Sonja Engelhard, Michael U. Kumke,
Hans-Gerd Löhmannsröben

OPQS – Optical Process and Quality Sensing. Exemplary applications in the beer brewing and polyurethane foaming processes

first published in:
Analytical and Bioanalytical Chemistry. - ISSN 1618-2650. -
384 (2006), 5, p. 1107 - 1112
doi: 10.1007/s00216-005-3364-4

Postprint published at the institutional repository of Potsdam University:
In: Postprints der Universität Potsdam :
Mathematisch-Naturwissenschaftliche Reihe ; 4
http://opus.kobv.de/ubp/volltexte/2007/1219/
http://nbn-resolving.de/urn:nbn:de:kobv:517-opus-12191

Postprints der Universität Potsdam
Mathematisch-Naturwissenschaftliche Reihe ; 4

OPQS – Optical Process and Quality Sensing. Exemplary applications in the beer brewing and polyurethane foaming processes

Sonja Engelhard, Michael U. Kumke, Hans-Gerd Löhmannsröben

University of Potsdam, Institute of Chemistry & Interdisciplinary Center of Photonics, Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm, Germany

Abstract

Optical methods play an important role in process analytical technologies (PAT). Four examples of optical process and quality sensing (OPQS) are presented, which are based on three important experimental techniques: NIR absorption, luminescence quenching, and, as novel method, photon density wave (PDW) spectroscopy. Addressed is the evaluation of four process and quality parameters eminent in the beer brewing and polyurethane (PU) foaming processes, namely the content of ethanol and oxygen (O_2) in beer, the determination of biomass, and the elucidation of cellular structures of PU foam from a pilot production plant.

Keywords: process analytical technology, beer, biomass, foam analysis, NIR spectroscopy, fluorescence quenching, photon density wave spectroscopy

Introduction

Process analytical technologies (PAT) and its sibling process analytical chemistry (PAC) play an ever increasing role in product-process optimisation strategies [1-3]. An augmented demand on effective product control calls for advanced analytical tools that allow real-time or short term monitoring and precise control of product quality along a production chain in factories and in the field. A recent report predicts that, partly due to the PAT initiative, the

market for process spectroscopic information will grow from 178 Mio$ in 2004 to 232 Mio$ in 2010, with an average annual growth rate of more than 5% [4]. In comparison to other analytical techniques, optical spectroscopy provides a wealth of inherent advantages, such as high sensitivity and selectivity, good temporal and spatial resolution, potential for remote, non-invasive sensing, etc. [5]. It is therefore obvious that optical methods constitute important elements of PAT. The three fundamental light-matter interaction mechanisms - absorption, scattering and luminescence - can be used in optical process and quality sensing (OPQS) to gain the desired information on parameters describing the quality and the quantity of a certain product or process. Presently, a major part of analytical methods for process control works off-line, which means that sampling has to be performed and a separate analysis is carried out in a laboratory. The contribution of on-line analysis in process control is growing, but only a very limited number of applications can be considered as fully in-line.

We are interested in OPQS in order to characterize key parameters of selected chemical and biotechnological properties and processes. OPQS activities are part of our efforts to bring advanced optical, often laser-based, techniques from the research environment into practise. Frequently, this is accomplished in a three-step procedure. First, laboratory investigations with well-known reference materials serve to establish and validate an optical method. Secondly, still in the laboratory, real-world samples are studied. Finally, measurement campaigns are conducted in production and process environments, such as a brewery, bio-production unit, soil remediation plant, etc.

In the present paper, four examples of OPQS will be presented, which reflect the fundamental light-matter interaction mechanisms, namely absorption, luminescence and scattering. Addressed are the determination of ethanol and oxygen (O_2) contents in beer, the measurement of biomass in a bioreactor, and the evaluation of cellular structures of

polyurethane (PU) foam from a pilot production plant [6-8]. For the latter two tasks, a novel spectroscopic technique was employed, namely photon density wave (PDW) spectroscopy [9]. Our other OPQS work, not reported here, relates to fibre-optical sensing (FOCS), e. g. for the detection of CO_2 and micro-bead probing of O_2 in living animal and plant cells [10-13], as well as to environmental monitoring. In the latter field, diffuse reflectance (DR) and laser-induced fluorescence (LIF) spectroscopy as well laser-based ion mobility (IM) spectrometry are employed to characterize the properties of soils and soil contaminations, [14-16], and tuneable diode laser (TDL) spectroscopy is used for isotope-selective sensing of soil-respired CO_2 [10,17].

Experimental

The methods and materials employed in this study have been described before so that here only reference to our earlier publications is given. The determination of O_2 and ethanol contents in beer by luminescence quenching and NIR absorption spectroscopy, respectively, has been addressed in [6, 8]. Principles and practice of PDW spectroscopy were outlined in [9], and the application of this method to biomass and PU foam monitoring were reported in [6, 7]. Experimental details can also be found in a recent dissertation [18].

