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Performance of a simple UV LED light source in the capillary electrophoresis of inorganic anions with indirect detection using a chromate background electrolyte

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Light emitting diodes (LEDs) are known to be excellent light sources for detectors in liquid chromatography and capillary electromigration separation techniques, but to date only LEDs emitting in the visible range have been used. In this work, a UV LED was investigated as a simple alternative light source to standard mercury or deuterium lamps for use in indirect photometric detection of inorganic anions using capillary electrophoresis with a chromate background electrolyte (BGE). The UV LED used had an emission maximum at 379.5 nm, a wavelength at which chromate absorbs strongly and exhibits a 47% higher molar absorptivity than at 254 nm when using a standard mercury light source. The noise, sensitivity and linearity of the LED detector were evaluated and all exhibited superior performance to the mercury light source (up to 70% decrease in noise, up to 26.2% increase in sensitivity, and over 100% increase in linear range). Using the LED detector with a simple chromate–diethanolamine background electrolyte, limits of detection for the common inorganic anions, Cl^-, NO_3^-, SO_4^{2-}, F^- and PO_4^{3-} ranged from 3 to 14 μg L^{-1}, using electrostatic injection at −5 kV for 5 s.

Introduction

Capillary electrophoresis (CE) has been used extensively as a technique for the determination of small inorganic anions.[1,2] The most commonly used detection technique for non-UV absorbing anions has been indirect photometric detection at wavelengths in the UV region. Indirect detection was first used in CE for anions by Hjerten *et al.*,[3] and is based upon the displacement on a charge for charge basis of a UV absorbing probe anion in the background electrolyte (BGE) by the sample analyte anions, producing negative peaks on a high absorbance background as the analytes pass through the detection window. From this early work the development of indirect UV detection as a universal detection method has led to the establishment of CE as a viable alternative to ion chromatography for the simultaneous determination of inorganic anions.

By far the most common absorbing probe anion used for the detection of inorganic anions is chromate.[4–7] Almost all the studies carried out using a chromate as a probe anion have utilised a BGE pH of between 8 and 9, with monitoring of absorbance at a wavelength of 254 nm (predominantly due to the use of mercury and deuterium lamps with filters as light sources within many commercial instruments).[3,8–15] However, chromate actually absorbs approximately 50% more strongly at 371 nm than at 254 nm, and so improved results, particularly in terms of sensitivity, should be obtained if the absorbance was monitored at this higher wavelength. Combination of this improvement in sensitivity with a stable, low noise light source can therefore be expected to yield substantial improvements in detection limits.

The use of light emitting diodes (LEDs) as light sources for photometric detection in CE has been investigated by a number of workers and these have been shown to exhibit some benefits over traditional light sources, such as deuterium or tungsten lamps. These can be summarised as: generally low cost (typically under US$1, somewhat dearer for the UV LED), small size, high robustness and reliability, long lifetimes (in the order of 10^5 h), little heat production, good linearity of the emitted light intensity with current, suitability for operation in a pulsed regime at high frequencies (emission output stabilisation measured in ns), particularly stable light emission, and extremely low noise.

Tong and Yeung[16] were the first to report the use of both diode lasers and LEDs as light sources within an absorption detector system for CE. They investigated two LEDs at 660 and 565 nm, finding reduced noise levels and improved stability over commercial detectors. Tong and Yeung also illustrated how inorganic anions could be determined sensitively using permanganate as a probe anion in place of chromate, with a green 565 nm LED as light source.

Later work by Macka *et al.*[17] found that LEDs in general exhibit stable output and markedly lower noise than other light sources such as mercury, deuterium and tungsten lamps. Since detection limits in CE are determined using the ratio of signal to noise, this reduction in noise can result in significant reductions in limits of detection. Macka *et al.* investigated 6 different LEDs having emission wavelengths within the visible region, ranging from 563 to 654 nm, and demonstrated the potential of this approach by the detection of alkaline earth metal complexes of Arsenazo I. Metal ion and metal complex separations were also investigated by Butler *et al.*[18] who used a green LED (525 nm) for the direct detection of metal–PAR complexes and the indirect detection of alkali and alkaline earth metals using a BGE containing Pyronine G.

