Violet (405 nm) diode laser for laser induced fluorescence detection in capillary electrophoresis

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The first application of the violet diode laser for laser induced fluorescence (LIF) detection in capillary electrophoresis is reported. Operating at 405 nm with an output power of 5 mW, this laser offers many advantages over conventional red diode lasers operating at 635 nm. We demonstrate the suitability of the violet diode laser for LIF detection by detecting amino acids labeled with naphthalene-2,3-dicarboxaldehyde (NDA). Detection limits of 10 nM were achieved with a linear range of over three orders of magnitude.

1 Introduction

Laser induced fluorescence (LIF) detection represents the current state-of-the-art in terms of sensitivity for microcolumn separation techniques. In particular, LIF detection coupled to capillary electrophoresis (CE) has achieved detection limits approaching the single molecule level. 1 However, a number of drawbacks are associated with LIF detection. Primarily, conventional lasers such as the Ar+, He–Ne, and He–Cd are generally expensive and have limited lifetimes (~5000 h). These lasers are also relatively bulky, making them unattractive for miniaturized instruments. Finally, the sensitivity achieved with conventional lasers is limited by the instability of their output (flicker noise). 2

Semiconductor or diode lasers are a promising alternative to conventional lasers. Diode lasers are cheap, require no maintenance, and have lifetimes approaching 50 000 h. 3 These lasers are also extremely compact and can run off battery power. Further, the output stability of diode lasers is significantly better than that of conventional lasers (0.01% vs. 1% noise). 2 These properties suggest that diode lasers are ideally suited for LIF detection.

Unfortunately, diode laser output has been limited to the near-IR and more recently the red region (635 nm) of the electromagnetic spectrum. The red diode laser has been used successfully in a number of CE-LIF applications. 4–6 However, despite the advantage of low background fluorescence signals in this region of the spectrum, use of the 635 nm diode laser has been limited. Very few commercially available dyes are excitable in this region. Further, those that are available tend to be rather large molecules that are water insoluble and/or complicate the separations. Thus diode lasers emitting at much shorter wavelengths would be extremely desirable.

Efforts have been made to shorten the wavelengths of diode lasers through second harmonic generation (SHG). 7,8 Unfortunately, SHG can be a very inefficient process, with conversion efficiencies as low as 2.5 × 10−6. 9 For instance, Imasaka et al. frequency-doubled a 40 mW 830 nm diode laser using a LiNbO3 waveguide to produce blue light at 415 nm. 10 Unfortunately, they ultimately found that the 0.01 to 0.05 mW of output power was too weak to be useful for many LIF applications. 10 More promising results were achieved by Jansson et al. who focused a diode laser (848 nm, 150 mW) through a potassium titanyl phosphate (KTP) waveguide to produce 0.5 mW of blue light (424 nm). 8 Jansson et al. claimed that 0.5 mW was more than sufficient for LIF detection of derivatized amino acids in CE, and yielded detection limits of 5.9 nM. Similarly, diode-pumped Nd:YAG lasers operating at 532 nm have also been employed for detection in CE. 10 These lasers have sufficient power (several mW), are relatively small, and are now commercially available. However, these diode-based lasers have numerous shortcomings. Firstly, the use of non-linear optics adds substantially to the cost of these lasers. Secondly, the low SHG conversion efficiency requires rather large input power, adding both to the cost and to the size of these lasers. Thus these diode-based lasers exhibit only moderate advantages over conventional gas-based lasers.

The quest for a short-wavelength diode laser began in the late 1960s when it became evident that group III nitride materials (AIn, GaN, InN) possessed band gap energies capable of producing violet–blue light. 11,12 Corporations such as AT&T, IBM, and RCA recognized the potential for such devices and implemented research programs. However, most of these programs were later cancelled due to the inability to solve several key technological problems. Specifically, GaN substrates were highly defective due to poor crystal growth technology. It was not until the mid-1980s that these problems begin to be resolved, due in large part to the efforts of Isamu Akasaki at Nagoya University and Shuji Nakamura at Nichia Chemical Company (now Nichia Corporation). The technique of metalorganic chemical vapor deposition (MOCVD) was developed for depositing GaN films onto sapphire substrates. 13 These structures exhibited remarkable improvements in optical properties, ultimately leading to the development of high-brightness blue and green light emitting diodes (LEDs). 14