Results and Discussion

Application of OPQS in beer brewing

With more than 100 Mio L produced alone in Germany, beer is one of the major beverage products in Europe, and effective methods for optimization and control of process parameters are indispensable. Different optical methods are of interest to monitor relevant parameters during the various stages of the brewing process. For fermentation, the content of biomass and ethanol are important quality parameters. At the end in the bottling process, the O_2 concentration is crucial because it affects the taste and the shelf lifetime of the beer. Near-

infrared (NIR) absorption spectroscopy can be used to monitor the ethanol content during the fermentation process, and luminescence quenching can be applied for O_2 monitoring in-line the bottling process.

Determination of ethanol in beer with NIR absorption spectroscopy

Standard methods for the determination of the ethanol content of beer are density measurements after distillation, combination of density and sound velocity measurements, gas chromatography, or methods based on enzymatically catalyzed reactions. All of these methods are time-consuming and expensive, because of the necessary elaborate sample preparation and the required highly trained laboratory staff. On the other hand, NIR absorption measurements can be performed without sample preparation for the determination of ethanol directly in whole beer. Our approach, based on "interpretive spectroscopy", [19] has the advantage of a strictly reduced set of detection wavelengths, namely only two, and a simple calibration procedure. This compares favourably to other spectroscopic approaches, which use extensive chemometric data treatments as well as multiple detection wavelengths.

The NIR absorption spectrum of beer is dominated by the absorbances of water, ethanol and carbohydrates. Thus, for ethanol quantification the NIR properties of these beer ingredients have to be considered. Here, maltose, a main building block of dextrines as most important class of sugars in beer, has been used as a model compound for carbohydrates in cross-sensitivity evaluations. The NIR absorption spectra of aqueous solutions of ethanol and maltose were recorded and difference spectra to pure water were calculated. Exemplary, in Figure 1 such difference absorption spectra of water/ethanol- and water/ethanol/maltose-mixtures (reference materials) and an entire beer sample (real-world material) are shown.

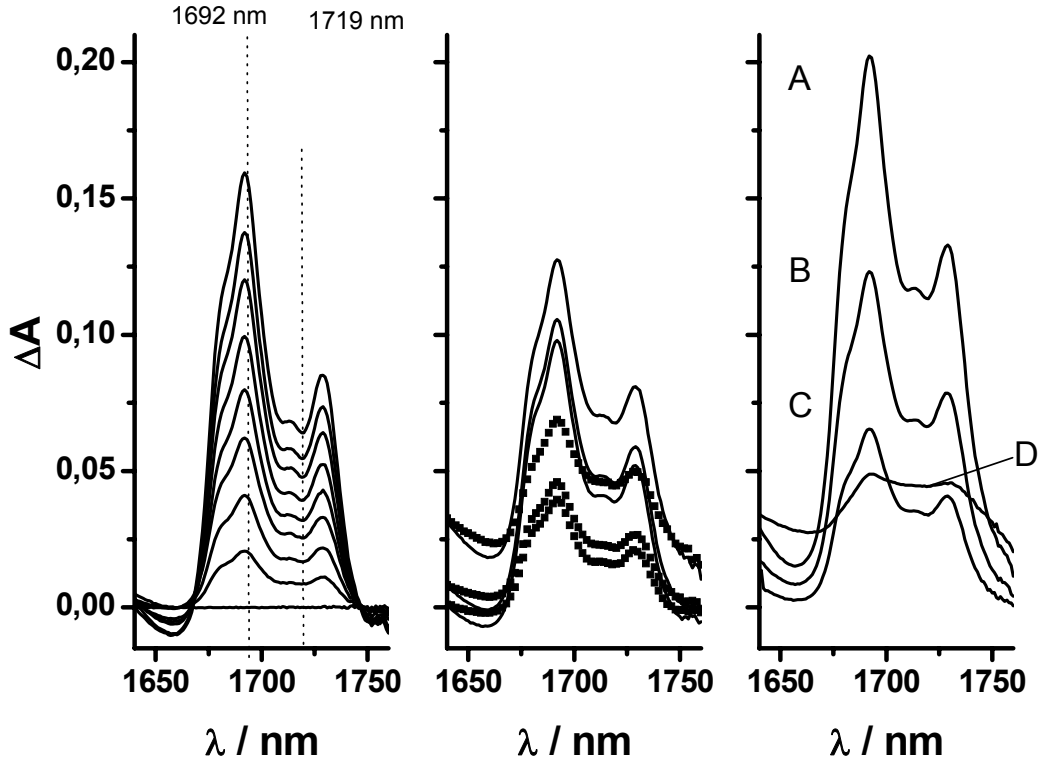

Figure 1: Difference absorption spectra of water ethanol–mixtures (left), water-ethanol-maltose mixtures (middle), and different types of beer (right). The spectra were recorded under ambient conditions at 293 K. The sample temperature was carefully controlled by a thermostat. The ethanol content was varied between 0 vol% < *c*(ethanol) <8 vol% with water as reference. The influence of maltose was checked for 0%, 1%, and 4% content and the difference absorption spectra are shown for two different ethanol concentrations 2 vol% (■) and 5 vol% . On the right side the difference absorption spectra of an "alcohol-free" beer (D) light beer (C, 2.5 vol%), a regular beer (B, 4.9 vol%), and a bock beer (A, 7.7 vol%) are compared.

