Liquid-phase broadband cavity enhanced absorption spectroscopy (BBCEAS) studies in a 20 cm cell

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Sensitive liquid-phase measurements have been made in a 20 cm cell using broadband cavity enhanced absorption spectroscopy (BBCEAS). The cavity was formed by two high reflectivity mirrors which were in direct contact with the liquid-phase analytes. Careful choice of solvent was required to minimise the effect of background solvent absorptions. Measurements were made on the broad absorber Sudan black, dissolved in acetonitrile, using a white LED light source and $R \geq 0.99$ cavity mirrors, leading to a cavity enhancement factor (CEF) of 82 at 584 nm. The sensitivity as measured by the minimum detectable change in the absorption coefficient (α_{min}) was 3.4×10^{-7} cm^{-1}. Further measurements were made on the strong absorber methylene blue dissolved in acetonitrile at 655 nm. A white LED was used with the $R \geq 0.99$ cavity mirrors, leading to a CEF of 78 and $\alpha_{min} = 4.4 \times 10^{-7}$ cm^{-1}. The use of a more intense red LED also allowed measurements with higher reflectivity $R \geq 0.999$ cavity mirrors, leading to a CEF of 429 and $\alpha_{min} = 2.8 \times 10^{-7}$ cm^{-1}. The sensitivity was limited by dark noise from the detector but nevertheless appears to represent the most sensitive liquid-phase absorption measurement to date.

Introduction

The quest for more sensitive absorption-based measurements has made great progress through the introduction of, initially, cavity ring down spectroscopy CRDS[1] and, more recently, cavity enhanced absorption spectroscopy CEAS[2,3] techniques. We would also like to highlight an early cavity-based liquid-phase study which appears to have been missed by most reviews of cavity-based techniques.[4] These have led to large increases in the effective path length of measurement, such that detection of certain species at the sub part-per-trillion (ppt) level is now possible.[5,6] Most of the cavity studies have reported measurements on gas-phase species as the scattering and absorption losses are significantly lower than measurements for liquid-phase species and thus a greater number of passes through the sample can be achieved. In principle, however, there are far more species of interest for study in the liquid phase.

Traditionally, absorption-based measurements in the liquid phase have been considered a very useful but relatively insensitive technique with limits of detection (LOD) in the nanomolar range for strongly absorbing species and standard experimental setups. Higher sensitivity absorption measurements have been recently achieved by increasing the path length of measurement through total internal reflection in liquid core waveguides (LCWs).[7,8] Typical path lengths of up to 5 m have been demonstrated. Liquid-phase measurements in the pico- to femto-molar range usually require the use of fluorescence-based techniques, which often are not applicable to many common analytes.

In the liquid phase, the number of cavity-based studies reported to date is fairly small. These include the CRDS-based

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measurements of Hallock et al.[9,10] Their first study[9] was made in a 21 cm cell, where the cavity mirrors were in direct contact with the liquid-phase analytes. They noted that careful choice of solvent had to be made as many of the common ones showed strong absorptions in the 620–670 nm region. Spectrophotometric grade cyclohexane, hexane, toluene and acetonitrile were identified as suitable solvents. Some very general measurements of sensitivity and LODs were given. In a later study, Hallock et al.[10] reported kinetic measurements on methylene blue in a 23 cm cavity formed by two mirrors, again in direct contact with the liquid-phase analyte. A simpler experimental setup was used, based on continuous wave (cw)-CRDS.

The more recent studies of Bahnev et al.[11] and van der Sneppen et al.[12,13] are all related to the application of CRDS to HPLC detection and have consequently used a much shorter 2 mm base path length cell.

Only one previous study where the liquid-phase analyte was in direct contact with the cavity mirrors has used CEAS. McGarvey et al.[14] reported CEAS measurements on the biomolecule bacteriochlorophyll a, made at 783 nm using high reflectivity mirrors in direct contact with the liquid sample in a 1.75 mm cavity.

CRDS requires the measurement of ring down times typically on the high nanosecond to microsecond timescale and hence need appropriate fast detection systems. These, along with the common use of pulsed laser systems, lead to CRDS setups being relatively expensive and experimentally complex. However, the technique provides a means for directly measuring the absorption coefficient (α) of an analyte. In contrast, CEAS requires the measurement of the time-integrated intensity output and thus slower and less expensive detection schemes can be used. The measurement of the absorption coefficient of an analyte does, however, require the reflectivity of the mirrors to be known, or more typically a separate calibration to be performed. The

experimental difficulty in using CRDS increases when experimental schemes employ short cavity lengths such as those used by van der Sneppen *et al.*[12,13] as these lead to shorter ring down times, which are worsened by the inherent high losses present in liquid-phase studies. These limit the range of analyte concentrations which can be studied before the ring down time becomes too short to measure.

Our recent liquid-phase measurements in a 2 mm cuvette,[15] and a 1 cm HPLC cell,[16] both using broadband CEAS (BBCEAS), have demonstrated sensitive measurements using a low-cost experimental setup and a simple experimental methodology. A natural extension to these studies is the use of a longer 20 cm path length cell where the mirrors are in direct contact with the liquid-phase analyte used. In principle this should allow more sensitive measurements to be made due to the longer base path length. Our previous measurements suffered from rather high cavity losses due to the use of a cuvette in the cavity, leading to only small improvements in the number of passes when higher reflectivity mirrors were used. The lack of additional optical components in the cavity should lead to lower cavity losses and a greater number of passes with higher reflectivity mirrors. An additional motivation was to investigate which wavelength regions were most suitable for study in common solvents.