A similar study was later carried out by Collins and Lu,[19] who investigated a red LED with a maximum emission wavelength of 660 nm. They detected uranium(VI) at a concentration of 23 μg L^{-1} using Arsenazo III as a pre-capillary complexing ligand. An LED-based visible wavelength absorbance detector in CE has also been investigated by Boring and Dasgupta,[20] who compared the performance of the LED detector with zinc, cadmium and mercury lamps. The LED used had a maximum emission wavelength at 605 nm (orange). They found that comparable noise levels were obtained for the LED and the cadmium and zinc lamps, although the cadmium and zinc sources were operated with a wider slit. The above study and others utilising visible and NIR LEDs as detector light sources in CE are the subject of a recent review by Malik and Faubel.[21]

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In contrast to all the previously used LEDs that emit in the visible spectrum, the use of LEDs as UV light sources has not yet been reported for CE absorption detection systems, nor to our knowledge for any column liquid chromatography or electromigration capillary separation technique. Only for fluorescence detection recently has there been a report on the use of a UV LED operated in a pulsed regime for fluorescence detection of labelled amino acids.[22]

Therefore, the present study examines the application of such a light source to the indirect absorbance detection of inorganic anions using a standard chromate BGE. The LED used in this study had an emission maximum at 379 nm, which matches closely the absorbance maximum for chromate. To achieve the lowest possible detection limits, the BGE used in this work was prepared in such a way as to eliminate the presence of interfering co-anions from either the added buffer or EOF modifier.[23,24]

Experimental

Instrumentation

A Waters Capillary Ion Analyser (Milford, MA, USA) was used either with the original detector (fitted with a mercury lamp and a 254 nm filter) or an in-house built UV LED-based detector, as described by Macka *et al.*[17] In brief, the LED was accommodated in place of the original mercury lamp and equipped with a 2 mm diameter circular slit positioned on the bulb of the LED, and powered by a standard stabilised laboratory power supply using a resistor (180 Ω) in series to give a current of 30 mA. All other parts including the capillary optical interface, the detector and associated electronics were of the original Waters CIA CE machine. Separations were performed using a Polymicro (Phoenix, AZ, USA) fused silica capillary (58 cm × 75 μm, length to detector 50 cm). The LED used was a 5 mm domed lens 10 degree UV LED with optical power of 1 mW obtained from Optosource (Marl International Ltd., Ulverston, Cumbria, UK). The emission spectrum was measured with an Ocean Optics S 1000 diode array fibre-optics visible spectrophotometer (purchased through LasTek, Thebarton, SA, Australia) and showed an emission maximum at 379 nm and a spectrum half width of 12 nm. Absorption spectra of the chromate BGE and other spectrophotometric measurements were determined using a Cary UV-Vis-NIR spectrophotometer (Varian, Australia) with a 1 cm pathlength quartz cell. The spectrum of chromate was registered using a 0.387 mM solution of Na_2CrO_4 in 50 mM NaOH.

Reagents

All chemicals used were of analytical-reagent grade. Chromic trioxide, diethanolamine (DEA), potassium chloride, (KCl) potassium dihydrogenphosphate, and didodecyldimethylammonium bromide (DDAB), were obtained from Aldrich (Milwaukee, WI, USA). Sodium sulfate, sodium nitrate, sodium nitrite and sodium fluoride were obtained from Fluka (Buchs, Switzerland). Water used throughout the work was treated with a Millipore (Bedford, MA, USA) Milli-Q water purification system.

