The Good Vibrations of Beer. The Use of Infrared and UV/Vis Spectroscopy and Chemometry for the Quantitative Analysis of Beverages

Oliver Klein[a], Andreas Roth[a], Fabian Dornuf[a], Otmar Schöller[b], and Werner Mäntele[a]

[a] Institut für Biophysik, Fachbereich Physik, Johann Wolfgang Goethe-Universität Frankfurt am Main, Max-von-Laue-Straße 1, D-60438 Frankfurt am Main, Germany

[b] Innovectis GmbH, Altenhöfer Allee 3, D-60438 Frankfurt am Main, Germany

Reprint requests to Prof. Dr. Werner Mäntele. E-mail: maentele@biophysik.uni-frankfurt.de

Z. Naturforsch. **2012**, *67b*, 1005 – 1015 / DOI: 10.5560/ZNB.2012-0166
Received June 17, 2012

Dedicated to Professor Heribert Offermanns on the occasion of his 75th birthday

Infrared spectroscopy in combination with a specially developed attenuated total reflection (ATR) flow cell and multivariate analysis was used for the quantitative analysis of beer and other beverages. IR spectra of samples were obtained in the range from below 1000 cm^{-1} to 4000 cm^{-1} and subjected to a multivariate analysis based on calibration sets with laboratory reference standards. In the case of beer, this calibration set included 240 beer samples spanning the entire range of ethanol content, extract and CO_2. Based on this calibration, an infrared and UV/Vis spectroscopy-based sensor for the quick and quantitative quality control of beer was developed and subjected to extensive tests in breweries. This sensor meets and exceeds all requirements from brewers for the routine control in the production and bottling. Its use for other beverages, for example wine, juices or apple wine, requires only another set of calibration data for the specific beverage.

Key words: Infrared Spectra, Attenuated Total Reflection, Multivariate Analysis, Chemometry, Beer

Introduction

Infrared spectroscopy is sensitive to changes of bond lengths and bond angles concomitant with the normal modes of molecules. As such, it is highly sensitive to the structural properties of molecules, their protonation and hydration states. Out of the entire spectral range termed mid-infrared (MIR), 4000 to 500 cm^{-1}, the range from approx. 2000 to 800 cm^{-1} is diagnostically most relevant, since it contains all stretching modes from bonds among C, N and O atoms and all -C–H, -N–H and -O–H bending modes. The fingerprint region (approx. 900 – 1300 cm^{-1}) is highly sensitive to local conformations and thus ideally suited for the precise identification of organic molecules, even if they are closely related.

Quantitative information on concentrations can also be obtained from IR spectroscopy, although these applications need the high signal-to-noise ratio of Fourier transform infrared (FT-IR) spectroscopy and have not been extensively applied in the early days of IR spectroscopy.

Aqueous solutions or suspensions have long been considered difficult to analyze because the high absorbance of water, which, in spite of its comparatively low molar absorption coefficient (approx. 16 $L \cdot mol^{-1} \cdot cm^{-1}$), leads to high absorbance (approx. 1.5) even for thin (10 μm) layers due to the high concentration (55 mol L^{-1}). Thin-layer transmission cuvettes with solutions/suspensions at high concentrations have been long considered complicated to handle; thus, mid-infrared analysis has not made a career in industrial *in-line* process analysis. However, it is fully established in laboratory quality control.

Near infrared (NIR) spectroscopy uses overtone ($\Delta\nu = \pm 2, 3, \ldots$) transitions which are much weaker but easier to measure due to the higher path length, strong light sources and sensitive detectors. This advantage is balanced by low selectivity and speci-

ficity due to overlapping bands, in particular the broad and featureless, but nevertheless intense OH overtones from water. A further problem of NIR spectroscopy is the temperature-dependent occupancy of the higher excited vibrational levels, thus necessitating excellent thermostating for aqueous samples.

Analytical application of mid-infrared spectroscopy for *in-line* process analysis and quality control requires robust sample interfaces that are easy to handle and do not require specific skills. Attenuated total reflection (ATR) spectroscopy, a technique that uses evanescent waves along with total reflection in a light guide, a so-called internal reflection element (IRE), has long been used for samples of low transmission. It offers the possibility for easy sample access and the design of flow cells, for example for medical applications.

An important application of liquid analysis is the characterization and quality control of beverages, in particular of beer. With a consumption of more than 100 L per capita and year in Germany, Beer is one of the most relevant foodstuffs in Bavaria and luxury foodstuff outside.

Beer consists of water, ethanol, sugars, soluble proteins, traces of bitter substances, natural colorants and dissolved CO_2. In addition, it may contain suspended yeast cell particles that cause turbidity. Parameters that require strict control during the fermentation process, after bottling and immediately before delivery, are ethanol, extract, and CO_2 as well as eventually pH, oxygen content, color and turbidity. In particular, a strict control of the ethanol content of beer is required by law, by tax regulations or for religious reasons, and extract content needs to be controlled for dietary reasons.

Up to now, beer analysis is performed by measuring global rather than specific parameters. Simple and unprecise methods are used, involving ample manpower instead of automated procedures.

Beer analysis is frequently performed by measuring the speed of sound and the specific density. Both depend on ethanol and extract content, but in the opposite direction, *i. e.* ethanol decreases density and extract increases density, and both ethanol and extract increase the speed of sound. Quality control with these techniques represents a multistep analysis and requires different procedures and instruments [1, 2]. A complication with the present techniques for analysis is that suspended particles (for example yeast) and dissolved CO_2 lower the precision of the measurement of the speed of sound and of the density. Thus, degassing and filtration procedures have to be applied before density and speed of sound can be measured at a precision sufficient to calculate ethanol and extract content [1, 2]. Needless to say that precise thermostating is required as well.