Experimental setup and measurement procedures

A schematic of the experimental setup is shown in Fig. 1. The output from a high intensity 1 W LED (Luxeon O star, white or red) was collimated into a 20 cm liquid-tight cavity formed by two high reflectivity mirrors which were in direct contact with the liquid-phase analytes. The mirrors were mounted into a custom mirror holder unit which allowed simple mirror attachment and removal. Perfluoro polymer O rings (Kalrez) were used to form a liquid-tight seal. The holder unit was joined to the main body of the cavity by stainless steel bellows. Three micrometer screws attached to the bellows allowed fine adjustment of each mirror

during cavity alignment. The body of the cavity was constructed from 2 mm thick stainless steel tubing which had an internal diameter of $\sim$25 mm. The volume of the cavity was 98 cm^3. Stoppered ports on the top and bottom of the stainless steel tubing allowed the cavity to be filled and emptied. Some of the experiments required the use of $R \geq 0.99$ mirrors, 25 mm diameter, radius of curvature (roc) = -50 cm, with a bandwidth $\approx$ 420–670 nm (Layertec, Germany). Other experiments utilised higher reflectivity $R \geq 0.999$ mirrors, 25 mm diameter, roc = -1.5 m (Laseroptik, Germany). These had a narrower bandwidth of $\sim$100 nm and covered the range 600–700 nm. The mirrors were cleaned daily before the start of experiments using the drag drop method to remove any residue present from the contact with liquid-phase analytes. For measurements with the $R \geq 0.999$ mirrors, a red Luxeon O star 1 W LED was used, as the intensity from the white LED at the detector was insufficient for use with this mirror reflectivity combination. The light exiting the cavity was focussed into a 600 μm diameter, 1 m length fibre optic cable (Thorlabs, UK) which was connected to a compact CCD spectrograph (Avantes AVS2000, The Netherlands). The spectrometer operated over the range 200–850 nm with a spectral resolution of $\sim$1.5 nm. The lack of thermoelectric cooling of the CCD sensor resulted in relatively high levels of dark noise and restricted the use of long integration times. The maximum integration time that could be used with acceptable noise was $\sim$3 s.

The typical alignment procedure for the cavity was through the iterative adjustments of the front and back mirrors to maximise the amount of light that leaks out, hence maximising the output of the LED reaching the spectrograph at a given integration time. The 20 cm cell was then filled with a blank solvent solution (typically acetonitrile). This resulted in a large decrease in the intensity of light reaching the spectrograph due to absorption and scattering by the solvent. The integration time was increased appropriately to ensure that the signal from the LED was significantly above the dark noise level, but not high enough to saturate the detector. Typical integration times for the $R \geq 0.99$ mirror set with the white LED were $\sim$30 ms, whilst
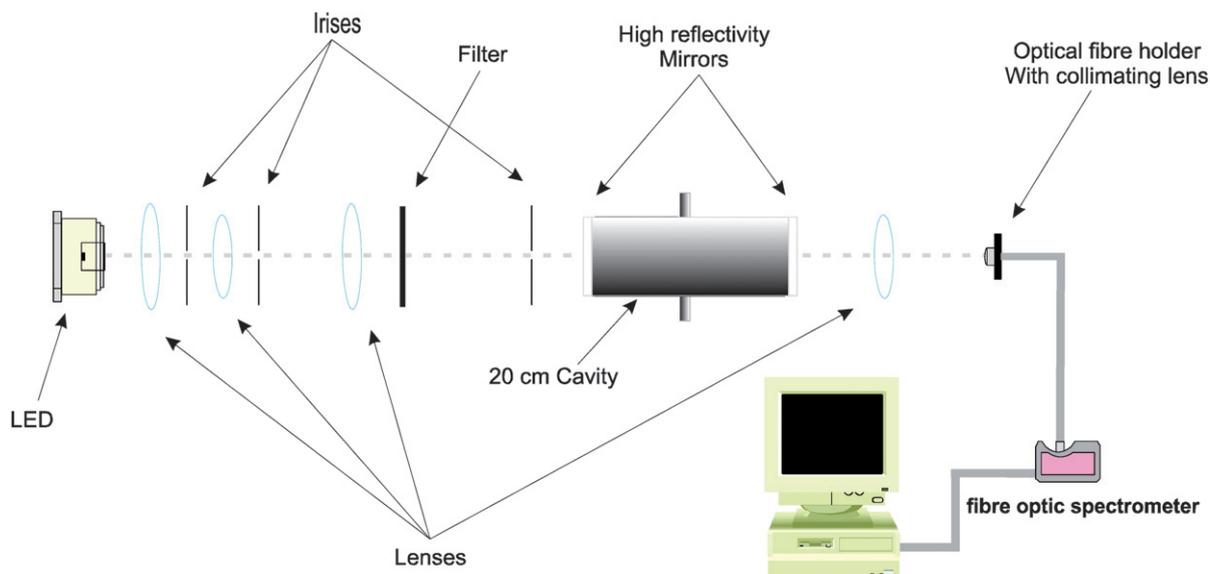

Fig. 1 Schematic of the experimental setup for liquid-phase BBCEAS measurements in a 20 cm cell.

those with the $R \geq 0.999$ mirrors and the red LED needed longer integration times of $\sim$2.5 s. The front and back cavity mirrors were then further adjusted iteratively to maximise the output reaching the detector, before a background spectrum was recorded. One hundred microliters of a known concentration of the analyte under study were added to the solvent using a Gilson pipette and typically allowed to mix for 10 min before a sample spectrum was recorded. The spectrometer was connected to a personal computer *via* a USB cable and spectral data were recorded using the Avasoft program.

Experimental methodology

As noted earlier, one of the main disadvantages of CEAS-based techniques is that unlike CRDS experiments the absorption coefficient (α) cannot be directly calculated and instead must be obtained through a separate calibration. For the liquid-phase BBCEAS experiments reported in this study the calibration and the experimental methodology could be performed in a straightforward manner. The calibration could be used to obtain in the first instance the cavity enhancement factor (CEF) or the number of passes made within the cavity. Once the effective path length had been calculated, the sensitivity from the minimum detectable change in the absorption coefficient α_{min} could also be calculated.