Procedures

New capillaries were conditioned with 100 mM NaOH, methanol and water before any analysis took place. BGEs were prepared using a previously described procedure.[23] In brief, 5 mM chromic acid solution was prepared by titrating the required amount of chromic trioxide with DEA to pH 9.2 (final concentration of DEA approximately 20 mM). The electrolyte was degassed and filtered using a 0.45 μm nylon membrane filter from Gelman Laboratories (Michigan, USA) prior to use. A 0.5 mM solution of DDAB was prepared as the electro-osmotic flow (EOF) modifier. The capillary was prepared for coating by flushing with NaOH (10 mM for 1 min at −0.5 bar pressure on the detection side), after which a coating of DDAB was applied by flushing the capillary (0.5 mM for 1 min) prior to analysis. The capillary was then flushed for a further 1 min with water to remove any excess EOF modifier and finally rinsed with the BGE before injection of the samples. Total rinse time prior to injection was 3 min. Electrokinetic injection of the analytes was used at −5 kV for 5 s. Analysis was performed at a separation voltage of −25 kV.

Linearity data were obtained as described previously[17,25] by flushing a series of chromate solutions made by serial dilution of a stock solution of chromate prepared in (and diluted with) 50 mM NaOH to ensure that chromate was the only absorbing species present. Absorbance measurements at both 254 and 379 nm were performed by flushing the capillary with water, followed by the standard chromate solution, then stopping the flow and measuring the absorbance under static conditions. The absorbance of each test solution was measured in triplicate. Absorbances were measured in order of increasing standard concentration to minimise possible carry-over errors.

Results and discussion

Effective pathlength

Fig. 1 shows an overlay of the chromate absorption spectra from 200 to 500 nm with the emission line of a standard mercury lamp and the emission spectrum of the UV LED. As can be seen, 254 nm is well-removed from the maximum absorption wavelength of chromate under alkaline conditions. Under the above conditions values for molar absorptivities of $\varepsilon = 2.58 \times 10^3$ (254 nm), 3.96×10^3 (371 nm) and 3.80×10^3 l mol^{-1} cm^{-1} (379.5 nm) were obtained. These values show that there is a 47% increase in the molar absorptivity at 379.5 nm compared with 254 nm.

In indirect detection, the limit of detection (LOD) is given by:[15]

$$\mathrm{LOD} = \Delta A/(\mathrm{TR}\varepsilon b_{\mathrm{eff}}) \qquad (1)$$

where ΔA is the baseline noise in absorbance units, ε is the molar absorptivity of the probe anion, b_{eff} is the effective pathlength of the detection cell and TR is the transfer ratio (*i.e.* the number of moles of the probe displaced by one mole of the analyte). TR is maximised through correct choice of probe and BGE conditions. Eqn. (1) shows that LOD should be decreased at higher values of ε, provided that ΔA does not increase.

In our previous papers[17,25] we have described a simple practical method for the determination of b_{eff} and the linearity range of on-capillary photometric detectors in CE. Both parameters are useful in describing the efficiency of the optical

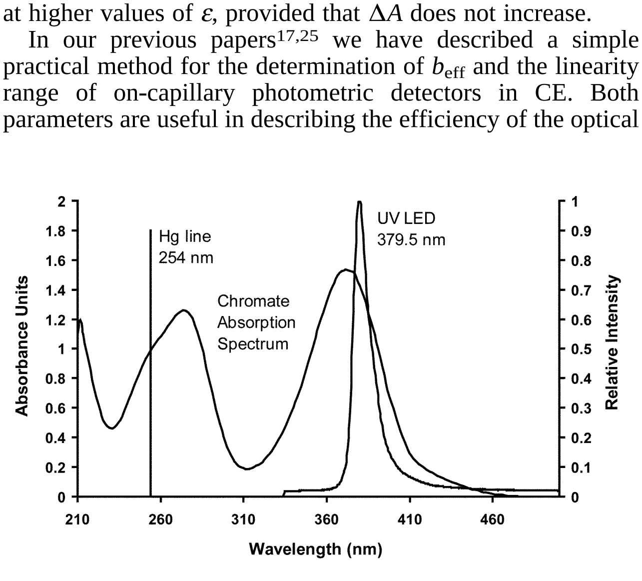

Fig. 1 Overlay of the chromate absorption spectra with the line emission wavelength of a standard mercury lamp and the emission spectrum of the UV LED.