Near-infrared spectroscopy has successfully been used for the determination of the ethanol content in beverages [3 – 5]. However, NIR determination of ethanol in beer also requires precise thermostating, degassing and filtration as well as transfer of the liquid into special cuvettes. At present, no technology is available that allows the analysis of the most important parameters of beer right from the bottle, without any preparation and pretreatment.

A mid-infrared (MIR) spectrum of beer contains the full information on all molecular ingredients (except for homonuclear and thus IR-inactive molecules such as O_2). However, due to the overlap of vibrational modes from different molecules, chemometric methods have to be applied that use absorbance values at different wavelengths. The combination of IR spectroscopy with multivariate analysis has demonstrated the great selectivity and specificity of vibrational spectroscopy for the analysis of complex matrices such as different body fluids [6 – 19].

Here we present an IR-based analysis method for complex solutions and suspensions such as beer and other beverages. The design of a flow-through attenuated total reflection (ATR) cell for process analysis is reported, and the IR spectra of beer are analyzed. Using a calibration set established from beers spanning the entire range of parameters, we report an analysis technique which can be used *in-line*, *i. e.* during production and bottling and for the final quality control in breweries.

The measurement of color and turbidity presently also relies on simple and unprecise techniques. In many breweries, the color is compared “by eye”, *i. e.* visually, with a color table or measured with a single-wavelength photometer. Turbidity is controlled “by eye” only instead of measuring apparent absorption or light scattering. We complement here the IR-based analysis of beer constituents with the quantitative analysis of color and turbidity with a flow cell. Both, IR and UV/Vis analyses are combined to determine the unique fingerprint of a beer and to guarantee the desired parameters in the quality control process.

Experimental Section

Infrared spectroscopy

Infrared spectra of beer were recorded with an attenuated total reflection (ATR) measuring cell specifically developed for this purpose (see Results and Discussion, Fig. 1). The cell was coupled to a Tensor FTIR spectrophotometer (Bruker Optics, Ettlingen/Germany). Alternatively, coupling to a compact FTIR spectrophotometer Alpha (Bruker Optics, Ettlingen/Germany) or to other FTIR spectrophotometers is possible. For data acquisition, 100 interferometer scans at a resolution of $2\,cm^{-1}$ were recorded in the double-sided/forward-backward mode and averaged before Fourier transformation. Spectra were calculated from 900 to $4000\,cm^{-1}$ using a Blackman-Harris-3-Term apodization function and a zero filling factor of 2. The OPUS software (Bruker Optics, Ettlingen/Germany) was used to record and display the spectra.

Software for brewery use

A software specific for breweries was designed which provides a user interface for the beer analysis. It uses a DLL (dynamic link library) interface provided by Bruker (Ettlingen/Germany) to control the instrument and to collect spectra. The software guides the user through the procedure, from tapping bottles to the data evaluation. The user is not directly involved in recording spectra.

UV/Vis spectroscopy

A measuring cell was designed for broadband spectral measurements in the visible spectral range. It consists of a flow cell with quartz windows and a path length of 1 cm connected to the outlet of the IR-ATR cell and is equipped with light fiber connectors and collimators to obtain a parallel beam in the range of the flowing sample. A high-power white light LED was used as source and a CCD multichannel spectrometer (Microparts, Dortmund/Germany) for detection (420 – 700 nm). For a beer spectrum, 40 individual spectra were averaged at a resolution of 3.5 nm per pixel.

Multivariate calibration

The principal component analysis was done using the PLS-toolbox for MATLAB from Eigenvektor Research (Wenatchee, WA/USA). The prediction models for the quantitative analysis of beers presented in this manuscript were created using the software package QUANT2 by Bruker (Bruker Optics, Ettlingen/Germany).

Reference analysis in breweries

The beers selected for calibration of the IR spectroscopic analysis were obtained from more than 30 different breweries. The content of ethanol, extract and CO_2 of these beers used for calibration had been determined in the internal quality control laboratories by established and commercially available techniques, *i. e.* measurement of the speed of sound and the gravity of degassed and filtered beer for ethanol content and for extract, and the expansion method for CO_2 [1]. In some cases, near IR measuring data for ethanol were available. Data on the color of beers were available from single-wavelength spectral measurements and from "guess by eye" determinations using an EBC color table.

Results and Discussion

Infrared and UV/Vis flow cell and analysis system

The setup used for the spectroscopic analysis of beverages is shown in Fig. 1a. Fig. 1b shows the design of a flow cell which makes use of the principle of attenuated total reflection (ATR) and is coupled to an FT-IR Instrument. It uses an internal reflection element (IRE) made from ZnS with IR transmission from approx. $4000 - 800\,cm^{-1}$ (Fig. 1b and position 5 in Fig. 1a). Based on the dimensions and the angle of incidence, 7 reflections on the top side of the IRE are obtained. The evanescent wave emerging from the IRE into the liquid to be analyzed penetrates to approx. $1.5\,\mu m$ (decay to $1/e$ at $1600\,cm^{-1}$). The IR beam thus only probes a thin layer along the crystal surface; with 7 reflections, the effective path length is $7 \times 1.5\,\mu m = 10.5\,\mu m$. This guarantees sufficient transmission even in regions of strong background absorbance from water around $1650\,cm^{-1}$.

The flow cell is designed to yield laminar flow for flow rates of up to approx. $100\,mL\,min^{-1}$. Higher flow rates lead to turbulences and can be used to "clean" the cell from residual liquid and particles left from the previous analysis.

At the cell outlet, further analytical devices can be coupled as flow cells to analyze, for example, for color, for turbidity or oxygen content. We have added a UV/Vis flow cell designed for the measurement of color and turbidity which makes use of a collimated beam from a white light-emitting diode (LED) and a CCD spectrophotometer (Fig. 1c, see also Experimental Section). A flow measuring device at the IR cell outlet is used to control the flow rate.

