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Short communication

Towards functional group-specific detection in high-performance liquid chromatography using mid-infrared quantum cascade lasers

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Abstract

A distributed feedback quantum cascade laser was applied for the first time as a powerful light source for mid-infrared (MIR) detection in liquid chromatography. Fructose and glucose in red wine were separated with an isocratic HPLC system, which was connected to a custom-made flow cell. This flow cell was constructed of two diamond windows with adjustable spacing and two hollow wave-guides for guiding the incoming and outgoing light. The HPLC column based on an ion-exchange resin with calcium(II) counter ion was run at 80°C with 0.04% formic acid as the mobile phase. Under these conditions the carbohydrates could not be completely separated from the organic acids also present in wine. However, the emission of the laser at 1067 cm^{-1} matches the absorption maximum of fructose and glucose, whereas the organic acids do not absorb appreciably at this wavenumber. Thus group-specific detection could be achieved. Additionally, the optical path length could be increased from 25 to 125 μm, which is very promising in gaining enhanced sensitivity compared to Fourier transform IR detection.

Keywords: Detection, LC; Quantum cascade laser IR detection; Infrared detection; Carbohydrates

1. Introduction

Infrared spectroscopy is of great interest to analytical chemistry as all organic compounds absorb in the mid-infrared region. Mid-infrared (MIR) absorption bands are generally well resolved and can be related to defined vibrational transitions of specific functional groups. Therefore MIR spectra provide a high qualitative information content of the analytes under investigation. However, in the case of coupling high-performance liquid chromatography (HPLC) to infrared detection the absorption of infrared radiation by the mobile phase results in strong spectral interferences especially in the case of aqueous eluents. Using Fourier transform (FT) IR spectrometry the optical path length must be in the low micrometer range (typically between 10 and 50 μm) to be able to perform measurements in the water window of the fingerprint region between 1600 and 950 cm^{-1}. The consequence of the short optical path length is a limitation in sensitivity, so for higher sensitivity a light source with higher intensity is of great interest. The recent developments in quantum cascade laser (QCL) technology in the MIR region are very

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promising in this context. A QCL [1,2] is an unipolar semiconductor laser where the light generation is based on intersubband transitions within the conduction band (or valence band). Only one type of carrier is involved in the light emitting process. Conventional semiconductor lasers are bipolar devices where the light generation is based on the recombination of electrons from the conduction band and holes from the valence band across the band gap. Therefore, the semiconductor material determines the emission wavelength. In contrast, the emission wavelength of a QCL is determined by the thickness of the alternating layers of different semiconductor materials. The thickness of the individual layers is in the region of the deBroglie wavelength which restricts the motion of the electrons perpendicular to the layers. This quantum confinement effect determines the emission wavelength, which can be designed in a wide range. Up to now, the emission wavelength of QCLs is in the range of 3.5 μm up to 19 μm. Thus quantum cascade lasers are micro structured, small in size and can be mass-produced, leading to inexpensive products. Due to the rapid growth and intensive research efforts in this technology further development, such as lasers covering several spectral regions in the MIR will be commercially available in the future.

QCLs have been already used successfully for gas absorption measurements [3–5] and photo-acoustic spectroscopy [6]. QCLs can also be used for absorption measurements in liquid phase as already shown by the example of phosphate analysis using a flow injection system [7]. In this application it was demonstrated that by using a room temperature QCL optical path lengths of more than 100 μm could be used, even in the case of aqueous matrices. Furthermore, using room temperature QCLs the signal-to-noise ratio was improved by a factor of 50 compared to a state-of-the-art Fourier transform spectrometer. Consequently, further application of QCL detection in liquid phase, especially in the field of liquid chromatography, are of interest due to the potential high sensitivity of QCL detection and the chemical information provided which opens the path to record functional group-specific chromatograms. In the current study an available liquid nitrogen cooled distributed feedback QCL was used to selectively measure carbohydrates in the chromatographic separation of red wines.