On the basis of spectral evaluations, the difference in the absorbance of the first overtones of the CH-absorption bands at 1692 and 1719 nm ($\Delta A_{1692\text{-}1719}$) was identified to be best suited for the quantitative determination of ethanol in beer. At 1692 nm ethanol displays a significant absorption maximum, while maltose absorption is equal at 1692 and 1719 nm. From the difference $\Delta A_{1692\text{-}1719}$ the ethanol content of beer can thus best be determined independent from carbohydrate ingredients. To our knowledge, this is the first time that such an approach

has been undertaken. As real-world samples, 34 different beers were investigated. The ethanol content ranged from 0.4 vol% (light beers) up to 7.7 vol% (bock beers). The beer samples were analysed for their ethanol content using NIR interpretive spectroscopy as well as enzymatic analysis and oscillating tube densiometry (standard analytic procedures).

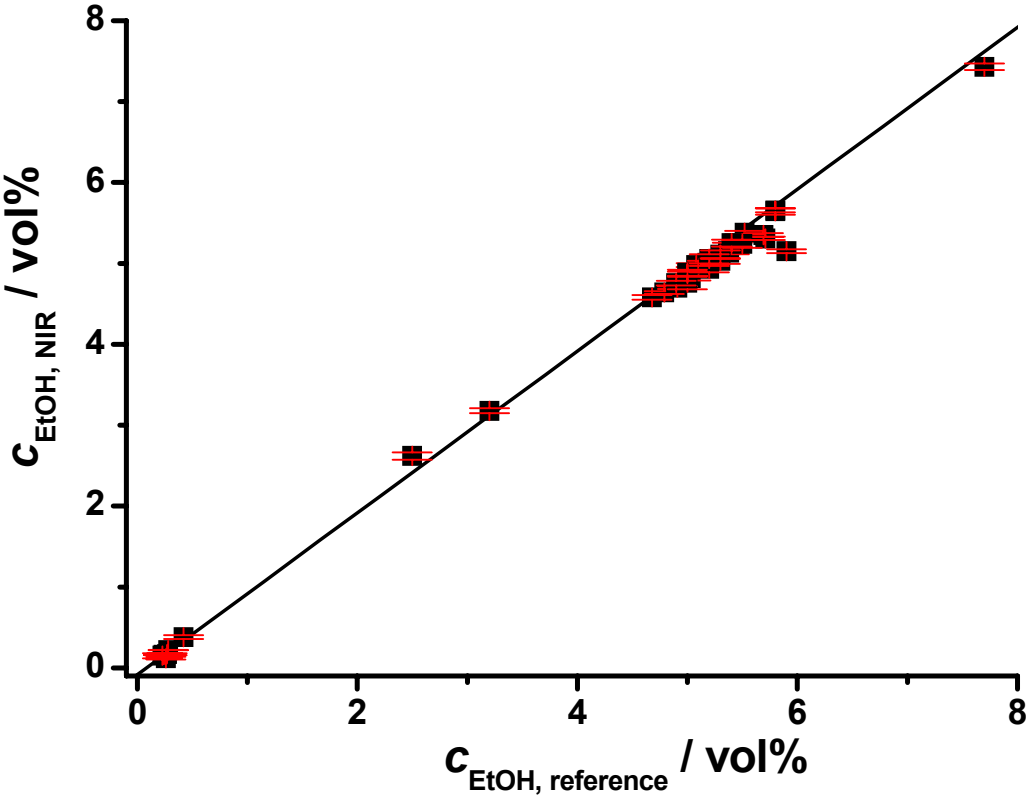

Figure 2: Comparison of the results of the determination of ethanol in beer using NIR difference absorption spectroscopy, $c_{EtOH,NIR}$, and standard analytical methods, $c_{EtOH,reference}$. Tested as real- world samples were 34 different commercial beers.

In Figure 2 the results of NIR and standard analytic procedures are compared. Obviously, the results are in an excellent agreement, which demonstrates the potential of NIR interpretive spectroscopy for ethanol determination directly in a broad range of different beers.

Due to the significant advances achieved, NIR spectroscopic ethanol analysis of beer and other beverages has within the last few years reached commercial realization. Several analysing systems are now available for in-line or in-bottle measurements, providing exactly the tremendous advantages of optical techniques outlined above [20]. It is expected that NIR analysers will gain considerable importance in quality control of beverages.

