A New Quantum Cascade IR-Laser Online Detector: Chemical-Sensitive Size-Exclusion Chromatography Measurement at Unprecedented Low Levels

Jennifer M. Kübel, Carlo Botha, Aleksandra Bucka, Johannes Höpfner, Henrik Zimmermann, Matthias Godejohann, and Manfred Wilhelm*

Online chemically sensitive detectors for size exclusion chromatography (SEC) through coupled setups based on infrared (IR) (or NMR) spectrometers present new possibilities through unprecedented levels of polymer detail with respect to molecular weight and chemical composition. Herein, a new external cavity quantum cascade laser (EC-QCL) mid-IR spectrometer as a chemically sensitive online detector for SEC is custom-designed, built, and tested. This unique spectrometer features multiple broadly tunable EC-QCL sources, which can be operated in continuous wave and pulsed mode, accompanied with balanced liquid nitrogen cooled mercury cadmium telluride (MCT) detectors and a new custom-built transmission flow cell. Automated data analysis is done with a self-written MATLAB code. The limit of detection (LOD) is measured online, coupled with SEC chromatography, where on average, one carbonyl functionality in 530 000 g mol^{-1} at chromatographic conditions for SEC could be detected. It is possible to detect 0.46 µg (LOD) PMMA, which is approximately a factor of 30 lower than that reported for SEC-Fourier transform infrared.

Quantum cascade lasers (QCLs) are one of the most exciting recent developments in mid-infrared (IR) spectroscopy as they create new possibilities that are not feasible with standard Fourier transfrom infrared (FTIR) instruments. The main advantage of QCLs is the much higher spectral power density, up to a factor of 10^4 improvement in photons per wavenumber, cm^{-1}, relative to a standard thermal emitter (globar) source. This

Dr. J. M. Kübel, C. Botha, A. Bucka, Dr. J. Höpfner, Prof. M. Wilhelm
Institute of Chemical Technology and Polymer Chemistry
Karlsruhe Institute of Technology (KIT)
Engesserstraße 18, 76131 Karlsruhe, Germany
E-mail: manfred.wilhelm@kit.edu
H. Zimmermann
neoplas control GmbH
Walther-Rathenau-Straße 49a, 17489 Greifswald, Germany
M. Godejohann
MG Optical Solutions GmbH
Industriestraße 23, 86919 Utting/Ammersee, Germany

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/marc.201900228.

enables new applications in fields such as sensor development.[1–3] Practical considerations such as their robustness, (relatively) low price, low weight, and compact size (for distributed feedback QCLs, where the wavelength selection is built into the QCL source itself, instead of external to the source) also allow for field sensing applications not possible with an FTIR instrument including reaction monitoring,[4] environmental monitoring of H_2S,[5] clinical diagnostics (breath analysis, biofluid analysis),[6–8] and greenhouse gas detection in the atmosphere[9] measured over distances up to 50 m or even in flight.[10,11]

We previously published work on an online setup combining size exclusion chromatography (SEC) and FTIR (SEC-FTIR)[12,13] and with a first prototype QCL spectrometer from Bruker Optik GmbH (Ettlingen, Germany, abbreviated as QCLS-B) used as an online chemically sensitive detector for SEC. The measured limit of detection (LOD) was compared with results from a high-end research IR spectrometer (Vertex 70 FTIR spectrometer from Bruker Optik GmbH).[14] These measurements were performed in series and allowed for a direct comparison between the two instruments. Both the Vertex 70 and the QCLS-B spectrometer face the challenge that SEC requires dilute injection concentrations of about 0.2 wt% to achieve good analyte separation with respect to hydrodynamic volume, while IR absorption is proportional to the sample concentration. As this is an online coupled technique, the time for the IR measurements is also limited (e.g., 15 s) to retain the resolution in the SEC size separation. Comparable method development work was done by Lendl and co-workers where they coupled liquid adsoption chromatography, LAC, to a QCL setup to measure sugars in beverages[15] and later stand-alone QCL setups were used to measure biological applications such as proteins in aqueous solutions,[16,17] glucose levels,[6] and multianalyte blood analysis.[18] Analog correlation between chemical composition and molecular weight are currently conducted via SEC-NMR indicating the needs for the acquisition of correlated information.[19–22] Previous results[14] showed that the LOD measured for the QCLS-B was about a factor of four better than the Bruker Vertex 70 FTIR, even though a liquid nitrogen