2. Experimental

2.1. Chemicals and samples

Glucose and fructose standard solutions were prepared by dissolving a defined quantity of each compound [concentration, α-D-(+)-glucose anhydrous 96%, Aldrich; D-(−)-fructose 98%, Aldrich] in distilled water. Standards were stabilised by addition of sodium azide in order to prevent microbiological growth. Three red wine samples were drawn from wineries from Lower Austria. The samples were filtered with a disposable syringe filter (0.45 μm pore size, Millipore) prior to injection.

2.2. Chromatography

The HPLC set-up consisted of a Merck–Hitachi L7100 isocratic pump (flow-rate 0.6 ml/min) and a Rheodyne 7725 injection valve (injection volume 50 μl). An in-line filter and a 4 mm×3 mm guard column (Phenomenex Carbo-Ca^{2+}) were used in order to protect the 300 mm×7.8 mm analytical column (Phenomenex Rezex 8% Ca. Monos.). The stationary phase of both the guard and the analytical column was a sulfonated styrene–divinylbenzene anion-exchange resin with calcium counter ion (particle size 8 μm, cross linkage 8%). Distilled and ultra purified water (18 MΩ) with 0.04% formic acid (98–100%, Merck) at a pH value of 3 was used as mobile phase. To keep the column temperature at 80°C a modified glass tube connected with a thermostated water bath was used.

2.3. Quantum cascade laser, operation and data acquisition, flow cell

The QCL used in these experiments was a distributed feedback (DFB) laser [8] emitting at 1067 cm^{-1} (full width at half maximum <0.5 cm^{-1}). The emission spectrum (Fig. 3, spectrum A) of the QCL was measured with an FT-IR spectrometer (Nicolet 860). The AlGaAs based QCL was operated in pulsed mode operation with a pulse length of 100 ns and a pulse repetition rate of 30 kHz (duty cycle 0.3%). The laser was mounted in a flow cryostat and cooled with liquid nitrogen. The instantaneous pulse power of the laser was in the range of 250 mW.

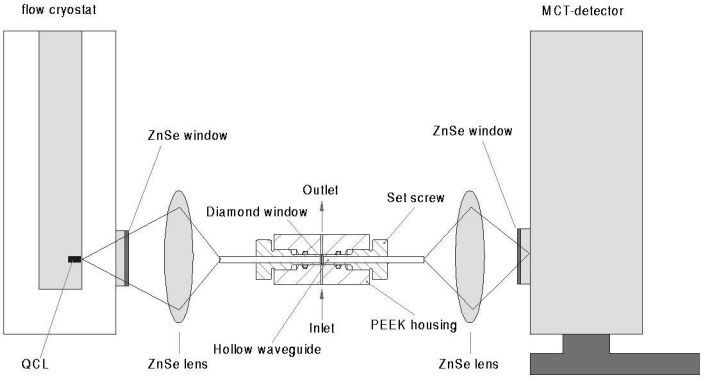

Fig. 1. Sketch of the fibre optic flow-through cell as used for laser measurements.

The flow cell (Fig. 1) was constructed using two diamond windows (diameter: 1.6 mm) with adjustable spacing from 50 to 250 μm and two hollow wave-guides (outer diameter: 1.2 mm, inner diameter: 1 mm, length: 20 mm each) to guide the incoming and outgoing light. At the path length of 125 μm as used in all experiments the flow cell had a total internal volume of 0.25 μl. The laser beam was collected with a Zn–Se lens (diameter: 50.8 mm, focal distance: 38.1 mm) and focused into the hollow wave-guide of the flow cell. After probing the sample the light was collected by the second hollow waveguide and focused by an additional Zn–Se lens (diameter: 10 mm, focal distance: 10 mm) onto a 250 μm×250 μm sized liquid nitrogen cooled mercury cadmium telluride (MCT) detector (EG&G J15D12). A preamplifier (EG&G PA101) (amplification 1000×) was used to amplify the MCT signal before feeding it into a lock-in amplifier (EG&G 5210).The lock-in signal, proportional to the transmission of the flow cell, was used for data evaluation. The transmission of the laser light through the absorption cell was recorded and afterwards transformed into absorbance. All data were edited with GRAMS/32 spectral note base (Galactic Industries).