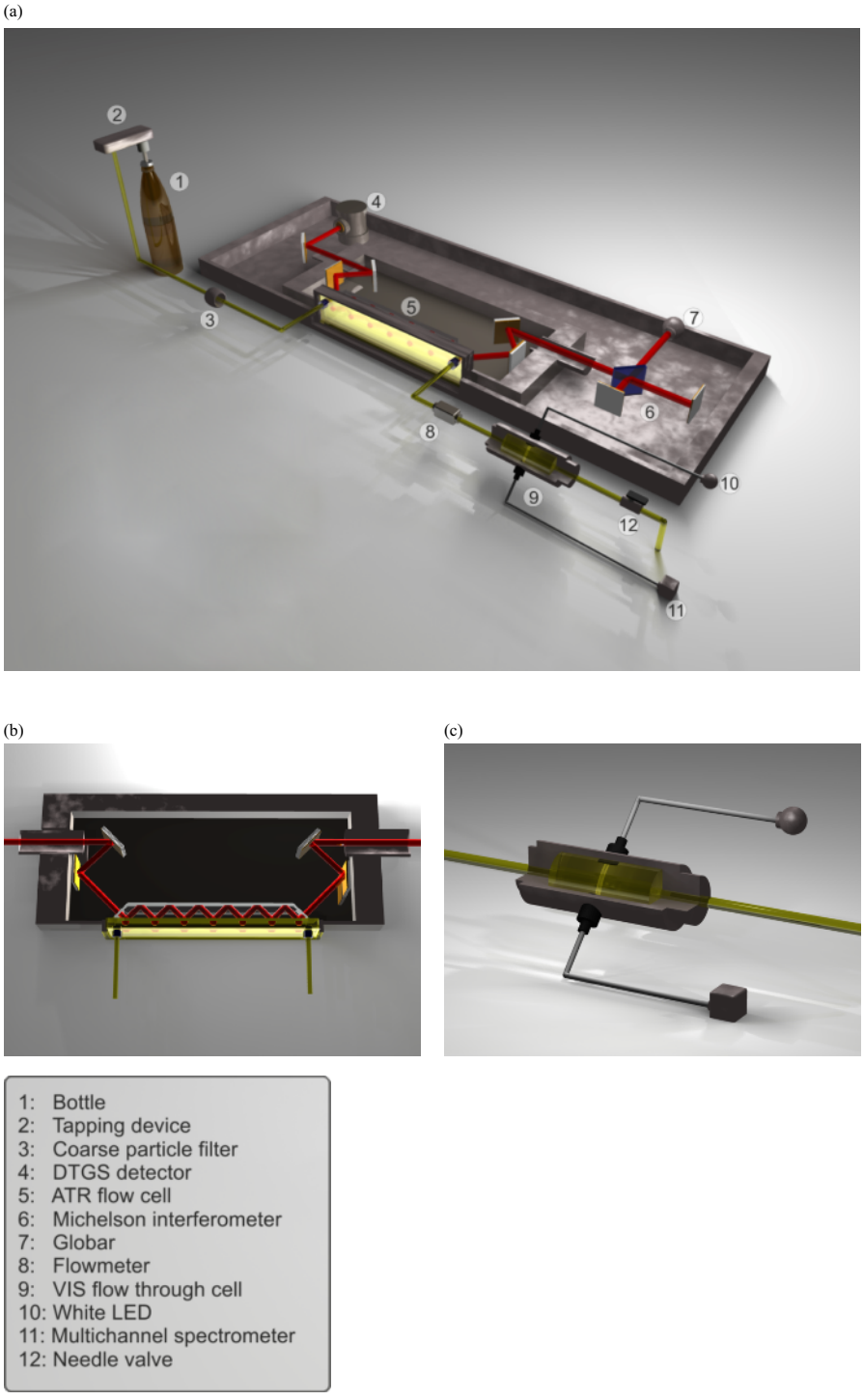

Fig. 1 (color online). Schematic view of the spectroscopic setup for the analysis of beverages. a) Infrared ATR and UV/Vis flow cell system integrated into an FT-IR spectrophotometer; b) detailed top view of the ATR flow cell; c) detailed view of the UV/Vis flow cell.

The parameters easily accessible for MIR spectroscopy of beer are ethanol, extract molecules, and CO_2. In the case of CO_2, a direct access to the content of the bottle or can has to be made to avoid loss of CO_2. The analyzer system shown in Fig. 1a uses a mechanical tapping device (position 8 in Fig. 1a) which punches a hole through the cap of a bottle (or through the bottom of a can). The tapping device is sealed with a rubber seal to the cap of the bottle or the bottom of the can and kept under N_2 pressure ($3-4\times10^5$ Pa). After punching a hole, the beer is directly transferred to the cell without degassing, *i. e.* without a loss of CO_2. A coarse particle filter (position 3 in Fig. 1a) is inserted between the cutting device and the flow cell to avoid the passage of metal particles which may appear in the liquid upon cutting the cap and which could destroy the IRE surface. Since the liquid to be analyzed could contain suspended particles (such as a naturally-turbid beer or a wheat beer with yeast cells), we have mounted the crystal sideways: Particles that are not moved by the stream of the liquid are going to sediment downward, and air or CO_2 bubbles are going to move upward, both outside the range probed by the evanescent wave.

This sample interface can be used for almost any liquid throughout a wide range of viscosities. We have developed similar cells with different IRE sizes for the analysis of blood [7] and for dialysis liquid [6] at flow rates from some μL min^{-1} up to 1 L min^{-1}.