Determination of yeast cell density during beer fermentation

Another important quality parameter of the brewing process is the biomass concentration c_{bio} (e.g., expressed as mass/volume) or cell number density ρ_N. Different optical techniques are used for the monitoring of cell growth parameters during fermentation, e. g., standard turbidimetry or fluorescence spectrometry. Turbidity is determined by the attenuation of light, which is caused by combination of absorption and scattering processes. Extensive calibration models have to be used, if turbidimetry would be applied at high c_{bio} or ρ_N. The fluorescence of intrinsic microbial compounds, like NADH, is dependent on cell physiological conditions. Evaluation of fluorescence data for ρ_N determination has to be based on chemometric models. Both approaches thus require tedious, extensive dilution and calibration steps and are hardly suitable for in-line applications. A promising approach is to use light scattering for biomass determination during fermentation. A major point of concern in the application of light scattering methods is their dependence on changes of the optical properties of the medium, e. g., changes of the absorption coefficients of sample constituents. It is therefore necessary to separate absorption and scattering coefficients, which becomes possible with photon density wave (PDW) spectroscopy. In PDW spectroscopy, the temporal and spatial propagation of intensity-modulated light in multiply scattering media is evaluated. In the simplest approach, only modulation at a fixed frequency in the kHz regime is used. The corresponding inexpensive electronic equipment is suitable for routine process analysis. The time-dependent

part of the signal detected from the re-emitted light, I_{AC}, which is proportional to the photon density, is given by:

$$I_{AC} \propto \frac{1}{r} \cdot \exp\left(-r \cdot \sqrt{3 \cdot \mu_a \cdot \mu_s'}\right) \quad (1)$$

with absorption coefficient μ_a and reduced scattering coefficient μ_s'.

However, determination of scattering properties can only be performed if the absorption of the medium is known. This can be overcome with high-frequency, either fixed or scanning, PDW spectroscopy (typically in the 0.02 - 1 GHz regime) which enables evaluation of frequency-dependent intensity decrease and phase shift of re-emitted light. The work presented here, employing single frequency measurements with a modulation frequency of 105 MHz, is a compromise between the usage of routine and elaborate research equipment.

In our experimental set up two optical fibres, one to guide the intensity-modulated excitation light into the sample and the other, separated by distance *r*, to collect the re-emitted light and guide it to the detector, are used.

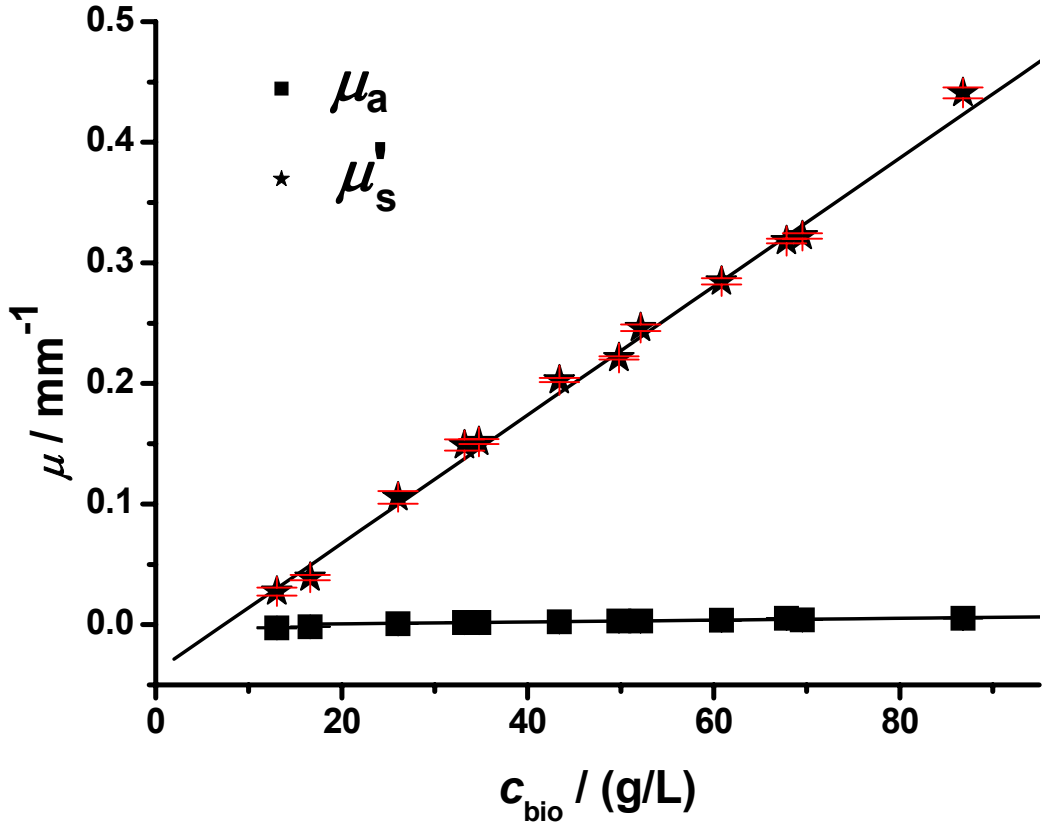

Figure 3: Absorption and effective scattering coefficients, μ_a and μ_s', vs. biomass concentration c_{bio} in a buffered brewer's yeast suspension in the absence of other beer ingredients. Results from PDW spectroscopy with diode laser excitation (λ_{ex} = 638 nm) and fixed modulation frequency (f_{mod} = 105 MHz).

