

Sensitive Method for the Kinetic Measurement of Trace Species in Liquids Using Cavity Enhanced Absorption Spectroscopy with Broad Bandwidth Supercontinuum Radiation

Ssegawa-Ssekintu Kiwanuka,[†] Toni Laurila,^{*,†} and Clemens F. Kaminski^{*,†,‡}

Department of Chemical Engineering and Biotechnology, University of Cambridge, Pembroke Street, Cambridge CB2 3RA, U.K., and Friedrich Alexander University Erlangen-Nuremberg, School for Advanced Optical Technologies, Paul-Gordan-Str. 6, 91052 Erlangen, Germany

A novel spectrometer for the rapid and sensitive detection of liquid phase analytes at trace concentrations is presented. Broad bandwidth supercontinuum radiation was coupled into a linear optical cavity incorporating an intracavity liquid-sample cuvette. Cavity enhanced absorption spectra of trace species covering more than 300 nm were acquired on time scales of milliseconds. Single shot acquisition times of 10–50 ms are demonstrated here. The effective absorption path length exceeds 2 m in sample volumes measuring 2.7 mL. A key feature of the instrument is that it can be calibrated using cavity ring-down spectroscopy without the requirement of changing the optical alignment. The sensitivity of the instrument is exemplified by measurements of trace concentrations of dye molecules and nickel sulfate dissolved in water. A minimum detectable absorption coefficient of $9.1 \times 10^{-7} \text{ cm}^{-1} \text{ Hz}^{-1/2}$ at 550 nm was obtained. The capability to capture broad bandwidth absorption spectra on short time scales permits kinetic studies of liquid phase reactions. We demonstrate this by recording the oscillatory behavior of a Belousov–Zhabotinsky reaction.

Many chemical processes demand fast and accurate detection of low concentrations of liquid phase analytes. Biomedical applications, the detection of environmental pollutants and industrial process control all require chemical analysis, often at very high sensitivity. Absorption techniques are commonly used analytical tools in these fields as they are both quantitative and nonintrusive. The sensitivity of such techniques is relatively low compared to fluorescence based methods but can be enhanced by orders of magnitude through multiple pass configurations, for example, cavity ring-down spectroscopy (CRDS)¹ and cavity enhanced absorption spectroscopy (CEAS).² Diode lasers provide a convenient tunable source for CEAS and CRDS, but the bandwidth over

which they are tunable is often limited for use in gas phase sensing.³ For this reason, broader bandwidth sources such as light emitting diodes^{4,5} and filament lamps⁶ have been employed as excitation sources for broad bandwidth CEAS measurements in the liquid phase; however, because of their incoherent nature, it is difficult to efficiently couple their output into optical cavities. Supercontinuum (SC) radiation sources, on the other hand, emit an extremely broadband spectrum (>1000 nm) of high spatial coherence,⁷ permitting tight focusing, flexible beam control and thus many new modalities of optical sensing.^{8–10} Furthermore, they permit efficient coupling into cavities.¹¹ The technique, supercontinuum cavity enhanced absorption spectroscopy (SC-CEAS), has been successfully demonstrated in the gas phase.^{11,12} For liquid phase measurements a variant of the technique has been demonstrated, which employs evanescent field absorption through the use of a total internal reflection prism placed in a folded cavity geometry.^{13,14} In the present work, supercontinuum radiation was used for the first time to detect chemical species in the liquid phase using a linear optical cavity. The system is simple to implement and robust, and calibration of the path length can be easily performed through measurement of the cavity ring-down time with a blank sample inserted into the cavity (e.g., pure solvent). The paper demonstrates the performance of the tech-

* To whom correspondence can be addressed. E-mail: tkk25@cam.ac.uk (T.L.); cfk23@cam.ac.uk (C.F.K.). Phone: +44 (0)1223 (3)30133. Fax: +44 (0)1223 334796.

[†] University of Cambridge.

[‡] Friedrich Alexander University Erlangen-Nuremberg.