Infrared spectra of beer

Fig. 2 shows a typical IR spectrum of a Pilsener beer measured in the flow-through mode and directly accessed from the bottle, *i. e.* kept under approx. 3×10^5 Pa pressure during the measurement. A water spectrum has been subtracted. The spectrum shows distinct absorbance lines and bands throughout the spectrum which can be assigned to individual ingredients. Bands appearing negative in the spectral range around 3300 and 1650 cm^{-1} are due to oversubtraction of water bands (a typical beer contains only 95 % water; subtraction of 100 % water thus generates negative bands).

The dominating line in the spectrum is the narrow band at 2343 cm^{-1}which can be assigned to the antisymmetric stretching mode of CO_2 dissolved in the liquid. This band is slightly shifted with respect to the band of gaseous CO_2 at approx. 2350 cm^{-1}. Contributions from ethanol and extract molecules are found in the 900 – 1500 cm^{-1} range and between 3000 and 2700 cm^{-1}, although strongly overlapping. They represent the "fingerprint" of a beer which can be used for identification and quantification of the ingredients.

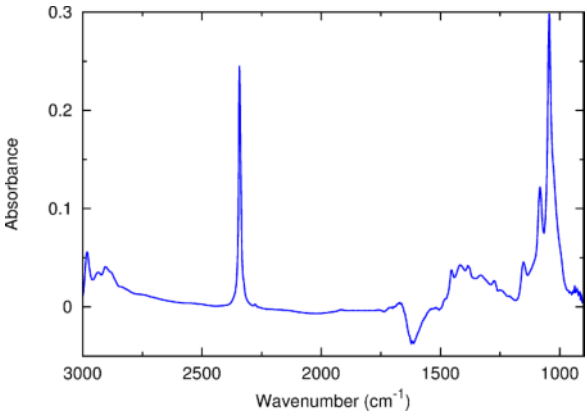

Fig. 2 (color online). Typical infrared spectrum of a Pilsener beer. A spectrum of water obtained with the same cell has been subtracted.

Singular value decomposition

Using the method of principal component analysis (PCA), the number of independent components in the spectral data was determined. The first six principal components were calculated ordered by their contribution to the total variance of the absorption values. As can been seen in Fig. 3, the first three principal components (PC 1 – 3) together account for 98 % of the spectral variations of beer. In other words, 98 % of the information of one single spectrum can be reconstructed by using only the first three principal components. Based on this fact, beer can be assumed as a system of mainly three independent components plus water.

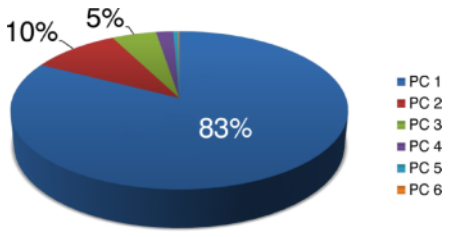

Fig. 3 (color online). Contributions of the first six principal components to the variability of a beer spectrum calculated by PCA.

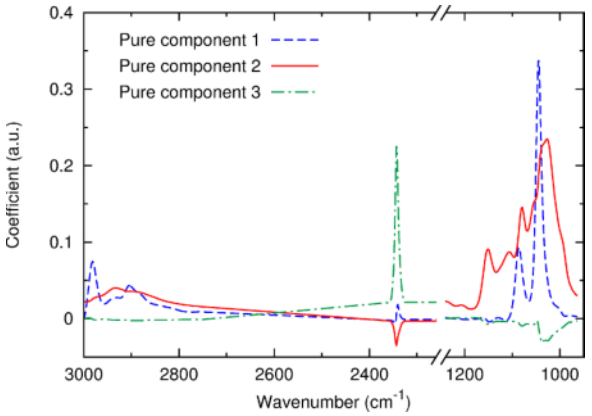

Fig. 4 (color online). Pure components obtained from PCD of beer spectra.

To identify these three components, a further calculation was done to determine the pure components. Considering the information on the concentration together with the spectral data of the analyzed beer samples, a pure component decomposition (PCD) allows to reconstruct the spectra of the independent components. The results are shown in Fig. 4.

Fig. 5 shows the spectra of a pure glucose solution, of pure ethanol and of dissolved CO_2. The band structures observed for these model solutions match very closely those of the pure components extracted by pure component decomposition shown in Fig. 4. They can also be identified in the beer spectrum shown in Fig. 2. However, it is clear from this figure that the overlap of vibrational modes from ethanol and extract requires a robust multivariate analysis to obtain a quantitative determination of the concentrations.

Multivariate analysis

The concentrations of absorbing ingredients are related to absorption values at specific wavelengths by Lambert-Beer's law. In case these absorption bands do not overlap, an absorption measurement at one wavelength would be sufficient to predict the concentration of one substance; this procedure is termed univariate analysis. In rather good approximation, this is the case for the determination of the CO_2 content.

Usually in a multi-component mixture the absorption bands of the different ingredients overlap. This is the case for ethanol and extract. An analysis of the infrared spectra of beers thus requires multivariate calibration to account for absorption values at multiple wavelengths. It is thus necessary to measure the absorption of samples with known concentrations at many different wavelengths. The absorption values at close-by wavelengths will be linearly dependent, thus leading to a prediction model which will only predict the concentrations of the known samples correctly, and will result in a poor prediction of concentrations in new, unknown samples. In order to overcome this problem, the amount of data used to create the prediction model has to be reduced.

Principal component regression (PCR) uses linear combinations of absorption values determined by the criterion of maximum variance. The first principal component thus describes the maximum variance of the absorption values. The second principal component describes the maximum variance of the absorption values with the constraint that it does not correlate with the first principal component, and so on.