cooled MCT detector is installed on the FTIR Vertex spectrometer and a far less sensitive (balanced) LiTaO$_3$ detectors was used in the first prototype QCLS-B. The QCLS-B was based on a single external cavity QCL (EC-QCL), which has the lowest system noise when operated in single wavelength mode.[14] This highlights the complementary nature of QCL spectrometers and standard FTIR instruments. While QCL sources cover a limited range of wavelengths (e.g., 200 cm^{-1}) at higher sensitivities, FTIR instruments provide information on the entire mid-IR spectrum (e.g., 400–4000 cm^{-1}), but at a lower sensitivity. A practical example of the advantage of using both instruments is that this combination could be used to identify an eluting polymer in SEC through FTIR and also quantify the end groups or functional groups present in that polymer as a function of the polymer molecular size with the EC-QCL.

This first result led to the decision to develop an entire new EC-QCL-IR spectrometer to overcome limitations, especially on the detector side. This new spectrometer contains multiple EC-QCL sources and is equipped with balanced liquid nitrogen cooled MCT detectors (**Figure 1**). This is a worldwide unique spectrometer designed to be used for the coupled SEC measurement while still retaining full flexibility for future applications. This spectrometer additionally features a new specially designed flow cell for the coupled SEC-EC-QCL measurement enabling the performance of this new EC-QCL spectrometer to be compared with the results from the QCLS-B and FTIR instruments. The purpose of this publication is to introduce this spectrometer and present the first results proving its unique sensitivity with respect to measurements correlating chemical information and molecular weight.

All SEC separations were performed on an Agilent 1260 Infinity system (Waldbronn, Germany) running on tetrahydrofuran stabilized with BHT at a flow rate of 1.0 mL min^{-1}. An auto sampler injected 500 µL of a 5 g L^{-1} solution (2.5 mg sample) onto a semipreparative 10 µm particle size Linear M SDV column (300 × 20 mm inner diameter) from Polymer Standards Service GmbH (Mainz, Germany). The reader is referred to our previous work for information on the QCLS-B experimental setup and the online coupled SEC-FTIR setup.[12–14] The EC-QCL spectrometer was designed to house up to eight EC-QCL sources (four per box, two boxes) in a single instrument using an automated beam switch to access all the sources. The EC-QCL spectrometer is operated using a custom written Labview program that continually monitors the spectrometer safety interlocks and provides a single interface to control the laser, digitizer, filter wheel, detectors and data output. Currently, one MIRcat-QT-system equipped with three collinear ultra-broadly tunable EC-QCL sources from Daylight Solutions (San Diego, CA,

USA) able to operate in both continuous wave (up to 300 mW) and pulsed mode are installed. A chiller is used to maintain the laser source temperature at 19 °C. The beam path is largely covered with PVC pipes and is purged with N$_2$ for improved safety and to quickly remove IR absorbing species, for example, CO$_2$ or H$_2$O, from the beam path. In order to compensate for arbitrary fluctuations in laser intensity, it is advantageous to use balanced detection, in this case, based on liquid nitrogen cooled MCT detectors (D^* at 77 K = 9.2×10^9 and 1.1×10^{10} cm Hz$^{1/2}$ W^{-1} for the sample and reference detectors, respectively) with software control of the preamplifier gain custom built by Vigo System S.A. (Ozarow Mazowiecki, Poland) for this instrument. MCT detectors have an optimum region of maximum sensitivity and linearity at about 1 V and the adjustable preamplifier gain allows for reaching this target over the wide range of experimental conditions found in SEC-IR leading to improved experimental reproducibility. Pulsed operation of the laser provides the modulation required for the detectors, whereas a 1 kHz MC2000B optical chopper from Thorlabs (Munich, Germany) modulates the beam in continuous wave operation. A 4-CH 16-Bit 20 MS/s Digitizer from Adlink Technology Inc. (Taipei, Taiwan) is able to integrate either over the entire pulse or a part of it and the integrated instrument software control allows for convenient control of the digitizer settings.