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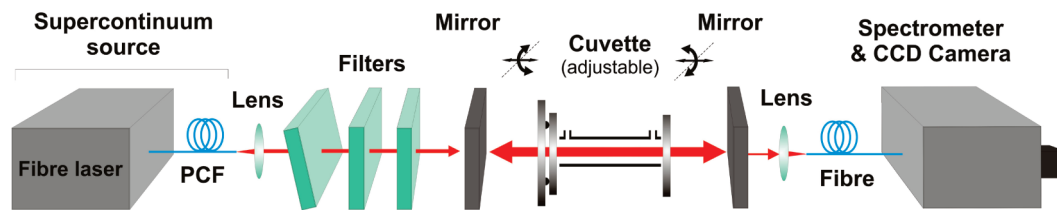


Figure 1. Experimental SC-CEAS setup for liquid phase studies. A supercontinuum radiation source consisting of a fiber laser and photonic crystal fiber (PCF) is coupled into the optical cavity formed by a pair of plano-concave mirrors. The liquid phase sample (2.7 mL) is contained in the 5.4 cm long intracavity cuvette. The spectrum of the transmitted light is detected by a grating spectrometer and CCD camera.

nique through spectrally resolved measurements of an oscillatory Belousov–Zhabotinsky (BZ) reaction as well as trace measurements of heavy metal and dye species in water.

EXPERIMENTAL SECTION

The experimental setup is shown in Figure 1. Output from a fiber laser (Fianium Femto Power-1060, 5 ps pulse width) operating at 1 MHz repetition rate was launched into a photonic crystal fiber (Crystal Fiber SC 5.0-1040 PCF) to generate supercontinuum (SC) light with a spectral bandwidth ranging from around 480 to 1800 nm. The average spectral power density was about $25 \mu\text{W}/\text{nm}$.¹⁵ The infrared portion of the emission was cut out with a short-pass filter (Comar 730 IK 25), and the visible light was subsequently coupled into a 30 cm long linear optical cavity which was formed by a pair of plano-concave cavity mirrors (Layertec, 0.5 m radius of curvature, 99.0% nominal reflectivity from 400 to 680 nm). The liquid sample was contained in a custom-designed 5.4 cm long intracavity cuvette. The cuvette features two cell windows and a plexi-glass cylinder. The windows are finely adjustable to ensure perpendicular alignment with respect to the supercontinuum light beam. The 2.7 mL cuvette contains an inlet and outlet allowing rapid sample exchange or measurement of flowing samples. The cuvette can be disassembled for cleaning without affecting the alignment of the cavity mirrors. The details of the cavity are discussed in the Supporting Information. The spectrum of the light transmitted through the cavity was recorded using a grating spectrometer (Andor Shamrock SR-303-i, 50 lines/mm grating) equipped with a cooled CCD camera (Andor iXon DV887ECS-BC, 512×512 pixels).

In CEAS the intensity of the transmitted light is recorded in the absence (I_0) and presence (I) of the sample from which the absorption coefficient α (cm^{-1}) can be calculated. At the low loss and high reflectivity limit, the absorption coefficient is given by⁶

$$\alpha(\lambda) = \left(\frac{I_0(\lambda)}{I(\lambda)} - 1 \right) \frac{1 - R(\lambda)}{d} \quad (1)$$

where d is the single-pass absorption length in the liquid sample (here $d = 5.4$ cm), I is the transmitted intensity in the presence of a sample (analyte and solvent), I_0 is the intensity in the absence of the sample (pure solvent), R is the effective mirror reflectivity, and λ is the wavelength. Compared to a single-pass absorption measurement, the path length, and thus sensitivity, is increased by a factor of $1/(1 - R)$.

Calibration. The calibration of the absorption length over broad wavelength ranges can be done by using known concentra-

tions of broadband absorbers, but this is restricted by the availability of such calibration species in some spectral regions of interest. An alternative method is to directly measure the reflectivity at a single wavelength or in a narrow wavelength range, e.g., using cavity ring-down spectroscopy (CRDS), and then the use of published data on the spectral response of the mirrors and solvent to extend the calibration over the required wavelength range. Furthermore, recent supercontinuum based approaches allow for the direct measurement of cavity reflectivity over broad spectral bandwidths.^{16–18}

