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Short communication

Generation of 275.4-nm UV output from a large-frame argon-ion laser for fluorescence detection in capillary electrophoresis

Steven J. Kok, Gerard Ph. Hoornweg, Ton de Ridder, Udo A.Th. Brinkman,
Nel H. Velthorst, Cees Gooijer*

Vrije Universiteit, Department of General and Analytical Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, Netherlands

Abstract

A standard, relatively old, large-frame argon-ion laser, which is available in many laboratories, was modified to produce output in the deep-UV (275–306 nm) region by installing a set of inexpensive, commercially available laser mirrors. The deep-UV output is generally applicable as excitation wavelength in the field of biochemical and environmental analysis. However, it has received little attention in the literature, probably because a complete laser set-up normally requires a high investment. The modification described, which includes a thorough cleaning of the Brewster windows of the laser tube and a careful alignment of the laser, is a good alternative to this investment. In daily operation, only some minor realignments are required. As an illustration of the applicability, excitation with the 275.4-nm line was used for laser-induced detection of native fluorescent aromatic sulphonates in capillary electrophoresis. © 1998 Elsevier Science B.V.

1. Introduction

Laser-induced fluorescence (LIF) detection is one of the most sensitive detection techniques for capillary electrophoresis (CE) [1]. We have used several types of laser systems for native LIF detection in CE, i.e. a continuous wave (CW) intracavity frequency-doubled argon-ion laser providing up to 100 mW at 257 nm, and a pulsed excimer dye-laser combination giving 25 mW of tunable emission which was used at 280, 290 and 325 nm [2,3]. As regards the laser source, the detectability achievable by a LIF set-up depends on three main factors: the wavelength of the applied laser line, the irradiance at the CE capillary and the duty cycle of the laser system. The selected wavelength mainly determines the background caused by sample interferences and impurities in the CE capillary material, the CE buffer and the applied optics. To quote an example, when using 257 nm for excitation, we could not use a lens-containing microscope objective for collection of emission light, because of its high impurity fluorescence. Furthermore, broad interfering peaks (which were probably due to humic acids present in the sample) were found in the electropherograms when using the CE–257-nm LIF set-up for environmental analysis. When using longer-wavelength (pulsed) excitation, the ratio (analyte signal/background interferences) was much better. Concerning the irradiance, applying only a fraction of the total laser output (5 mW for the CW and 0.7 mW averaged for the pulsed laser operated at 10 Hz) was found to give the best

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*Corresponding author.

signal-to-noise ratios. Apparently, if more laser power is used, the irradiance of the focused laser beam is so high that absorption saturation occurs, so that only the background level is increased. The main reason for concern regarding the applicability of pulsed lasers for detection in dynamic systems is their extremely low duty cycle (i.e. pulse duration times repetition rate) which, typically, is as low as 10^{-6}.

The above observations are in line with other studies demonstrating the appropriateness of laser excitation around 280 nm [4,5]. They show an improvement of up to two orders of magnitude in detection limits could be obtained by using (CW) 275.4 nm as excitation wavelength as opposed to 257 nm [4]. The usefulness of (CW) 275.4 nm as excitation wavelength for LIF detection in CE separation was further demonstrated for e.g. the native-fluorescence detection of proteins in single cells [6], biopharmaceuticals [7], catecholamines [8], nucleic acids [9], and DNA fragments [10], as well as for absorption detection [11]. Unfortunately, the investment required for such a complete new laser system is quite high (approx. US$150 000). In this communication we want to show that, even old, large frame argon-ion lasers, which presumably are widespread over various laboratories, can be modified to provide the same laser line. It only requires the installation of a relatively inexpensive (ca. US$2000) set of mirrors. To illustrate the performance of the modified laser, its suitability for CE–LIF was examined by analysing naphthalene sulphonates in standard solutions and waste water.

2. Experimental

2.1. Chemicals

The naphthalene sulphonates used in this study were purchased from Aldrich (Steinheim, Germany), Kodak (Darmstadt, Germany) and Merck (Prodotti Gianni, Milan, Italy). The individual compounds are indicated by the substituent positions on the naphthalene skeleton as follows: positions of NH_2 groups/positions of OH groups/positions of SO_3^- groups. The label 'zero' indicates the absence of a particular group. The borate buffer (pH 8.75) used

for CE was prepared from laboratory-distilled water and boric acid (J.T. Baker, Deventer, Netherlands). A solution of 12 g ammonium hydrogenfluoride ($NH_4F \cdot HF$, J.T. Baker) in 100 ml distilled water was used to clean the Brewster windows of the laser tube.