design of a CE photometric detector. If determined for two different detectors under the same BGE conditions and with the same capillary, these parameters will indicate quantitatively the relative performance of the two detectors. The better the optical design, the more light passes through the centre of the capillary and the closer the value of b_{eff} is to the actual capillary inner diameter. At the same time, the linearity will generally be better for those detectors exhibiting lower levels of stray light, and usually detectors exhibiting larger linear ranges will also show higher b_{eff} values.[17]

To determine b_{eff} a rearrangement of the Beer–Lambert law can be used to give the ratio of sensitivity to probe absorptivity. To obtain a valid estimation of b_{eff} the sensitivity of the detector must be obtained using an absorbance value well within the detector's linear range. Here, absorbance values below 50 mAU were used to determine sensitivity as this was known from our previous study[25] to be within the linear range of all detectors studied to date, even those exhibiting the worst linearity. Using a 75 μm capillary, values for b_{eff} of 54.28 and 42.29 μm for the Waters mercury lamp detector and the UV LED based detector, respectively, were obtained using this method. The smaller b_{eff} value for the LED detector can be explained by the fact that the LED is not a perfectly monochromatic light source, and the (effective) absorptivity of chromate when detected with the LED will be an average of all absorptivities over the range of wavelengths emitted by the LED, weighted for the relative emission intensities. Therefore, the effective absorptivity of the chromate as detected by the LED is likely to be somewhat lower than when measured with a monochromatic source at 379 nm and will lead to a lower effective pathlength. This also explains why the improvement in sensitivity achieved in this study is somewhat smaller than that expected from a simple comparison of chromate absorptivities at 254 and 379 nm.

Detector linearity

When using indirect detection it is known that operation of the detector outside its linear absorbance range will compromise accurate quantification. Therefore, it was necessary to determine the linear range of the LED-based CE detector in this study. This was again carried out using the method described previously.[25]

Sensitivity data were calculated from the measured absorbances of the chromate standard solutions and were plotted against chromate concentration (Fig. 2(a)) and background absorbance (Fig. 2(b)). The concentration (or absorbance) of the chromate solution at which sensitivity decreased by more than 5% was used to define the upper limit of detector linearity. It was found that detector linearity was maintained up to a chromate concentration of 50 mM for the Waters CIA instrument equipped with the UV LED as the light source. This corresponded to an upper absorbance limit of 0.375 AU. The upper limit of detector linearity for the same instrument with a standard mercury detector was only 10 mM (0.175 AU). Since the probe concentration for indirect UV detection is generally below 10 mM (for chromate typically between 2 and 5 mM) both detectors were deemed suitable for this application, but there is a significantly larger reserve in maximal applicable background absorbance for the LED detector and this would be particularly useful for analysis of high ionic strength samples.