The first step in the calibration was to obtain a single pass spectrum of the analyte to be studied. This could be performed in a standard 1 cm path length cuvette with a tungsten halogen light source and the same Avantes AVS2000 spectrometer. The concentration of the solution was typically a factor of 2000 higher than in the BBCEAS experiments. An absorption spectrum was obtained by recording a background spectrum with just the solvent in the cuvette, I_0, followed by the sample spectrum, I, and then the calculation of the absorbance from ABS = $\log_{10}(I_0/I)$. The Beer–Lambert law was then used to scale the peak of the absorption spectrum to the concentration used in the cavity and also the 20 cm cavity path length. This gave the single pass absorbance under cavity conditions ABS_{sp}. The cavity enhanced absorption spectrum was obtained by first recording a background solvent only spectrum in the 20 cm cavity. A sample spectrum was then recorded and the absorbance spectrum calculated. The value of the cavity enhanced absorbance at the peak wavelength of the single pass spectrum was measured. This gave the cavity absorbance ABS_{cav}. The CEF, at this peak wavelength, could be calculated as the ratio of ABS_{cav} to ABS_{sp}. The CEF value at any particular wavelength in the experimental range could likewise be calculated from the cavity absorbance $ABS_{cav}(\lambda)$ divided by the single pass absorbance at cavity concentrations $ABS_{sp}(\lambda)$. The value of α_{min} could be calculated by dividing 2.303 times the one standard deviation (1σ) absorbance noise on a given spectrum, by the effective path length (l_{eff} = CEF $\times$ 20 cm).

The limit of detection (LOD) for an analyte could be calculated in two ways. The usual method for analytical studies is to perform an error-weighted linear regression through a plot of absorbance *versus* injected concentration, where three separate measurements are made for each concentration. The LOD is then calculated from dividing the 3σ value of the intercept by the molar extinction coefficient for the analyte (ε). An alternative 'spectral method' makes use of the 3σ noise on the baseline of an absorption spectrum and thus the LOD could be calculated from dividing $3\alpha_{min}$ by 2.303ε.

Some new experimental challenges arose as a result of the 100-fold increase in the base path length from our previous measurements in a 2 mm cuvette. The background absorption from the solvents used, at certain wavelengths, was far more significant than in the 2 mm cuvette. These were due to high CH and OH overtone vibrations found in most common solvents. Single pass absorption measurements on a range of common solvents in a 10 cm path length cell were recorded with a commercial double beam spectrometer (Jasco V630), between 500 and 700 nm, at 1.5 nm resolution, to identify the location of these overtone vibrations. The reference background was air. These spectra are shown in Fig. 2. Although the absolute values of the absorbances were small in the 10 cm cell, the use of the double beam spectrometer allowed the absorption features to be clearly seen. Water had the broadest absorption, presumably due to the 4th overtone of the OH stretch. The other solvents displayed clear absorption features above $\sim$610 nm, due to the 5th overtone of the CH stretch. It was even possible to see the absorption due to the weaker 6th overtone of the CH stretch at $\sim$550 nm. The position of the peaks was consistent with the liquid-phase CH overtone spectra of similar organic compounds recently measured by thermal lens spectroscopy.[17] The presence of the absorption features meant that the maximum number of passes at these wavelengths would be greatly reduced, especially for higher reflectivity mirrors. Consequently, additional preliminary cavity measurements were performed on the staining dye Sudan black with the $R \geq 0.99$ mirror set and spectrophotometric grade hexane, acetonitrile, diethyl ether and ethanol, in an attempt to identify the most suitable solvent. These absorption spectra are shown in Fig. 3. It can be seen by comparing with the single pass spectrum of Sudan black, that significant spectral distortion occurs at $\sim$620 nm and 550 nm as a result of the 5th and 6th overtone CH vibration limiting the cavity enhancement at these wavelengths. Additional distortions arose from the ripples in reflectivity profile of the $R \geq 0.99$ mirror set.
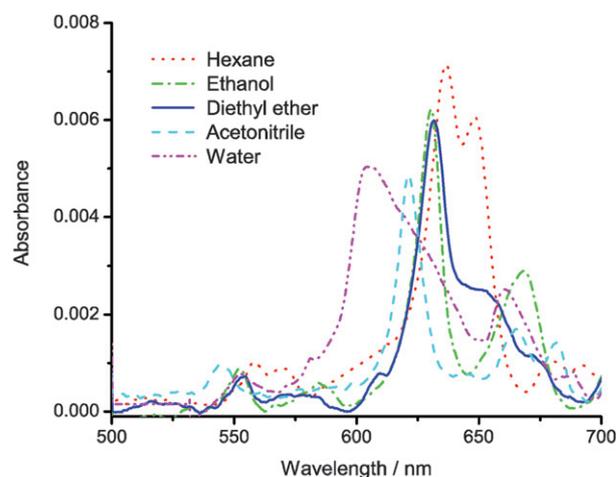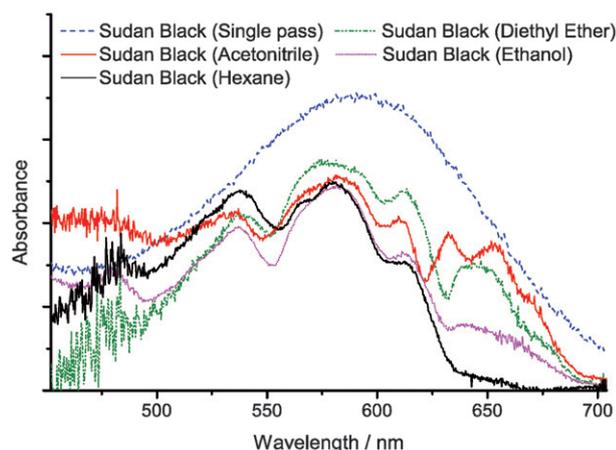

Fig. 2 Single pass absorbance *versus* wavelength spectra of hexane, acetonitrile, diethyl ether, ethanol and water, recorded in a 10 cm path length cell with a double beam spectrometer.

Fig. 3 Scaled absorption spectra of Sudan black dissolved in acetonitrile, hexane, diethyl ether and ethanol, recorded in the 20 cm cavity with the $R \geq 0.99$ mirror set.