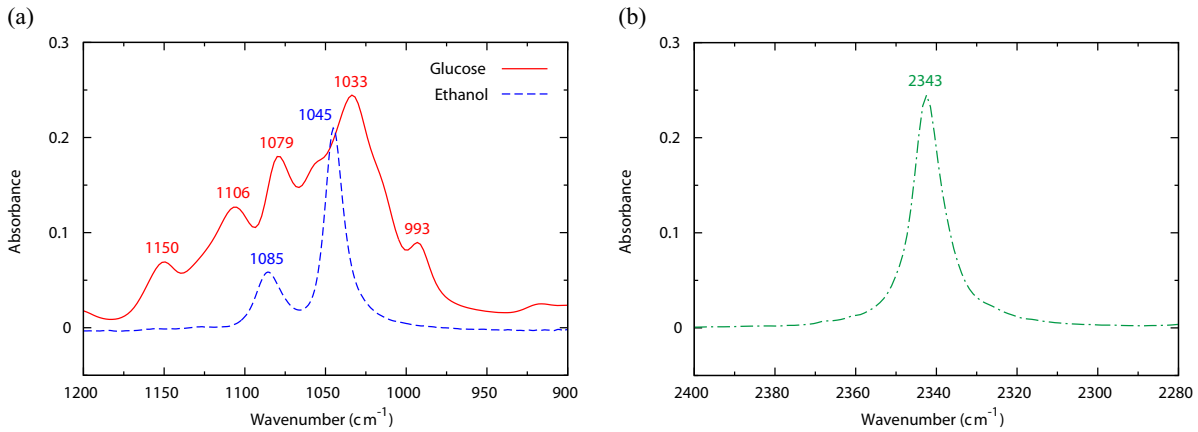

Fig. 5 (color online). Infrared spectra of the major components detected in beer: a) ethanol and glucose; b) CO_2.

These principal components are the ones used instead of the measured spectra to create the prediction model. The drawback of PCR is that the principal components are selected only because they create variability in the absorption values, and the information on the concentrations of the ingredients is not used. There is a certain risk that some principal components are of low relevance for the prediction of the specific ingredient. In order to solve this problem, we have used partial least squares regression (PLS). PLS uses components which maximize the covariance between the concentrations and the absorption values. The first component thus does not contain the strongest differences between the spectra, but the most relevant as related to the concentration values. This procedure leads to better predictions with fewer components.

Quantitative infrared analysis of beer

In order to facilitate a quantitative analysis of different beers with respect to the content of ethanol, extract, and CO_2 allowing independently varying concentrations, we have established a calibration set consisting of more than 100 different beer types from more than 30 breweries. All these beers had been analyzed by conventional methods with state-of-the-art techniques, either in the brewery laboratory or in central brewery reference laboratories.

Table 1 shows the range of concentrations and the precision requirements for different ingredients of beer. These values represent standards that are desired by the breweries, although many analysis techniques used up to now yield results of lower precision.

Apart from some exotic strong beers with an ethanol content slightly above 10 %, these values cover more than 99.9 % of the beer brands worldwide, even the non-alcoholic beers allowed for religious reasons. The calibration set also contains classical mixtures of beer and sweet lemonade ("Radler") in different variations. Exotic but fashionable mixtures, such as wheat beer with banana juice or beer with red wine were excluded as decided by an *ad-hoc* beer ethics commission formed by the authors.

Table 1. Required range of ingredient concentration and precision for an IR-based analysis.

Ingredients	Range	Precision required
Ethanol	0 % – 10 % v/v	±0.025 % v/v
Dissolved CO_2	0 % – 1 % w/w	±0.005 % w/w
Extract	0 % – 10 % w/w	±0.025 % w/w

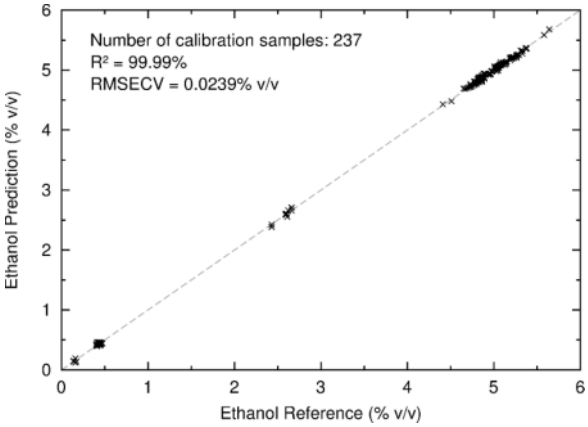

Fig. 6. Cross validation for ethanol contents of beers.

This calibration set included 247 beer variants, of which 237 were finally used for calibration. The quality of the analysis is demonstrated in the form of a cross validation, where the "real" concentration obtained from a reference method is drawn on the abscissa, and the concentration obtained from multivariate analysis of the IR spectra is drawn on the ordinate.

Fig. 6 shows the cross validation for the ethanol content. The different beers cluster in different ranges of ethanol content. Each dot represents a specific beer. In the ethanol range of 4.5 to 5.5 %, normal draft beers, "Spezial", "Lager" and "Pilsener" are found. Some light beers, light wheat beers and the "Radler" mixtures made with normal beer cluster around 2.5 % ethanol, and many of the so-called non-alcoholic beers cluster close to below 0.5 %, the legal limit of ethanol for "non-alcoholic" beers in Germany. Below that, there are few non-alcoholic beers made with different procedures to remove ethanol, and "Radler" mixtures made with non-alcoholic beers. Only a few exceptions of beers are found outside these clusters.

A correlation analysis for these clusters yields an excellent linear relation, with an R^2 of 99.99 % and a root-mean square error of cross validation (RMSECV) of 0.0239 %.