Baseline noise and detection limits

As can be seen from eqn. (1), two important factors for obtaining low LODs in indirect detection in CE are the molar absorptivity of the probe and the magnitude of the baseline noise. Another major parameter is TR, which is maximised when the electrophoretic mobilities of the probe anion and the analyte anions are similar and when there are no other (competing) anions in the BGE.[5–7] Such competing anions can be introduced into the BGE by incorrect choice of the buffer or the EOF modifier. In the present study, it was decided to employ semi-permanently coated capillaries which can be used without the addition of any EOF modifier to the BGE. In addition, the BGE was buffered with a counter cationic buffer, in this case DEA, so as not to introduce any co-anions into the system.[5–7] The capillary was pre-coated using DDAB as described by Melanson *et al.*,[24] who found that DDAB formed more stable coatings on capillary walls than CTAB, and thus could be used to semi-permanently pre-coat the capillary and therefore not be required as a component of the BGE. Under these conditions test mixtures of common inorganic anions were separated and detected using both the standard 254 nm mercury lamp detector and the 379 nm UV LED-based detector. Baseline noise levels and limits of detection (signal to noise ratio of 3) were determined. Since limits of detection in CE are dependent on injection mode, the same injection parameters were used throughout to permit valid comparison of the two detectors. These data are listed in Table 1, together with the manufacturers' quoted detection limits (at 254 nm) when using their recommended BGE and injection conditions.[26] Table 1 shows that the detector noise was up to 70% lower with the LED-based detector at 379 nm than with the mercury lamp at 254 nm. Combined with the increased sensitivity for chromate obtained at 379 nm, this resulted in a decrease in detection limits of an order of magnitude.

Qualitative analysis of water samples

To illustrate a potential application that benefits from the improved sensitivity of the LED-based detector, a number of

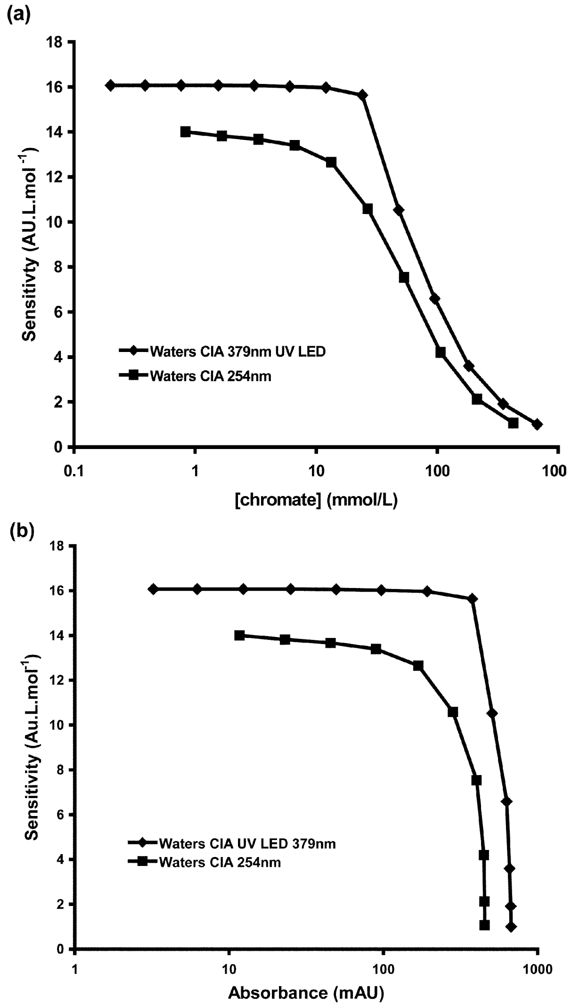

Fig. 2 Sensitivity *versus* probe concentration (a) and *versus* absorbance (b) plots for testing solution of chromate with detection at 254 nm and 379 nm. For other conditions see text.

Table 1 Baseline noise values and approximate detection limits for common anions using indirect detection with a chromate BGE at 379 nm (LED source) and 254 nm (mercury lamp source)

	UV LED 379 nm		Mercury lamp 254 nm		Mercury lamp 254 nm literature[a]	
Noise[b]/mAU	0.024		0.060		—	
Linearity limit/AU	0.375		0.175		—	
Effective pathlength/μm	42.3		54.3		—	
Detection limits	μg L^{-1c} (±SD)[d]	mM[c] (±SD)[d]	μg L^{-1c} (±SD)[d]	mM[c] (±SD)[d]	μg L^{-1}	mM
Chloride	5 (0.4)	0.14 (0.01)	60 (5)	1.7 (0.13)	46	1.3
Nitrate	9 (0.7)	0.15 (0.01)	120 (9)	2 (0.15)	84	1.4
Sulfate	14 (0.5)	0.15 (0.01)	190 (7)	2.0 (0.07)	32	0.3
Fluoride	3 (0.3)	0.16 (0.02)	120 (12)	5.5 (0.60)	84	4.4
Phosphate	5 (0.4)	0.04 (0.005)	70 (7)	0.7 (0.07)	41	0.4