By comparing with a single pass spectrum of a known concentration of Sudan black, the CEF as a function of wavelength could be calculated for each solvent between 450 and 670 nm. It was found that acetonitrile (spectrophotometric grade, Fisher Scientific, UK) offered the highest CEF value, which is the greatest transparency over the largest wavelength range with the $R \geq 0.99$ mirrors, and consequently it was chosen as the solvent for subsequent measurements. Use of diethyl ether, which had similar transmission properties, was ruled out as a result of its high volatility, which caused condensation of the solvent on the external face of the cavity mirrors. Fig. 4 shows a plot of the CEF as a function of wavelength recorded with Sudan black dissolved in acetonitrile in the 20 cm cavity. Also shown is the CEF plot obtained from earlier experiments on Sudan black dissolved in hexane in a 2 mm cuvette.[15] The variation of the CEF values in the 2 mm cuvette studies are essentially due to variations in the reflectivity profile of the $R \geq 0.99$ mirrors, whilst the variation in CEF values in the 20 cm cavity
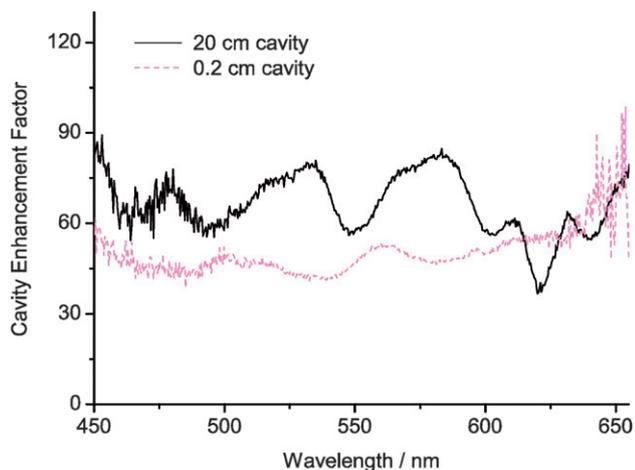
Fig. 4 CEF as a function of wavelength in a 20 cm cavity (Sudan black dissolved in acetonitrile) and a 2 mm cuvette (Sudan black dissolved in hexane).

also contain the effect of the overtone solvent absorptions which significantly reduce the CEF value at particular wavelengths.

Choice of analytes

The two analytes chosen for study were the dye molecules Sudan black and methylene blue. Sudan black had already been studied in the 2 mm cuvette and was chosen as it had a very broad absorption spectrum covering most of the visible spectrum and thus could be used to demonstrate the wavelength coverage of BBCEAS in the 20 cm cell. The second analyte needed to have a high molar extinction coefficient so that the measured LOD would be as low as possible; also its peak wavelength of absorption needed to coincide with the output of the red LED, so that it could be studied with the higher reflectivity $R \geq 0.999$ mirror set. After some experimentation with a number of strongly absorbing dyes, methylene blue was identified as the most suitable candidate for study as it was sufficiently soluble in acetonitrile and also its peak absorption wavelength of 655 nm was far enough removed from the background solvent absorption peak of acetonitrile.

Results

Liquid-phase BBCEAS measurements have been carried out in a 20 cm cavity for two different analytes dissolved in acetonitrile, at the peak absorption wavelength, using appropriate LEDs and high reflectivity mirror sets.

Table 1 summarises the measurements made in terms of the analyte studied, the reflectivity of the mirror set used, the CEF or number of passes obtained for each analyte, the wavelength of measurement, the calculated α_{min} values for each measurement, the concentration of sample used for the calculation of CEF and α_{min}, an estimation of the LOD for each analyte, obtained by two independent means, namely a spectral method (LOD_s) and a regression method (LOD_r), and finally the molar extinction coefficient, ε, of the analyte at the wavelength of measurement.
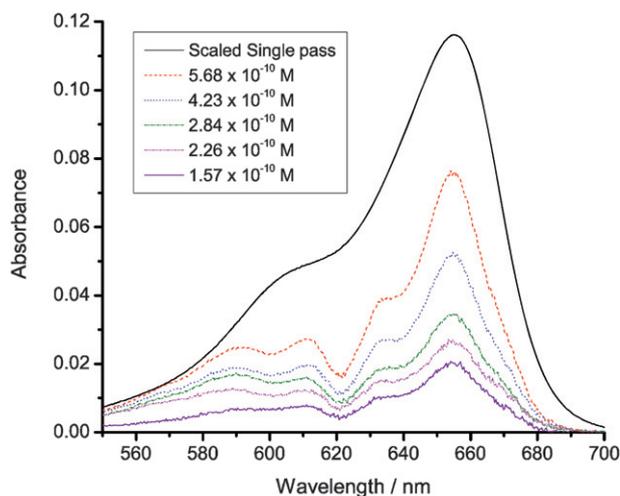
Methylene blue

Fig. 5 shows representative absorption spectra recorded over a ~150 nm wavelength range in a single measurement for methylene blue using the white LED and the $R \geq 0.99$ mirror set. When compared to the scaled single pass measurement, the absorption effect due to the solvent can be observed at ~620 nm.

Fig. 6 shows plots of absorbance *versus* concentration for methylene blue, obtained with the white LED and the $R \geq 0.99$ mirror set. The methylene blue measurements were made at 655 nm and a range of concentrations from ~0.1 nM to ~6 nM. Three replicate measurements were made at each concentration and the error bars for each concentration represent the standard deviation of the measurements. The plot can be broken into two parts. The data in the inset show measurements in the range ~0.1 nM to ~1.5 nM, which show a linear dependence of the absorbance on the concentration. The measurements at higher concentrations, up to ~6 nM are non-linear.