2.4. Determination of the optical path-length

To determine the effective optical path of the flow cell FT-IR spectroscopic measurements on a Bruker IFS-66 spectrometer were performed. Instead of Zn–Se a K–Br lens (diameter: 50.8 mm, focal distance: 38.1 mm) was used here to focus the parallel IR beam of the spectrometer in the hollow waveguide. The optical path length of the flow cell was then determined by measuring the absorbance of the ν-C=O vibration of a 1% (v/v) acetic acid isobutyl ester in tetrachlorethylene (background: tetrachlorethylene). Since tetrachlorethylene is highly transparent in the MIR region, all measurements could be carried out using the FT-IR spectrometer even at increased optical path lengths. A standard flow cell employing CaF_2 windows with an optical path length of 50 μm (determined from interference fringes of the single beam spectrum of the empty flow cell) was used for reference. The ratio of absorbance values was then multiplied by the path length of the reference cell to calculate the path length of the flow cell.

2.5. Fourier transform infrared detection, operation and data acquisition

In order to verify the achieved chromatographic separation the HPLC set-up was connected to a flow cell incorporating an attenuated total reflection (ATR) element for recording FT-IR spectra in the spectral range between 1900 and 900 cm^{-1}. Thus it was possible to identify all substances as they were eluting the column as well as to check the purity of the peaks. For these experiments a Bruker Equinox IFS 55 FT-IR spectrometer equipped with a horizontal ATR cell (Dura SamplIR, SensIR Technologies) and liquid nitrogen-cooled MCT detector were used. As the ATR element a nine-bounce diamond crystal with an active area of 4.3 μm in diameter was used.

The laboratory-made flow cell [9] had a total internal volume of 5 μl. All spectra were recorded at 8 cm^{-1} resolution from 1900 to 800 cm^{-1} (128 scans co added, scanner velocity: 100 kHz, apodisation: Blackman-Harris-3-term). All data were edited with GRAMS/32 spectral note base (Galactic Industries).

2.6. Reference measurements

All reference measurements were carried out with a commercially available enzymatic test kit (R-Biopharm, Darmstadt, Germany). The test is based on the formation of nicotinamide adenine dinucleotide phosphate, which can be determined at the wavelength of 334 nm. For liquid handling transfer pipettes (volume: 10–100 μl and 100–1000 μl, Brand) were used. Absorbance values were measured using an UV–Vis diode array spectrometer (HP 8452A, Hewlett-Packard) equipped with a 3.5-ml quartz cuvette with 1 cm optical path length.

3. Results and discussion

3.1. Preliminary experiments using FT-IR detection

In addition to fructose and glucose red wine contains acetic acid, citric acid, ethanol, glycerol, lactic acid, malic acid, and tartaric acid as further major and minor constituents. As can be seen in the traces from a chromatogram obtained by FT-IR spectroscopy (Fig. 2) the peaks of the target analytes fructose and glucose were not fully resolved. Glucose overlapped with tartaric and malic acid and fructose partly overlapped with acetic acid. However, as illustrated in Fig. 3, at the wavelength of the available laser (1067 cm^{-1}) organic acids do not exhibit significant absorbances. This showed that by focusing on this wavelength the group-selective detection of carbohydrates was feasible also in the presence of organic acids. The selected laser matches the bands attributed to ethereal and hydroxylic νC–O of carbohydrates, which appear in the region between 1200 and 1030 cm^{-1}, whereas organic acids do not contribute appreciably in the spectral region between 1100 and 1000 cm^{-1} [10]. It was the aim of the experiments shown below to demonstrate the

