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On-line hyphenation of quantum cascade laser and capillary electrophoresis

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Abstract

We report the first successful hyphenation of a Fabry Pérot quantum cascade (QC) laser to a capillary electrophoresis system. This involved use of a dedicated IR-transparent flow cell, made of CaF_2, constructed by means of SU-8 based lithography and low temperature wafer bonding techniques. Adenosine, guanosine, xanthosine and adenosine-5′-monophosphate were separated in a borate-containing separation electrolyte (10 mM, pH 9.3). Functional group (carbohydrate) detection was accomplished by use of the 1080 cm^{-1} emission line of the available QC-laser. The assessable optical path length could be increased, from the normally available 10–15 μm in CE-FTIR analyses, to 60 μm using this powerful mid-infrared laser and aqueous solutions.

Keywords: Quantum cascade laser; Capillary electrophoresis; Mid-infrared detection; On-line hyphenation; Aqueous samples

1. Introduction

Rapidly growing interest in chemical separation research is focused on micro-scale techniques such as capillary liquid chromatography (LC) and capillary electrophoresis (CE). The popularity of CE arises from its ability to separate a wide range of substance, including compounds with similar functional groups, efficiently and rapidly. CE is an electrically driven separation process generally performed in fused silica capillaries (i.d. in the range 20–100 μm) using aqueous buffers. CE has been adapted for the separation of an immense range of compounds, including small inorganic ions and large proteins and peptides. Laser-induced fluorescence (LIF) spectroscopy is currently the most sensitive technique for detecting CE-separated compounds [1], although UV detection systems are most commonly used.

In the mid-infrared region almost all organic compounds exhibit characteristic vibrational patterns, thus providing a sensitive means of fingerprint identification and quantification. However, nearly all compounds absorb in the mid-IR region, which may be disadvantageous when working with complex mixtures. Thus, hyphenation with a separation system prior to mid-IR detection is often necessary for the determination of specific organic compounds in mixtures. Hyphenation may be performed either off-line, employing sophisticated solvent elimination approaches, or on-line, utilising short optical path lengths [2,3]. A frequently cited shortcoming of on-line hyphenation is that a shorter optical path length will inevitably lead to lower sensitivity according to Lambert–Beer's law. When applying mid-IR light with aqueous, biologically interesting compounds the intense water absorption may hamper its use, predominantly due to the strong absorption arising from the O–H bending vibration of water around 1640 cm^{-1}. With respect to these shortcomings, quantum cascade (QC) laser technology offers significant improvements. QC-lasers are powerful, mid-IR light sources, providing the ability to increase the optical path length,

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and hence improve sensitivity [4,5]. In QC-lasers photon emission occurs within the conducting band via intersubband transitions, in contrast to normal semi-conducting diode lasers where photon emission is due to the recombination of electrons and holes in p–n junctions. These intersubband transitions can be controlled by careful bandstructure engineering at monomolecular resolution, thereby forming an artificial semi-conducting crystal with a heterostructure of alternating "well" and "barrier" layers, grown by molecular beam epitaxy (MBE) techniques. Faist et al. presented the first quantum cascade laser in [6]. The intersubband nature of the optical transitions has several key advantages. First, the laser emission wavelength is primarily a function of the thickness of the quantum well (QW) layers. Hence, the wavelength can be tailored over a wide spectral region, essentially the entire mid-IR and into the far-IR, using the same semi-conducting material, in contrast to normal lasers, where the chemical characteristics of the active material determine the emission wavelength. Secondly, the multistage cascade nature of the QW permits electrons to be recycled as they remain inside the conducting band, contributing in every cycle to the gain and photon emissions. Consequently, each injected electron above the laser threshold value can, in principle, generate n_p photons, where n_p is the number of active regions in the laser. This leads to very high quantum efficiencies and optical power, both of which are proportional to n_p. Rapid progress in heterostructure semi-conducting research soon led to the development of QC-lasers that can function in continuing mode (cw) at cryogenic temperatures as well as at room temperature in pulsed mode, covering the mid- to far-IR region [7,8]. The research focus then shifted to the development of new laser waveguide designs allowing cw-operation at room temperature [9,10] and different heterostructure materials providing shorter wavelengths and multiple cascade operation [11,12]. Within a decade of their invention QC-lasers started to become commercially available [13].