Fig. 7 shows representative absorption spectra recorded over a ~40 nm wavelength range in a single measurement for methylene blue using the red LED and the $R \geq 0.999$ mirror set. The transmission through the cavity was much lower than with the

Table 1 Summary of the results obtained in terms of analyte, the reflectivity of the mirrors, the CEF value, the wavelength of measurement λ, the minimum detectable change in absorption α_{min}, the concentration of sample used for the calculation of CEF and α_{min}, the LOD of the analyte calculated by both the spectral method (LOD_s) and the regression method (LOD_r) and the molar extinction coefficient ε at the wavelength of measurement

| Analyte	$R \geq$	CEF	λ/nm	α_{min}/cm^{-1}	Conc./M	LOD_s/M	LOD_r/M	ε/M^{-1} cm^{-1}
Methylene blue	0.99	78	655	4.4×10^{-7}	4.2×10^{-10}	7.1×10^{-12}	3.2×10^{-11}	7.9×10^4
Methylene blue	0.999	429	655	2.8×10^{-7}	6.0×10^{-10}	4.6×10^{-12}	1.2×10^{-11}	7.9×10^4
Sudan black	0.99	82	584	3.4×10^{-7}	1.1×10^{-9}	1.8×10^{-11}	1.6×10^{-10}	2.5×10^4

Fig. 5 BBCEAS absorbance *versus* wavelength spectra of methylene blue in acetonitrile in the range 550–700 nm, and concentrations between 1.57 and 5.68 × 10^{-10} M, obtained with the white LED and the $R \geq 0.99$ mirror set. A scaled single pass spectrum of methylene blue is also shown.
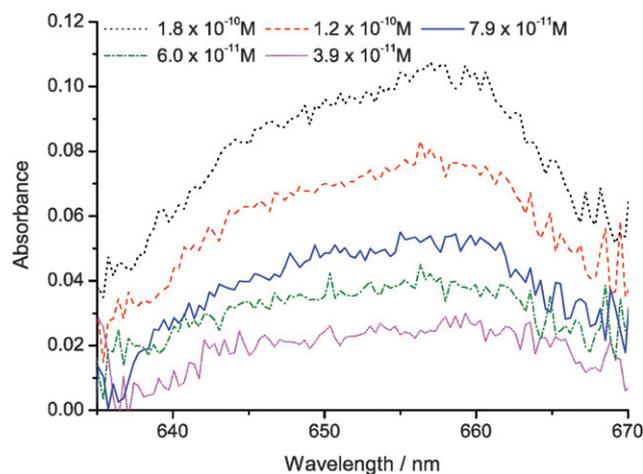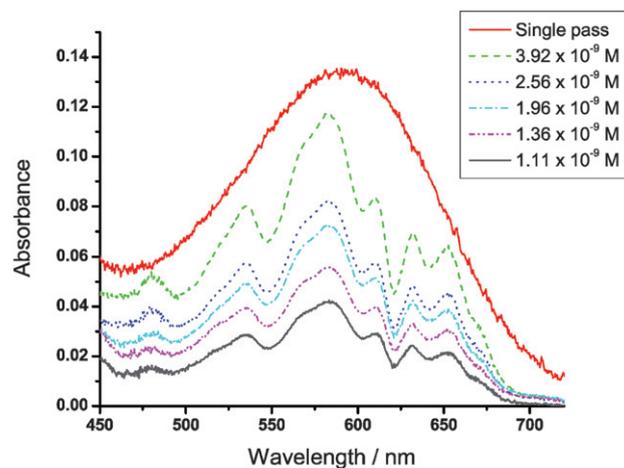

Fig. 7 BBCEAS absorbance *versus* wavelength spectra of methylene blue in acetonitrile, in the range 630–670 nm, and concentrations between 3.9 × 10^{-11} M and 1.8 × 10^{-10} M, obtained with the red LED and the $R \geq$ 0.999 mirror set.
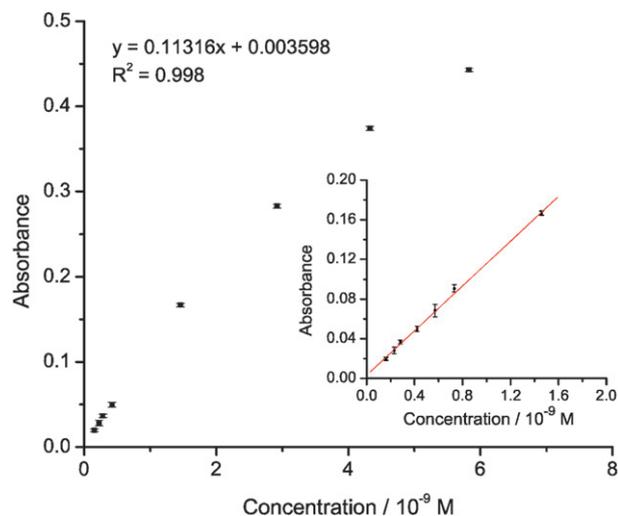
Fig. 6 Absorbance *versus* concentration plot for methylene blue at 655 nm, in the range ~0.1 nM to ~6 nM, obtained using the white LED and the $R \geq 0.99$ mirror set. The inset shows the measurements in the linear range from ~0.1 nM to ~1.5 nM. The error bars represent the 1σ error limit of three replicate measurements at each concentration. The equation on the diagram refers to an error-weighted linear fit to the measurements shown in the inset.

$R \geq 0.99$ mirrors, and consequently the use of much longer integration times (~2.5 s) resulted in the visible effects of dark noise on these absorption spectra.

Fig. 8 BBCEAS absorbance *versus* wavelength spectra of Sudan black in acetonitrile in the range 450–700 nm, and concentrations between 1.11 × 10^{-9} M and 3.92 × 10^{-9} M, obtained with the white LED and the $R \geq$ 0.99 mirror set. A scaled single pass spectrum of Sudan black is also shown.

Sudan black

Fig. 8 shows representative absorption spectra recorded over a ~250 nm wavelength range in a single measurement for Sudan black using the white LED and the $R \geq 0.99$ mirror set. When compared to the scaled single pass measurement, background

absorption due to the solvent can be observed at ~550 nm, ~620 nm, and ~490 nm whilst the other ripples were caused by the variation in the reflectivity profile of the $R \geq 0.99$ mirror set.