In the mid-1990s, Nichia Corporation pumped an unprecedented 1.5% of its annual revenue into Nakamura’s research. In 1995, Nichia achieved room temperature pulsed operation of an InGaN-based laser diode with an emission wavelength of 410 nm. In 1996, Nakamura et al. demonstrated room temperature continuous-wave (cw) operation of InGaN laser diodes, but lifetimes were limited to 1 s due to the generation of large amounts of heat. 15 Further optimization of the InGaN structure led to the reduction of the threshold current and voltage, initially increasing lifetimes to the tens of hours and then up to nearly 10 000 h in 1997. In October of 1999, Nichia launched the commercial sale of their InGaN-based violet laser diode. 16 The diode operates at 405 nm with a power output of 5 mW and has an estimated lifetime of 2000 to 5000 h. It is anticipated that this laser will revolutionize technologies such as laser printing, telecommunications, and high-density data storage. For instance, Pioneer Electronics Corporation has already demonstrated an optical disk that holds 27.4 Gbyte (enough to store 4 h of high-definition video) of data using the Nichia diode. Pioneer Electronics plans to use this technology for the next generation of DVD players. Current DVDs have a capacity of 8.54 Gbyte,
not nearly enough to store an adequate length of high-definition video.

We report here the first application of the violet diode laser for laser induced fluorescence detection in capillary electrophoresis. We demonstrate the detection of amino acids labeled with naphthalene-2,3-dicarboxaldehyde (NDA). NDA is a commonly used fluorogenic label for primary amines and amino acids.\textsuperscript{17}

2 Experimental

2.1 Apparatus

A P/ACE 2100 (Beckman Instruments, Fullerton, CA, USA) equipped with an LIF detector was used for all experiments. Data acquisition and control was performed using P/ACE Station software (Beckman) for Windows 95 on a 486 PC. Untreated silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 \( \mu \)m, outer diameter of 365 \( \mu \)m, and a total length of 57 cm (50 cm to the detector) were used. The laser beam was coupled to the LIF detector through a 1.5 m multi-mode fiber patchcord with a 100/140 \( \mu \)m (core/cladding) diameter and SMA 906 connectors (Oz Optics Inc., Carp, ON, Canada). Fluorescence was collected through a 500 nm band pass filter, with a 25 nm full-width-at-half-maximum (Omega Optical, Brattleboro, VT, USA). The data acquisition rate was 5 Hz with a rise time of 1 s. Fluorescence spectra were obtained on a Shimadzu RF-5301 PC Spectrofluorometer (Columbia, MD, USA) controlled by RF-530XPC Personal Fluorescence software on a Pentium PC. Laser power measurements were performed with a Newport 835 optical power meter (Irvine, CA, USA).

2.2 Violet diode laser construction

The laser diode (shown in Fig. 1) was purchased from Nichia Corporation (Tokyo, Japan). Individual diodes lase at a wavelength of 405 ± 10 nm. This particular diode was rated by the manufacturer to emit at 400 nm with an output power of 5 mW, under an operating current of 48 mA at 25 °C, with beam divergences in the parallel (\( \theta _p \)) and perpendicular (\( \theta _c \)) directions of 7.5° and 32.3°, respectively. A ±6 V power supply was employed and delivered a current of 47.4 mA, resulting in an output of 3.5 mW (less than typical power, discussed below). An automatic power control (APC) function was integrated into the circuit to monitor light output through a photodiode and adjust the operating current accordingly to maintain constant output power.

The laser diode was placed into an aluminum housing and a focusing lens was mounted approximately 1 mm from the diode head. The focusing lens was plano-convex with a 5 mm diameter and 6 cm focal length. The diode housing was then inserted into a finned aluminum heat sink, onto which a fiber optic bulkhead was mounted (SMA 905/906 receptacle; Amphenol Canada Corp., Scarborough, ON, Canada). The distance of the diode head with respect to the bulkhead was then optimized to achieve optimal focusing into the fiber, resulting in a transmission efficiency of approximately 30%.

2.3 Reagents

All solutions were prepared in Nanopure 18 MΩ water (Barnstead, Chicago, IL, USA). Buffers were prepared from reagent grade boric acid (BDH, Darmstadt, Germany) and the pH was adjusted using sodium hydroxide (BDH). Sodium dodecyl sulfate (98%) was obtained from Aldrich (Milwaukee, WI, USA). The amino acid standard mixture designed specifically for fluorescence detection (Cat.# A 2161) was purchased from Sigma (St. Louis, MO, USA). The solution contained 25 \( \mu \)M of \( \alpha \)-amino acids except for cysteine at 12.5 \( \mu \)M. Individual \( \alpha \)-amino acids for peak identification were also obtained from Sigma (Cat # LAA-21). Naphthalene-2,3-dicarboxaldehyde (NDA) was purchased from Molecular Probes (Eugene, OR, USA) and sodium cyanide was obtained from Sigma. NDA was dissolved in A.C.S. grade acetonitrile (Fisher, Pittsburg, PA, USA).