The correlation analysis for CO_2 in beer is shown in Fig. 7. Only two clusters are evident, those of the "Lager" and "Spezial" type draft beers with a CO_2 content around 0.55 % and those of the "Pilsener"-type beers with a somewhat higher CO_2 content. It is also evident that the determination of CO_2 content

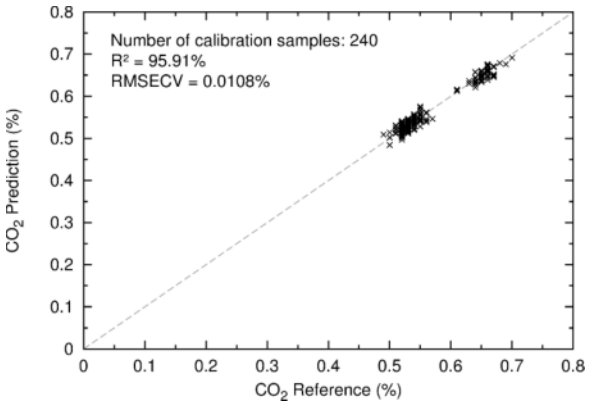

Fig. 7. Cross validation for CO_2 contents of beers.

is performed in breweries only coarsely. The infrared method would allow a much better accuracy if the reference analysis would be more accurate.

The cross-validation for the extract is shown in Fig. 8. The clusters represent rich, malted beers (also malt beers that are non-alcoholic) at the high end of the scale, the Lager, Spezial and "Pilsener"-type beers around 4 % and diet beers (for diabetics) at the low end of the scale. The correlation is excellent ($R^2 = 99.93\,\%$) and the RMSECV is 0.0269 %.

We would like to emphasize that the goal of this study was the quantitative determination of beer ingredients with only *one single* calibration, in contrast to the conventional analysis practiced in breweries and brewery reference laboratories where individual calibrations are used for each type of beer. Furthermore, we would like to emphasize that the RMSECV values

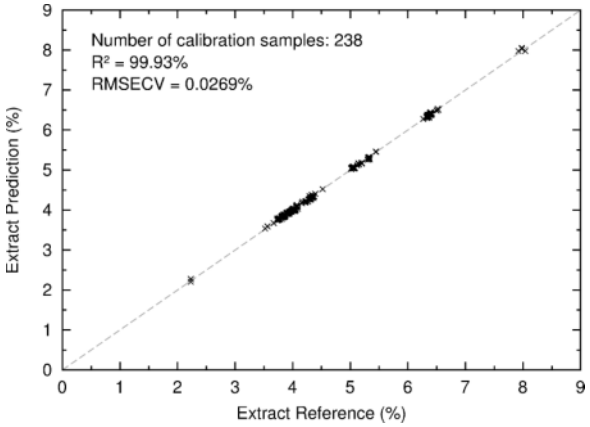

Fig. 8. Cross validation for the extract of beers.

Table 2. Achieved accuracy and precision by IR-based analysis.

Ingredients	Accuracy achieved	Precision achieved
Ethanol	±0.02 % v/v	±0.01 % v/v
Dissolved CO_2	±0.01 % w/w	±0.004 % w/w
Extract	±0.03 % w/w	±0.013 % w/w

reported stand for absolute accuracy. The precision is much higher, as determined by repeatedly measuring the same beer and comparing the error. As shown in Table 2, the standard deviation obtained from 10 subsequent measurements of one beer is only 0.01 %. Thus, the absolute accuracy of the spectroscopic method demonstrated here could be much higher if the reference analysis used for the calibration set would be at higher accuracy. Nevertheless, the desired by breweries precisions shown in Table 1 are already achieved and even exceeded.

Replacement of the full spectral width by individual wavelengths

The application of IR spectroscopy for the analysis of beer is presently based on FT-IR spectrophotometers, where the full spectral range is recorded. Although these instruments are compact and affordable, the future application aims at the use of infrared lasers at wavelengths specific for one or more analytes. Quantum cascade lasers (QCL) as single mode lasers have been available as OEM components for more than 10 years, and, with external cavity (EC-QCL), can be tuned over a wide wavelength range [21]. We have demonstrated in other applications that quantum cascade lasers in combination with photoacoustic detection or with ATR flow cells and optical detection can be used to determine analyte concentrations at precisions as high as FT-IR spectroscopy or even higher, because of the high spectral density of these lasers [20 – 23].

The broad spectral information obtained here from the IR spectra allows to determine the number and the exact emission maximum of individual wavelengths by taking only the most relevant ones, finding a balance between the number of wavelengths used and the precision desired. This determination forms the base for the selection of single-wavelength lasers to be used for beer analysis.

As shown in Table 3, three measuring wavelengths (1149, 1088 and 1045 cm^{-1}) are necessary in order to quantitatively determine the ethanol content of beer

Table 3. Detection of ethanol, extract and CO_2 at selected wavelengths.

	Wavelengths (cm^{-1})	Accuracy (%)
Ethanol	1149 1088 1045	0.10
Extract	1065 1045 993	0.18
CO_2	2343	0.019

at 0.1 % accuracy. One of these ($1045\,cm^{-1}$) can be shared with extract determination, where three wavelengths are necessary to obtain 0.18 % accuracy. Finally, only one laser centered at $2345\,cm^{-1}$ would be necessary to detect CO_2 at perfect accuracy, much better than presently available in breweries. Overall, an optical setup including six single-wavelength QCL lasers could form the basis of a sensor.

Color measurements

The color impression that our vision has of a beer is due to the molecules extracted from the malted grains and from the hop added for the bitter taste. These are mostly riboflavins absorbing at approx. 450 nm and carotenoids absorbing at around 520 nm. The color impression is further influenced by turbidity caused by residual yeast particles, in particular with "naturally turbid" beers or wheat beers.