Discussion

Results have been obtained for liquid-phase BBCEAS measurements in a 20 cm cavity with the analytes methylene blue and Sudan black dissolved in acetonitrile. This represents the first time that BBCEAS has been applied to liquid-phase analytes in such a long base path length cavity. The main figures of merit obtained were values of the CEF, the α_{min}, and also the LODs by two independent methods. These are discussed after initial comments on some of the experimental challenges presented by this study and the general applicability of the technique. This is followed by a comparison with previous cavity studies in which the mirrors were in direct contact with the liquid-phase analytes, and finally some suggestions as to how the experimental setup could be improved are made.

The motivation for making this study arose from trying to make ultra-sensitive absorption measurements on liquid-phase analytes using BBCEAS by using a long base path length cell. Experimental challenges arose from solvent absorptions at selected wavelengths. Hallock et al.[9] had hinted at the problems raised by the choice of solvent and had made suggestions for measurements in the 620–670 nm range, but no information was given for measurements at shorter wavelengths. Our preliminary studies with a range of solvents showed that absorptions due to high overtone CH and OH stretches either precluded their use in measurements in the 600–650 nm range or introduced restrictions for the wavelength range in which the maximum number of passes could be achieved. The long effective path length of ~16 m with the $R \geq 0.99$ mirror set also accentuated differences in the transmission of the solvents at other wavelengths. It is recommended to use the highest purity spectrophotometric grade solvent that can be afforded. Acetonitrile which was the solvent chosen for use in this study had significant absorptions at ~620 nm and ~550 nm due to the 5th and 6th CH overtones. The widths of the absorptions were ~20 nm and ~30 nm respectively. This represents ~20% of the visible spectrum (400–700 nm) where it will be difficult to obtain a large number of passes. The higher CH overtones (e.g. the 7th overtone at ~490 nm) should get progressively weaker but will have a greater effect if higher reflectivity mirrors are used. The applicability of this technique is consequently limited for those analytes where the peak absorption coincides with the CH overtones. However, most liquid-phase analytes which absorb in the visible have broad absorption spectra with peak widths >50 nm, and even if the BBCEAS spectra are distorted by solvent absorptions the technique can still be usefully applied.

The CEF values obtained with the $R \geq 0.99$ mirror set for methylene blue (CEF = 78) and Sudan black (CEF = 82) are approximately the same. The small difference is most probably due to the variation in reflectivity profile of the mirror set, as a function of wavelength. It should be noted that the CEF values are calculated for the absorption maximum of an analyte in a given solvent. The CEF will be different at other wavelengths due to the wavelength reflectivity profile of the mirrors and solvent absorptions. A change of solvent will also affect the reflectivity profile due to a change in refractive index. Higher analyte concentrations will also reduce the CEF value. Comparing these values with those obtained using the 2 mm cuvette,[15] one can see that the best value obtained in that study (CEF = 52, with the $R \geq 0.99$ mirror set) is significantly lower, which is good evidence to show that, as expected, the background cavity losses are considerably reduced in this study. This is further confirmed when comparing the CEF values obtained using the higher reflectivity $R \geq 0.999$ mirror set. For the 2 mm cuvette study the highest CEF value was only 104, that is, only a two-fold increase in the number of passes on increasing the mirror reflectivity ten-fold, which was attributed to the limiting interface losses from the cuvette. For the present study, measurements with the $R \geq 0.999$ mirror set were made on methylene blue and a CEF value of 429 was obtained. This is a ~5.5-fold increase in the number of passes on increasing the mirror reflectivity ten-fold. However, it is still lower than what would be expected from gas-phase measurements, for which the number of passes is given by $(1 - R)^{-1}$. That is, $R \geq 0.999$ mirrors should result in ≥1000 passes. This, however, assumes that background scattering and absorption losses are insignificant; also, the stated reflectivity of mirrors is quoted for contact with air. When the mirrors are in contact with a liquid the actual reflectivity can decrease significantly, especially for higher reflectivity mirrors.

The sensitivity of the BBCEAS measurements as determined by the α_{min} value has been calculated for all the measurements in this study. For the experiments on methylene blue and Sudan black with the $R \geq 0.99$ mirror set, values of 4.4×10^{-7} and 3.4×10^{-7} cm^{-1} have been obtained. The difference in the values arises from the fact that the spectral intensity of the white LED used for these measurements was significantly higher at 584 nm (the peak absorption wavelength for Sudan black) than at 655 nm (the peak absorption wavelength for methylene blue). Consequently, shorter integration times could be used for the Sudan black measurements, which resulted in lower levels of dark noise from the CCD detector and thus a lower α_{min} value. Comparing these values with those obtained from the 2 mm cuvette,[15] it can be seen that the current values for the $R \geq 0.99$ mirror set are approximately 100-fold better, which is what one might expect given that the base path length is 100 times longer, and the CEF values are slightly higher. However, the lower background losses in the 20 cm cavity allowed these values to be obtained with a white LED, whilst the best values with the 2 mm cuvette required the use of a higher intensity red LED. If the best values with the white LED in the 2 mm cuvette are compared, then the present values are approximately 500 times better. The differences increase to a factor of approximately 1400, when the measurements with the $R \geq 0.999$ mirror set and the red LED are compared. The greater losses in the 2 mm cuvette setup resulted in far worse α_{min} values than obtained with the $R \geq 0.99$ mirror set, whilst in the 20 cm cell the lower cavity losses allowed a ~5.5-fold increase in the number of passes, which compensated for the increased noise from a longer integration time and actually resulted in a small improvement in the α_{min} value compared to the $R \geq 0.99$ mirror set.

The LOD for all the analytes has been calculated by two independent methods. The spectral method used the α_{min} value calculated from the 10 successive absorption spectra, whilst the

regression method obtained the LOD from the error limit on the intercept of a plot of absorbance *versus* concentration, and knowledge of the effective path length of measurement and molar extinction coefficient of the analyte. It is clear that the LOD values obtained by the regression method are substantially higher than those obtained by the spectral method. On further investigation it appeared that the replicate measurements introduced a new source of error due to the mechanical stability of the optical cavity. Emptying and refilling the cavity resulted in small changes in the cavity alignment leading to small changes in the measured absorbance. It was not possible to adjust the cavity mirrors to get exactly the same cavity alignment for each replicate measurement and thus this variation becomes the limiting factor in obtaining the LOD by the regression method. We would suggest that the LODs obtained by the spectral method are a fairer indication of what is achievable with the BBCEAS setup.