Shown in Fig. 3 are the coefficients μ_a and μ_s' vs. biomass concentration c_{bio} in a buffered brewer's yeast suspension determined with PDW spectroscopy using 639 nm diode laser excitation light. It is obvious that at the wavelength chosen, μ_a is very small and only slightly dependent on the biomass content. In contrast, an excellent linear correlation between μ_s' and c_{bio} is found. This underlines that PDW spectroscopy can be readily applied for in-situ monitoring of the cell growth during beer fermentation by analysing the effective scattering coefficient μ_s' without dilution or sample preparation. No transfer to brewing facilities has

been made yet. Currently, we are preparing to integrate a PDW spectroscopic unit into larger-scale (25 – 100 L) photobioreactors for monitoring of the production of, e. g., phototropic green algae [21].

In-situ measurement of O_2 concentrations in beer bottles

In order to guarantee highest taste and quality standards, residual dissolved oxygen concentration, $c(O_2)$, in bottled beer should be lower than 0.1 mg/L. Standard procedures for the determination of oxygen in beer bottles are based on electrochemical methods and a sampling step is required before the measurement, which includes opening of the bottle and transferring the beer into a cell containing a Clark electrode. Both steps are labour intensive and a source for errors. A real on-line method that allows the determination of oxygen during the bottling process is highly wanted. Luminescence probes for the determination of gaseous and dissolved oxygen are readily available. The measurement principle is based on the dynamic luminescence quenching of a sensor dye by O_2. This dye is usually a transition metal complex embedded in a O_2-permeable polymer matrix. Frequently used transition metal complexes contain ruthenium or platinum as metals and porphyrin or other organic ligands [13,22]. The luminescence lifetime of these metal complexes used are in the order of μs and can be measured effectively using phase modulation detection schemes. Since the quenching mechanism is purely dynamic in nature, the decrease in luminescence decay time is directly related to the concentration of oxygen. The data can be evaluated according to the well-known Stern-Volmer equation for dynamic quenching:

$$\frac{\tau_0}{\tau} = 1 + K_{SV} \cdot c(O_2) \qquad (2)$$

(τ_0, τ, luminescence decay time in the absence and presence of O_2; Stern-Volmer constant K_{SV} = k_q*τ_0, with the bimolecular quenching rate constant k_q). It is noted that k_q usually is slightly

temperature dependent, so that temperature variations have to be accounted for in luminescence quenching measurements.

In principle, also determination of the luminescence intensity would be amenable for the determination of the oxygen concentration. Compared to time-resolved measurements, however, intensity measurements, particularly when performed in biologically active environments, are prone to experimental artefacts, such as fluctuations of lamp intensity, bleaching of the luminescence probe or fouling of the sensor material itself, and are therefore less preferable.

Suitable excitation sources are intensity modulated laser diodes or LEDs. The luminescence decay time is determined from the change in the phase angle θ and the demodulation of the luminescence relative to the excitation light. The luminescence decay time is give by

$$\tau = \frac{\tan\theta}{2 \cdot \pi \cdot f_{\mathrm{mod}}} \tag{3}$$

with the modulation frequency f_{mod}.

With equations (2) and (3), the oxygen concentration can be directly determined from the phase shift of the signal. For in-bottle measurements, modified beer bottles with sensor spots at the bottom were used (sensor bottles, cf. Fig. 4). The sensor spot, which contains the luminescence probe, can be accessed from outside the bottle by a simple optical Y-fibre, which is used to direct the excitation light to the sensor spot and the luminescence light to the detector. By variation of dissolved O_2 concentration, calibration of the sensor was performed with aqueous solutions of ethanol (5 vol%) as reference. A linear correlation between phase angle θ and oxygen concentration was found for the concentration interval 0 mg/L < $c(O_2)$ < 5 mg/L, which encompasses the targeted concentration range.