[a] BGE = 4.7 mM Na_2CrO_4/4.0 mM TTAOH/10 mM CHES/0.1 mM calcium gluconate, applied voltage = −15 kV, injection = hydrostatic at 10 cm for 30 s, detection = Hg lamp at 254 nm.[26] [b] Baseline noise determined from multiple analysis (n = 3). [c] Injection = electrostatic at −5 kV for 5 s. [d] SD determined from multiple consecutive analysis of standard solutions (n = 20).

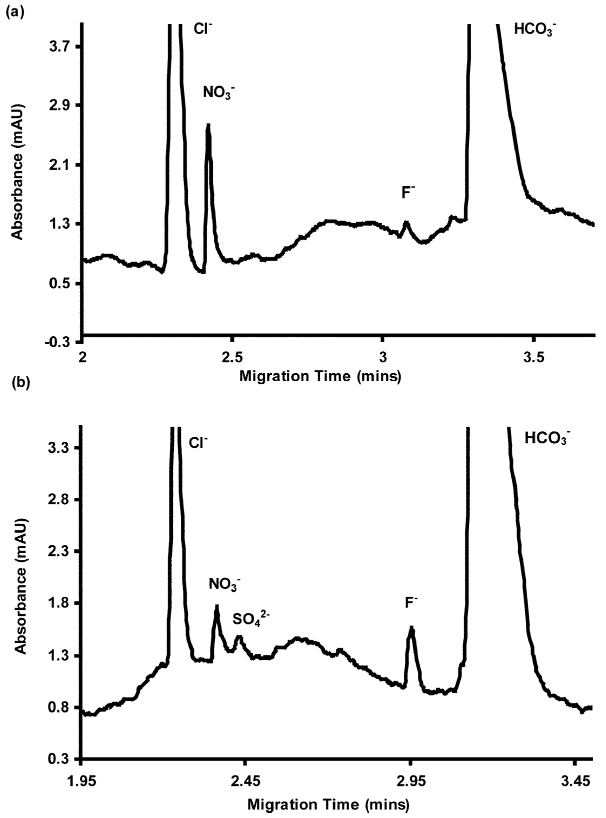

Fig. 3 Analysis of real water samples. Electropherograms of (a) river water and (b) mineral water with detection using 379 nm LED. For other conditions see Experimental.

water samples were screened for the presence of trace anions. Typical sample electropherograms for (a) a river water, and (b) a mineral water sample are shown in Fig. 3. As can be seen from Fig. 3, the presence of trace levels of NO_3^-, SO_4^{2-} and F^- was evident in the samples, which also contain higher concentrations of Cl^- and HCO_3^-. Quantification of these trace anions using a single point calibration at 25 μg L^{-1} indicated NO_3^- to be present at 140 μg L^{-1} in the river water sample and 40 μg L^{-1} in the mineral water. SO_4^{2-} was determined to be 20 μg L^{-1} in the mineral water, with F^- found to be 5 μg L^{-1} in the river water sample and 13 μg L^{-1} in the mineral water.

Conclusions

UV LEDs provide a potential low cost alternative to commercial mercury and deuterium light sources in absorbance detectors for chromatography, electrophoresis and related techniques. If, as in this case, the LED has an emission maximum that closely matches the absorption maximum of the indirect detection probe, LODs can be improved significantly, due to improvements in both detector sensitivity and baseline noise. Similar improvements in detection parameters can also be expected for direct photometric detection, but to make this option widely attractive, LEDs with emission wavelengths in the low-UV spectral region must become available commercially.

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