To characterize the color of a beer, breweries use EBC (European Brewery Convention) units that are related to the extinction measured at 430 nm:

$$\mathrm{EBC} = E_{430\,\mathrm{nm}} \times 25$$

Typical Pilsener beers may have colors at 5 – 10 EBC, dark beers being somewhat higher (15 – 30 EBC). There are some "Black beers" that exhibit colors at more than 100 EBC.

In most breweries, simple color charts are used for a comparison of the color of a beer "by eye" to determine an EBC value. Others use single- or dual-wavelength spectrophotometers with a measuring wavelength at 430 nm and eventually a reference wavelength in the red part of the spectrum (700 nm). The color is measured after degassing and complete removal of CO_2 and after filtration through particle filters with 0.45 μm pore sizes. As for turbidity, there are no standards apart from visual comparison.

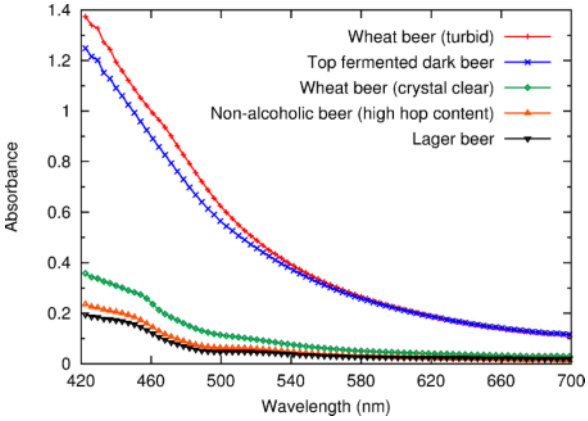

Fig. 9 (color online). Vis absorbance spectra of different beers.

The analysis of beer color and turbidity in one step with a flow-through cell, *i. e.* without degassing and particle filtration, is shown in Fig. 9. The spectra shown here are composed of light scattering curves with additional absorbance contributions in the 440 – 550 nm range. The scattering curves coarsely follow a λ^{-4} relation, which indicates Rayleigh and Rayleigh-Gans-Debye scattering from particles smaller than the wavelength. The structures in the scattering curves in the 440 – 520 nm range are due to flavin and to carotenoid absorption.

Fitting of the spectra in the range 700 to 420 nm to a λ^{-4} function with exclusion of the ranges from 420 to 450 nm and 500 to 550 nm yields scattering curves that can be used to determine light scattering properties and thus to form a "turbidity index" (data not shown) that can be used to compare different beers or the same beers from different production batches; this generates a standard to guarantee constant turbidity. Subtraction of the scattering curves from the measured spectral values yields EBC values.

In the calibration set used for the infrared analysis, only a few beers with a quantitative reference analysis of the color were available due to the "visual" determination made in most breweries. Fig. 10 shows the cross validation of the colors for these beers. The correlation is excellent ($R^2 = 99.95$), and the RMSECV is below 0.6 EBC units. This demonstrates that an *in-line* measurement of color and turbidity is possible in a flow system and without further sample preparation.

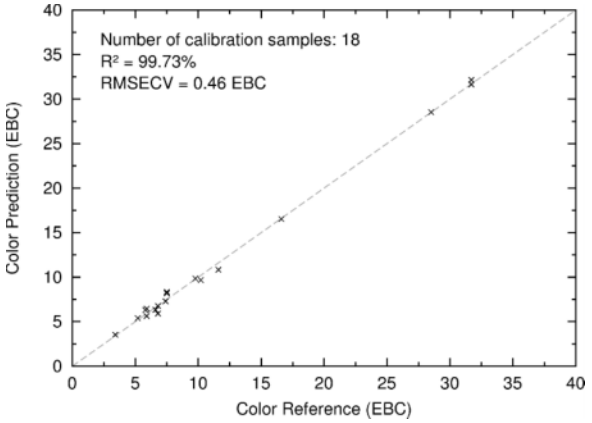

Fig. 10. Cross validation for the color of different beers.

Conclusions and Outlook

We have presented here an infrared-based method for the quantitative and highly precise analysis of beer which is superior to the presently available techniques and methods:

- it measures independently ethanol, extract and dissolved CO_2 on the basis of their molecular fingerprints;
- it measures specific parameters instead of "global" parameters such as gravity or speed of sound;
- it does not need any sample preparation, degassing or filtration;
- the handling is simple and can be performed by non-experts in a brewery;
- the measuring time is less than two minutes per sample, including the time for a turbid flow (approx. 100 – 150 mL) to clean the flow cells, and a laminar flow of 50 – 100 mL for the recording of the spectra and the multivariate analysis to obtain the concentrations;
- no rigid thermostating is required;
- direct sampling from bottles or cans is possible;
- sampling from fermentation tanks through tubing can be performed *in-line*, *i. e.* during the process;
- full precision is obtained even with very turbid beers;
- all beers can be analyzed with one single calibration;
- if necessary, precision can be further increased by introducing a calibration specific for one beer type or for groups of beers;
- even very low ethanol traces can be detected.

In combination with visible light spectroscopy, parameters such as color and turbidity have also been characterized and, together with the parameters obtained from IR spectroscopy, they form the "fingerprint" of a beer, which can be used to uncover and prevent forgery and false declaration of beers, which are both quite common. In particular, beers with a false declaration may be sold on big fairs.