QC-lasers have primarily been adapted for use as chemical gas sensors for detecting and monitoring toxic and dangerous compounds, including various pollutants and exhaust emissions [14]. Their potential utility as powerful light sources in the condensed phase has only recently been demonstrated [5,15].

In this study the on-line hyphenation of a QC-laser to a CE separation system is presented for the first time. Functional group detection of carbohydrate-containing analytes, dissolved in the separation electrolyte solution, was achieved utilizing a custom-built IR-transparent flow cell, with an optical path length of 60 μm, and a Fabry Pérot QC-laser emitting light at 1080 cm^{-1}.

2. Experimental

The entire experimental set-up, including the QC-laser system, the data acquisition system and the CE system are schematically illustrated in Fig. 1.

2.1. Quantum cascade laser system

A Fabry Pérot QC-Laser (S1839a21, Alpes Lasers, Neuchâtel, Switzerland) with an emission maximum at 1080 cm^{-1} was used throughout the experiments. The laser was placed in a dedicated laser housing (Alpes Lasers) equipped with an air- or water-cooled Peltier element. Before it was sealed, the laser housing was filled with dry nitrogen gas and silica gel pads. A temperature controller (TC51, Alpes Lasers) was included to allow precise monitoring of the laser's temperature. The laser operation was controlled by a specially fabricated laser driver (QUANTA-BP, Laser Components GmbH, Olching, Germany), triggered by a function generator (Model 33120A, Agilent Technologies, Palo Alto, California, USA). The laser was operated at −30 °C with a pulse current of 4.5 V, a pulse frequency of 20 kHz and a pulse duration of 50 ns. With the selected operating conditions and according to the specification of Alpes Lasers, a peak power of 1 W is obtained which results in an emitted average power of 1 mW. A home-made software package (Sagittarius v. 3.0) [16] was developed enabling, in combination with a computer interface, control of the pulse duration and amplitude as well as the A/D conversion of the laser signal.

2.2. Data acquisition system

The light from the laser was collimated and focused by means of two gold-coated parabolic mirrors (focal length: 43 mm) onto the developed IR-transparent flow cell (see Fig. 1). Subsequently, using the same type of mirrors, the remaining light was collimated and focused onto a photovoltaic mercury cadmium telluride (MCT) detector element (KMPV12-0.1-J1/100 MHZ Kolmar Technologies, Newburyport, MA, USA). The detector signal was processed using a Boxcar Averager (SR250, Stanford Research Systems, Sunnyvale, California, USA). Ten thousand pulses, triggered by the function generator, were averaged within a 10 ns gate window in the laser pulse. Using this system a new value could be recorded and stored every second.

2.3. Capillary electrophoresis system

The CE system was constructed in-house, consisting of untreated fused-silica capillaries (i.d. 50 μm, o.d. 375 μm, Polymicro Technologies, Phoenix, Arizona, USA), a high voltage power supply (Brandenburg, Thornton Heath, UK) and two platinum electrodes. The total capillary length was 51 cm, with the IR detection cell 30 cm from the injection end. The applied voltage at the injection end of the capillary was +16 kV, resulting in currents of around 54 μA. The surface of new capillaries was activated by rinsing with 1 M NaOH, 0.1 M NaOH, distilled water (each for 5 min) and electrolyte solution (10 min). The capillary was washed between runs, when necessary, using 0.1 M NaOH, distilled water and electrolyte solution (each for 3 min). Hydrodynamic sample