2.2. Laser systems

An Innova 100A-15 (Coherent, Palo Alto, CA, USA) laser tube, with an (older generation) CR-10 power supply was used. The tube had been in use for a prolonged period of time, and the cathode inside the tube was seen to be sagging due to ageing, so that it would soon block the laser bore. To prevent this, a construction was made inside and outside the laser tube housing, to put the complete laser head upside down. This operation appears to increase the tube lifetime considerably.

The all-lines UV output in the 332–368 nm wavelength region as a function of the laser tube current for the above laser system is shown in Fig. 1. The all-lines output of the laser could be separated into the various wavelengths by an Applied Photophysics (London, UK) laser filter monochromator with a throughput of 51% in the region of interest. The new 275–308 nm high-reflector mirror (type 0160-495-00, planar) and the output-coupler mirror (type 0166-853-00, 0.8% at 275.4 nm and 1.3% transmission at 300–308 nm, radius 2.5 m) were purchased from Coherent.

2.3. CE system

CE separations were performed as described in a previous paper [12] using a Prince injection and high-voltage system from Lauerlabs (Emmen, Netherlands) and a 90 cm (74 cm to the detector)× 75 μm I.D. capillary from Polymicro Technologies (Phoenix, AZ, USA). Pressure-driven injection was applied. The laser beam was focused on the capillary by a 3-cm focal-length lens. Emission was collected by a reflecting 25×N.A. 0.4 microscope objective (Ealing, Holliston, MA, USA) and projected on the entrance slit of a MonoSpec 18 spectrograph (Scientific Measurement Systems, Grand Junction, CO, USA) equipped with a 600 gr/mm holographic grating. The Model 1420 intensified linear diode array detector (EG&G, Princeton, NJ, USA) cover-
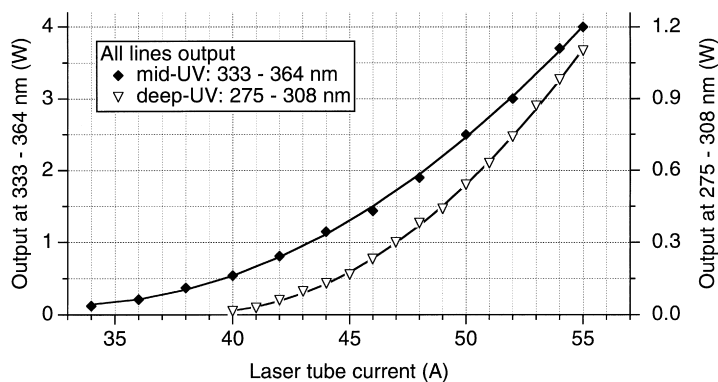
Fig. 1. Total output of the large-frame argon-ion laser as a function of laser tube current using standard mid-UV mirrors (♦, left-hand vertical axis) and the new deep-UV mirror set (▽, right-hand vertical axis).

ing the spectral range of 311–512 nm was controlled by a Model 1460 OMA III console. The three-dimensional data so obtained were further processed as described elsewhere [2] by using the IGORPRO (WaveMetrics, Lake Oswego, OR, USA) software programme, run on an Apple Macintosh computer.

3. Results and discussion

Since both the mid-UV and deep-UV output of an argon-ion laser are based on the production of Ar^{2+}, first of all the performance of the laser system concerned should be checked. For our instrument this performance is shown in Fig. 1; as can be seen, despite the prolonged use the specified value of 3 W output in the mid-UV region is easily reached. To generate the required deep-UV output, the new mirror set was installed.

In the initial alignment procedure three steps can be distinguished. (i) Optimization of the alignment in the visible (514.5 nm) region. (ii) Exchange of mirrors to obtain mid-UV output. Since the emission gain inside the laser cavity is very sensitive to losses by contamination, the Brewster windows of the laser tube had to be cleaned carefully with methanol and an ammonium hydrogenfluoride solution. Since ammonium hydrogenfluoride is an aggressive reagent for synthetic materials, only wooden-shaft cotton swabs (Q-tips) should be used and the mirrors should be cleaned only with methanol, to protect their coatings [13]. The alignment was optimized for

maximum output. (iii) Exchange of mirrors for the new 275–308 nm high reflector mirror and output coupler mirror. The gain for the deep-UV wavelengths will be less than for the typical visible or mid-UV lines. Therefore, the alignment of the laser cavity was much more critical. In fact, it took us several hours to find an alignment that resulted in any laser output at all. However, once a laser beam had been formed, optimization of the alignment was straightforward.