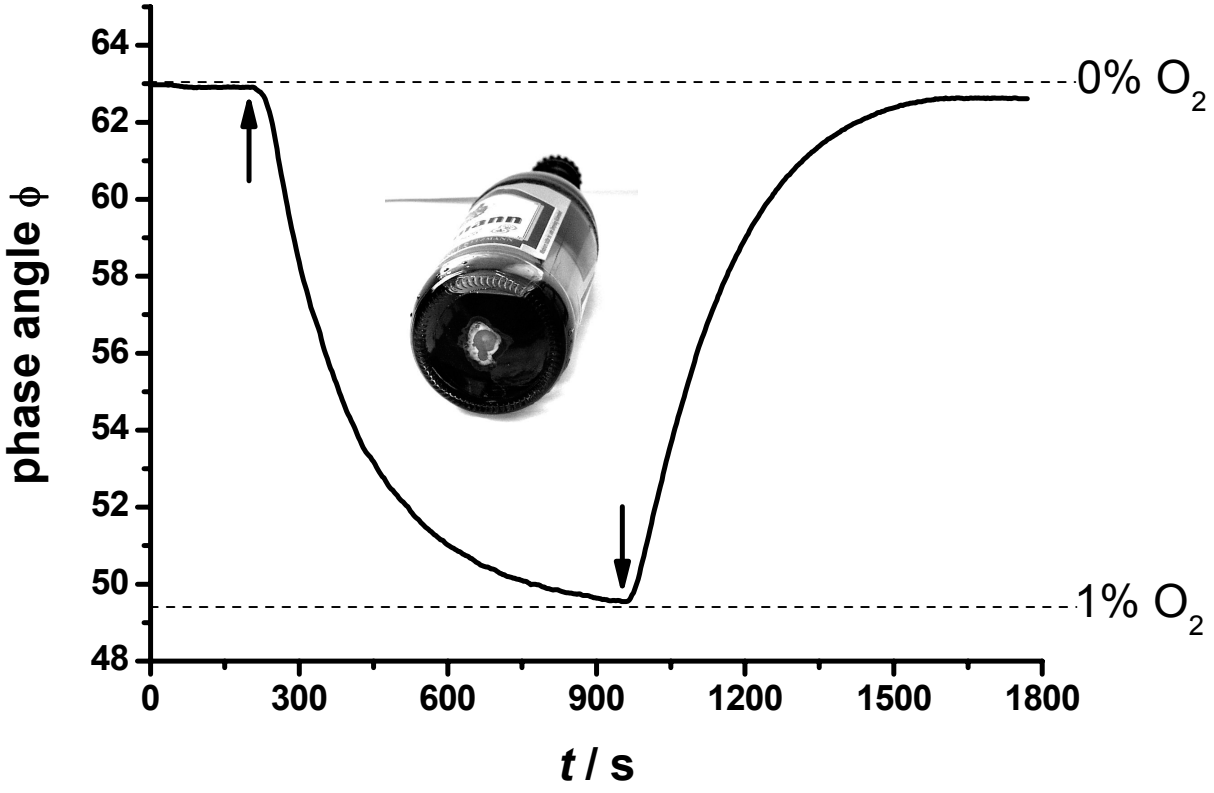

Figure 4: Temporal evolution of the O_2 content in an entire beer gassed with pure N_2 and a 1% O_2/N_2-mixture (inset: beer bottle with sensor spot). Shown is the dependence of the phase angle on the oxygen concentration. At the points marked with arrows the gas mixture was changed.

Shown in Fig. 4 are the laboratory results of phase angle measurements in an entire beer gassed with a 1% O_2/N_2-mixture. The good quality of the data and the reproducibility of the measurements are obvious. Such measurements were also performed in the brewery, were sensor bottles were filled and capped in the regular bottling plant. With the sensors employed, the overall response time of measurements in aqueous ethanol solutions was ca. 1 min, which increased, probably due to the presence of beer ingredients, to ca. 10 min in whole beer (cf. Fig. 4). Certainly, this can be improved by optimisation of the polymer matrix properties. On the other hand, the slow response is of no disadvantage if one wants to secure thermal equilibration of the sample.

Clearly, the main advantage of the luminescence quenching method is that it can be performed in-situ, as exemplified here with oxygen measurements in bottled beer. It is pointed out that luminescence based O_2 detection is increasingly used in other areas, such as in water industries, coastal technologies, ocean observation, etc. It is now commercially available for the control of oxygen concentration in food and beverages [23]. The optical technique successfully competes with traditional electrochemical detection schemes.

Evaluation of cellular structures of polyurethane (PU) foam

In our last example we discuss the application of PDW spectroscopy for the determination of structural parameters of PU foams. In particular, we were interested in the diameter of the foam cells that are formed during foaming. The cell sizes are an important parameter for mechanical and thermal foam properties. In standard procedures cell diameters are determined by counting the number of formed cells along a pre-defined section under a microscope. Recently, also x-ray tomography assisted methods have been used. However, these methods are very expensive and time-consuming and not suitable for in-line applications to control the foaming process.