In order to increase the dynamic range while keeping the detectors in their best working point neutral density filters are applied. This additional control over the intensity reaching the detectors is obtained by means of two rotating filter wheels, each with a set of six neutral density IR filters, where one rotating filter wheel is set in the main beam path and a second one directly before the reference MCT detector. The intensity
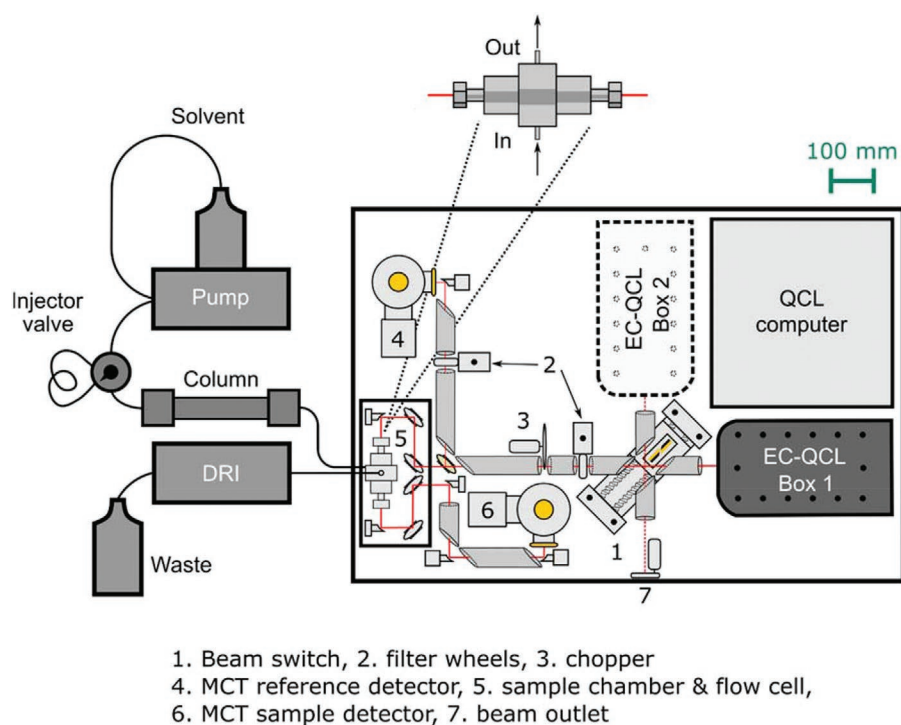

Figure 1. Schematic illustration of the EC-QCL spectrometer interior design with identification of the key components; see Figure 2.

1. Beam switch, 2. filter wheels, 3. chopper
4. MCT reference detector, 5. sample chamber & flow cell,
6. MCT sample detector, 7. beam outlet

**ADVANCED
SCIENCE NEWS**
www.advancedsciencenews.com

**Macromolecular
Rapid Communications**
www.mrc-journal.de

profile generated by a QCL source is in the mW range and has approximately a Gaussian shape with respect to its intensity as a function of frequency with a fairly steep decline toward the edges of the source meaning that the incident power is a strong function of the wavenumber. The maximum emitted power reached at least 300 mW for each of the EC-QCL sources when operated in continuous mode. The overall high QCL power is advantageous, allowing for longer SEC-cuvette path lengths and consequently higher sensitivities. However, the laser source might still overload the MCT detector and the filter wheels prevent that possibility while still retaining control and flexibility.