In a number of large-scale brewery tests, we have collected data on the robustness of the analysis process and on the handling by untrained personal. In these tests, the analysis time for one sample was reduced by a factor of 20 – 30 as compared to the existing techniques. Precision was maintained over months of testing, and recalibration was not required. Cleaning of the fluidic system and of the IR cell is performed by tapping a bottle of water every 20 – 30 bottles of beer or every 2 – 3 hours, whatever comes first. This procedure is also used to define the background spectrum $I_0(\lambda)$. If this procedure is followed, no deposits on the IRE are formed. In one case, the cell was, by mistake, left drying with a beer filling over the weekend. The dried extract could be easily removed by rinsing and cleaning with mild detergents.

The IR-based measuring method can be easily adapted to other beverages like wine, juices, cider, soft drinks, or others. In this case, a calibration matrix needs to be established with a number of samples with a reference analysis, which should be as precise as possible. An urgent application is the determination of ethanol in fruit juices formed by fermentation during storage to prevent its use for children. In medical applications, we have recently demonstrated the potential of this technique for the determination of compounds in the dialysis liquid during hemodialysis, where glucose, urea, lactate and creatinine can be measured *in-line* and used for the monitoring of the parameters of the patient and its detoxification [6].

Finally, a replacement of the IR spectrophotometer by quantum cascade lasers is possible. At present, the costs for QCL are prohibitively high for such a replacement. However, experts predict a drastic decrease for laser prizes and increase of performance in the next years.

Acknowledgement

The authors appreciate the valuable discussions with Prof. H. Offermanns in the course of this research and development project. We would like to thank E. Winter, Institut

für Biophysik, for excellent mechanical engineering. We acknowledge the help of Centec GmbH to establish a calibration set with reference beers from different breweries and for the possibility to test the sensor in the "Binding" brewery in Frankfurt/Main. Funding from the Technologiestiftung Hessen is gratefully acknowledged.

[1] MEBAK *Brautechnische Analysemethoden* (Ed.: H. Pfenninger), Band II, **2002**, p. 87.
[2] MEBAK *Brautechnische Analysemethoden* (Ed.: H. Pfenninger), Band II, **2002**, p. 88.
[3] B. Osborne in *Encyclopedia of Analytical Chemistry* (Ed.: R. A. Meyers), John Wiley & Sons, Chichester, **2000**, pp. 1 – 14.
[4] F. D. Barboza, R. J. Poppi, *Anal. Bioanal. Chem.* **2003**, *377*, 695 – 701.
[5] D. Cozzolino, H. E. Smyth, M. Gishen, *J. Agric. Food Chem.* **2003**, *51*, 7703 – 7708.
[6] A. Roth, F. Dornuf, O. Klein, D. Schneditz, H. Hafner-Gießauf, W. Mäntele, *Anal. Bioanal. Chem.* **2012**, *403*, 391 – 399.
[7] A. Roth, Dissertation, Department of Physics, Johann Wolfgang Goethe-Universität Frankfurt am Main, Frankfurt am Main **2012**.
[8] G. Hosafci, O. Klein, G. Oremek, W. Mäntele, *Anal. Bioanal. Chem.* **2007**, *387*, 1815 – 1822.
[9] M. Brandstetter, A. Genner, K. Anic, B. Lendl, *Analyst* **2010**, *135*, 3260 – 3265.
[10] E. Diessel, P. Kamphaus, K. Grothe, R. Kurte, U. Damm, H. M. Heise, *Appl. Spectrosc.* **2005**, *59*, 442 – 451.
[11] G. Janatsch, J. D. Kruse-Jarres, R. Marbach, H. M. Heise, *Anal. Chem.* **1989**, *61*, 2016 – 2023.
[12] P. S. Jensen, J. Bak, S. Ladefoged, S. Andersson-Engels, *Spectrochim. Acta, Part A* **2004**, *60*, 899 – 905.
[13] H. M. Heise, R. Marbach, T. Koschinsky, F. A. Gries, *Appl. Spectrosc.* **1994**, *48*, 85 – 95.
[14] P. Bhandare, Y. Mendelson, R. A. Peura, G. Janatsch, J. D. Kruse-Jarres, R. Marbach, H. M. Heise, *Appl. Spectrosc.* **1993**, *47*, 1214 – 1221.
[15] G. Budinova, J. Salva, K. Volka, *Appl. Spectrosc.* **1997**, *51*, 631 – 635.
[16] R. Vonach, J. Buschmann, R. Falkowski, R. Schindler, B. Lendl, R. Kellner, *Appl. Spectrosc.* **1998**, *52*, 820 – 822.
[17] H. M. Heise, G. Voigt, P. Lampen, L. Küpper, S. Rudloff, G. Werner, *Appl. Spectrosc.* **2001**, *55*, 434 – 443.
[18] K. Z. Liu, R. A. Shaw, A. Man, T. C. Dembinski, H. Mantsch, *Clin. Chem.* **2002**, *48*, 499 – 506.
[19] D. Rohleder, G. Kocherscheidt, K. Gerber, W. Kiefer, W. Köhler, J. Möcks, W. Petrich, *J. Biomed. Opt.* **2005**, *10*, 031108.
[20] M. Pleitez, H. v. Lilienfeld-Toal, W. Mäntele, *Spectrochim. Acta, Part A* **2012**, *85*, 61 – 65.
[21] A. Hugi, R. Terazzi, Y. Bonetti, A. Wittmann, M. Fischer, M. Beck, J. Faist, E. Gini, *Appl. Phys. Lett.* **2009**, *95*, 061103.
[22] Daylight Solutions Inc., San Diego, CA (USA): Tunable Mid-IR External-cavity Pulsed Über Tuner™ Lasers. http://www.daylightsolutions.com/assets/003/5257.pdf (accessed 06/17/2012).
[23] Block Engineering Inc., Marlborough, MA (USA): LaserTune™ IR Source. http://www.blockeng.com/products/lasertune.pdf (accessed 06/17/2012).