The linear dynamic range for the BBCEAS measurements was nearly two orders of magnitude for the experiments carried out using the $R \geq 0.99$ mirror set. For larger absorbance values, the response became non-linear as shown in Fig. 6, but in principle could still be quantified using a calibration curve. The non-linear behaviour at higher concentrations was attributed to the increasing absorbance of the analyte, resulting in a lower number of passes and subsequently a reduction in the effective path length.

Table 2 summarises some of the figures of merit from this study such as the lowest value of α_{min} obtained, the lowest LOD obtained, and the molar absorption coefficient for the particular analyte. These are compared with corresponding data where available, from the small number of reported previous liquid-phase cavity studies where the analyte was in direct contact with the cavity mirrors. Comparing with the results from this study, it can be observed that the mirror reflectivities used in the previous studies were generally much higher. The best figures of merit for this study were obtained with the $R \geq 0.999$ mirror set but the values obtained with the $R \geq 0.99$ mirror set were only slightly worse. The lowest α_{min} value obtained in this study is 2.8×10^{-7} cm^{-1} which is the lowest reported value to date for a liquid-phase measurement, making these the most sensitive liquid-phase absorption measurements reported. It should be pointed out that α_{min} is directly proportional to the base path length, and at 20 cm, the base path length used in this study, is amongst the longest used to date. It is most similar to those used in previous studies by Hallock *et al.*[9,10] and it is with these studies that closest comparison should be made. The first study by Hallock *et al.*[9] made CRDS measurements in a 21 cm base path length cell. A wide range of analytes were studied but firm figures, such as the range of concentrations used, were only given for copper acetate dissolved in acetonitrile. The sensitivity of the experimental setup was determined from a calibration plot of absorbance *versus* concentration and also considering the standard deviation in the ring down time as a function of time in a sample of pure acetonitrile. This led to a value of $\alpha_{min} = 1 \times 10^{-6}$ cm^{-1}, which is about four times higher than our best value and was obtained using a far more expensive experimental setup and considerably more complex experimental methodology.

The second study by Hallock *et al.*[10] used a simpler experimental setup based on cw-CRDS in a 23 cm cavity, using a red laser diode. It was mainly concerned with demonstrating ultra-trace kinetic measurements on methylene blue, but the sensitivity of the technique was stated to be $\sim 3.3 \times 10^{-7}$ cm^{-1}. This is similar to our best value and although the experimental setup is cheaper than the first study by Hallock *et al.*[9] it is still substantially more expensive than the setup used in this study as an acousto optic modulator (AOM) had to be used as a fast shutter to facilitate ring down of the cavity, and fast response detection was still needed. The other studies in Table 2 used much shorter base path lengths and consequently the listed α_{min} values are much higher than those obtained in this study. Table 2 also lists the best LOD value for the analytes studied, where available. For this study the lowest LOD of 4.6 pM was obtained for methylene blue with the $R \geq 0.999$ mirrors. To our knowledge this represents the lowest reported LOD for a liquid-phase absorption measurement. Hallock *et al.*[9] state that LODs of 10 pM could be achieved with their experimental setup for strong absorbers with molar extinction coefficients of 1×10^5 M^{-1} cm^{-1} but no actual measurements on suitable analytes were reported. The second Hallock *et al.* study[10] did not report an LOD for their kinetic measurements on methylene blue, whilst the other studies in Table 2 have much higher reported LODs as a result of much shorter base path lengths.

One advantage of the BBCEAS experiments as shown in Fig. 5 and Fig. 8 is that experiments can be made over a wide wavelength range in a single measurement, usually limited by the bandwidth of the cavity mirrors used or the bandwidth of the light source. The measurements on methylene blue and Sudan black with the white LED and the $R \geq 0.99$ mirrors covered a wavelength range of ~ 150 nm and ~ 250 nm respectively. This allows the full spectral profile of the analyte to be obtained in a single measurement. By comparison, the previous liquid-phase

Table 2 Comparison between this study and previous selected liquid-phase cavity studies as a function of technique, the mirror reflectivity, base path length, the wavelength of measurement λ, the lowest value of α_{min}, the minimum LOD for an analyte, and the molar extinction coefficient ε for that analyte

Study	Technique	Mirror reflectivity	Base path length/cm	λ/nm	α_{min}/cm^{-1}	LOD/M	ε/M^{-1} cm^{-1}
This work	BBCEAS	0.999	20	655	2.8×10^{-7}	4.6×10^{-12}	7.9×10^4
Hallock *et al.*[9]	CRDS	0.9998	21	620–670	1×10^{-6}	$\sim 1 \times 10^{-11}$	$\sim 1 \times 10^5$
Hallock *et al.*[10]	CRDS	0.9998	23	655	3.3×10^{-7}	—	—
Bahnev *et al.*[11]	CRDS	0.9998	0.2	532	1.6×10^{-4}	2.5×10^{-9}	5.4×10^4
van der Sneppen *et al.*[12]	CRDS	0.99996	0.2	532	1.0×10^{-5}	1.5×10^{-8}	1.4×10^4
van der Sneppen *et al.*[13]	CRDS	0.9995	0.2	355	5.0×10^{-5}	7.5×10^{-8}	1.02×10^4
McGarvey *et al.*[14]	CEAS	0.99998	0.175	783	3×10^{-6}	2×10^{-10}	6×10^4
Yao *et al.*[18]	LCW	—	450	540	1×10^{-5}	5×10^{-10}	2.5×10^4

cavity studies listed in Table 2 were predominantly CRDS measurements and, aside from Hallock *et al.*,[9] were carried out at a single wavelength.