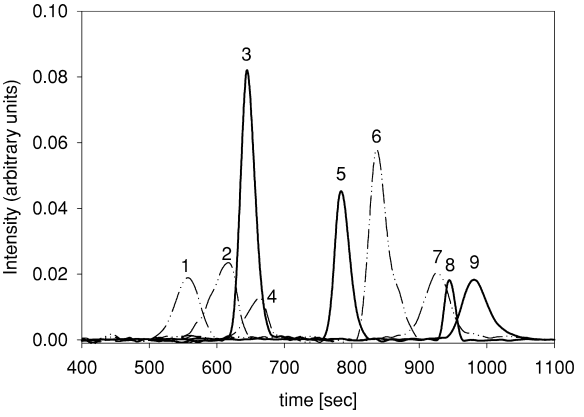

Fig. 2. HPLC–FT-IR traces of standard solutions containing 3 mg/ml of the major and minor constituents of wine (ethanol and glycerol 10 mg/ml). To visualise the achieved separation all traces were calculated with respect to the absorption of the analytes by integrating the appropriate peak areas of the spectra (integration limits are given in parentheses). 1, Citric acid (1272–1158); 2, tartaric acid (1167–1037); 3, glucose (1181–955); 4, malic acid (1138–1059); 5, fructose (1181–955); 6, acetic acid (1332–1207); 7, lactic acid (1159–1065); 8, glycerol (1123–1066); 9, ethanol (1123–1066).

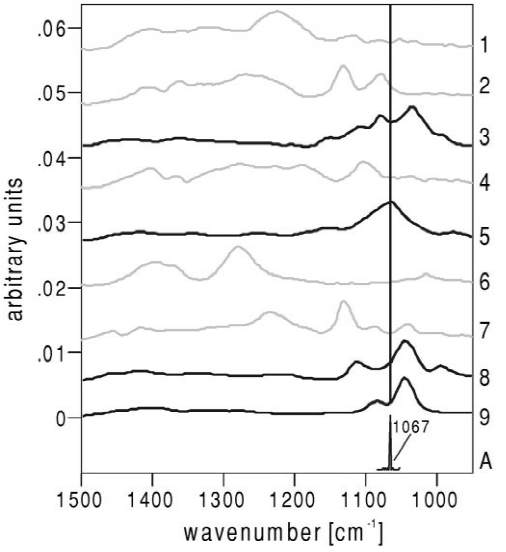

Fig. 3. Spectra of standard solutions containing 3 mg/ml of each compound (ethanol and glycerol 10 mg/ml) recorded with the ATR cell after elution from the column. 1, Citric acid; 2, tartaric acid; 3, glucose; 4, malic acid; 5, fructose; 6, acetic acid; 7, lactic acid; 8, glycerol; 9, ethanol; A, QCL (for better visualisation an appropriate offset was used).

potential of QCL detection in HPLC on the example of selective sugar analysis in wines.

3.2. HPLC–QCL measurements

3.2.1. Optimisation of the optical path length

The optical transmission of the flow cell filled with water is in the range 1/1000, therefore the laser power is damped by three orders of magnitude by the absorbance of water. The electronic noise of the detection system was determined by masking the laser beam and was found to be in the range of 500 μV. Then an optimum between gain of analyte signal by increasing the path length and the noise had to be found. The optical path was adjusted such that the detector output after amplification reached 300 mV, which corresponded to a signal-to-noise-ratio of approximately 600. The optical path length was measured to be 125 μm and the transmission of the water filled cell was 0.15%. The absorption of 1 mg/ml of the analyte fructose as eluting from the column caused a decrease of 5 mV in signal intensity. This corresponds to a signal-to-noise-ratio of 10. However, it has to be mentioned, that when using a liquid nitrogen-cooled QCL the experimental set up is more complex than in the case of a room temperature QCL (which was not available to us) as the laser had to be mounted inside an evacuated flow cryostat and additional lenses were needed to bring the light to the place of measurement. Furthermore the electronics available for these experiments were not designed to minimize electronic noise.