After completing this procedure, daily maintenance only requires minor adjustments of the alignment after the warm-up period of the laser tube. The Brewster windows should be cleaned with ammonium hydrogenfluoride approximately once a week. The all-lines output created in the 275–308 nm region as a function of the laser tube current is also depicted in Fig. 1; comparison of the two curves shows that, at a reasonable laser tube current of 48 A, a mid-UV output of 2.0 W corresponds with 400 mW in the deep UV. Contrary to other approaches wherein deep-UV output is obtained by frequency-doubling of fundamental visible or dye-laser lines, the present modification does not negatively affect the stability; it is the same as in the mid-UV, typically 0.5–1%. Since our final aim was to use only the 275.4 nm output, a laser filter monochromator was placed in the beam. A typical diagram of the output for the four individual laser lines thus obtained as a function of the laser tube current is shown in Fig. 2, together with the output spectrum at high current. By comparison with Fig. 1, it can be
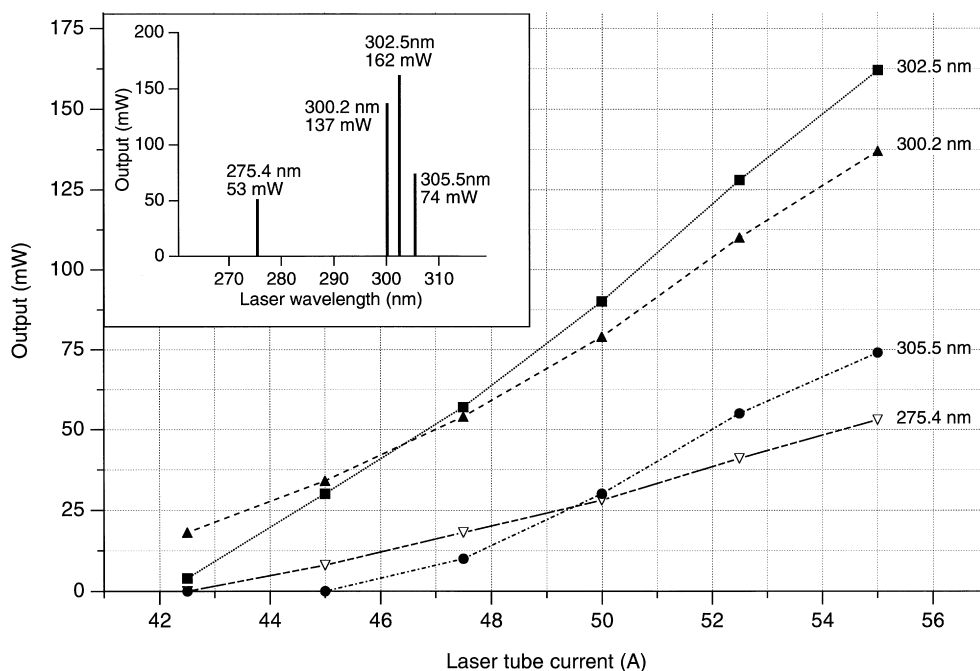
Fig. 2. Output of the modified set-up of four individual lines selected by means of a laser filter monochromator. The insert shows the output spectrum at a laser tube current of 55 A.

stated that for the generation of an output of 10 mW at 275.4 nm, a laser producing at least 1 W in the mid-UV is required. The outputs of the several lines show a different dependency on the laser tube current; this probably reflects that distinctly different electronic states of the doubly charged argon ions are involved.

To evaluate the performance of the 275.4-nm laser line for CE–LIF, the fluorescence signal-to-noise ratios of several naphthalene sulphonates (NSs) were determined as a function of laser intensity. It was found that using 3 mW (less than 10% of the available output) gave the best overall results. The detection limits (expressed as concentrations injected) for a representative group of NSs characterized by distinct differences in emission maxima are given in Table 1. The results are compared with those obtained earlier [2,3] by pulsed excitation at

Table 1
Concentration detection limits (in ng/ml; S/N=3) in CE of selected NSs in standard solutions, applying different lasers for excitation

Analyte	$\lambda_{em, max}$ (nm)	Concentration detection limits for	
		CW (275.4 nm; 3 mW)	Pulsed (280 nm; 0.7 mW)
0/0/2	340	1	30
0/0/1,5	340	2	30
0/4,5/2,7	370	8	30
2/0/1	420	1	8
0/1/4	430	6	3
7/0/1,3	450	0.75	1
3/0/2,7	460	0.75	1
0/7/1,3	460	1	2
8/0/2	480	1	4

280 nm. For most compounds, the CW laser gives distinctly better results, especially for those emitting in the short-wavelength region. For NS emitting at longer wavelengths the improvement is rather small and in one case even absent. This is probably due to a higher susceptibility of these compounds to photo-degradation [3]. Presumably, somewhat better results could be obtained using less than 3 mW excitation intensity, but this phenomenon was not further studied.