This unique spectrometer was designed with an exchangeable sample chamber mounted on three kinematic seats allowing for exchanging the sample chamber for diff erent experimental setups with reproducible alignment. The sample chamber (Figure 1) is designed for online SEC detection and features guide mirrors and a set of off-axis parabolic mirrors (OAPs) to focus the beam to the center of the flow cell, beam diameter of ≈1 mm, with the aid of IR active alignment cards. The sample chamber is purged with N_2 and has a liquid sensor that automatically shuts off the laser source in case of a solvent leak. The flow cell (**Figure 2**) is designed for direct IR absorption spectroscopy measurements in transmission and features custom wedge-shaped ZnSe windows (10 mm diameter, 2 mm thick, 30 arc min wedge) with a 2–13 µm antireflection coating on the sides not in contact with the SEC solvents. These windows are mounted on hollow rods that can be pulled out to create an adjustable IR path length of 2–10 mm with sets of Kalrez o-rings to prevent leaks. The IR beam path passes through the center of the hollow rods. The advantage of an adjustable path length was established previously as the maximum increase in the QCL path length is a function of the absorption coefficient.[23] Previous work on variable path length transmission IR flow cells provided a starting point for the current design, but were further modified to meet the needs of SEC, specifically to minimize flow disturbances and too long residence distribution times.[24,25] The liquid enters the flow cell after exiting the SEC separation column from the bottom of the flow cell and flows perpendicular to the IR beam. The flow cell volume depends on the position of the rods and has a minimum volume of ≈275 µL with an IR path length of 3.8 mm. In comparison, the SEC-FTIR setup could only be realized with a 6-reflection attenuated total reflection flow cell with an estimated IR path length of about 60 µm.[12,13] Consequently, the EC-QCL has a factor of 60 increase in path length. The EC-QCL flow cell volume is comparable to that used in the SEC-FTIR setup (170 µL)[13] and is matched to SEC semipreparative columns. In

principle, there is a clear path forward to use analytical SEC columns with an EC-QCL flow cell designed for a smaller total volume with the use of the installed off-axis parabolic mirrors. The current EC-QCL flow cell does affect the SEC resolution. The flow cell increases the eluting polymer full width half maximum (FWHM) by 4.7%, peak tailing by 14% and a peak delay of 0.14 mL relative to control measurements measured on an identical sample with only the differential refractive index detector in use directly after the SEC column, and this broadening is considered acceptable within the experimental margin of error of SEC.

A self-written MATLAB data processing program was developed to uniformly process data from the setup in multiple steps (see Figure S1, Supporting Information, for description). The EC-QCL spectrometer data is recorded at one frequency over time. The first steps in this program are to perform a baseline correction and denoising of the data. Automated peak recognition is used to identify the location and width of absorption peaks, which were then masked in order to fit a second-order polynomial to the remaining data. The second-order polynomial

a)

b)

SEC eluent

100 mm
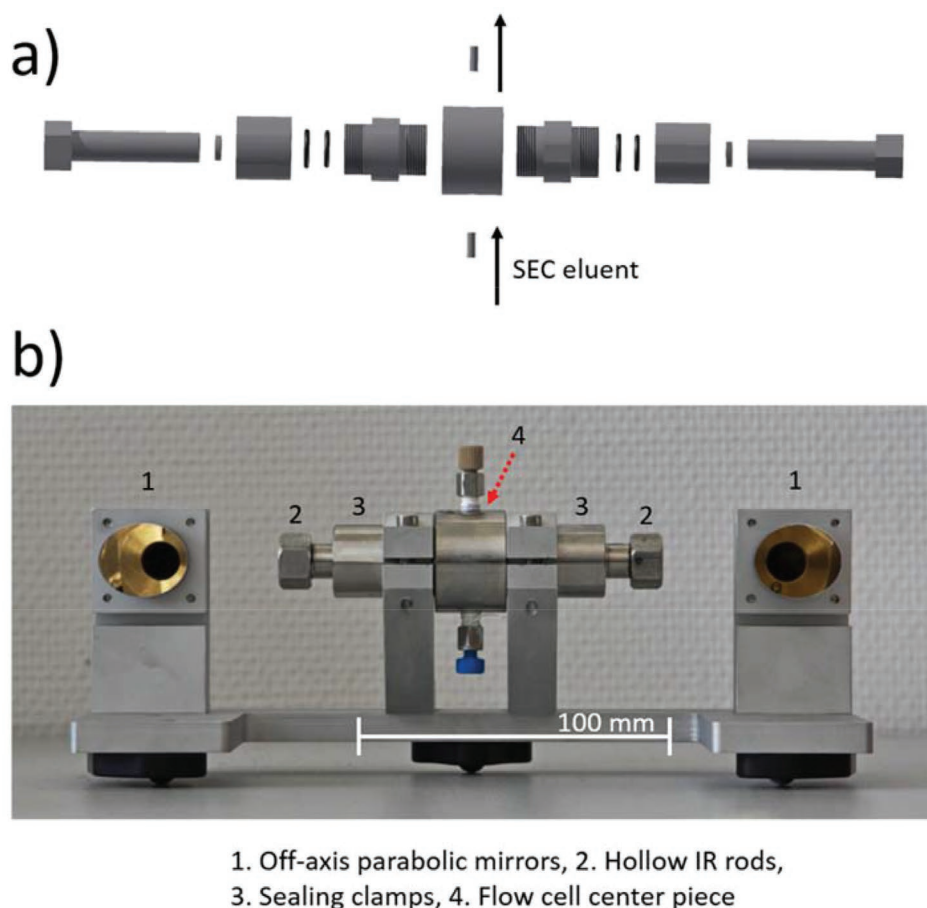
1. Off-axis parabolic mirrors, 2. Hollow IR rods,
3. Sealing clamps, 4. Flow cell center piece