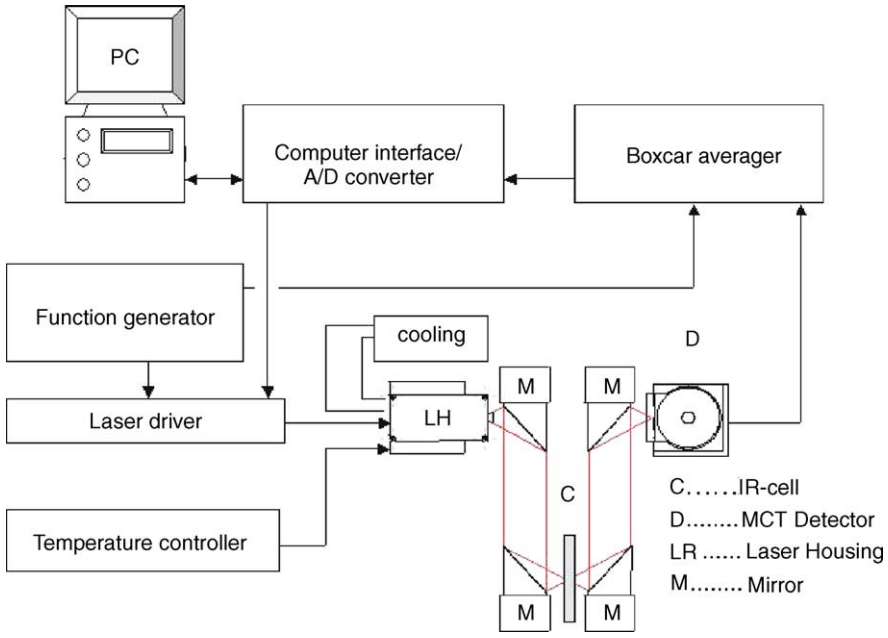

Fig. 1. Schematic view of the experimental set-up including the optical, separation and data acquisition systems.

injection was accomplished by manually elevating the inlet side of the capillary 23 cm for 20 s.

2.4. The flow cell unit

The production of the IR-transparent CaF_2 flow cells has been described in detail elsewhere [17], but a number of modifications to the published procedure were introduced, and are briefly described here. The flow channel was formed by two streaks (100 μm wide and ∼30 μm thick) of epoxy-based SU-8 photoresist (Microchem Corp., Newton, MA, USA) on each CaF_2 disc, producing a 150 μm wide channel with an optical path length of 60 μm. Aluminium foil was glued to the top-surface of the flow cell, providing an optical aperture. The fused silica capillary was carefully cut to ensure that the terminus of the capillary was perpendicular to its axis and that the surrounding polymer was not frayed. The flow cell was placed in a cell-holder and commercially available o-rings (1.10 mm × 0.5 mm × 0.30 mm, Rudolf Flume Technik GmbH, Essen, Germany) were used to ensure a tight connection between the flow cell and the cut capillary ends. Fig. 2 shows a microscopic view of the cell in the cell-holder.

2.5. Chemicals

Adenosine (>99%), guanosine (>98%,) and adenosine-5′-monophosphate disodium salt (>99%) were purchased from Fluka (Buchs, Switzerland) and xanthosine (>99%) from Sigma–Aldrich (St. Louis, MO, USA). The separation electrolyte consisted of 10 mM borate (pH 9.3) dissolved in 0.2-μm filtered HPLC water (Fluka, Buchs, Switzerland). Five millimolar stock-solutions of the individual analytes were prepared and dissolved in the separation electrolyte. Before introduction into the CE system, all solutions were degassed for 15 min in an ultrasonic bath.

2.6. Reference measurements

Reference FTIR spectra of all analytes dissolved in the separation electrolyte were obtained by a Bruker IFS 88 spectrometer (Bruker Optik GmbH, Germany) equipped with a MCT detector (J-15D16 Judson, Montgomeryville, PA, USA) and a commercially available CaF_2 flow cell based on transmission with a 44 μm optical path length. The spectra were recorded, with the separation electrolyte serving as background, between 4000 and 700 cm^{-1}, at 8 cm^{-1} spectral resolution, with 500 co-additions and applying a Blackman-Harris 3-Term apodization function.

The emission wavelength of the QC-laser was experimentally verified. For this purpose the black body radiation source of a Bruker Equinox 55 spectrometer (Bruker Optik GmbH, Germany) was substituted for the QC-laser. The spectra were recorded between 1400 and 900 cm^{-1} with a spectral resolution of 2 cm^{-1} using an MCT detector (KMPV11-1-LJ2/239 Kolmar Technologies, Newburyport, MA, USA). One hundred twenty-eight scans were co-added and a Blackman-Harris 3-Term apodization function was applied.