3.2.2. Calibration with standard solution

Standards containing 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/ml of each carbohydrate were prepared and injected in triplicate. The resulting transmission was recorded as a function of time. After conversion into absorbance units, the recorded peaks corresponding to glucose and fructose were integrated and a linear regression was calculated. Fructose: $y=0.1003x+0.0131$, $R^2=0.9991$, glucose: $y=0.0702x+0.007$, $R^2=0.9993$. The precision of the method was evaluated by the standard deviation of the method s_{xo}, calculated as the ratio of the residual standard deviation, s_y, and the slope (according to ISO 8466-1). The standard deviation was found to be 120 μg/ml for the determination of glucose and 130 μg/ml for fructose. The limit of detection, estimated as the threefold standard deviation was then found to be 360 μg/ml glucose and 390 μg/ml fructose, respectively.

3.2.3. Wine samples

Finally, the contents of glucose and fructose were measured in a real complex matrix such as red wine. The separation of an Austrian wine sample with laser detection is shown in Fig. 4. The HPLC–QCL results compared with the reference method are listed in Table 1. The values obtained by the newly developed method are in good agreement with the values found by the reference method.

3.3. Further perspectives

The spectral density in watt/wavenumbers of QCLs is several orders of magnitude higher than the one of a black body radiation source as used in standard FT-IR spectrometers. Therefore, these light sources can be regarded as a solution to the problems resulting from strong solvent absorption which has limited the spectral region accessible for MIR detection and restricted the optical paths to a few micrometres only. An additional attractive feature of QCLs is their small size. Basically it is possible to produce an array of QCLs, each QCL lasing at different wavenumbers [11] and to integrate them in

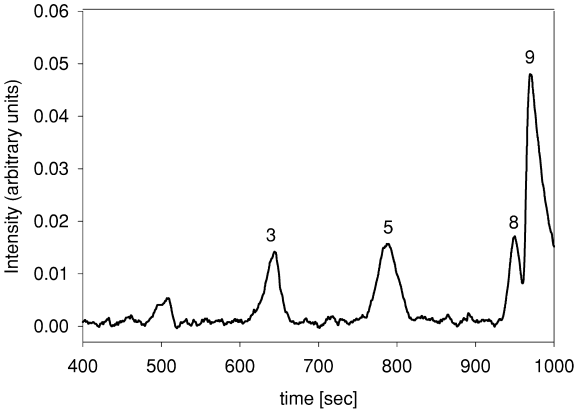

Fig. 4. Functional group-specific chromatogram sensitive to carbohydrates of a real Austrian red wine (sample A) detected by the QCL at 1067 cm^{-1} (3, glucose; 5, fructose; 8, glycerol; 9, ethanol).

Table 1
Glucose and fructose contents of three Austrian red wines determined by HPLC–QCL

	A[a]	B[a]	C[a]	SD[b] (mg/ml)
Glucose (mg/ml)	1.18 (1.04)	0.52 (0.35)	2.41 (2.25)	0.13
Fructose (mg/ml)	1.13 (1.03)	0.55 (0.44)	4.04 (3.91)	0.12

[a] Reference data in parentheses.
[b] Standard deviation of the newly developed method according to ISO 8466-1 (1990).

one optical set-up. This technology therefore holds promise to perform quasi simultaneous multi-wavelength recordings during a chromatographic separation. Recently it was also shown that based on the same technology used to produce QCLs that MIR detectors can be fabricated [12]. Therefore further miniaturization and integration of the whole set-up will be possible by incorporating light source, flow cell and the detector on-chip.

4. Conclusion

This paper reported the first application of a mid-infrared quantum cascade laser for group-specific laser detection in liquid chromatography. Using a laser wavelength of 1067 cm^{-1} glucose and fructose could be quantified in real samples (red wine) even though the sugars partly co-eluted with organic acids. Due to the ongoing, intensive research efforts in the field of quantum cascade laser technology and the recent commercial availability of room temperature QCLs [13] it can be expected that QCLs will find broad applications in analytical chemistry. Especially in the field of chromatography the possibility to add functional group-specific QCL detection to other detection schemes like mass spectrometry is definitely intriguing despite the fact that the sensitivity achieved by mass spectrometry is very unlikely to be matched by QCL detection based on absorption measurements.

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