Figure 2. a) Expansion drawing of the flow cell (see Figure 1). A wedged ZnSe window was attached to the angled end of the hollow rod to seal the flow cell. b) Photograph of the EC-QCL flow cell mounted in a holder with the off-axis parabolic mirrors. This holder fits into the SEC-EC-QCL-IR sample chamber using kinematic seats for reliable alignment.

was then subtracted from the entire data set. The regression coefficient for the second-order baseline drift correction is calculated and, if necessary, other polynomial functions can be used to improve the fit. In order to allow the laser source to thermally stabilize, the first 10 min of the data set were also masked. The data is converted to absorption A using the Lambert–Beer law, $A = -\ln(I/I_0)$, where I is the measured intensity and I_0 is the baseline intensity obtained from the drift correction.[14]

The noise in the data is reduced through smoothing in the SEC (time) dimension where a boxcar filter and a Gaussian filter were employed. The filter widths were automatically calculated in the MATLAB program so that the FWHM of the analyte peak relative to the raw data is limited to a maximum 10% increase. Typical filter widths were 30 s or less for narrow Đ samples, different values were explored, however, it was found that using very large filter widths, for example, 100 s, resulted in over smoothing of the data as well as increasing the FWHM in the SEC dimension. This control on the increase in the FWHM was introduced in the code to prevent over-processing of the data. The signal to noise ratios, SNR, of the analyte peaks were determined by the MATLAB code from the analyte peak maximum (signal) and the noise, N, was calculated from the standard deviation ($\sigma = N$) of the data collected over a period of 10 min after the SEC system peak after baseline subtraction. This region was selected because the approach of the polymer solution toward the IR flow cell caused additional pressure fluctuations, contributing to higher signal fluctuations before polymer elution.

An example of the achievable sensitivity measured on the new EC-QCL spectrometer is shown in **Figure 3** for a blend of polystyrene (M_w = 21.2 kg mol^{-1}, Đ = 1.02) and poly(methylmethacrylate) homopolymers (M_w = 199 kg mol^{-1}, Đ = 1.02) where 4.2 µg PMMA and 4.3 mg PS, having a 1:1000 difference in injected mass, was injected onto the column. In this example, the EC-QCL was operated in pulsed mode (5% duty cycle, 100 kHz) at a single wavelength, 1730 cm^{-1}, to measure the carbonyl stretching vibration of PMMA. For the data shown in Figure 3, the SNR of the raw data for the PMMA fraction was 15 and using 20 s Gaussian filter (one sigma) increased the SNR to 53, at a 10% increase in FWHM. In general, filters require an even data point spacing to prevent the possibility of artefacts, which was not yet achieved in the QCL data output rate and is currently under investigation. Furthermore, it should be noted that the IR absorption of PS and THF is slightly different, consequently the baseline correction fails at this PS concentration around the elution volume of the PS, V_e = 51–56 mL, resulting in a negative peak. Thus, the entire Gaussian filter trace is not shown in Figure 3.

It is important to mention that a priori information is required about the sample prior to analysis, that is, what chemical species are present in the sample. In the case of the results presented in Figure 3, prior to analysis it should be known that PS and PMMA are present in the sample.