A general comparison can also be made with liquid core waveguide studies.[7,8] Taking a representative study on the determination of nitrite using a 4.5 m liquid core waveguide,[18] the stated LOD is 0.5 nM, $\varepsilon \approx 2.5 \times 10^4$ M^{-1} cm^{-1} at 540 nm for the azo dye product used to determine nitrite concentration, then a calculated $\alpha_{min} \approx 1 \times 10^{-5}$ cm^{-1} is obtained. This is significantly higher than the values obtained in this study and is actually quite close to those obtained in our 1 cm base path length HPLC-BBCEAS study.[16] If the experimental setup from this study could be incorporated into a flow injection analysis (FIA) apparatus to determine nitrite then an LOD of ∼18 pM could be obtained with the $R \geq 0.99$ mirrors. However, the sample volume of our setup is much higher than typically used in FIA setups.

One of the most important uses of sensitive spectroscopic techniques is in the quantitative retrieval of unknown concentrations of chemical species. This forms the basis of established techniques such as differential optical absorption spectroscopy (DOAS), which has recently been demonstrated using LED-BBCEAS for atmospheric species.[19] This study has focussed on the measurement of low known concentrations of a given analyte. The methodology used could also be applied to the retrieval of unknown concentrations of a single analyte, by dividing the BBCEAS spectrum by the CEF profile for a given solvent, obtained with a broad absorber such as Sudan black, and knowledge of the cross-section of the analyte. If multiple analytes were present then retrieval techniques similar to those used in DOAS could be employed.

Although this study has demonstrated the most sensitive liquid-phase absorption measurements to date, it is possible to improve the experimental setup in several ways. The simplest method for improving the sensitivity of the measurements would be to increase the base path length of the cavity. This would, however, also increase the volume of the cavity and make it less suitable for analytes which were only available in small quantities and also FIA setups, where <1 mL sample volumes are typically used. This disadvantage could be addressed by using narrower diameter tubing to form the cavity, and also smaller diameter mirrors. The use of 2 mm diameter tubing for example, would reduce the volume of a 20 cm path length cell from ∼100 mL to ∼600 μL. Alignment of the cavity would, however, become considerably more challenging due to increased scattering of the intracavity beam by the cavity walls. Appropriate reduction of the beam diameter by suitable optics would be needed to minimise this.

Further improvements could be achieved either through changing the CCD spectrograph used and/or increasing the intensity of light transmitted through the cavity. As noted earlier, the uncooled CCD detector used in this study suffered from large amounts of dark noise at longer integration times, resulting in poorer than expected results. A more expensive thermoelectrically cooled CCD detector would largely eliminate this problem.

A cheaper alternative could be to use a higher intensity light source. Already, more powerful 5 W white LED light sources are available for low cost. Provided this extra photon flux could be collimated into the cavity this would lead to shorter integration times being used and thus less dark noise. A similar result would be achieved if more efficient collimation of the light source and alignment of the cavity to create greater cavity transmission were possible.

A final method for improving the sensitivity of the measurements would be to use higher reflectivity cavity mirrors to increase the number of passes through the cavity and thus the effective path length. These would only be effective in regions removed from background solvent absorptions. Analysis of the results from Hallock *et al.*[9] show that they achieved ∼3000 passes in their study with $R \geq 0.9998$ mirrors, which is about seven times greater than that achieved in this study. The use of higher reflectivity mirrors would, however, reduce the transmission through the cavity and so would have to be implemented with some of the other improvements suggested above, to be fully effective. It would also be advisable to use mirrors where the design specification takes into account the direct contact between the analyte and the mirrors. The absorption and scattering by the solvent will also limit the maximum mirror reflectivity which can be usefully employed. Analysis of the single pass solvent absorption spectra in Fig. 2 suggests that the minimum loss per pass in a 20 cm cavity is ∼2 × 10^{-4}, which would imply that use of mirrors with reflectivities greater than $R \geq 0.9999$ are unlikely to lead to significant improvement in the number of passes and consequently LODs.

The applicability of the BBCEAS technique would benefit from extension into both the UV and near infrared (NIR) regions as a greater number of analytes could potentially be studied. We are currently exploring measurements in both these regions. This will require the use of high reflectivity mirror sets which cover the UV and NIR regions as well as suitable light sources. The background solvent absorption would be stronger in the NIR as lower CH and OH overtone vibrations are present in this region, whilst measurements in the UV region would be hindered by greater amounts of absorption by solvent impurities, solvent scattering and the onset of electronic transitions in the solvent, but these potential problems may well provide an alternative use for this technique as a sensitive means for studying weak absorptions in solvents, which may be of interest to both experimentalists and theoreticians.

Conclusions

High sensitivity liquid-phase absorption measurements using a low-cost experimental setup and simple experimental methodology based on BBCEAS in a 20 cm cavity has been demonstrated. The solvent was in direct contact with the cavity mirrors, and measurements were made on the analytes Sudan black and methylene blue, dissolved in acetonitrile. Background solvent absorptions due to high overtone vibrations resulted in significant distortion of the absorption spectra at selected wavelengths, and resulted in some restrictions on the choice of analytes. Measurements were made with a white LED and an $R \geq 0.99$ mirror set on Sudan black (between 450 and 700 nm) and methylene blue (between 550 and 700 nm), whilst the use of a red LED also allowed measurements on methylene blue (between 635 and 670 nm) with a higher reflectivity $R \geq 0.999$ mirror set. In the wavelength regions removed from the solvent absorptions the cavity losses were much lower than the previous

measurements with a 2 mm cuvette, whilst with the $R \geq 0.999$ mirror set, a CEF value of 429 was achieved. The best value of α_{min} of 2.8×10^{-7} cm^{-1} was obtained with the $R \geq 0.999$ mirror set, although this value was restricted by dark noise from the long integration time needed, and was only marginally better than the figures obtained with the $R \geq 0.99$ mirror set. Nevertheless, in comparison to previous studies, this value represents the most sensitive liquid-phase absorption reported to date. Likewise, the best LOD value for methylene blue of 4.6 pM is, to our knowledge, the lowest recorded detection limit for a liquid-phase absorption measurement. Improvements to the experimental setup could be made through the use of smaller sample volumes, a thermoelectrically cooled CCD detector, a higher intensity light source, the use of higher reflectivity cavity mirrors and extension of the wavelength range to the UV and NIR.

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