2.4 Derivatization procedure

The derivatization procedure used was adapted from that of Ueda et al.\textsuperscript{18} To 700 \( \mu \)l of borate buffer (100 mM, pH 9.5) in a 1.5 ml polypropylene vial, 100 \( \mu \)l of sodium cyanide solution (10 mM in borate buffer) and 100 \( \mu \)l of the amino acid standard mixture (25 \( \mu \)M each, 2.5 \( \mu \)M after 1/10 dilution) were added and mixed. Next, 100 \( \mu \)l of NDA solution (1 \( \mu \)M in acetonitrile) was added and the vial was capped, shaken, and the reaction was allowed to proceed at room temperature for 30 min. The reaction mixture was injected directly into the capillary or diluted accordingly with borate buffer.

2.5 Separations

New capillaries were pre-treated with 0.1 M NaOH for 10 min before use. A 5 min pressure rinse (138 kPa) with the separation buffer (100 mM borate at pH 9.5 with 50 mM SDS) was performed before each injection. Injections were executed hydrodynamically (3.45 kPa) for 3 s. Separations were performed under an applied potential of 15 kV with a voltage ramp time of 30 s.

3 Results and discussion

3.1 Separation of NDA-labeled amino acids

NDA reacts with primary amines in the presence of cyanide to form 1-cyano-2-substituted-benz[f]isoindole (CBI) derivatives.\textsuperscript{17} These derivatives cannot be adequately separated by conventional CZE, thus micellar electrokinetic chromatography (MEKC) was employed. Displayed in Fig. 2 is the separation of a standard mixture of NDA-labeled amino acids in 100 mM borate–50 mM SDS buffer at pH 9.5. Baseline separation was achieved, except for leucine (Leu) and phenylalanine (Phe) which were only partially resolved. Di-labeled amino acids such as lysine (Lys) and cysteine have very low quantum efficiencies.\textsuperscript{17} This explains the very small peak observed for lysine and the absence of a cysteine peak. Efficiencies approaching 1 000 000 plates m\(^{-1}\) were achieved.
The separation of a standard mixture of NDA-labeled amino acids. Experimental conditions: 100 mM borate of NDA-labeled glycine in pH 9.5 borate buffer. The bold arrow indicates the point of excitation.

3.2 Detection limits

A linear detector response was achieved over 3 orders of magnitude (25 nM to 2.5 μM; slope of log response vs. log concentration plot = 1.03 ± 0.07). Detection limits were determined through serial dilution of the reaction mixture with 100 mM borate buffer at pH 9.5. A detection limit of 10 nM (S/N = 3) was determined for each amino acid, except lysine and cysteine which were not detected at these levels. The sensitivity achieved was slightly poorer than that quoted by Ueda et al. (0.24 nM) using the 442 nm line of the He–Cd laser and comparable to that obtained by Jansson et al. (5.9 nM) employing a frequency-doubled diode laser at 424 nm.

The fluorescence excitation spectrum of NDA-labeled glycine is shown in the inset of Fig. 2. This spectrum possesses sharp excitation bands at 440 and 420 nm. However, NDA–glycine is still excited strongly at 400 nm (in borate buffer, pH 9.5)—approximately ¾ the intensity of the 420 nm band and ¾ the intensity of the 440 nm band. Though excitation of NDA-labeled amino acids at 400 nm seems less than ideal, filtering of the excitation light from the broad emission band (450–525 nm) is facilitated. In this study, a somewhat narrow 500 nm (FWHM = 25 nm) band pass filter was used. Thus further optimization using a broader band pass filter may have resulted in sensitivity gains.

A laser output power of 3.5 mW was used in this study instead of the typical 5 mW. Firstly, Jansson et al. observed no increase in S/N beyond 0.3 mW of output power due to photobleaching of the NDA-labeled amino acids. In our study, the fiber optic coupling of the laser to the detector was measured to be 30% efficient, resulting in only 1 mW reaching the capillary. Thus this power output is more than adequate for this application. Finally, operating the laser diode at lower power can increase the lifetime of the diode. However, for applications requiring higher laser power, the operating current can easily be increased to achieve maximum power.

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6 References

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