Mid-IR Quantum Cascade Lasers as an Enabling Technology for Analytical Chemistry

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Abstract: After providing an overview on the state-of-the-art of measuring condensed phase samples by quantum cascade lasers (QCL), this presentation will focus on protein analysis. The qualitative analysis of mid-IR spectra of aqueous protein solutions provides information on the secondary structure elements present in the proteins under study. In this work an external cavity (EC)-QCL was used for performing transmission measurements of different aqueous protein solutions in a CaF_2 flow cell with optical paths up to 75 µm. The strong temperature dependence of the ν_2-band of water overlapping the amide I absorption band (~1650 cm^{-1}) required a temperature stability of +/- 0.005°C. Successful determination of the secondary structures of albumin and β-lacto-globulin was achieved in the low g/L concentration range.
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1. General characteristics for the analysis of liquids using mid-IR spectroscopy

The spectroscopic situation encountered when measuring analytes in solution is different in many aspects from gas phase spectroscopy. Whereas sharp ro-vibrational lines are observed in the gas phase, broad and often overlapping absorption bands are typically present in condensed (liquid) phase. As a consequence, to achieve the required selectivity, different approaches as compared to gas phase spectroscopy have to be realized. Using a tunable laser source, such as an EC-QCL, direct quantitative analysis is possible when using chemometric approaches (e.g. partial least squares (PLS) calibration) for data analysis. This concept has been successfully applied for quantitative analysis of several blood parameters in human plasma and serum [1-2]. Another possibility for achieving the required selectivity is to add a sample pretreatment step prior to measurement. Such sample pretreatment steps can consist of a selective liquid-liquid extraction step, as shown in the new ASTM test method D7678-11 for measuring oil-in-water, a pH modulation in the determination of carbon dioxide in sugar containing liquids, or in performing on-line separation steps like high-performance liquid chromatography [3-5]. In condensed phase the matrix, being in many cases water, does have a very strong absorbance in the mid-IR spectral range. As a consequence the possible optical path-length is limited to a few micrometers only. For instance, when attempting to measure the amide I band (mainly due to ν-C=O of the amide moiety) of proteins in aqueous solutions using an FTIR spectrometer comprising a thermal light source, the path-length has to be shorter than typically 8 micrometers as the amide I band almost completely overlaps with the bending vibration of water (ν_2) centered at 1645 cm^{-1}.

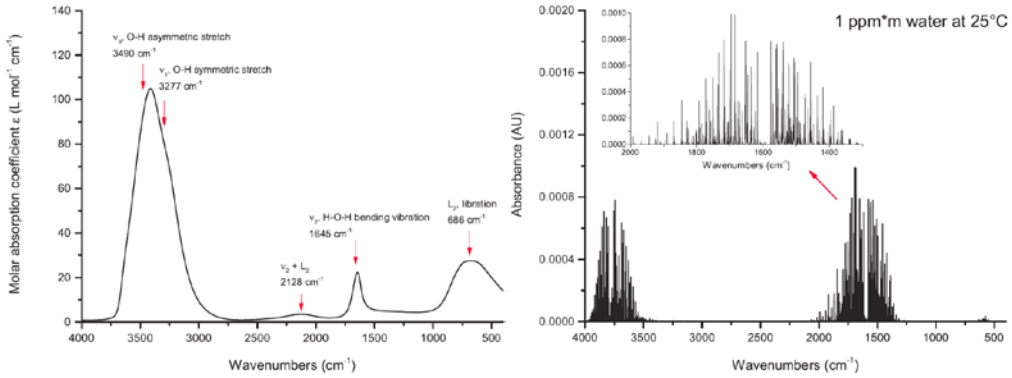

Fig. 1 Mid-IR absorption spectrum of liquid (left) and gaseous water(right).