The above results suggest that the thirty-fold analyte preconcentration step, involved previously to analyse NSs in water samples [2], is in fact not needed if, instead of the pulsed excimer-dye laser, the CW 275.4-nm LIF system is used. To check this, a waste water sample (that had been subjected to clean-up without enrichment by solid-phase extraction [14]) was injected. The electropherogram of Fig. 3 shows a large number of peaks. On the basis of their migration times and emission spectra two of these could be identified, viz. as a monosulphonated naphthalene (9.4 min; probably NS 0/0/2) and a disulphonated aminonaphthalene (16.3 min; probably NS 7/0/1,3). None of the other peaks in the

electropherogram can be assigned to NSs because emission was found at significantly shorter wave-lengths (ca. 300 nm) than is usual for NSs (340–480 nm). The concentrations of NS 0/0/2 and 7/0/1,3 in the waste water were 13 ng/ml and 9 ng/ml, respectively. These results show that the CE–275.4-nm LIF set-up is well suited for environmental analysis.

4. Conclusions

By installing a commercially available set of rather inexpensive laser mirrors suitable for the 275–308 nm wavelength region, a standard large-frame argon-ion laser could be modified to produce suffi-cient deep-UV output. The modified set-up enables selective excitation with a high duty cycle. Thorough cleaning of the Brewster windows of the laser tube with methanol and ammonium hydrogenfluoride, and careful alignment of the laser cavity was essential to create optimum results. Once the mirrors were installed, the set-up needed little further attention. The 275.4 nm line was successfully used for the
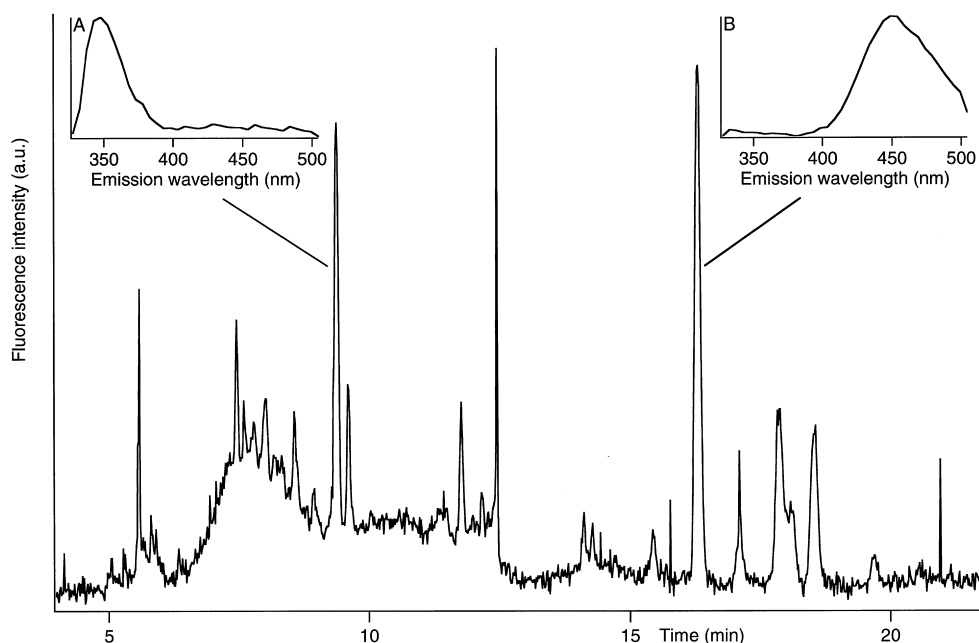

Fig. 3. Electropherogram of a waste water sample, recorded using a borate buffer of pH 8.75, after clean-up (no enrichment) by means of off-line solid-phase extraction. The emission spectra of the identified peaks at 9.4 min (A) and 16.3 min (B) are given as inserts.

analysis of waste water samples by means of CE with LIF detection without the need of analyte preconcentration. We feel that the present communication will stimulate other researchers to modify their available mid-UV producing argon-ion lasers at relatively low cost to enable LIF detection with deep-UV excitation.

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