The most effective test of this instrument is to determine the LOD. To calculate the LOD, a series of measurements were made on blends of polystyrene homopolymer (M_w = 80.2 kg mol^{-1}, Đ = 1.06) and a polystyrene-b-poly(methyl methacrylate) block copolymer (2.92 mol% PMMA, M_w = 92.6 kg mol^{-1}, Đ = 1.09) such that the total injected mass on the column was kept at 2.5 mg, but the total amount of PMMA was reduced. This ensured similar conditions on the column for separation, but tested the LOD of the EC-QCL, defined as the injected mass of PMMA at which a SNR of 3 is measured (**Figure 4**). As seen in this figure, the LOD measured on the coupled SEC-EC-QCL is 0.46 µg PMMA, which is approximately a factor of 30 lower than that reported for the SEC-FTIR.[14] When converted to an equivalent single carbonyl group in a polymer, this corresponds to one carbonyl group in about 530 000 g mol^{-1} measured at high diluted chromatographic conditions. These are the first results from this unique IR-spectrometer illustrating its potential to measure, for example, single end groups in a polymer chain, as this rare functionalization is of high importance in case surface absorbance is intended. The strength of this result can be further put into perspective by comparing the measured LODs for SEC-FTIR and SEC-MR-NMR (measured on polystyrene in CHCl₃), which were reported to be 13.4 µg and 110 µg, respectively, and are reproduced in Figure 4.[14,21] Therefore, each of the coupled SEC-IR and SEC-NMR techniques offers unique advantages where SEC-FTIR and SEC-MR-NMR
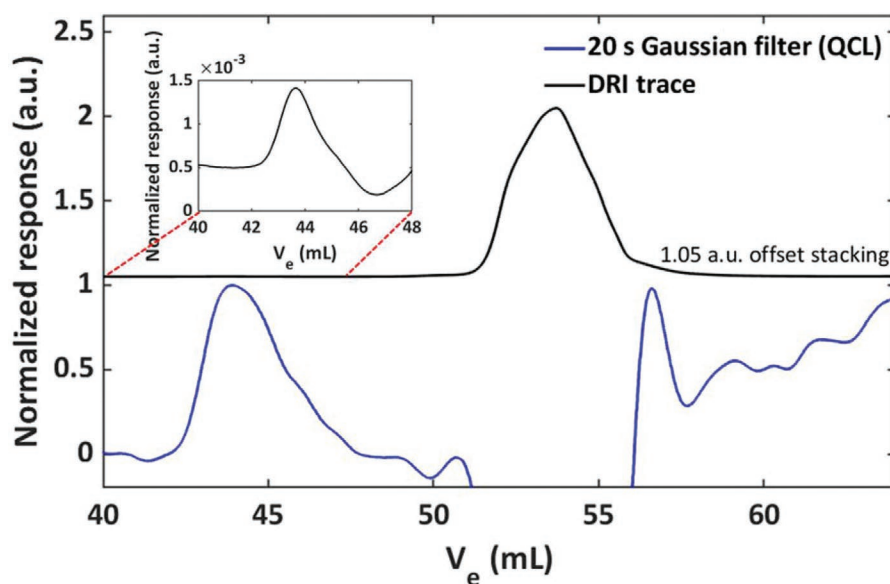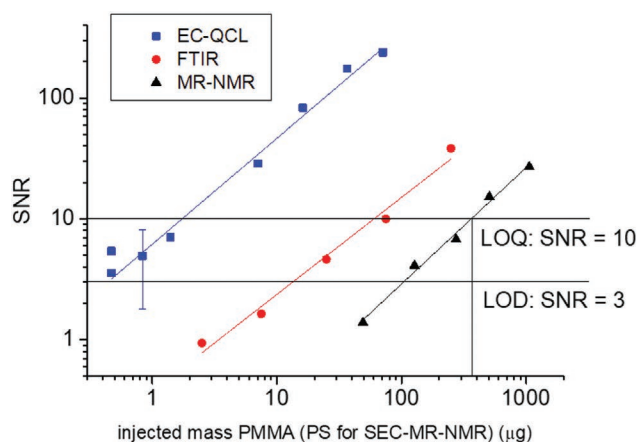

Figure 3. Elution profile raw data (EC-QCL and DRI) measured with the online SEC-EC-QCL-IR coupled setup using the EC-QCL spectrometer operating in pulsed mode (5% duty cycle, 100 kHz) at 1730 cm^{-1} (single wavelength). The inset is a 1000× magnification of the DRI trace, in the region where the PMMA fraction elutes. The measured sample is a blend of polystyrene (M_w = 21.2 kg mol^{-1}, Đ = 1.02) and poly(methylmethacrylate) homopolymers (M_w = 199 kg mol^{-1}, Đ = 1.02) where 4.2 µg PMMA and 4.3 mg PS, having a 1:1000 difference in injected mass, was injected onto the column.

**ADVANCED
SCIENCE NEWS**
www.advancedsciencenews.com

**Macromolecular
Rapid Communications**
www.mrc-journal.de

	LOD (µg)	LOQ (µg)
MR-NMR	110	369
FTIR	13,4	60.9
EC-QCL	0,46	1.75

Figure 4. The measured limit of detection (LOD) for the SEC-EC-QCL coupled setup using the EC-QCL spectrometer measured on a blend of polystyrene and polystyrene-*b*-poly(methyl methacrylate), with a total mass of 2.5 mg, where the injected mass of PS-PMMA was continually reduced until a SNR of 3 was reached. The measurements were made in pulsed mode (5% duty cycle, 100 kHz, single wavelength) at 1730 cm⁻¹. A 30 s boxcar filter was applied to the data. For comparison, published LOD results on our coupled SEC-FTIR[14] and SEC-MR-NMR[21] are shown to illustrate the sensitivity limits of each coupled technique. The LOD (SNR = 3) and the limit of quantification (LOQ; SNR = 10) are included in the graph to show the instrument capabilities. Data for the SEC-FTIR[14] and SEC-NMR[21] are reproduced with permission. A typical trace of the blend for the LOD and LOQ determination is illustrated in Figure S2, Supporting Information. Data for the SEC-FTIR and SEC-NMR, are reproduced with permission.[14,15,21,22] Copyright 2018, Wiley-VCH, respectively.

measure full spectra as a function of the polymer size at lower sensitivities and SEC-EC-QCL measures only over limited wavelengths at a substantially higher sensitivity. Future efforts are concentrated on improving the sensitivity on all three coupled SEC techniques.

The presented EC-QCL spectrometer is a unique research instrument. The new SEC detector based on QCLs is highly relevant to industry and academic research due to its multifold improvement in detection. A QCL-based SEC detector at a reasonable cost can be built as well using distributed feedback QCLs (DFB-QCLs), which are significantly smaller and less expensive than an EC-QCL source, but only cover a small wavelength of typically 2 cm⁻¹ and up to 10 cm⁻¹ with temperature and/or laser current tuning. For applications where a limited number of known wavelengths are required, a compact SEC detector with DFB-QCLs can provide this SEC-IR information at a lower investment cost. An SEC detector based on DFB-QCLs is similar to the use of DFB-QCLs already used in new clinical diagnostic or other field applications where measurements of dilute, but IR active, species at high sensitivity are required under field conditions.

In conclusion, the EC-QCL was introduced here for the first time as a unique world class EC-QCL spectrometer and evaluated in its first application as an online chemically sensitive IR based detector for SEC. The 30 times lower measured LOD confirmed the relevance of this technique for measuring unique details of the molecular construction of a polymer as a function of the polymer molecular weight, which were not previously available. In this case, on average one carbonyl group in 530 000 g mol⁻¹ under SEC conditions at 0.2 wt% was achieved. Furthermore, a semipreparative column was employed in this work, as the initial idea was to push the limits of the EC-QCL spectrometer. In future developments, the semipreparative column will be replaced with an analytical SEC column due to

the unique sensitivity of the spectrometer. This development parallels QCL sensor developments in other fields showing the importance and commercial relevance of new QCL sources in spectroscopy. These tunable IR-lasers will significantly impact vibrational spectroscopy for both SEC and LAC detection systems.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

Keywords

[1] J. Faist, *Quantum Cascade Lasers,* Oxford University Press, Oxford, UK **2013**.

[2] G. Wysocki, R. Lewicki, R. F. Curl, F. K. Tittel, L. Diehl, F. Capasso, M. Troccoli, G. Hofler, D. Bour, S. Corzine, R. Maulini, M. Giovannini, J. Faist, *Appl. Phys. B* **2008**, *92*, 305.

[3] R. F. Curl, F. Capasso, C. Gmachl, A. A. Kosterev, B. McManus, R. Lewicki, M. Pusharsky, G. Wysocki, F. K. Tittel, *Chem. Phys. Lett.* **2010**, *487*, 1.

[4] J. Wagner, R. Ostendorf, J. Grahmann, A. Merten, S. Hugger, J. P. Jarvis, F. Fuchs, D. Boskovic, H. Schenk, *Proc. SPIE* **2015**, *9370*, 937012.

[5] H. Moser, W. Pölz, J. P. Waclawek, J. Ofner, B. Lendl, *Anal. Bioanal. Chem.* **2017**, *409*, 729.

[6] T. Vahlsing, H. Moser, M. Grafen, K. Nalpantidis, M. Brandstetter, H. M. Heise, B. Lendl, S. Leonhardt, D. Ihrig, A. Ostendorf, V. Deckert, *Proc. SPIE* **2015**, *9537*, 953713.

[7] T. Rubin, T. Von Haimberger, A. Helmke, J. Lock, M. Stockmann, K. Heyne, *Photonics* **2016**, *3*, 31.

[8] A. Schwaighofer, M. Brandstetter, B. Lendl, *Chem. Soc. Rev.* **2017**, *46*, 5903.

[9] L. Zhang, G. Tian, J. Li, B. Yu, *Appl. Spectrosc.* **2014**, *68*, 1095.

[10] A. P. M. Michel, J. Kapit, M. F. Witinski, R. Blanchard, *Appl. Opt.* **2017**, *56*, E23.

[11] R. Kormann, R. Königstedt, U. Parchatka, J. Lelieveld, H. Fischer, *Rev. Sci. Instrum.* **2005**, *76*, 075102.

[12] T. F. Beskers, T. Hofe, M. Wilhelm, *Macromol. Rapid Commun.* **2012**, *33*, 1747.

[13] T. F. Beskers, T. Hofe, M. Wilhelm, *Polym. Chem.* **2015**, *6*, 128.

[14] S. Morlock, J. M. Kübel, T. F. Beskers, B. Lendl, M. Wilhelm, *Macromol. Rapid Commun.* **2018**, *39*, 1700307.

[15] T. Beskers, M. Brandstetter, J. Kuligowski, G. Quintas, M. Wilhelm, B. Lendl, *Analyst* **2014**, *139*, 2057.

[16] M. R. Alcaráz, A. Schwaighofer, C. Kristament, G. Ramer, M. Brandstetter, H. Goicoechea, B. Lendl, *Anal. Chem.* **2015**, *87*, 6980.

[17] A. Schwaighofer, M. Montemurro, S. Freitag, C. Kristament, M. J. Culzoni, B. Lendl, *Anal. Chem.* **2018**, *90*, 7072.

[18] M. Brandstetter, L. Volgger, A. Genner, C. Jungbauer, B. Lendl, *Appl. Phys. B* **2013**, *110*, 233.

[19] W. Hiller, P. Sinha, M. Hehn, H. Pasch, *Prog. Polym. Sci.* **2014**, *39*, 979.

[20] M. Cudaj, J. Cudaj, T. Hofe, B. Luy, M. Wilhelm, G. Guthausen, *Macromol. Chem. Phys.* **2012**, *213*, 1833.

[21] J. Höpfner, K.-F. Ratzsch, C. Botha, M. Wilhelm, *Macromol. Rapid Commun.* **2018**, *39*, 1700766.

[22] C. Botha, J. Höpfner, B. Mayerhöfer, M. Wilhelm, *Polym. Chem.* **2019**, *10*, 2230.

[23] M. Brandstetter, C. Koch, A. Genner, B. Lendl, *Proc. SPIE* **2014**, *8993*, 89931.

[24] P. A. Flowers, S.-A. Callender, *Anal. Chem.* **1996**, *68*, 199.

[25] H.-Y. Chen, Y.-T. Long, *Anal. Chim. Acta* **1999**, *382*, 171.