UVC LEDs for Fixed Wavelength Detection in HPLC

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HPLC is a separation technique typically used for protein purification, quality control in manufacturing, and biotechnological research. Nearly 80% of all known chemicals and materials can be identified with HPLC when utilizing a suitable detector. Detection of mixture of components is commonly done with spectroscopy (primarily absorption and sometimes, fluorescence). UV absorption spectroscopy is the most popular detection technology in HPLC not only because many analytes have high absorbance coefficients and therefore absorb strongly in the UV spectrum while solvents remain transparent at these wavelengths, but also due to the relatively low cost of measurement. Current HPLC detectors frequently use deuterium lamps as a light source because of the high light output in the UV wavelengths as well as excellent stability of light and relatively long life. However, advancements in light emitting diode (LED) technology are providing for alternative solutions that match or exceed the performance of such traditional ultraviolet C-range (UVC) lamps, while also reducing system costs for instrument manufacturers. UVC refers to ultraviolet light in the range from 200-280 nm. UVC LED solutions have enabled HPLC manufacturers to replace deuterium lamps and offer lower cost detectors for fixed wavelength applications.

Improving the performance of UVC LEDs

While LEDs offer several advantages in footprint, ease of alignment, and productivity for HPLC detectors, the adoption of UVC LEDs (250-280 nm) had lagged to date. This was largely due to the poor lifetime of the earliest commercialised devices which were based on sapphire substrates. The drive to improve lifetime has led to the development of new lattice matched substrates for the fabrication of UVC LEDs. Emerging Aluminium Nitride (AlN) based UVC LEDs are showing progress in power output and device lifetime for these applications. The increase in performance has been driven by the relatively recent development of high quality, single-crystal AlN substrates which allow the growth of pseudomorphic AlGa x N device layers with very low defect densities. These low defect densities coupled with adaptation of visible LED manufacturing techniques has resulted in improved power and reliability of the LEDs.

Figure 1 shows a comparison of an AlN-based UVC LED surface versus a sapphire-based UVC LED surface. The image shows the atomically flat, low defect density layers of the AlN-based UVC LED.

New UVC LEDs are being fabricated on lattice matched AlN substrates, and this results in ten thousand fold fewer defects compared to LEDs fabricated on sapphire substrates. A large number of defects can reduce the efficiency of a LED and increase the possibility of material deterioration—more quickly degrading output power and reducing the device lifetime.

Performance comparison of deuterium lamps versus AlN-based UVC LEDs

HPLC manufacturers select deuterium lamps because of the high stability of light output through the duration of a measurement as well as the relatively high light output of the lamps at their HPLC-relevant UV wavelengths. However, AlN-based UVC LEDs offer more irradiance at a specific wavelength in the deep UV range than the broader spectrum deuterium lamp (Figure 2). At 255 nm, a key line from the mercury lamp spectrum around which methods have been developed for HPLC detection, UVC LEDs exhibit ten times more irradiance. This higher irradiance of the LED is especially advantageous in fluorescence, where the signal strength is proportional to the source intensity and enables detection of compounds to the picogram levels.

Another key benefit of LED-based detectors is stability of light. The stability of light output is defined as fluctuations in light output over short periods of time (i.e., the duration of a measurement). High light output stability of a UV light source in HPLC ensures detection of lower concentrations...
of compounds. Since even a miniscule fluctuation in light intensity can cause an error in measurement, stability of the light intensity in tens of ppm (parts-per-million) is required for the HPLC light sources. Fluctuation is evaluated by peak-to-peak intensity variations within a 30 second time window, and averaged over 15 minutes or longer. A comparison [1] of UV lamps indicates that deuterium lamps have the best stability (i.e. lowest fluctuation) when compared with other conventional UV lamps. AlN-based UVC LEDs exceed the stability of high-end deuterium lamps with a peak-to-peak fluctuation lower than 50 ppm (0.005%) (Figure 3). The superior stability (lower fluctuation) of UVC LEDs leads to lower detection limits in HPLC.

Deuterium lamps require 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. LEDs offer longer life and instantly turn on, ensuring that LED lifetime is not wasted in warm-up. Lifetime considerations are significant in HPLC systems, as frequent light source replacement could lead to laboratory bottlenecks and extra costs. A standard 30W deuterium lamp has an average lifetime of about 3,000 hours. Depending on operating conditions, UVC LED matches or exceeds the lifetime of a deuterium lamp (see Figure 4).

Deuterium lamps are bulky and have a large electrical power requirement (20-30 W). In addition, they require expensive power supplies, and housing is required to store the lamp. This adds significantly to the total system cost. Historically, these lamps have not been easy to replace and align, which restricted design capabilities, today however lamps do come pre-aligned and are replaced with relative ease. Deuterium lamps also generate heat, which can affect thermally labile samples.

LEDs typically utilise less complex power supplies and do not generate forward heat. Furthermore, the emission from LEDs can be readily coupled to an optical fibre which is a benefit to applications where the flow cell needs to be isolated. These advantages are allowing manufacturers to develop fixed wavelength systems with UVC LEDs for their light source.

UVC LEDs benefit fixed wavelength detection in HPLC

UVC LEDs offer the sensitivity of a high-end deuterium lamp while reducing the overall instrument cost and size for fixed wavelength detection. For end users that require options for a single or few fixed wavelengths, manufacturers can optimise their laboratory real estate with smaller units.

For fixed wavelength HPLC systems, the largest difference in cost generally stems from the initial purchase cost, as this includes both the light source and any ancillary equipment. An HPLC system using an LED detector would require a power supply, photodiodes, and a beam splitter. The typical HPLC detector with LEDs is significantly less expensive to build. In contrast, an HPLC system that uses a deuterium light source requires more costly equipment to build. The necessary
power supply is much more expensive, and a housing is required to store the lamp. In addition, deuterium lamps are broad-spectrum sources, emitting light across many wavelengths in the UV range. This necessitates the use of expensive filters and monochromators for fixed wavelength HPLC detection. As a result, a typical system is 4-6 times more expensive than an LED based system. Figure 5 shows a typical instrument design using a deuterium lamp (a) versus one incorporating a UVC LED (b).

With high performance UVC LEDs these applications can now benefit from improved wavelength specificity, long lifetime, and light stability. For applications that only require a few specific wavelengths, manufacturers can develop more compact, less costly instruments without sacrificing performance. For existing instruments using arrays of LEDs for other wavelengths, manufacturers can now access high performing chips in the deep UV spectrum - where a majority of all molecules absorb - opening doors to address more applications.

In conclusion, systems using UVC LEDs can match and sometimes exceed the performance of systems using UV lamps, while at the same time delivering higher efficiency and reduced costs for fixed wavelength applications. The comparable performance allows manufacturers to offer longer instrument life, higher reliability, and increased productivity while reducing overall costs for the end user. These new devices are driving innovations in instrument design for the life sciences to address key market trends around productivity, cost reduction, and miniaturisation.

References: