ULTRA HIGH FLEXIBLE UV-VIS RADIATION SOURCE AND NOVEL DETECTION SCHEMES FOR SPECTROPHOTOMETRIC HPLC DETECTION

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ABSTRACT

The concept and performance of the first multi-wavelength DUV-LED-based HPLC absorption detector are presented. In single wavelength mode and with optical referencing, the limit of detection (LOD) is comparable to conventional state-of-the-art HPLC detectors. In multi-wavelength operation mode – currently up to 8 wavelengths and currently without optical referencing - the LOD is about 10x higher. Stray light is dependent on the operation mode, either negligible or totally controlled in the electronic/digital world. MUX and DEMUX methods are used to separate chromatographic signals and allows keeping the detector configuration simple and yet flexible.

KEYWORDS: Deep UV LED, Optical Grating, UV-Detection, Liquid Core Waveguide Flow Cell, HPLC

INTRODUCTION

High-performance liquid chromatography (HPLC) is an analytical technology widely used for the separation and analysis of small molecules as well as biomolecules in Pharma, Food, Environmental and many other industries [1]. Absorption spectrophotometry has been, and still is, the industry standard for detection in HPLC. Recent advances in HPLC column technology have led to low flow-rate HPLC (1-100µL/min) and put higher demands in spectrophotometric HPLC signal detection because the volume of the detector flow cell required is relatively low (<<1µL) and Lambert-Beer's law mandates the path length of the detector flow cell be long in order to obtain a high absorbance signal. Today Deuterium (D_2) discharge lamps are the only suitable light sources for UV-absorption detectors. D_2-lamps are relatively stable, but very bulky and hot, and have a large electrical power requirement (20-30W).

Over the recent years, significant improvements in material quality and device design resulted in the demonstration of Deep UV Light Emitting Diodes (DUV-LEDs) capable to deliver mW of optical output power [2]. Key emerging applications for DUV-LEDs include water and medical device sterilization and epoxy curing [3]. Because of their small size, small emitting area and low power consumption, DUV-LEDs are gaining increased interest as light sources for miniature (portable) bio-analytical optical measurement systems [4,5]. (DUV-)LEDs do not only support miniaturization plans, e.g., in optical HPLC detection; in fact they have the potential to enable novel detection concepts and detector configurations because of their very fast turn-on and turn-off time. DUV-LEDs are commercially available in the wavelength range from 240nm to 400nm.

GRATING LAMP DETECTOR

The concept of the new 'Grating Lamp Detector' or 'Wavelength Source Spectrophotometer' is fairly simple. It is in principle reversing the optics of today's photodiode-array HPLC spectrophotometers. A linear array of DUV-LEDs is spatially arranged such that the wavelengths of interest are diffracted and combined by an optical grating into a common exit, e.g. into a miniaturized liquid core waveguide (LCW) detector flow cell. The grating lamp can be used as a very flexible light source, e.g., in combination with a photodiode array based spectrophotometer module. Wavelengths not required are simply switched off to improve the stray light performance and/or to prevent damage of photo-degradation sensitive sample. Or it can be part of a new, multiple wavelength – single photodiode detector concept (Fig.1). Multiplexing and demultiplexing techniques, such as time or frequency or code division multiplexing, are then used to separate chromatographic signals.

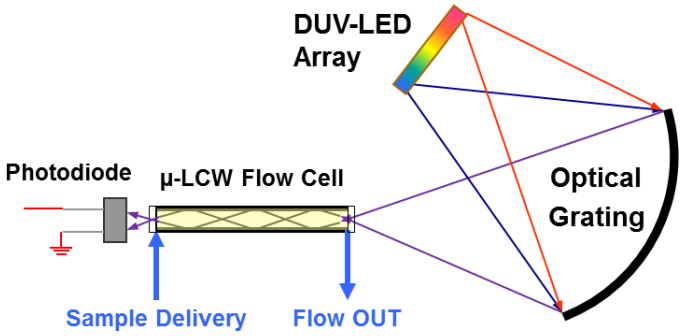

Figure 1: *Light from a custom built DUV-LED array (8 wavelengths; 250nm-355nm in 15nm steps; 5mm long) is diffracted and focused by an optical grating into a micro LCW flow cell. A single photodiode monitors the change in transmission caused by the sample eluted from an HPLC separation column (not shown).*

DUV-LEDs are known to have side bands in the visible. The optical grating is acting, in addition, very efficiently as an optical filter (Fig.2). Only the wavelength of interest is passing the detector flow cell.