An Ultimate fiber optic UV-Detector (Dionex, Sunnyvale, California, USA) set at 210 nm and an ELDS Pro 1.0 laboratory data system (Chromatography Data systems, Kungshög, Sweden) were used to register the UV electropherograms. On-line UV detection was achieved by removing the polyimide coating from a short segment of the fused silica capillary and placing it in the optical path of the UV detector. The UV reference measurements were performed under the

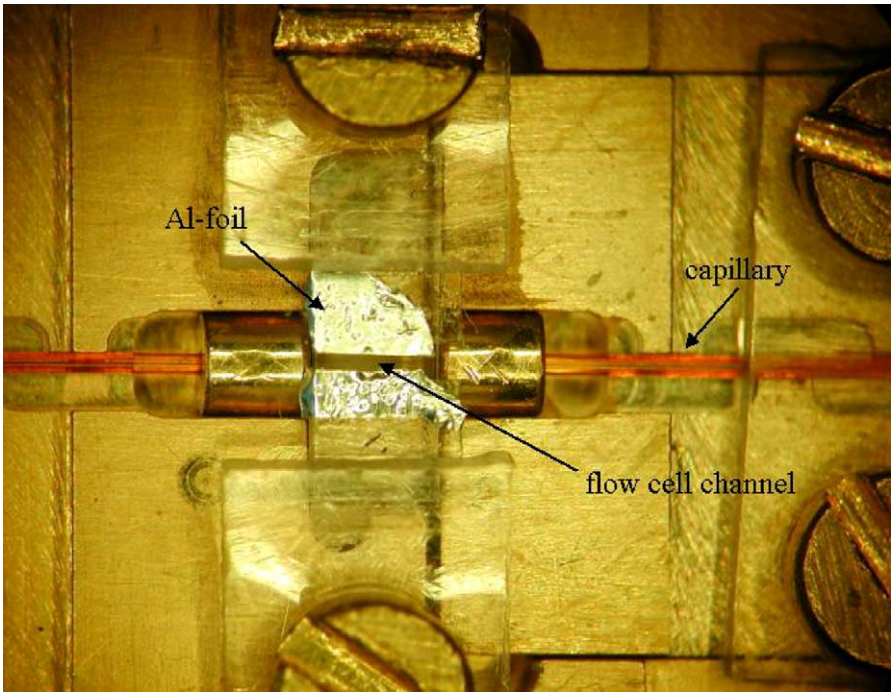

Fig. 2. Microscopic view of the custom-built IR-transparent flow cell and cell-holder.

same experimental conditions as those used when the QC-laser provided the light source.

3. Results and discussion

3.1. CE-QCL recordings

Fig. 3 shows a typical electropherogram illustrating the on-line, real-time registration from a CE separation utilizing a QC-laser as a light source for mid-IR detection. The analytes separated were adenosine, guanosine, xanthosine and adenosine-5′-monophosphate (AMP), each with a concentration of 5 mM. The signal from the bulk sample, i.e. the electroosmotic flow, is easily detected as a sharp negative peak in the electropherogram. Also included in this figure is the electropherogram from a CE separation, recorded under similar separation conditions, but with the QC-laser exchanged for a UV detection light source. Both detectors give comparable electrophoretic peak shapes, although the

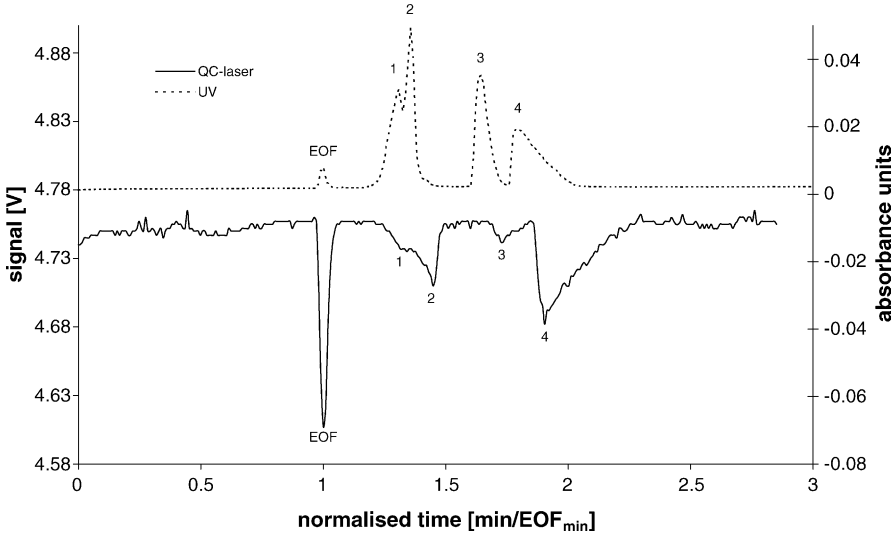

Fig. 3. On-line CE-QCL electropherogram (solid line) and a CE-UV electropherogram (dotted line), recorded under similar conditions, of (1) adenosine, (2) guanosine (3) xanthosine and (4) AMP each with a concentration of 5 mM separated with CE. Applied voltage, 310 V cm^{-1}; capillary inner diameter, 50 μm; length to the detection point, 30 cm; separation electrolyte, 10 mM borate buffer at pH 9.3; hydrodynamic injection by manual elevation.