In this work the pulsed SC source permits convenient calibration of the effective absorption path length in the liquid sample using CRDS. The same light source can be used for calibration without altering the experimental alignment. The photon residence time in the cavity, i.e., the effective absorption length divided by the speed of light, can be determined using CRDS over a narrow spectral range. This calibration can be extended to cover larger bandwidth by using the mirror reflectivity and solvent absorption data. With the 99.0% reflective cavity mirrors and the cuvette filled with ultrapure water, a 2.50 ± 0.02 m absorption length at 550 nm was obtained. The use of much higher mirror reflectivity mirrors, such as those typically employed in gas phase measurements, where reflectivity values of 99.995% and higher are common,¹¹ would not yield better sensitivity here because the intracavity losses are dominated by the solvent absorption and scattering.¹⁹ Furthermore, such high reflectivities are not available in the visible range over the extreme bandwidth we require here. The optimum mirror reflectivity for given intracavity losses can be determined

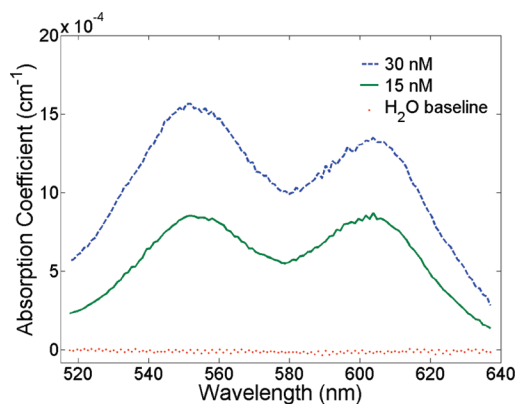


Figure 2. Absorption spectra of Alexa Fluor 633 Hydrazide in $18 \text{ M}\Omega \text{ cm}^{-1}$ ultrapure water at 15 and 30 nM concentrations. The spectra were acquired using 50 ms single-shot exposure time. Averages of 20 single-shot spectra are shown here corresponding to 1 s total acquisition time. The spectral resolution of the spectrometer was 1.1 nm.

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Table 1. Comparison of Liquid Phase Absorption Techniques Using Linear Optical Cavities^a

| | ref | method | R | L_{abs} (cm) | λ (nm) | t_{meas} (s) | α_{min} (cm ⁻¹) | $\alpha_{\text{min},\Delta\nu}$ (cm ⁻¹ Hz ^{-1/2}) |
|----|--------------------------------------|--------|---------|-----------------------|----------------|-----------------------|---|--|
| 1 | this work | CEAS | 0.99 | 5.4 | 400–700 | 10.0×10^{-3} | 9.1×10^{-6} | 9.1×10^{-7} |
| 2 | Alexander ²² | CRDS | 0.9998 | 2.3×10^{-3} | 628 | | 5.4×10^{-3} | |
| 3 | Bahnev et al. ²³ | CRDS | 0.9998 | 0.2 | 532 | | 1.6×10^{-4} | |
| 4 | Fiedler et al. ¹⁹ | CEAS | 0.99 | 1 | 607 | 5 | 2×10^{-5} | 4.5×10^{-5} |
| 5 | Hallock et al. ²⁴ | CRDS | 0.9998 | 21 | 620–670 | | 1×10^{-6} | |
| 6 | Hallock et al. ²⁵ | CRDS | 0.9998 | 23 | 655 | | 3.3×10^{-7} | |
| 7 | McGarvey et al. ²⁶ | CEAS | 0.99998 | 0.175 | 783 | 1×10^{-3} | 7×10^{-6} | 2.2×10^{-7} |
| 8 | Seetohul et al. ²⁷ | CEAS | 0.999 | 20 | 630–670 | 2.5 | 2.8×10^{-7} | 4.4×10^{-7} |
| 9 | van der Sneppen et al. ¹⁴ | CRDS | 0.99996 | 0.2 | 532 | | 1.0×10^{-5} | |
| 10 | Xu et al. ²⁸ | CRDS | 0.9997 | 1.0 | 607 | | 1.0×10^{-5} | |

^a The table shows the cavity enhanced method used: reflectivity of cavity mirrors R , single-pass sample absorption length L_{abs} , measurement wavelength range λ , total measurement time t_{meas} , minimum detectable absorption coefficient α_{min} , and the minimum detectable absorption coefficient normalised by the measurement bandwidth $\alpha_{\text{min},\Delta\nu}$.

from eq 6 of ref 19. More details on the calibration are discussed in the Supporting Information.

