a single second dimension column at a time. One way to address this issue is to run the 2nd-D columns in tandem in order to increase sample throughput, which in turn decreases the overall separation time. One such system has been recently developed in our research group. Zhu et al. [17] published this work showing continuous and comprehensive online analysis of intact proteins while obtaining over 500 protein peaks from an E. Coli lysate. This system re-equilibrated several 2nd-dimensional columns while running separations on the rest. However, the major hurdle proved to be the detection aspect of the system due to no commercial systems available to detect the tandem columns.

Waters (Waters 2488) [18] offers a commercial multichannel UV/Vis detector, but there are only two channels. This system works in conjunction with common 2D-HPLC systems that store fractions and may have a maximum of two separation columns but would not accommodate our needs. Other multichannel/multiplexed detection systems have been developed over the years including several capillary electrophoresis (CE) coupled to laser induced fluorescence detectors (LIF) [19–22] but due to cost and other system requirements, these were not an option. A literature search into multichannel absorbance detection, our preferred method of detection, showed very few multichannel absorbance detectors to have been constructed due to the limited demand of such a detector. Until now, a seven channel [23] absorbance detector was reported by our group and a 96-channel detector was commercialized by now a disbanded company CombiSep. Both of these absorbance systems were paired with CE similar to the LIF systems listed above.

In this work, we develop a 12-channel UV absorbance detector to meet this need. The detector consists of a deuterium lighthouse, a flow cell assembly (13 individual flow cell channel fitted with a 13-photodiode-detection system), and a data acquisition and monitoring terminal. A UV light beam from a deuterium lamp is monochromated and focused to the entrance of an optical fiber and then split into 13 beams (1 as reference and remaining 12 to the LC separation columns). Transmittance from each column is measured by a photodiode, converted to absorbance and acquired by the monitoring terminal. The detector has background noises at tens of μAU level and a linear range over three orders of magnitude. To demonstrate the feasibility and capabilities using this detector for 2D-HPLC, we construct an updated 2D-HPLC system using this detector and show the 2D separation results.

2. Experimental

2.1. Reagents and materials

Fused-silica capillaries and the furcated-fiber optic cable were purchased from Polymicro Technologies Inc (Molex, Phoenix, AZ). Trifluoacetic acid, methanol and acetonitrile and other reagents used were obtained from EMD Chemicals, Inc. (Gibbstown, NJ). Water was purified by a NANO pure infinity ultrapure water system (Barnstead, Newton, WA). Ball lens and optical filters were purchased through Edmond Optics (Barrington, NJ). Printed circuit boards (PCB) were designed in house and manufactured by OSHPark (Portland, OR). The deuterium light source, an Apex Monochrometer Illuminator, was produced by Newport (Irvine, CA), while a comparative capillary-based absorbance detector (Linear UVis 200), was manufactured by Linear Instruments (Reno, NV).

2.2. UV absorbance detector design

The detector itself consists of a deuterium lighthouse, a flow cell assembly, a 13-channel flow cell fitted with a 13-photodiode detection system, and a data acquisition and monitoring terminal as depicted by the three dimensional rendering (Fig. 1). The machined optical fiber assembly is shown in Fig. 1A including the flow cell, furcated fiber optic cable, and printed circuit board with its electrical components. The optical fiber assembly consists of a custom 13-furcated fiber integrated into a machined and anodized aluminum box with the 13 individual flow cells (one for each of the 12 columns and the last for reference signal detection) integrated into a single piece, which bolts to the top of the housing for the furcated optical fiber. Each individual flow cell comprised of two PEEK fittings, a ball lens and a custom PEEK ball lens holder. The total dimensions of the system including the lighthouse are 30 cm × 17.5 cm × 50 cm (l × w × h respectively) while the machined optical fiber assembly dimensions are 20 cm × 5 cm × 20 cm.

Fig. 1B depicts the optical path and includes Apex Monochrometer Illuminator, that was used throughout this research as the lighthouse. The light originates from a deuterium lamp in the lighthouse where it is collimated, travels through a bandpass filter, and is focused on the fiber optic bundle housed within the flow cell assembly. The light then