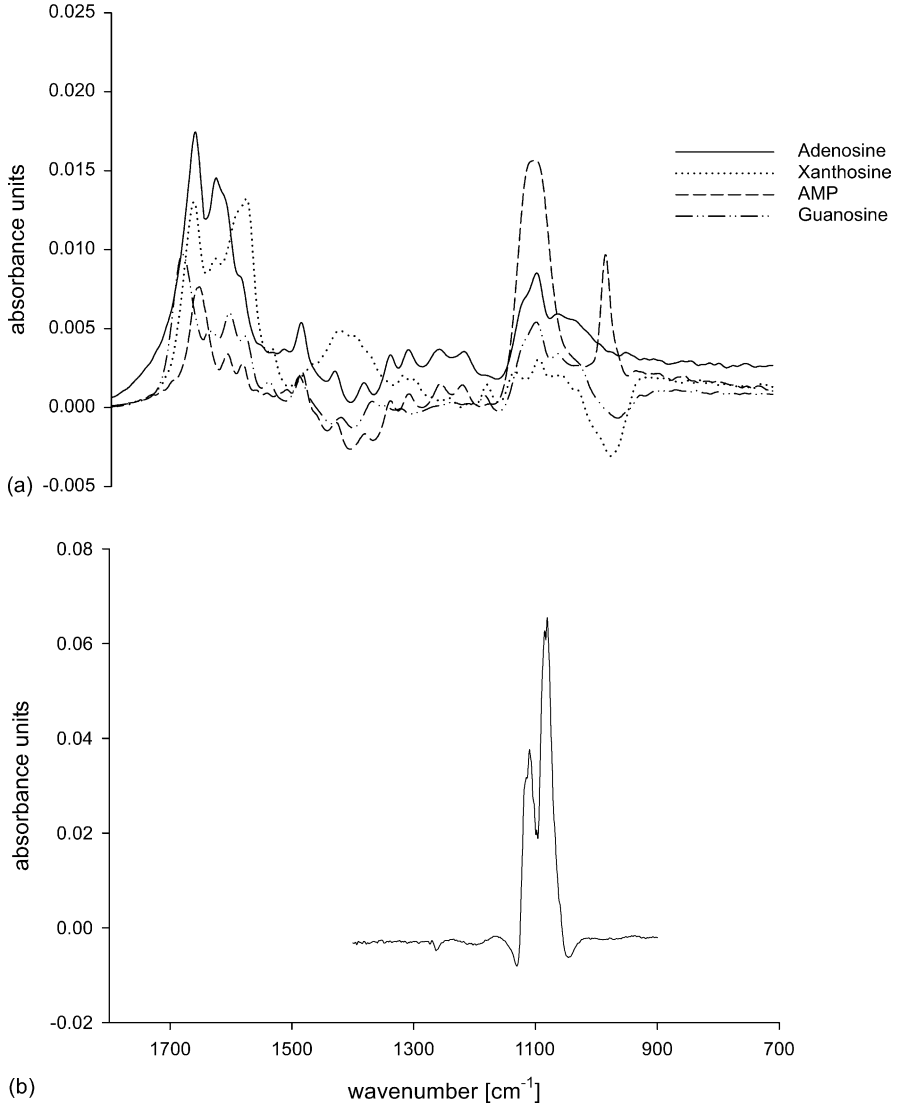

Fig. 4. (a) FTIR reference spectrum (1800–700 cm^{-1}) of adenosine (solid line), guanosine (dashed-dotted line), xanthosine (dotted line) and AMP (dashed line), each at a concentration of 5 mM with the separation electrolyte serving as background. Spectral resolution, 8 cm^{-1}; 500 co-additions. (b) QC-laser emission spectrum recorded between 1400 and 900 cm^{-1} with a spectral resolution of 2 cm^{-1}; 128 co-additions and applying a Blackman-Harris 3-Term apodization function.