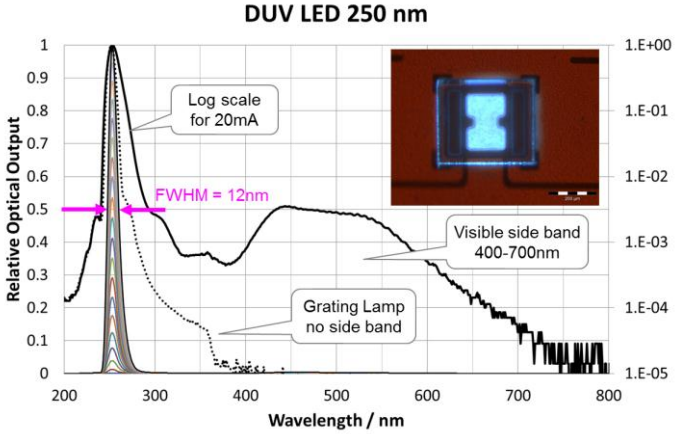

Figure 2: *Spectral power distribution of '250nm' DUV-LED (black solid line) and spectral power distribution entering the detector flow cell (black dotted line) after the grating. The side band in the visible is very efficiently suppressed. The 'true' center wavelength of the DUV-LED (252nm) is forced to the detectors 'design' wavelength (250nm). The picture in the figure shows the emitting area of the '250nm' LED pixel on the LED-array which is about 150µm x 250µm.*

Stray light - typically limiting the linear dynamic range of an absorption detector - is dependent on the multiplexing mode either totally negligible or simply controlled in the electronic / digital world. Lifetime (drop in optical output power) and short term stability (relatively large temperature coefficient for the optical output power) are known to be an issue of DUV-LEDs. The Grating Lamp Detector has a built-in optical reference to compensate efficiently source intensity fluctuations. The new detector concept is also very flexible in space. It allows, e.g., to perform parallel LC (multiple columns and multiple flow cells) at very small pitch without the need of additional optical elements.

EXPERIMENTAL

The new Grating Lamp Detector concept was benchmarked against the 1290 Infinity Diode Array HPLC detector and the 1100 series Variable-Wavelength HPLC detector G1314A (both Agilent Technologies, Waldbronn). A DUV-LED array was obtained from Sensor Electronic Technology Inc. as a custom built device. 8 single LED chips - 8 wavelengths starting from 250nm to 355nm in 15nm steps - were attached and wire bonded on to a custom made ceramic substrate. The LED-Array, the optical grating and the detector flow cell were precisely aligned to each other as laid out in Figure 1 and mounted in a common housing. DUV-LEDs were driven and controlled by an FPGA-board from National Instruments. The FPAG-board was also used to readout the digitalized photocurrent signal (24 bits) of a Hamamatsu photodiode. The photodiode in turn was connected to the readout electronics of the 1290 Infinity Diode Array Detector; slightly modified to allow single photodiode readout. LabVIEW FPGA programming was used to implement time, frequency and code division multiplexing methods in order to separate the different chromatographic signals (different wavelengths).

Sample, chromatographic conditions, and detector settings: **A.** Limit of detection: Anthracene (167 pg/µL) and Toluene (1.67µg/µL) were dissolved in Methanol and Acetonitrile. Separation column 4.6x50mm Zorbax SB-C18 1.8µm, column temperature 36°C. Injection volume 3µL. Mobile phase: Water and Acetonitrile; isocratic 35% Water, 65% Acetonitrile. Flow rate = 1.2mL/min. Detector flow cell 2.5µL/10mm. Detector settings: same wavelength, same response time = 1sec; same optical bandwidth = 7nm for both detectors. **B.** Linear Dynamic Range: similar to ASTM E685-93 [6]. Two stock solutions Caffeine in Water 500mg/L and 50mg/L. Mobile phase: Water (%A) and Water + Caffeine (%B). Two step gradients starting from %B 0 to 100 in steps of 10. Flow rate 1mL/min. No column. Temperature 25°C. Evaluation of plateau of each gradient step. Detector flow cell 2.5µL/10mm. Detector settings: as **A.**

RESULTS AND DISCUSSION

In single wavelength (continuous wave) mode, and with optical referencing, the limit of detection (LOD) is comparable to conventional state-of-the-art HPLC detectors (Fig.3).