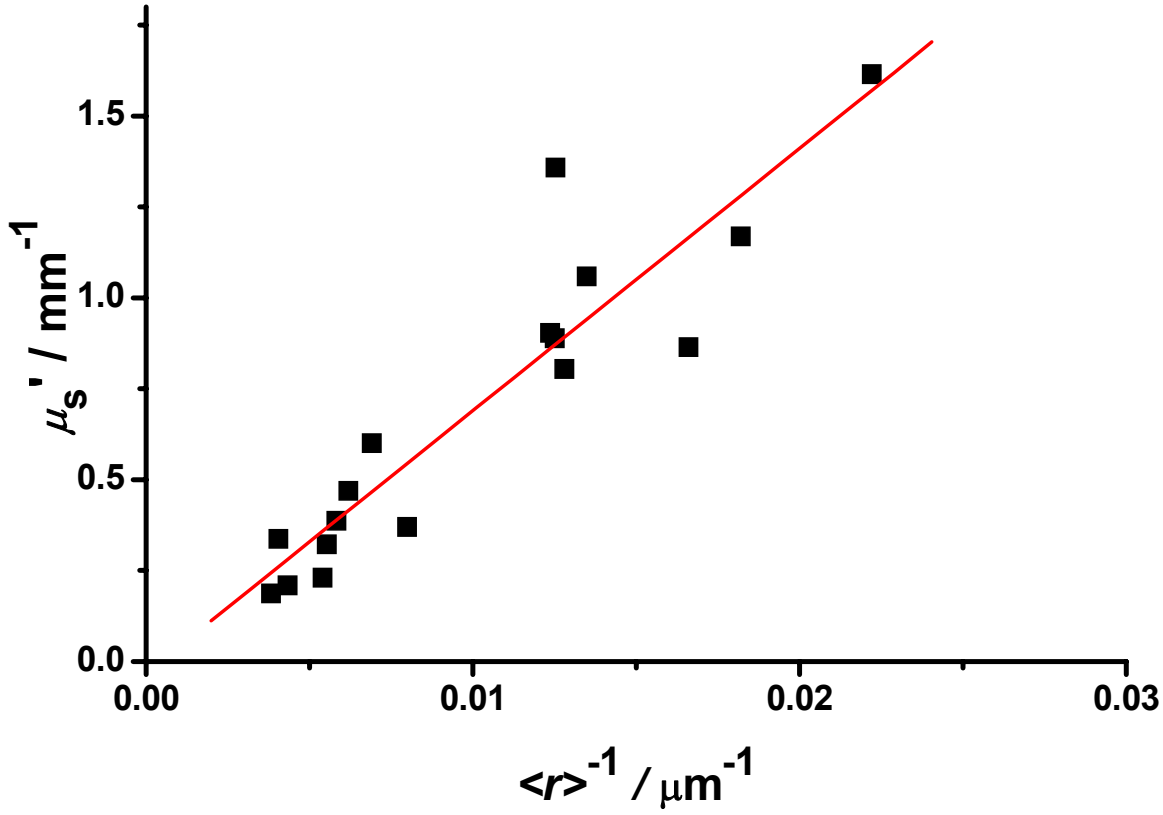

Figure 5: Effective scattering coefficients, μ_s', vs. the reciprocal average diameter, $\langle r \rangle^{-1}$ of PU foams. Results from PDW spectroscopy with diode laser excitation (λ_{ex} = 638 nm) and fixed modulation frequency (f_{mod} = 105 MHz).

The attenuation of light in PU foams was measured with PDW spectroscopy and the absorption and reduced scattering coefficient were determined. According to Mie theory, the effective scattering coefficient μ_s' is directly related to the size of the scatterers. For the limiting case of a monomodal size distribution of relatively large scatterers and in the simplest approach, a linear correlation between μ_s' and the inverse size is expected. However, for PU foams a broad distribution of cell diameters is characteristic, and μ_s' thus relates to an average cell diameter $\langle r \rangle$. In our experiments 20 different PU foams, production samples from the Bayer AG and considered as real-world materials, were investigated. The cell diameters were determined by the standard procedure based on counting and sizing of cells under a

microscope. A good correlation between μ_s' and the reciprocal average diameter, $\langle r \rangle^{-1}$, was found and is shown in Figure 5. From our results it is clear that the determination of μ_s' is an excellent alternative for the determination of cell diameters in foams. The effective scattering coefficients are obtained from non-invasive measurements in real-time. PDW spectroscopy has therefore high potential for process integration and monitoring of the foaming process itself.

Summary

The experimental techniques employed here, namely NIR absorption, luminescence quenching and PDW spectroscopy, have proven to be valuable tools for OPQS. It is of interest to note that, particularly in the context of PAT, these methods vary strongly in their maturity. NIR absorption spectroscopy is well-established in a variety of sectors including pharmaceutical and food industries. [24]. It appears that only during the last years NIR analysis of ethanol content in entire beers has made such progress that the technique is now marketed and finding its way into breweries. Luminescence quenching spectroscopy for O_2 determination has long been an experimental tool mainly in research environments. The method has great potential for in-vitro and in-vivo imaging of intracellular processes and is thus expected to be of great impact in the life sciences [12]. Lately, the luminescence quenching technique is being introduced, also on a commercial level, into processing facilities, e. g. in water industry [25]. For PDW spectroscopy the situation is somewhat different in that, aside from biomedical investigations, up to now only very few applications have been reported. However, the technique provides a number of unique opportunities, such as calibration-free separation of absorption and scattering processes, accessibility of strongly turbid media, etc., that makes it highly attractive in many fields of OPQS.