QC-laser and UV-detector provide transmission peaks and absorption peaks, respectively.

3.2. Functional group detection

Fig. 4a presents reference FTIR spectra of the analytes in the region between 1800 and 700 cm^{-1}. All four analytes contain a furanose ring-structure, absorbing in both the UV and IR regions, as well as a carbohydrate group that only absorbs in the IR. The absorptivities differ, but all analytes absorb light at around 1080 cm^{-1}, primarily due to the C–O–H motions of the carbohydrate group. In addition to the carbohydrate, the AMP also contains a phosphate group, absorbing at around 980 cm^{-1}. Fig. 4b shows the emission spectrum of the QC-laser. The QC-laser emission has a broad peak between 1055 and 1127 cm^{-1}, hence it is suitable for the naturally large, condensed-phase absorption peaks. Consequently, a specific functional group (the carbohydrate) of the carbohydrate-containing analytes separated by the CE system can be detected using the available QC-laser.

3.3. Estimation of the signal-to-noise ratio

The signals from the four CE-separated analytes were investigated with respect to their signal-to-noise (S/N) ratios, calculated from the baseline noise and their respective peak heights. The results are presented in Table 1. For comparison, the same concentration of the individual analytes was measured in a FTIR spectrometer using a commercial flow cell with an optical path length of 44 μm. The analytes were dissolved in the electrolyte solution and the background spectrum was obtained from the electrolyte solution. The highest

Table 1
Signal-to-noise estimates

Analyte	QCL	FTIR
Adenosine	2.4	9.5
Guanosine	4.4	6.0
Xanthosine	1.8	3.4
AMP	6.6	17.6

Table 2
Linear regression data

Analyte	Slope (mAU mM^{-1})	Intercept (mAU)	r^2
Adenosine	0.342	0.475	0.96
Guanosine	0.744	0.240	0.96
Xanthosine	0.284	0.170	0.91
AMP	0.931	1.126	0.99

r^2 = regression coefficient, number of calibration points = 5.

peak in the region between 995 and 1150 cm^{-1}, i.e. the region where the laser emission occurs, was compared to a reference background spectrum in the same region. Again, the S/N ratios were calculated from the baseline noise and respective peak heights. The results are included in Table 1.

As recently reported [18], the commercially available electronic systems (i.e. the laser starter kit from Alpes laser) generate a significant amount of electronic noise and, consequently, the operating conditions for the QC-laser were not entirely satisfactory. However, modifications allowing 50-fold improvements in S/N ratios have been reported recently for the detection of phosphate [5] using a home-made and no longer available set-up, and the authors see no reason why similar improvements should not be possible for determining the analytes determined here when the commercially available electronic systems become more stable.

3.4. Quantification

To obtain figures of merit for the calibration of the presented method the concentration of the injected sample was varied between 2 and 5 mM for adenosine (0.53–1.34 g L^{-1}), guanosine (0.57–1.42 g L^{-1}), xanthosine (0.57–1.42 g L^{-1}) and AMP (0.69–1.74 g L^{-1}). Exceeding these upper concentration regions resulted in overload in the CE system. The resulting transmission peaks were recorded as functions of time. After conversion into absorbance units, the peak heights were measured and the linearity of their relationship with the concentration of the corresponding analytes was calculated by regression analysis.

The results are presented in Table 2.

4. Conclusions

The first preliminary results obtained utilizing a Fabry Pérot QC-laser as a powerful, mid-IR light source in a CE separation system are presented. The on-line hyphenation maintains the spatial profile acquired in the CE separations. The poor signal-to-noise ratio obtained is primarily a result of electrical noise arising in the laser housing. When the connection between the QC-laser and the electrical contact is improved by the manufacturers we are convinced that the S/N will increase sharply. Nevertheless, the results achieved indicate the potential utility of QC-lasers, as tuneable, tailored mid-IR light sources, providing new detection opportunities in capillary electrophoresis. When production costs are reduced the scope for deploying arrays of QC-lasers or QC-lasers with multiple cascade operation will further boost interest in this field and the authors are convinced that research into and the application of QC-lasers will continue apace.

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