RESULTS AND DISCUSSION

Two dye molecules, Alexa Fluor 633 Hydrazide (Invitrogen) and Rhodamine 6G (Rh6G, Sigma Aldrich), which are commonly employed in antibody labeling and cell tracing, for example, were used to characterize the performance of the spectrometer. Figure 2 shows absorption spectra of Alexa Fluor dissolved in ultrapure (18 M Ω cm⁻¹) water at 15 and 30 nM (nanomolar (nM); 10⁻⁹ mol L⁻¹) concentrations. A linear response ($R^2 = 0.9982$) was obtained for Rh6G concentrations ranging from 25 to 200 nM with an estimated detection limit (3σ) of 200 ± 70 pM corresponding to a minimum detectable absorption coefficient of 9.1×10^{-7} cm⁻¹ Hz^{-1/2} for a 10 ms signal integration time on the CCD camera. This sensitivity is comparable with the best reported liquid-phase absorption results obtained but with the valuable advantage of broad spectral coverage (see Table 1). The broadband nature of SC-CEAS permits the deconvolution of complex, overlapping spectra from which the components of complex mixtures can be recovered. Furthermore, as the analyte is not in contact with the cavity mirrors, day-to-day operation and maintenance of the spectrometer is greatly simplified. Thus no mirror cleaning nor realignment is required upon sample exchange. Furthermore, the liquid handling cuvette is made of low cost and disposable components thus avoiding any risk of sample cross contamination between measurements. The required sample volume (2.7 mL) is small and could be reduced to less than 0.5 mL without loss in sensitivity. Further details of cavity and cuvette design are included in the Supporting Information.

Broadband CEAS permits dynamic measurements of chemical reactions. Using evanescent field absorption in a folded cavity arrangement, we have in the past performed broadband SC-CEAS of electrochemical reactions at repetition rates up to 600 Hz (10 μ s single-shot exposure time).¹⁴ Here we demonstrate the capability of the simple linear-cavity absorption technique to monitor the kinetics of complex reactions by time-resolved measurements of the Belousov–Zhabotinsky reaction.²⁰ The BZ reaction has been called a “chemical clock reaction” exhibiting periodic oscillations which can be monitored in time.²¹ The reaction studied here involves the reduction of cerium(IV) ions to cerium(III) by malonic acid and the oxidation using bromate(V) ions back to cerium(IV). The Ce⁴⁺ oxidation state is yellow in color, whereas Ce³⁺ is colorless, therefore oscillations in color occur which correlate with Ce⁴⁺ concentration.

The oxidation step of the reaction involves bromomalonic acid, an autocatalytic intermediate, which is critical to the self-propagating oscillations. For spectroscopic analysis of the reaction, ferroin indicator is used as it exhibits a stronger color change from red to blue upon oxidation in the reaction step (further information in the Supporting Information, in particular see eq S2).

In this work, the BZ reaction was monitored over a period of 6 min using 50 ms exposure time per spectral reading. Figure 3 shows 1000 single-shot spectra ranging from 400 to 620 nm, acquired at 2.75 Hz repetition rate. Higher acquisition rates are clearly possible at 50 ms exposure time but not required here as the oscillation period of the reaction was measured to be 44 s (see Figure 3). The changes in spectra permit the relative proportions of ferroin in its unoxidized (red) and oxidized form (blue) to be detected at any time point during the reaction. This is a clear advantage of the multiplexing capability of the technique. The ultimate acquisition speed is limited by the shot to shot intensity variation of the SC pulses. The extreme nonlinearity of SC generation processes causes significant shot-to-shot spectral and intensity variations. However, in a previous publication we showed the effect of spectral averaging on the measurement precision of absorption spectra.¹⁵ In gas phase measurements, an average of 120 independent spectra gave a measurement precision of 2.6%, corresponding to an averaging time of just over 1 ms. In this work we have averaged up to 50 individual spectra. The long-

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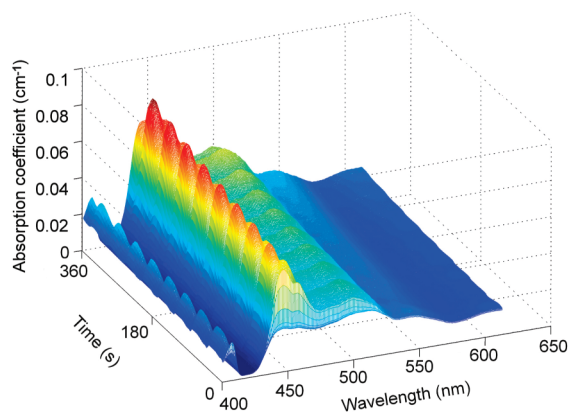