References

1. Workman J, Koch M, Veltkamp DJ (2003) Anal Chem 75: 2859 – 2876

2. Dünnebier G, Bamberg A (2004) Chem Ing Tech 76: 1467 - 1474

3. Kueppers S, Haider M (2003) Anal Bioanal Chem 376: 313 - 315

4. Process spectroscopy market (2005) Spectroscopy Europe 17: 6

5. Gurden SP, Westerhuis JA, Smilde AK (2002) Aiche J 48: 2283 - 2297

6. Engelhard S, Gehmlich K, Schael F, Löhmannsröben HG (2001) in: Sensorik, R. Erb, S. Heiden (eds), Spektrum Akademischer Verlag 43 – 46

7. Raffel B, Löhmannsröben HG, Schael F, Reich O, Engelhardt S (2002) Deutsche Patentanmeldung Nr. 10243665.7, 20.9.2002, Deutsches Patent- und Markenamt, München

8. Engelhard S, Löhmannsröben HG, Schael F (2004) Appl Spectroscopy 58: 1205 - 1209

9. Reich O, Löhmannsröben HG, Schael F (2003) Phys Chem Chem Phys 5: 5182 – 5187

10. Hörner G, Lau S, Löhmansröben HG (2004) Proc SPIE 5544: 47 – 54

11. Schmälzlin E, van Dongen JT, Klimant I, Marmodée B, Steup M, Fisahn J, Geigenberger P, Löhmannsröben HG (2005) Biophys J, submitted for publication

12. Monson E, Brasuel M, Philbert MA, Kopelman R (2003) In Biomedical Photonics Handbook, Vo-Dinh T (ed). CRC press, Boca Raton

13. Demas JN, DeGraff BA, Coleman PB (1999) Anal Chem News Feat 793A – 800A

14. Schultze RH, Lemke M, Löhmannsröben HG (2004) in: Laser in Environmental and Life Sciences, P. Hering et al. (eds), Springer Verlag 79 - 98

15. Beitz T, Laudien R, Löhmannsröben HG, Schultze RH (2004) Proc SPIE 5547: 16 – 24

16. Lemke M, Fernández-Trujillo R, Löhmannsröben HG (2005) Sensors 5: 61 – 69

17. Hörner G, Lau S, Kantor Z, Löhmansröben HG (2004) Analyst 129: 772 - 778

18. Engelhard S (2003) (PhD Thesis) Optische Verfahren zur Qualitätskontrolle von Bier und zur Zellzahlbestimmung in *S. Cerevisiae* Kultivierungen, University of Potsdam

19. Workman JJ (1996) Appl Spectrosc Rev 31: 251 - 320

20. See, for example, a) Anton Paar – Lab density & concentration meter / Alcohol Meters; online in Internet: http://www.anton-paar.com/density-meter/lab-density_cxsn-5nuflp.en.0.jsp [May 10th, 2005] b) Alcohol Analyser; online in Internet: http:// www.nirtech.zip.com.au/alcoholanalyser.htm [May 10th, 2005], c) McNab Food + Beverage Industry Systems – Overview, online in Internet: http:// www.themcnab.com/food_beverage.htm [May 10th, 2005], d) FOSS – Beer; online in Internet: http:// www.foss.dk/c/p/solutions/customersegments/Beer/default.asp [May 10th, 2005]

21. Puls O, Scheibenbogen K (1998) Adv Biochem Eng 59 : 123 – 152

22. Jorge PAS, Caldas P, Rosa CC, Oliva AG, Santos JL (2004) Sensors and Actuators B 103: 290 - 299

23. See, for example, a) PreSens GmbH – Precision Sensing; online in Internet: http:// www.presens.de/html/start.html [May 10th, 2005], b) Hac-Lange – United for Water Quality; online in Internet: http://shop.hach-lange.com/shop/action_q/news/news_id/100/lkz/II/spkz/en/TOKEN/KLUqbTzupU8HaeTIHp6D9VryRK0/M/SOMy8Q [May 10th, 2005], c) Fiber Optic Oxygen Sensors; online in Internet: http:// www.oceanoptics.com/products/foxysystem.asp [May 10th, 2005], d)Submersible sensors – Oxygen Optodes 3830/3975/3930 – Aanderaa Instruments; online in Internet: http://www.aanderaa.no/render.asp?ID=156&segment=46&session= [May 10th, 2005].

24. Blanco M, Villarroya I (2002) Trac-Trends Anal Chem 21:240 - 250

25. Meller G (2004) Intern Environ Technol 14: 18-19