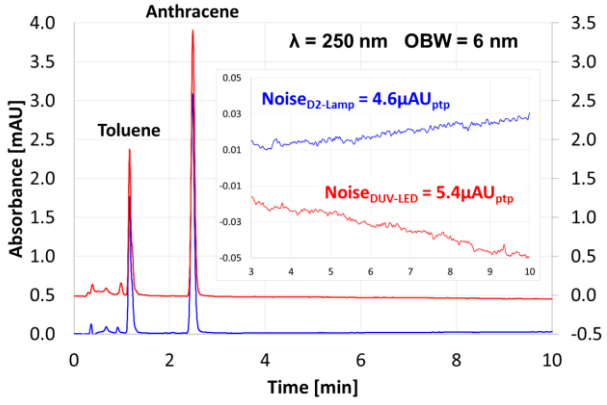

Figure 3: *Limit of detection in comparison; e.g., Anthracene dissolved in Methanol. Amount injected = 500pg. RED: Grating Lamp Detector in single wavelength, continuous wave mode; LOD at SNR =3 is 2pg. BLUE: Conventional HPLC detector; Agilent G1314A; LOD is also 2pg. Both detectors were operated* ***with optical reference****.*

In multi-wavelength operation – currently up to 8 wavelengths and without optical referencing - the LOD of the Grating Lamp Detector is about 10-25x higher (Fig.4). Part of the performance loss in MUX mode is, of course, a consequence of using a multiplex method. However, in single wavelength mode without optical reference the loss in performance for the present setup is already 10x mainly due to the relatively large temperature coefficient of the optical output of the DUV-LEDs.

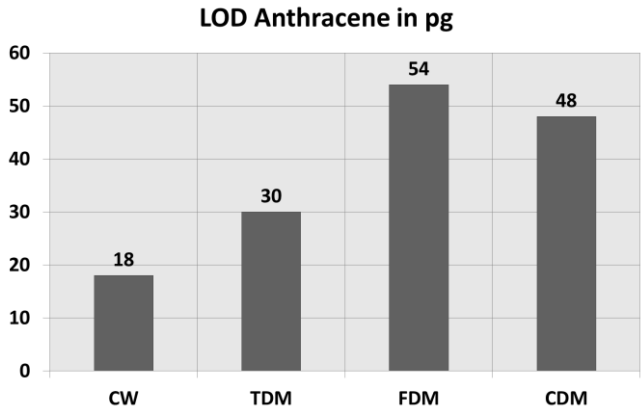

Figure 4: ***Limit of Detection*** *in pg of the new Grating Lamp HPLC detector for Anthracene at λ =250nm* ***without optical reference****.*

CW = Continuous Wave (single wavelength);
TDM = Time Division Multiplexing;
FDM = Frequency Division Multiplexing;
CDM = Code Division Multiplexing.

The new detector concept has a clear advantage over conventional detectors when comparing the upper limit in absorbance. While conventional HPLC detectors typically reach saturation already at about 3.5 Absorbance Units (AU), the Grating Lamp Detector saturates around 6 AU (Fig.5).

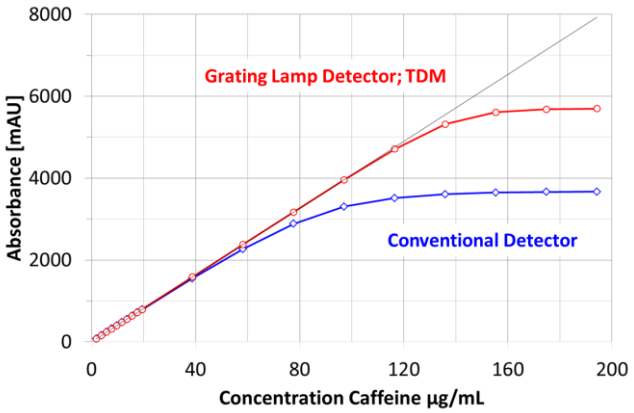

Figure 5: Caffeine linearity test at λ=265nm. While conventional detectors are typically limited in their ***Linear Dynamic Range*** *by stray light (A_{sat}= 3.5AU), the new Grating Lamp Detector is currently limited by its capability to resolve small photocurrents. A_{sat} = ~6AU in CW and TDM mode. The Grating Lamp Detector saturates around 4.5AU in FDM operation mode (not shown) due to the current performance limitations of the Lock-in amplifiers used. Conventional HPLC detector = Agilent 1290 Infinity Photodiode Array Detector.*

SUMMARY and CONCLUSION

The concept and performance of the 1st DUV-LED-based multi-wavelength detector has been demonstrated. The grating lamp detector is, to our knowledge, the first HPLC detector that uses MUX-DEMUX techniques to separate chromatographic wavelength signals. The Signal-to-Noise Ratio (SNR) in single wavelength mode is comparable to state-of-the-art detectors but requires a 10-100x improvement in multiplexing mode to be competitive. DUV-LED technology is a relatively new technology. Lower wavelengths (<250nm) are still in the research phase but are important for scientific instrumentation and currently not covered by DUV-LED technology.

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