Figure 3. Oscillating Belousov–Zhabotinsky reaction recorded using SC-CEAS. The periodic transition between Fe(II) and Fe(III) oxidation states is easily observed in a closed (unstirred) cuvette. Spectra (1000 single-shot) with 250 nm spectral bandwidth were acquired at 2.75 Hz rate using 50 ms exposure time. The period of oscillation of the medium is 44 s and is clearly visible. Initial concentrations in 1 M H₂SO₄ solvent were potassium bromate = 0.17 M, potassium bromide = 0.26 M, malonic acid = 0.83 M, ceric ammonium nitrate = 0.008 M, ferroin indicator = 1.0 M.

term drifts, on the other hand, were insignificant for averaging times of up to around 100 s.

The high sensitivity of the technique makes it useful for measurements of toxic compounds at trace concentrations. Many heavy metal compounds are currently difficult to detect and require specialized and expensive equipment that may be difficult to calibrate. An example is nickel sulfate (NiSO₄, Sigma Aldrich) which is generally considered to be toxic to humans at concentrations higher than 5 mg/L.²⁹ In order to detect such low concentrations, techniques such as atomic absorption spectrophotometry or gravimetric methods are usually employed.³⁰ We demonstrate that SC-CEAS offers an efficient alternative for its measurement. Figure 4 shows the absorption spectra of 10 and 20 mg/L concentrations of NiSO₄ acquired using SC-CEAS. The water baseline is also shown in Figure 4 from which a limit of detection of 0.5 ± 0.2 mg/L for NiSO₄ was estimated.

CONCLUSIONS

A broadband spectrometer using a supercontinuum source and cavity enhanced absorption spectroscopy has been developed and applied to liquid phase measurements. Detection sensitivities at picomolar levels were obtained in the visible range for representative absorbers with signal integration times in the lower millisecond range. Liquid samples can easily and quickly be ex-

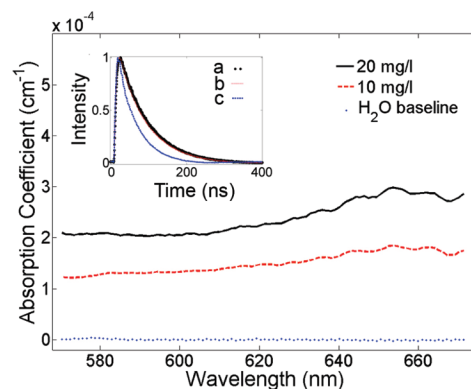


Figure 4. Absorption spectrum of 10 and 20 mg/L nickel sulfate in 18 MΩ cm⁻¹ ultrapure water. The single-shot acquisition time was 50 ms and the spectrum shown above is an average of 50 individual single-shot spectra corresponding to 2.5 s total measurement time, with a 1.1 nm spectrometer resolution. Inset: Ring-down traces recorded using a 70 nm bandpass filter centered at 550 nm and shown here with normalized intensity. The ring-down times are used to calibrate the absorption length. (a) Ring-down trace of the cavity formed by the two 99.0% reflective plano-concave mirrors alone; (b) ring-down trace of the cavity with two cell windows (i.e., no liquid sample in the cuvette) in place; (c) ring-down trace with ultrapure water (solvent) in the cuvette.

changed during measurements for fast, convenient analysis of multiple samples or coupling to complex fluid handling systems. The setup is straightforward and allows for high sensitivity and rapid acquisition of broad bandwidth spectra, from which components in mixtures can be identified and conveniently quantified via the in-built calibration capability.

The sensitivity of the technique could be further improved by optimizing the length of the intracavity cuvette. Reducing the internal diameter of the cuvette would enable smaller sample volumes. The availability of more affordable and compact SC sources, based on microchip pump laser³¹ and conventional fiber technology,³² for example, can lead to cost-effective, versatile, and highly sensitive instruments.

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SUPPORTING INFORMATION AVAILABLE

Cavity and cuvette design; calibration details, cavity ringdown spectroscopy; and Belousov–Zhabotinsky reaction. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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