Such short optical path-lengths present an important drawback concerning the practical usability of FTIR spectroscopy. In this respect mid-IR quantum cascade lasers can make a remarkable difference. In this presentation the challenges and opportunities for measuring liquids by mid-IR laser technology will be outlined. Special emphasis will be given to the direct analysis of proteins in aqueous solution by using an EC-QCL system.

2. EC-QCL for the measurement of proteins in aqueous solution

The exact location and shape of the amide I band of proteins is highly informative concerning the secondary structure elements present in the protein under study. This is due to the characteristic molecular interactions between adjacent carbonyl double bonds of the amides like hydrogen bonding, dipole-dipole interactions and alike [5].

In this study we employed a pulsed EC-QCL (daylight solutions, San Diego, CA) covering a spectral range from 1570 to 1730 cm^{-1} with a maximum pulse power of 800 mW in the center of the emission curve, as well as a flow cell containing 1 mm thick CaF_2 windows and a PTFE spacer to adjust the optical path to 50-75 µm. Fig 2 left shows a single beam spectrum of a 75 µm water film. Despite of the strong ν_2-band of water enough intensity was provided by the EC-QCL to perform measurements across the whole tuning range of the laser. Nevertheless, due to the strong temperature dependence of location and shape of the ν_2-band (fig. 2 right), a temperature controlled flow cell with a temperature stability of +/- 0.005°C had to be used. Absorption spectra were calculated using the single beam spectrum of distilled water as reference.

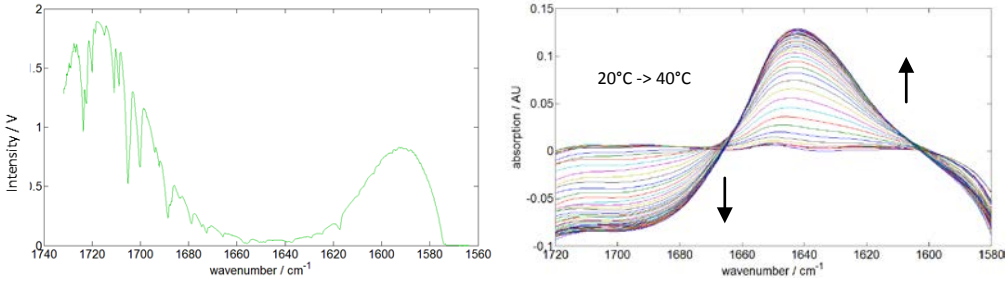

Fig. 2 Single beam spectrum through a 75 µm film of water as measured with the EC-QCL (left). Temperature dependence of the ν_2-band of water with a background taken at 20°C (right).

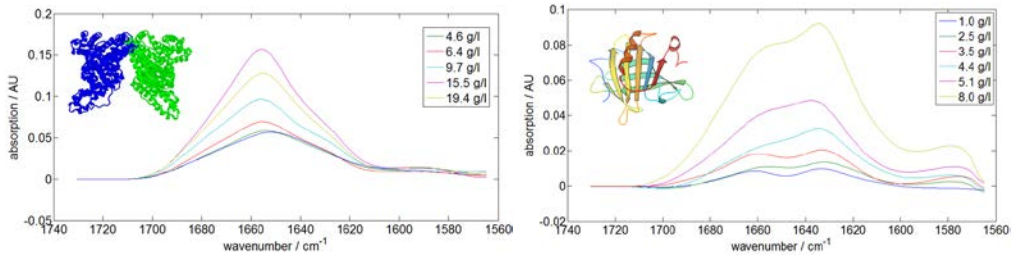

Fig. 3 EC-QCL spectra of albumin (left) and β-lacto-globulin (right).

Albumin and β-lacto-globulin were chosen as two model proteins which have a clearly distinct secondary structure. Whereas in albumin the α-helical structure is prevailing, β-sheet is the dominating secondary structure element in β-lacto-globulin (see also inserts in fig. 3). The recorded EC-QCL spectra confirm the dominating secondary structure elements of these two proteins, which is in agreement with literature [6].

4. References

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